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par

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**Improving the success of the critical peri-partum transition period in
small ruminants: access to physiological and metabolic disturbances
and prospection of effects of natural products to prevent them**

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PERIOD IN SMALL RUMINANTS: ACCESS TO PHYSIOLOGICAL AND
METABOLIC DISTURBANCES AND PROSPECTION OF EFFECTS OF NATURAL
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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
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Research Advisor:

Ph.D., DVM. José Augusto Bastos Afonso

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*"Start by doing what is necessary, then do what is possible;
and suddenly you are doing the impossible"*

Saint Francis of Assisi

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SUMMARY

The *peri-partum* period is a critical time for small ruminants because nutritional requirements are very high and feed intake is often limited. Nutritional diseases, such as pregnancy toxemia, can develop. Farmers try to limit these problems. β -glucan and saponin-based plant extracts have been shown to have favourable effects on animal production: immune-modulation for the former and reduced methane production and modification of rumen fermentation patterns for the latter. However, little is known about their effects on metabolism. Therefore, the general objective was to better understand the effects of 1) β -glucan injections and 2) dietary saponin, on metabolism and rumen fermentation in small ruminants during the transition period. The influence of β 1,3-glucan (i.m. injections, 1ml/animal/week for 60 days) on the metabolic profile of 14 Santa Inês ewes in the last weeks of gestation was studied. Two groups of ewes were formed in late gestation: a glucan group (G, n=7) and a control group (C, n=7). Nutritional requirements during the experiment were covered. Blood samples were collected throughout the study. Blood samples were analysed for metabolites, ions and enzymes. All ewes were clinically healthy. Glucose, L-lactate, non-esterified fatty acids and β -hydroxybutyrate peaked at parturition ($p<0.05$). Aspartate aminotransferase (AST) was higher in C vs. G ($p<0.05$) and there was a similar trend for fructosamine and albumin ($p<0.10$). Urea tended to be higher in G vs. C ($p<0.10$). In conclusion, the changes with time were consistent with a change in physiological status (gestation/lactation). β 1,3-glucan appeared to protect muscle and liver because AST levels were lower than in controls and it did not negatively affect metabolism. The influence of a dietary saponin-based additive (25g saponin/animal/day) on the metabolic and rumen fermentation profile of Saanen and Alpine dairy goats during two different metabolic situations: metabolic neutrality (mid-lactation) and metabolic imbalance (pregnancy/lactation) was studied in two experiments. Experiment 1, used mid-lactating goats (Saponin group (S), n=10; Control group (C), n=10). The 6 week experiment was divided into: 1 week of C diet, 4 weeks either S or C and 1 week C. Experiment 2, used peri-parturient goats (Saponin group (S), n=12; Control group (C), n=12). The 7 week experiment was divided into: 1 week C diet, 4 weeks either S or C (stopping at parturition) and 2 weeks C. Nutritional requirements during the experiments were covered. Blood samples and rumen fluid were collected throughout both experiments. Blood samples were analysed for metabolites, ions and enzymes. All the goats were clinically healthy. There was no effect of saponin in experiment 1 on animal husbandry measures, plasma metabolites and ruminal fermentation ($p>0.05$). The ruminal acetate to propionate ratio tended to be affected by treatment ($S < C$; $p=0.057$). There was no effect of saponin in experiment 2 on blood metabolites ($p>0.05$), except for plasma urea ($S > C$; $p=0.054$). Total protozoa numbers tended to be affected by treatment ($S > C$; $p<0.10$). Most of the animal husbandry, plasma and ruminal variables showed a time effect around parturition ($p<0.05$). In conclusion, dietary saponin during mid-lactation or the *peri-partum* period had little effect on metabolism and ruminal fermentation. The trends observed in blood urea, total protozoa count and acetate/propionate ratio could be potentially beneficial. Lastly, the potential for saponin to influence ruminal fermentation may depend on the level, the period of administration and the type of diet. β -glucan and saponin did not have a negative effect on metabolism and it would be interesting to conduct further work in animals suffering from pregnancy toxemia. Studies could also be conducted on the possible immune actions of β -glucan and saponin.

Key-words: Metabolism; β 1,3-glucan; Saponin; Goat; Ewe; *Peri-partum*.

RESUME

Le péri-partum est une période critique chez les petits ruminants car les besoins nutritionnels sont élevés et l'apport alimentaire souvent limité. Des maladies nutritionnelles comme la toxémie de gestation peuvent survenir et doivent être évitées. Des produits d'origine naturelle comme le β -glucane ou des extraits de plantes à base de saponine peuvent favoriser sur la production : modulation immunitaire dans le 1^{er} cas, réduction des émissions de méthane et modifications du profil fermentaire dans le 2nd. Cependant, leurs effets sur le métabolisme sont mal connus. L'objectif était de mieux comprendre les effets 1) d'injections de β -glucane et 2) de saponine alimentaire, sur le métabolisme et la fermentation ruminale de petits ruminants pendant la transition gestation/lactation. L'influence du β 1,3-glucane (1ml IM/animal/semaine pendant 60 jours) sur le profil métabolique de brebis Santa Inês a été étudiée en fin de gestation (groupes Glucane (G, n = 7) et Contrôle (C, n = 7)). Les besoins nutritionnels étaient couverts. Les métabolites, électrolytes et enzymes plasmatiques ont été dosés. Toutes les brebis étaient cliniquement en bonne santé. Le glucose, le L-lactate, les acides gras non-estérifiés et le β -hydroxy-butyrat ont atteint un pic à la parturition ($p < 0,05$). L'aspartate aminotransférase était plus élevée dans le groupe C vs G ($p < 0,05$) avec une tendance similaire pour la fructosamine et l'albumine ($p < 0,10$). L'urémie tendait à être plus élevée dans le groupe G vs C ($p < 0,10$). En conclusion, l'évolution dans le temps était cohérente avec une modification du statut physiologique (gestation/lactation). Le β 1,3-glucane a semblé protéger le muscle et le foie, les taux d'AST étant inférieurs à ceux des contrôles. Il n'y a pas eu d'effet négatif sur le métabolisme. L'influence d'un additif alimentaire à base de saponine (25g/animal/jour) sur le profil métabolique et de fermentation ruminale de chèvres Saanen et alpines a été étudiée dans deux expérimentations : équilibre (mi-lactation) ou déséquilibre (gestation/lactation) métabolique. Dans la 1^{ère}, des chèvres en mi-lactation ont été utilisées (groupes Saponine (S, n=10) et Contrôle (C, n = 10)). Les 6 semaines ont été divisées en 1 semaine de régime C, 4 semaines soit S soit C et 1 semaine C. Dans la 2^{nde}, des chèvres péri-parturientes ont été utilisées (groupes Saponine (S, n = 12) et Contrôle (C, n = 12)). Les 7 semaines ont été divisées en 1 semaine de régime C, 4 semaines soit S soit C (arrêt à la parturition) et 2 semaines C. Les besoins nutritionnels étaient couverts. Du sang et du contenu ruminal ont été prélevés dans les deux cas. Les métabolites, les ions et les enzymes plasmatiques ont été dosés. Toutes les chèvres étaient cliniquement saines. Dans l'expérience 1, il n'y a eu aucun effet de la saponine sur les paramètres de production, les métabolites plasmatiques et la fermentation ruminale ($p > 0,05$). Le rapport acétate/propionate du rumen a eu tendance à être réduit ($S < C$; $p=0,057$). Dans l'expérience 2, il n'y a pas eu d'effet de la saponine sur les métabolites sanguins ($p > 0,05$), sauf pour l'urémie ($S > C$; $p=0,054$). Le nombre de protozoaires a eu tendance à être augmenté ($S > C$, $p < 0,10$). La plupart des variables de production, plasmatiques et ruminales ont présenté un effet temps autour de la parturition ($p < 0,05$). En conclusion, la saponine alimentaire en mi-lactation ou en péri-partum a eu peu d'effets sur le métabolisme et la fermentation ruminale. Les tendances observées pour l'urémie, le nombre de protozoaires et le rapport acétate/propionate pourraient être potentiellement bénéfiques. L'effet de la saponine sur la fermentation ruminale pourrait dépendre de la dose, de la période d'administration et du type de régime. Le β -glucane et les saponines n'ont pas eu d'effets négatifs sur le métabolisme. Il serait intéressant de réaliser d'autres études chez des animaux atteints de toxémie de gestation ou d'évaluer les actions immunitaires des deux produits.

Mots-clés: Métabolisme; β 1,3-glucane; Saponine; Chèvre; Brebis; Péri-partum.

RESUMO

O período do periparto é um momento crítico para os pequenos ruminantes devido às necessidades nutricionais serem muito elevadas e também devido à ingestão de alimentos que muitas vezes é limitada. As doenças nutricionais, como por exemplo, a toxemia da prenhez, pode desenvolver-se, no qual os criadores tentam evitar esses problemas de diversas maneiras. O medicamento β -glucana e o extrato de planta a base de saponina demonstraram ter efeitos favoráveis na produção animal: imuno-modulação para o primeiro e uma redução da produção de metano e modificação dos padrões de fermentação ruminal para esta última. No entanto, pouco se sabe sobre os seus efeitos sobre o metabolismo. Portanto, o objetivo geral foi entender melhor os efeitos de 1) injeções da β -glucana e 2) aditivo saponina, no metabolismo e fermentação ruminal em pequenos ruminantes durante o período de transição. Estudou-se a influência do β 1,3-glucana (injeções i.m. 1 ml/animal/semana por 60 dias) no perfil metabólico de 14 ovelhas Santa Inês nas últimas semanas de gestação. Dois grupos de ovelhas foram formados no final da gestação: um grupo glucana (G, n=7) e um grupo controle (C, n=7). Requisitos nutricionais durante o experimento foram cobertos segundo o período metabólico estudado e amostras de sangue foram coletadas ao longo do estudo. As amostras de sangue foram analisadas quanto a metabolitos, íons e enzimas. Todas as ovelhas estavam clinicamente saudáveis. A glicose, o L-lactato, os ácidos graxos não esterificados e o β -hidroxibutirato atingiram o pico no parto ($p<0,05$). A aspartato aminotransferase (AST) foi maior em C vs. G ($p<0,05$) e houve tendência semelhante para a fructosamina e a albumina ($p<0,10$). A ureia apresentou uma tendência a ser maior em G vs. C ($p<0,10$). Em conclusão, as mudanças com o tempo foram consistentes com uma mudança no estado fisiológico (gestação/lactação). A β 1,3-glucana pareceu proteger o músculo e o fígado porque os níveis de AST eram mais baixos no grupo controle e não afetou negativamente o metabolismo. A influência de um aditivo à base de saponina na dieta (25g de saponina/animal/dia) sobre o perfil metabólico e de fermentação ruminal de cabras leiteiras Saanen e Alpina durante duas situações metabólicas diferentes: equilíbrio metabólico (meio da lactação) e desequilíbrio metabólico (gestação/lactação) foram estudados em dois experimentos. No experimento 1, utilizaram-se cabras de no meio de lactação (grupo saponina (S), n=10; grupo de controle (C), n=10). O experimento de seis semanas foi dividido em: uma semana de dieta C, quatro semanas S ou C e uma semana C. Experimento 2, utilizou cabras peri-parturientes (grupo Saponina (S), n=12; grupo Controle (C), n=12). O experimento de 7 semanas foi dividido em: uma semana de dieta C, quatro semanas de S ou C (até o momento do parto) e duas semanas de C. Os requisitos nutricionais durante os experimentos foram cobertos. Amostras de sangue e fluido ruminal foram coletados ao longo de ambos os experimentos. As amostras de sangue foram analisadas quanto aos metabolitos, íons e enzimas. Todas as cabras estavam clinicamente saudáveis. Não houve efeito da saponina no experimento 1 em medidas de produção animal, metabolitos plasmáticos e fermentação ruminal ($p>0,05$). Em relação acetato-propionato ruminal houve uma tendência a ser afetada pelo tratamento ($S<C$; $p=0,057$). Não houve efeito da saponina no experimento 2 nos metabolitos do sangue ($p>0,05$), com exceção da uréia plasmática ($S>C$; $p=0,054$). O número total de protozoários tendeu a ser afetado pelo tratamento ($S>C$, $p<0,10$). A maior parte das variáveis de produção animal, plasma e ruminal apresentou efeito de tempo no momento do parto ($p<0,05$). Em conclusão, a saponina na dieta durante o período médio de lactação ou no período periparto teve pouco efeito sobre o metabolismo e fermentação ruminal. As tendências observadas na ureia no sangue, na contagem total de protozoários e na relação acetato / propionato poderiam ser potencialmente benéficas. Por fim, o potencial para a saponina influenciar a fermentação ruminal pode depender do nível, do período de

administração e do tipo de dieta. A β -glucana e saponina não têm um efeito negativo sobre o metabolismo e seria interessante realizar um trabalho posterior em animais que sofrem da toxemia da prenhez. Estudos também poderiam ser realizados sobre as possíveis ações imunológicas da β -glucana e da saponina.

Palavras-chaves: Metabolismo; β 1,3-glucana; Saponina; Cabra; Ovelha; Periparto.

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	– Amino acids
ADF	– Acid detergent fiber
ADL	– Acid detergent lignin
AFNOR	– <i>Association Française de normalisation</i>
AGNE	– <i>Acides gras non-estérifiés</i>
AgroParisTech	– <i>Institut des sciences et industries du vivant et de l'environnement</i>
AGV	– <i>Acides gras volatils</i>
ALP	– Alkaline phosphatase
APP	– Acute phase protein
AST	– Aspartate aminotransferase
BCS	– Body condition score
BRMs	– Biological response modifiers
BT	– <i>Bilirubine totale</i>
BW	– Body weight
Ca	– Calcium
Ca ²⁺	– Ionized calcium
CAPES	– Coordination for the Improvement of Higher Education Personnel
CH ₄	– Methane
CK	– Creatine kinase
Cl ⁻	– Chloride
CP	– Crude protein
CT	– <i>Cholestérol total</i>
DM	– Dry matter
DMI	– Dry matter intake
DVM	– Doctor in veterinary medicine
ECF	– Extracellular fluid
ENVA	– <i>École Nationale Vétérinaire d'Alfort</i>
EU	– European Union
FFA	– Free fatty acids
g	– Gramme
GGT	– Gamma glutamyl transferase
GLUT4	– Glucose transporter 4
H ₂ O ₂	– Hydrogen peroxide
Hb	– Haemoglobin
HbA1c	– Glycated hemoglobin
HCO ₃ ⁻	– Bicarbonate
HDL	– High-density lipoprotein
Hp	– Haptoglobin
HR %	– Hygrometry
i.m	– intramuscular
ICF	– Intracellular fluid
ILs	– Interleukins
INRA	– <i>Institut National de la Recherche Agronomique</i>
K ⁺	– Potassium
Kg	– Kilogramme
LDL	– Low-density lipoproteins
LHD	– <i>Lipoprotéines de haute densité</i>
LPS	– Lipopolysaccharide

LS	– Least square
MFS	– Methylgreen formalin-saline solution
mg	– Milligram
Mg ³⁺	– Magnesium
mL	– Milliliter
MoSAR	– <i>Modélisation Systémique Appliquée aux Ruminants</i>
MOSs	– Mannan oligosaccharides
MY	– Milk yield
N	– Nitrogen
Na ⁺	– Sodium
NDF	– Neutral detergent fiber
NEB	– Negative energy balance
NEFA	– Non-esterified fatty acids
NH ₃	– Ammonia/ <i>Ammoniaque</i>
NK	– Natural killer
NO	– Nitric oxide
OM	– Organic matter
P	– Propionate
PAL	– <i>Phosphatase alcaline</i>
pH	– Potential hydrogen
PL	– <i>Production laitière</i>
PO ₄ ³⁻	– Phosphorus
PSMs	– Plant secondary metabolites
PT	– Pregnancy toxemia
PT	– <i>Protéines totales</i>
PV	– <i>Poids vif</i>
RMT	– <i>Ration mixed totale</i>
RNA	– Ribonucleic acid
s.c	– subcutaneous
SAS	– Statistical analysis software
T°C	– Temperature
TB	– Total bilirubin
TC	– Total cholesterol
TMR	– Total mixed ration
TNF	– Tumor necrosis factor
TP	– Total protein
TS	– Tea saponin
UFRPE	– <i>Universidade Federal Rural de Pernambuco</i>
UMR	– <i>Unité Mixte de Recherche</i>
VFA	– Volatile fatty acid
VLDL	– Very low-density lipoproteins
W	– Week
YE	– <i>Yucca schidigera</i> extract
βHB	– Beta-hydroxybutyric acid
%	– Percentage
°C	– Degree Celsius
μL	– Microliter

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1. GENERAL INTRODUCTION

Ruminants have been through a series of evolutionary steps influenced by environmental and climate shifts, and have adapted to different nutritional diets due to the increase in demand for food caused by the rise in the human population. In addition, they have shown extraordinary adaptive mechanisms for digestibility efficiency, regardless of the body condition and their ability to regulate water metabolism in drought conditions. Incorporation of relatively high proportions of easily degradable carbohydrates in the diet of ruminants increases the risk of ruminal/metabolic diseases. Today the increase in food demand caused by the rise in the human population has forced ruminant production systems to replace the fiber based diet by diets which use greater proportions of grains. The evolutionary responses to these new challenges will drive future research. Therefore, morphology, physiology, nutrition and evolution knowledge will help to minimize insults to ruminant health and to obtain the production efficiency required by the modern world (de Tarso et al., 2016).

Ruminants as a part of the livestock structure, occupy an important role in the present and future of the global system (Herrero & Thornton, 2013) and in the maintenance of an environmental equilibrium in wild habitats (Hungate et al., 1959 cited by de Tarso et al., 2016). The challenge of feeding a growing population, together with changes in consumption patterns will increase the demand for livestock products. Thus, considering that domestic ruminants are responsible for the production of 50% of the meat and 41% of milk in developing and developed countries, the understanding of ruminant biology has a substantial relevance for future food production (Steinfeld et al., 2006; McMichael et al., 2007; Hackmann & Spain, 2010; Steinfeld & Gerber, 2010). The production capability of domestic ruminants has changed over the past decades, either by massive improvements in genetics to follow the demand in consumption, or by the natural evolution throughout the centuries (Clauss et al., 2010).

Despite the fact that these feeding shifts might enhance production efficiency, they put a strain on ruminant physiology (Allen, 1997; Enemark et al., 2002; Dijkstra et al., 2012). Modern production systems include several practices and many dietary changes during different physiology situations (Banninka et al., 2012). The addition of rapidly fermentable non-structural carbohydrate lowers the proportion of ruminal fiber, changes the pattern of gas production, and increases the chances of metabolic and ruminal diseases (Enemark et al., 2002; Kleen et al., 2003). Recent studies found that long-term feeding of a high-concentrate diet provokes the accumulation of volatile fatty acids in ruminal fluid and colonic digesta, and

damages the colonic mucosa inducing cell apoptosis in lactating goats (Tao et al., 2014; Tao et al., 2015).

Although dietary changes can increase the risk of ruminal/metabolic diseases, the nutritional changes which have occurred during the past decades may cause drastic modifications to the future evolutionary adaptations of the digestive system (Clauss et al., 2003). Based on genetic/physiological evidence of individual animal variations in feed intake, studies are trying to enable genetic selection of ruminants for residual feed intake to improve production system efficiency (Herd & Arthur, 2009; Moore et al., 2009).

Small ruminants are known for their excellent ability to utilise fibrous feeds; however production effectiveness is generally lower in these species than in other farm animals. Therefore, attempts are made to supplement animal diets with feed additives that stimulate female productivity and increase milk production to ensure optimal offspring-rearing conditions (Milewski & Sobiech, 2009). The productive requirements of farmers (genetic selection and the use of intensive rearing systems) have increased the risk and the incidence of nutrition imbalances and metabolic disorders in herds. This is due to increasing nutrient requirements during gestation and lactation in response to increasing milk yield (González et al., 2000; Campos et al., 2010).

One of the goals of intensive farms is to limit and if possible, to predict nutritional and metabolic disturbances during critical physiological periods, such as the *peri-partum* period. This physiological phase in ruminants is particularly critical because in this period, the energy needs of pregnant animals are greatly increased and the ability to meet these demands is very limited. The high requirements in productive systems and intensive management in modern farms have increased the risk and incidence of nutritional imbalances and metabolic disturbances in sheep and goats during the *peri-partum* period. Moreover, this period is a phase where changes occur in animal metabolism, due to accelerated fetal growth, and to the production of colostrum, thus, characterizing a critical window for the occurrence of metabolic diseases and deficiencies. Many biological systems can be disrupted such as the reproductive and immune system, and the mammary gland (González et al., 2000; Campos et al., 2010).

A variety of useful alternatives to synthetic drugs in preventing and treating various diseases have the possibility to improve dry matter intake and production performance. One such natural stimulator, with probiotic and prebiotic properties, is *Saccharomyces cerevisiae* yeast, which has a wide spectrum of activity (Milewski & Sobiech, 2009). Indeed, the use of β -glucan produced from *Saccharomyces cerevisiae*, which belongs to a group of biologically active compounds, is also called biological response modifiers. Therefore, β -glucan is well

recognized in humans and animals for having an immunomodulatory action as its main characteristic. Several studies have shown that this component has immunostimulatory properties including antitumor and antibacterial activities. It can also reduce levels of cholesterol and triglycerides, normalize blood glucose levels, heal and rejuvenate the skin and it acts against stress (Akramienè et al., 2007; Novak & Vetvicka, 2009; Vetvicka & Vetvickova, 2010).

In recent years, there is an increasing interest in exploiting natural products such as phytogenic feed additives for livestock, due to the fact that they are an alternative strategy to replace antibiotic growth promoters. As pointed out by Wallace et al. (2002), consumers and health authorities in developed countries, especially in the European Union, have controlled the use of chemical feed additives, including antibiotics and ionophores in livestock feed since 2006 (Council Regulation (European Council) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition). Natural products used as feed additives must be harmless to animals, not leave toxic residues in animal bodies or animal products and they must be safe for the environment. Therefore, the purpose of this regulation is to establish a community procedure for authorizing the marketing and the use of feed additives. It also lays down the rules for the supervision and labelling of feed additives and premixtures in order to provide the basis for the assurance of a high level of protection of human and animal health and welfare, the environment, and “users and consumers” interests in relation to feed additives, whilst ensuring the effective functioning of the internal market (Wallace et al., 2002; Franz et al., 2005; Santoso et al., 2007; Vrublova et al. 2010).

At the present time, propylene glycol, a synthetic chemical, is the only compound regularly used by farmers, as a glucose precursor, in order to prevent metabolic diseases. Moreover, there is particular interest in finding another compound of natural origin with similar effects. Some plants, due to their complex compositions can be used as food additives with multiple biological activities. This is the case of saponin-based additives, currently mainly produced from *Yucca schidigera*. Saponins are common in a large number of plants and plant products. It has an important role in human and animal nutrition. Saponins have biological roles such as modifying membrane-permeability, immunostimulation and hypocholesterolemic properties and it has been found to have significant effects on growth and feed intake in animals. These compounds have been observed to reduce protozoa, to impair protein digestion and the uptake of vitamins and minerals in the gut and to act as hypoglycaemic agent. These compounds thus affect animals in both positive and negative ways (Das et al., 2012).

One of its impacts is to decrease methane emissions as previously demonstrated in ruminants, but there are also some beneficial effects on energy metabolism during periods of metabolic imbalance which need to be confirmed. In spite of the intensification of production systems and the development of production, researchers have begun to search for indicators that assess the metabolic status of animals in more detail. The purpose of techniques is to detect metabolic disorders as early possible, in order to rectify them immediately and avoid expensive veterinary treatment and a decrease in production (Lila et al., 2005; Silva Filho, 2016).

Several metabolic profiles exist (energy, proteins, lipids etc...). Each profile is based on a specific list of parameters which are measured in the blood. The study of metabolic profiling is a systemic approach, based on laboratory measurements of blood and rumen fluid parameters over a certain period. It reflects nutritional status and can be used to predict or diagnose clinical abnormalities and metabolic disorders in small ruminants (González et al. 2000; Caldeira et al. 2007; Balikci et al. 2007; Lima et al., 2016). In general, the use of metabolic profiles on farms has not been adopted extensively, as it is thought that blood parameters cannot predict the metabolic status of an animal without a characterization of its diet and phase and level of production (Caldeira et al, 2007).

The information provided by strategic metabolic indicators and body condition score, for example, can however provide a more substantial basis concerning the knowledge of the metabolic status of the small ruminant and therefore, diets can be adjusted and metabolic disorders prevented and production can be improved. In animal production, crucial consideration is given to rational feeding since this may contribute to improve the meat and dairy quality (Caldeira et al. 2007).

2. REVIEW OF THE LITERATURE

2.1 METABOLIC PROFILES

The criteria that were previously used to establish optimum nutrient intakes for ruminants were not always adequate. In the pregnant animal's body for instance, body weight (BW) has been used as a criterion for measuring nutrient adequacy. However, the change in weight of the fetus confuses the interpretation of a change in the BW of the female. In nutritional studies where short-term effects are of interest, the use of BW, milk production, or wool production is often not satisfactory as a measure of response. In order to demonstrate a response in these situations it is necessary to establish criteria that can be accurately measured and that are closely associated with the metabolic processes within the animal's body. Since most physiological processes in the animal body involve transport of metabolites by the blood, therefore metabolites could be helpful in detecting changes in types or rates of biochemical processes related to growth or productivity (Bowden, 1971).

Metabolic profile evaluation has been used for a long time in veterinary clinics. However, the metabolic profile became a animal husbandry term only in the 70's, where the metabolic constituents are used as an auxiliary method in the evaluation of productive and reproductive performance in production ruminants (Payne et al., 1970; Peixoto & Osório, 2007). Blood is the fluid mostly used to check the indicators of nutritional or metabolic status, both for the quality of information which it provides, and also the ease of obtaining samples. The use of blood parameters for assessing the metabolic state began with a group at the Institute of Research on Animal Diseases in England (Caldeira, 2005).

The mechanism that alters the blood metabolite levels of energy and protein in small pregnant ruminants have not been studied sufficiently yet (Thomas et al., 2001), especially when considering the racial factor, since many studies have been conducted in certain breeds of small ruminants such as the Santa Inês sheep, however, nothing has been done with Dorper sheep (Soares et al., 2014).

The biochemical variables of ruminant metabolic profile may be used to monitor and diagnose metabolic-nutritional adaptations and imbalances such as during the transition period (González et al., 2000; Caldeira, 2005; Caldeira et al., 2007). According to Russel (1991), the fastest method to evaluate the nutritional balance in small ruminants, at critical periods, is the determination of certain metabolites in the bloodstream. Therefore, the analysis of these

metabolites has been a valuable approach; however, their interpretation is quite complex due to the mechanisms that control blood levels of various variables and the large variation in these levels due to factors such as regional characteristics, breed, species, age, diet, physiological state, seasonality periods, nutritional management and the production system that are all taken into consideration. In addition, recent studies have shown that the peri-parturient period has a significant influence on the metabolic profile of serum biochemical parameters such as total proteins, globulin, creatinine, urea, cholesterol, triglycerides and plasma glucose (González et al., 2000; Soares et al., 2014). However, variations in blood metabolite concentrations can be caused by nutrient excess or deficiency in the diet, but there is also an interrelationship between nutrients, which can lead to errors of interpretation if metabolite variations are analyzed simply for an increase or decrease (Contreras & Phil, 2000).

2.1.1 Transition Period

The transition period between late pregnancy and early lactation presents an enormous metabolic challenge to the high-yielding dairy ruminant. This period from 3 weeks before to 3 weeks after parturition, is critically important to maintain health, production, and profitability of these animals. Most health disorders occur during this time, compared with other stages of the lactation cycle, relatively little is known about fundamental biological processes during the transition period. This period starts when a female goes from pregnancy and no lactation to non-pregnancy and lactation, leading to a stress due to severe and abrupt changes in their metabolism (Grummer, 1995; Drackley, 1999; Rabelo et al., 2005).

The transition period in ruminants is marked by intense physiological, hormonal, metabolic and anatomical changes. These changes are associated with a decrease in feed intake, high demands for nutrients and rapid fetal growth, which result in negative energy balance. Depending on the intensity the animals can be affected by metabolic disorders, resulting in decreased milk production, and reduced reproductive performance and increased herd culling rate (Drackley et al., 2005; Tharwat et al., 2013). The rapid onset of lactogenesis soon after parturition requires large amounts of nutrients; moreover feed intake is low in this period due to endocrine changes that may be particularly responsible for an accelerated decline of dry matter intake (DMI) that starts in the last weeks of pregnancy (Bell, 1995; Goff, 2006).

The regulation and coordination of lipid metabolism among adipose tissue, liver, gut, and mammary gland are key components of the adaptations to lactation. Lipid accumulation in the liver may contribute to health disorders and decreased milk production. Knowledge of key

elements of the control system are lacking, as is an understanding of the metabolic effects of hormones, growth factors, and cytokines that mediate stress. Recent evidence indicates that supplemental fats or restricted feed intake before parturition can induce a coordinated set of metabolic changes in the metabolism of long-chain fatty acids, including peroxisome β -oxidation, possibly mediated by peroxisome proliferator-activated receptors. Estimations of the mixture of fuels for metabolizable energy in ruminants during early *post-partum* period suggests that supply of amino acids and glycogenic compounds may be too low. Because dietary fat does not inhibit body lipid mobilization, during the early *post-partum* period supplemental fat may further imbalance the mixture of fuels and lead to decreased dry matter intake (Drackley, 1999).

A suboptimal transition from the late-gestation period to lactation can impair production and reproductive performance and cause economic losses. Due to the aforementioned, this period is seen as the most stressful time in the production cycle of a dairy ruminant. Therefore, an optimal transition requires a comprehensive understanding of the biochemical events occurring during the peri-parturient period and should decrease health problems and increase profitability of dairy ruminants (Drackley et al., 1999; Guo et al., 2007).

Most of the available data describing metabolism of dairy cows during the transition period is based on only a few measurements obtained over a large interval of time, such as a week or longer. However, measurements of blood metabolites are associated with great variations, indicating large fluctuations in metabolite profiles during the peri-parturient period (Drackley et al., 1999).

In small ruminants on the other hand, the transition period has gained little attention. Measurement of blood metabolites in sheep and goats should be performed more frequently to capture the dynamic changes in the peri-parturient period (Khan & Ludri, 2002a, b; Skotnicka et al., 2011; Santos et al., 2012; Silva et al., 2013).

The correct interpretation of a metabolic profile is indispensable to know the appropriate reference values for the region and the population. In the case where these values are not known, the reference values to be used should be for climatic zones and similar groups of animals (González & Scheffer, 2003).

2.1.2 Energy profile

Ruminants are obligate herbivores whose evolutionary success has, in large part, resulted from their pregastric, fermentative mode of digestion. This allows them to efficiently

utilize cellulose and other fibrous feed components, and get most of their protein requirements from the digestion of rumen microbes. However, it also ensures that more readily digestible, non-structural carbohydrates, including starches and sugars, are subjected to microbial fermentation in the reticulorumen before they can become available for amylolytic digestion and absorption in the small intestine. This means that in the recently fed as well as the post-absorptive state, ruminants must depend almost exclusively on gluconeogenesis in liver and to a lesser extent, kidneys, for their tissue glucose requirements (Bell & Bauman, 1997).

In ruminants, most of the carbohydrate feed is fermented in the rumen, giving rise to short-chain fatty acids: mainly acetate, propionate and butyrate. They represent the main source of energy (Kosloski, 2005). In the digestion process of these animals, only a small part of the glucose from the alimentary tract arrives in the bloodstream and most of it is oxidized by ruminal bacteria. The organ responsible for the synthesis of glucose is the liver which uses precursor molecules for gluconeogenesis. Propionic acid, is the main substrate accounting for approximately 50% of production of glucose, gluconeogenic amino acids (glutamine and alanine) and lactic acid contribute 25% and 15% respectively; moreover another important precursor is the glycerol (González, 2000). The importance of hepatic gluconeogenesis is highlighted by the fact that glucose requirements are more than doubled in late-pregnant ewes carrying twins, and increased 4-fold in genetically superior, lactating dairy cows compared to their non-pregnant or non-lactating counterparts (Bell & Bauman, 1997).

Endocrine changes directly influence the ruminant's energy profile, through a decrease in feed intake and an increase in the demands for nutrients, inducing lipolysis (Drackley, 1999). This mobilization occurs because of NEB and can trigger severe liver injury due to fatty infiltration, thus directly affecting energy production (Souza et al., 2004; Souto, 2013). The vital organs are extremely susceptible to energy deficits and this is correlated with low reproductive performance, a delay in age at puberty, an increase in the interval between parturition and the first ovulation and *post-partum* estrus, plus a reduction in conception rates and pregnancy rates in beef and dairy ruminants. Hypoglycemia depresses nervous activity and results in a reduction in GnRH secretion by the hypothalamus which induces reduced ovarian activity (LeBlanc et al., 2006).

The level of nutrient intake is associated with variations in the metabolism of fat in the body, especially the mobilization of fat deposits during low intakes. Related to this process are changes in several biochemical indicators which have been studied in high producing ruminants, the main metabolites associated with energy balance are glucose, fructosamine, non-

esterified fatty acids (NEFA) and β -hydroxybutyrate (β HB) (Bowden, 1971; Kida, 2003; Enemark et al., 2004).

2.1.2.1 Glucose

Glucose is one of the most widely used blood metabolites to determine the energy status of ruminants. However, some studies have shown a certain difficulty in exploiting the results because the homeostatic mechanism that controls blood glucose levels has become difficult to interpret objectively due to a link between nutritional status and glucose levels. In addition, many tissues use free fatty acids (FFA) and ketones bodies as an energy source, despite the fact that the liver of these animals has a high gluconeogenic function (Payne & Payne, 1987; Peixoto & Osório, 2007).

When it comes to the ruminant species, this metabolite is extremely important due to the central role it has in mammary gland function in supplying components such as carbon, hydrogen and oxygen for the synthesis of lactose. Moreover, the synthesis of lactose is responsible for controlling the volume of milk produced due to the high osmotic pressure it induces. Glucose plasma concentrations are stable during the transition period, with an increase only being seen during labor and immediately after parturition. This increase is linked mainly to rises in glucagon concentrations and glucocorticoids (Kunz et al., 1985; Vazquez-Añon et al., 1994).

In well-fed ruminants, the main precursor for hepatic gluconeogenesis is propionate, one of the major VFA byproducts of pregastric fermentation, which is absorbed via the ruminal epithelium into portal venous blood and almost quantitatively removed by the liver. The rate of ruminal production of propionate and other VFA is directly related to dietary intake of fermentable substrate; propionate synthesis is especially favored by fermentation of starches by amylolytic bacteria. Since the hepatic supply of propionate is a principal determinant of hepatic glucose synthesis; it is not surprising that in all classes of ruminant whole-body glucose production is highly correlated with digestible energy intake. As the supply of propionate dwindles, the importance of other glucogenic substrates, such as lactate, amino acids, and glycerol, increases (Bell & Bauman, 1997; Caldeira, 2005).

Blood glucose levels may be changed as a function of the availability of precursors for the synthesis of glucose. Low metabolizable energy intake can cause a reduction in propionate in the rumen, which is the main factor responsible for a decrease in glucose levels (Reynolds et al., 2003).

Monitoring and/or evaluating the energy status of ruminants via glucose has been identified in previous studies as an imperfect indicator due to its insensitivity to nutritional changes and its sensitivity to stress. Nevertheless glycemia could still be used in situations of severe energy deficit and in animals that are not pregnant and during lactation (Mundim et al., 2007). Previous studies on sheep and goats with pregnancy toxemia confirmed that glucose is not a reliable indicator of metabolic disorders when referring to this disease, since a common finding is that the animals the most affected had hyperglycemia and this was explained by a stress induced increase in cortisol levels (Santos et al., 2011; Souto et al., 2013).

At the end of pregnancy, glucose requirements are absolute and are not controlled by small ruminants when there is transfer of glucose from the dam to the offspring(s), the latter use it and glucose does not return to the maternal bloodstream. Moreover, when feed intake is too low to meet fetal energy demands, especially for females carrying twin fetuses, the dam uses its own body reserves to try to maintain the correct glucose balance (Bruère & West, 1993). In addition, the glycemic profile is the most important indicator during the last days of gestation as a predictor of fetal viability.

This information about glycemia has a great importance in the development of therapeutic protocols, especially by the intravenously administration of glucose in sick animals (Lima et al., 2012). Glucose in the pregnant goat and ewe is the major source of energy to the fetus(es). Therefore, pregnant goats are at high risk of developing pregnancy toxemia due to the rapid foetal growth (Bergman, 1993). The energy requirement of the pregnant goat increases by a factor of 1.5 when it carries one fetus and by a factor of 2 when it carries two fetuses (Pugh, 2005). Blood glucose levels in pregnant goats are generally low, because of fetal demand. There is little information in the literature addressing the occurrence of hyperglycemia in pregnant does. Studies showed that as the disease progresses in ewes, blood glucose and cortisol levels may increase due to fetal death. Wastney et al. (1983) suggested that hyperglycemia occurs because fetal death removes the inhibitory effect of the fetus on hepatic gluconeogenesis. Smith and Sherman (2009) referred to the existence of a marked hyperglycemia in terminal cases.

2.1.2.2 Non-Esterified Fatty Acids (NEFA)

In the ruminant, ketones are produced by intermediary metabolism and during the absorption of butyric acid from the rumen. In the *peri-partum* period the main source of ketones is fatty acids including those with short, medium and long chains. Of course, any compound (glucose, lactate, glycerol, amino acids, etc.) that can be converted to fatty acids and can be

considered a source of ketone, but the origin of ketones is considered to be fatty acids, either esterified or nonesterified (Kaneko et al., 2008).

A massive mobilization of NEFA from adipose tissue during and after parturition in high-yielding dairy ruminants is the metabolic hallmark of the transition from pregnancy to lactation (Bell, 1995). There is an abrupt shift in the metabolic demands from crucial nutrients (body reserves and fetal mass) to rapid mobilization of lipid and protein stores in support of the sudden onset of high milk production (Adewuyi et al., 2005).

NEFA are released into blood when adipose tissue is mobilized to supply the metabolic needs of the animal, primarily the need for energy. Although the quantity of NEFA in the blood of ruminants is small, it is an important factor in caloric homeostasis of the body. In other words, high levels of this metabolite reflect the magnitude of fat mobilization of body reserves, usually associated with the period of insufficient energy consumption, and are an effective way to evaluate the energy status of ruminants (Bowden, 1971; LeBlanc, 2010).

Increased lipolysis and decreased lipogenesis are stimulated by negative energy balance (BEN), observed when the animals do not meet their requirements through the diet, in addition to the high demands for fetal growth and milk production. It is necessary to use alternative sources for energy production through increased lipid mobilization, which is used as an energy source by many tissues, including the mammary gland and the liver (Bell, 1995; Grummer, 1995). Furthermore this mobilization results in decreases in body condition score and weight of animals, leading to endocrine and metabolic disturbances (Rodrigues et al., 2006; Artunduaga et al., 2011).

Circulating NEFA comes from the hydrolysis of triglycerides deposited in adipocytes and these metabolites are released and carried into bloodstream by albumin, which provides the necessary solubility for their circulation in the blood (Caldeira, 2005). Their accumulation due to the inability of the liver to metabolise them can trigger metabolic disorders. Accordingly, this will expose the animals to increased risks of diseases such as subclinical and clinical pregnancy toxemia, uterine diseases, decreased milk production and reduced reproductive performance (Pullen et al., 1989; Radostits, 2000). Consequently, the blood concentration of NEFA depends on the degree of fat mobilization in response to NEB (Van Saun, 2000).

An increase in NEFA concentrations is observed as pregnancy progresses and this provides some advantages to the pregnant ruminant, acting as a source of energy for dam metabolism and promoting the development of an insulin resistant state (Regnault, 2004). This mechanism reduces the utilization of glucose by the peripheral tissues, through the inhibition of the action of insulin, thus preserving and providing the glucose for placental and fetal

metabolism (Brockman, 1979; Regnault, 2004). An elevation in plasma NEFA occurs commonly during parturition, but it is observed before the animal presents symptoms of metabolic disease (Barakat et al., 2007). Therefore, previous studies reported that the level before parturition is an important tool to predict the mobilization of body reserves due to high energy demands at this stage. This situation allows early detection in ruminants at risk of developing disorders associated with NEB (Grummer, 2002; Souto et al., 2013).

Sheep in the early lactation period have higher energy demands when compared to pregnancy and the dry period due to milk synthesis (Abdelrahman et al., 2002; Karapehlivan et al., 2007). In the *post-partum* period, the rate of lipolysis overrides lipogenesis, providing greater amounts of NEFA to supply peripheral tissues. Bell (1995) noted that NEFA provide about 40% of the fat in milk during the first days of lactation. In ruminants, mammary uptake of NEFA depends on their circulating concentrations. It is important to realize that fatty acids in milk come from two sources, uptake from the circulation and synthesis within the mammary epithelial cells. The free fatty acids taken up from the circulation by the mammary gland are derived from circulating lipoproteins and NEFA that originate from respectively absorption of lipids from the digestive tract and from mobilization of body fat reserves (Adewuyi et al., 2005).

In the liver, NEFA metabolism depends on the availability of glucose and on the mobilization rate, because they may be completely oxidized for energy production or partially oxidized for the production of ketone or reesterified and stored as triglycerides. The ruminant liver has a limited capacity to export triglycerides as low density lipoproteins, so that high mobilization in relation to low exportation leads to hepatic steatosis and predisposes the body to metabolic disorders (Head & Gulay, 2001).

2.1.2.3 β -Hydroxybutyrate (β HB)

Ruminants usually have higher ketone body concentrations in blood plasma than monogastric animals due to postprandial production of β -hydroxybutyrate (β HB) in the ruminal epithelium. Even higher concentrations of ketone bodies are present in ruminants suffering from clinical or subclinical ketosis. A NEB during the transition period around parturition is regarded as the primary cause of the development of the disease and the development of hyperketonemia in small ruminants and cattle. Sustained hyperketonemia is probably the most characteristic biochemical sign of spontaneous clinical and subclinical ketosis of sheep, goats and cattle. In ketotic animals, when feed intake has ceased, ketone bodies are almost exclusively produced by the liver from β -oxidation of fatty acids (Schlumbohm & Harmeyer, 2004).

Ketone bodies are energy sources in the absence of carbohydrates and lipids in ruminants and their precursors are lipids and fatty acids from the diet, as well as fat deposits. Butyric acid is produced in the rumen and transformed in the epithelium of the pre-stomachs via acetoacetate to β HB (Wittwer, 2000; Wittwer et al., 2006). β HB assays are important clinical tools for assessing nutritional status and adaptation to the NEB (Chung et al., 2008). Amongst the ketones bodies (β HB, acetoacetate and acetone), β HB is the most widely used indicator of NEB due to its stability in serum, and from the fact that it is not influenced by stress and its blood concentration in NEB situations is not limited by the availability of a carrier (Caldeira, 2005). The severity and duration of NEB is reflected by the increase in circulating NEFA and β HB and the degree of the decrease in glucose concentrations (Drackley, 1999). The decreased DMI prepartum causes NEB and increases NEFA and β HB concentrations (LeBlanc, 2010).

Most ketone bodies produced are used as a source of energy for skeletal and cardiac striated muscles. In the case of the adrenal cortex, acetyl CoA which comes from acetoacetate is not only used as an energy source but also as a substrate for the synthesis of cholesterol and steroid hormones (Araújo, 2009). Some evidence suggests that there is a deleterious effect of FFA on the sensitivity and action insulin, moreover they act by inhibiting the secretion of insulin by the pancreatic beta cells and may also stimulate cellular apoptosis (Corrêa et al., 2010).

2.1.2.4 L-lactate

In healthy ruminants, the concentrations of L-lactate in the blood depend on diet and rumen function, physiological state (early pregnancy and lactation), physical activity and stress (Allison et al., 2008). Guideline values for normal ruminant serum lactate concentrations are between 0.5 and 2.0 mmol/L and for rumen fluid normal ranges between 1-20 mmol/L have been found (Moller et al., 1997).

Lactic acid is produced in significant amounts during microbial fermentation in the rumen, especially when the diet comprises of easily digestible carbohydrates such as starch and sugars. Under such feeding conditions the lactate concentration in rumen fluid may rise and the rumen pH decreases gradually. At this stage, the buffering capacity of rumen fluid is reduced, and at increasing lactate concentrations rumen acidosis is likely to develop. Lactic acid has two possible routes of transport out of the rumen: passage with rumen fluid to the abomasum

and small intestine for absorption or direct absorption through the rumen wall into the blood (Moller et al., 1997).

Physiological status also causes an increase in L-lactate at parturition which could be due to the presence of lactate anions originating from the production of lactic acid. This acid is derived from anaerobic glycolysis due to decrease tissue oxygenation. Lactic acid may affect the permeability of cell membranes, especially muscle cells and some enzymes may also leak into the bloodstream, particularly creatine kinase (Thrall, 2006; González & Silva, 2006).

L-lactate is produced by mammalian cells (Moller et al., 1997). L-lactic acidosis is relatively common, occurring primarily as a result of tissue hypoxia, but also due to drugs and toxins, inborn errors of metabolism, and underlying disease states. L-lactate is rapidly metabolized to become pyruvate by L-lactate dehydrogenase in the liver (Ewaschuk et al., 2005). Both D-lactate and L-lactate are produced in the rumen. When D- and L-lactic acid accumulate more rapidly than they are absorbed, rumen pH falls and rumen atony develops. Some L-lactate is absorbed and metabolized by the liver and other tissues, but D-lactate cannot be utilized and contributes significantly to the acid load of the body (Kaneko et al., 2008)

Previous studies have shown a lacticidemia condition which is compatible with metabolic acidosis that is observed in animals affected by pregnancy toxemia (Barakat et al., 2007; Santos et al., 2011). This observation is different from that detected by Ferris et al. (1969) who did not find an elevation in L-lactate in the blood and no significant metabolic acidosis in sheep affected by this disease. Lacticidemia seen in sheep with pregnancy toxemia is caused by ketonemia, which arises as a consequence of the exacerbated oxidation of NEFA, since this generates excessive production of ketone bodies (acetoacetate, acetone and BHB), and as these are anions have acid characteristics, which raise the gap anion and reduce the concentrations of HCO_3^- , Cl^- , Na^+ and K^+ , which is typical of metabolic acidosis. Associated with this disorder, dehydration is also observed in small ruminants and triggers the glycolytic process (anaerobic glucose), producing lactate from glucose, and contributing to the lacticidemia (Naylor et al., 1984; Kaneko et al., 2008).

2.1.2.2.5 Fructosamine

Fructosamines are stable ketoamines formed by the covalent non-enzymatic reaction of glucose with amino groups of proteins, mainly albumin (Armbruster, 1987; Kaneko et al., 2008). Fructosamine has been used to differentiate between persistent and transient hyperglycemia induced by stress, thus it is a stable ketoamina formed continuously by the

reaction between glucose and amino groups of circulating proteins and its blood concentration is controlled by the balance between synthesis and disposal of these protein compounds and glucose (Thrall, 2006; Kaneko et al., 2008). Therefore, nowadays the determination of fructosamine is considered to be the gold standard for monitoring glycemia and control in cases of human diabetes (Moraes et al., 2011).

Due to the importance of glucose in the intermediary metabolism and its relationship to amino acid and lipid metabolism, the measurement of the glucose supply to the body may also be a useful tool for monitoring the health and metabolic status. However, direct measurements only show the momentary blood glucose concentration, which is subject to rapid and frequent changes (depending on diurnal, dietary and individual factors). In human medicine, the measurement of a product of blood protein glycation (HbA1c and Fructosamine) has been established as an indicator of glycaemia over a long period of time (Armbruster, 1987; Filipovic et al., 2011). In addition, fructosamine levels represent a mean value of glycemia in the two weeks prior to its measurement (Kaneko et al., 2008).

As pointed out by Braun et al. (2010) there is a lack of research related to the concentration of fructosamine in small ruminants. On account of metabolic alterations during late pregnancy and early lactation in sheep, the analysis of fructosamine concentrations in blood reflects more accurately the changes in relation to the concentrations of proteins and glucose. Fructosamine concentrations provide an indirect index of average glycemia in human and monogastric animals. Although fructosamine has been proposed as a marker of hypoglycemia (Cantley et al., 1991) and hypoproteinemia (Stear et al., 2001) in sheep, they are not frequently used. Previous studies recorded in sheep and goats affected by metabolic disorders have showed different fructosamine concentrations when compared to healthy small ruminants.

Cantley et al. (1991) reported lower concentrations of fructosamine in pregnancy toxemic ewes compared to healthy ewes, suggesting persistent hypoglycemia due to pregnancy toxemia. On the contrary, other studies reported high values of fructosamine in ewes with pregnancy toxemia and in goats with diabetes during lactation (Santos et al., 2011; Yattoo et al., 2015).

Yattoo et al. (2015) have studied the prevalence of subclinical diabetes in a commercial flock of dairy goats in India and its interaction with milk quality. They observed higher levels of fructosamine and glycated hemoglobin in diabetes. These metabolic alterations may reflect the ineffective action of insulin and hyperglycemia, which causes glycosylation of proteins and thus, increases levels of glycated hemoglobin and fructosamine. Glycation of proteins and

receptors affect enzyme and hormone activity and thus, may have been the cause of the metabolic disturbance in subclinical diabetic goats.

In this context, the analysis of fructosamine is based not only on glucose, but also on total protein, albumin, cholesterol and urinary glucose. Indeed, in a study with Dorper sheep during the *peri-partum* period, it was possible to identify a negative linear profile of fructosamine concentration as gestation progressed and at the time of parturition its concentration was lower (Soares et al., 2014).

2.1.3 Lipid profile

During the first half of pregnancy amongst the metabolic processes, anabolism is predominant, e.g. increased hepatic production of triacylglycerol, intensified removal of lipids from the cardiovascular system, higher rate of fat deposition and a significant inhibition of lipolytic processes (Smith et al., 1998; Winkler et al., 2000). In the second half of pregnancy, catabolic processes become predominant. In this period, due to an increased resistance of target tissues (adipose tissue and muscles) to insulin and the stimulation of lipase by placental hormones, fatty acid mobilization from adipose tissue can be observed, increasing the free fatty acid concentration in the blood of pregnant females (Butte, 2000; Winkler et al., 2000;). This results in a preferential supply of glucose and amino acids for the fetus (Butte, 2000). The main precursor for lipid synthesis in the fetus is glucose because the transfer of lipids through the placental barrier is limited and restricted to the transfer of polyunsaturated fatty acids which are essential precursors of steroidal bioactive compounds and maintain the fluidity of cell membranes especially in the brain (Iriadam, 2007). An adaptation of this type can be observed in many species of mammals, e.g. in rats (Piccione et al., 2009), cows (Mohebbi-Fani et al., 2006) and humans (Butte, 2000). Similar lipid mobilization may also be observed in female sheep and goats in the early lactation period (Nazifi et al., 2002a; Piccione et al., 2009; Skotnicka et al., 2011).

Eighty per cent of the metabolites related to milk synthesis circulating in the blood are utilized by the secretory cells of the mammary gland, depending on the speed of incorporation of precursors into milk compounds (free amino acids, glucose and fatty acids). An increase in lipase activity of the mammary gland is induced by the reduction of lipogenesis and increased fatty acid release. The process is regulated by norepinephrine and epinephrine which provide the substrates for milk fat synthesis (Nazifi et al., 2002a).

In small ruminants, during late pregnancy, the blood lipid profile is characterized by increased concentrations of total cholesterol, triglycerides and lipoproteins (Piccione et al. 2009). Lipid profiles have been used to predict *peri-partum* diseases and circulating blood triglycerides contribute significantly to milk fat synthesis (Nazifi et al., 2002a).

2.1.3.1 Total Cholesterol (TC)

Cholesterol in animals may be of exogenous and endogenous origin, originating from feed or synthesized from acetyl-CoA respectively. It is synthesized from acetyl-CoA in the liver, the gonads, intestine, adrenal gland, and skin. The biosynthesis of cholesterol in the body is inhibited by the ingestion of exogenous cholesterol (González & Silva, 2006). In ruminants, cholesterol concentrations are altered by different factors such as diet, age, gender, breed, physiological status such as pregnancy and lactation and hepatic and biliary diseases (Özpınar & Firat, 2003).

The analysis of blood cholesterol may influence the reproductive performance of ruminants since it is a precursor of important steroid hormones such as progesterone (Brito et al., 2006; Campos et al., 2007; Pogliani et al., 2010). Therefore, decreased levels of cholesterol can lead to reproductive impairment (Godoy et al., 2004). Its decrease is usually related to liver failure, low energy diet, hyperthyroidism and the *pre-partum* period. From these indicators, it could be concluded that, in addition to the final stage of gestation, inadequate energy intake is responsible for reduced levels (Gonzales et al., 2000; González & Scheffer, 2003; Soares et al., 2014).

In early lactation, cholesterol values gradually increase and then decrease at the end of this period. This increase, generally during lactation period, has been attributed to the increased synthesis of plasma lipoproteins (González & Silva, 2006). As pointed out by Duffield et al. (2003) in their study with dairy cattle during the transition period, they assigned an increase of cholesterol due to a higher release of lipoproteins from the liver. In other words, the gradual increase in cholesterol and triglycerides in late pregnancy in sheep may be due to insulin levels, which play a direct role in adipose tissue metabolism during pregnancy and insulin responsiveness is significantly reduced in ewes during late pregnancy. The diminished responsiveness of the target tissue to insulin during late pregnancy predisposes the ewes to increase these metabolites and lipoprotein concentrations (Balikci et al., 2009).

2.1.3.2 Triglycerides

The main storage form of FFA is triacylglycerol, also called triglycerides, in which three FFA are esterified to a glycerol molecule. Triglycerides are even less water-soluble than FFA and therefore must be bound to proteins in complexes called lipoproteins for transport in plasma. Although most cells can synthesize triglycerides, liver, adipose, mammary gland, and the small intestine are particularly adept at it. Free fatty acid-CoA is considered to be the building block for triglyceride synthesis, also, it should be stated that there are two sources: FFA in the plasma and synthesized locally. Generally, physiological or pathological circumstances, such as starvation or diabetes, which promote high plasma levels of free fatty acids, inhibit FFA synthesis. Physiological circumstances that promote free fatty acid synthesis, such as eating a carbohydrate meal inhibits lipolysis in adipose tissue, which does not lead to an increase in plasma FFA (Kaneko et al., 2008).

At the onset of lactation, glucose contributes significantly to the synthesis of lactose by mammary gland alveoli, amino acids for the synthesis of casein and lactalbumin and fatty acids for milk fat synthesis. High milk yields in livestock require adequate nutritional balance, especially in early lactation. It is noteworthy that at 60 days *post-partum*, the goat reaches peak milk production, even if feed consumption is decreased, resulting in mobilization of body reserves to meet the high metabolic requirements.

Triglycerides are an important source of fatty acids for the synthesis of milk. Studies with dairy goats observed lower levels of blood triglycerides during the peri-parturient period which could be explained by the increase in milk production, reduced availability of fatty acids, the presence of lipolysis for energy and the higher amount of circulating triglycerides in the mammary gland and milk fat synthesis. In addition, the activity of lipoprotein lipase is increased in the mammary gland and decreased in adipose tissue (Nazifi et al., 2002a; Mundim et al., 2007).

Some additives as monensin have been shown to be effective in improving the energy status of peri-parturient dairy ruminants. They increase the activity of ruminal gram-negative bacteria, resulting in the production of more propionic acid (providing glucose), which provides the animal with more energy (Duffield, 2001) and improves energy status, induces better liver function, and higher export of triglycerides is expected. It appeared that less attention has been paid to the effect of these additives on serum concentrations of triglycerides and lipoproteins with regard to liver function. Measurement of triglycerides alone to assess the liver may not be sensitive enough, because some triglycerides and a number of their vehicles in the blood have

non-hepatic sources. Measuring serum lipoproteins in combination with triglycerides and cholesterol may help describe liver function and energy metabolism in peri-parturient ruminants (Mohebbi-Fani et al., 2006).

2.1.3.3 High Density Lipoproteins (HDL)

Since very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) all contain some triglycerides; all of them may play a role in increasing serum triglycerides. The major lipids of the VLDL molecule are triglycerides, whereas those of LDL and HDL are cholesterol and phospholipids. HDL makes up about 80% of serum lipoproteins (Mohebbi-Fani et al., 2006). The HDL are synthesized and secreted by the liver and the small intestine. These particles are the main particles implicated in a cholesterol transport system that returns excess cholesterol from peripheral cells to the liver for bile excretion and resynthesis of new VLDL particles (Bauchart, 1993).

HDL concentrations gradually increased in the first weeks of the *post-partum* period as well as TC and triglyceride concentrations (Nazifi et al., 2002b). Therefore, an increase in these metabolites during the lactation period could be explained by lipid mobilization which is triggered by glucagons or by increased synthesis of the aforementioned plasma lipoproteins (Cavestany et al., 2005; Sadjadian et al., 2013). The opposite effect was found by Manat et al. (2016) in that there was a decrease in TC and triglycerides from parturition until the 45th day *post-partum* indicating that the goats used the lipids to supply energy for milk production.

Likewise, this dynamic of TC and HDL after parturition shows that during the lactation period of sheep and goats, lipogenesis and esterification are reduced, and FFA mobilization is stimulated by an increase in norepinephrine and epinephrine secretion. The activity of lipoprotein lipase is increased in the mammary gland and decreased in adipose tissue. In addition, increased lipolysis around parturition is hormonally regulated and not the result of an energy deficit (Nazifi et al., 2002a; Nazifi et al., 2002b; Manat et al., 2016).

2.1.3.4 Total Bilirubin (TB)

Most of the bilirubin in the plasma is derived from the degradation of old erythrocytes by the reticuloendothelial system, especially in the spleen. The remaining bilirubin comes from the degradation of myoglobin, cytochromes, and immature red blood cells in the bone marrow. The hemoglobin released from erythrocytes is divided into the globin portion and the heme

group. After extracting the iron molecule, which is stored or reused, the heme group is converted in bilirubin. The bilirubin thus formed is called free bilirubin, which is transported to the liver bound to plasma albumin. This form, is also known as indirect bilirubin in the clinical laboratory and is not water soluble. Being liposoluble, it is not filtered by the renal glomeruli, and is not excreted in the urine. In the liver, bilirubin is detached from albumin and conjugated to glucuronic acid to form conjugated bilirubin. It is soluble in water and actively secreted by the smaller bile ducts and subsequently excreted in bile. Conjugated bilirubin cannot be reabsorbed in the gut, but the bacterial enzymes present in the ileum and colon can convert bilirubin into fecal urobilinogen, which is reabsorbed at a rate of 10 to 15% by the portal-to-liver circulation. Most of this urobilinogen is re-excreted through the bile although some of it can be excreted in the urine. The unabsorbed urobilinogen in the intestine is oxidized with stercobilinogen, a pigment responsible for the brown color of feces (González & Scheffer, 2003).

2.1.4 Protein profile

Overall body protein status is usually assessed through the levels of total proteins in the serum or plasma, which include two major protein fractions: albumins and globulins. The interpretation of the biochemical constituents of animals depends on the availability of reference or baseline values for the parameters since there are distinct species variations. For example, in humans, sheep, goats, rabbits, dogs, guinea pigs, and rats, albumin predominates over the globulins, while in horses and cows, the ratio of albumins and globulins is nearly equal, or the globulins tend to predominate (Swenson, 1993).

In addition to species differences, there is evidence that some physiologic factors, namely: age, body weight, hormones, sex, pregnancy, lactation, puerperal period, estrus, temperature season, nutritive state of animal (proper and adequate intake of protein or protein-building materials in the diet), and many conditions (severe burns, dehydration, hemorrhage, liver and kidney dysfunctions, the terminal stage of cancer and inflammatory processes) affect serum protein levels (Batavani et al., 2006).

There are a variety of continuous physiologic changes that the dam undergoes during pregnancy which represents the most anabolic period of the female life cycle. These occur with respect to the dam's blood composition, cardiovascular system, uteroplacental blood flow, and metabolic changes. In fact, the dam makes adjustments to provide an adequate supply of

nutrients for the development of the fetus while maintaining maternal homeostasis and preparing for lactation (King, 2000; Batavani et al., 2006).

During lactation, the mammary gland secretory cells utilize 80% of the blood-circulating metabolites for milk synthesis. Synthesis and composition of the milk depend on the rapid infiltration of precursors of milk compounds, including free amino acids, glucose and fatty acids into the mammary gland cells. Milk protein, lactose and fat are produced by mammary gland cells using these precursors. Changes in milk composition and a decrease in milk production have been reported when there is a decrease in the production of protein, lactose or fat (Radostits et al., 2007).

2.1.4.1 Total Protein (TP)

Blood proteins are mainly synthesized by the liver, and the synthesis rate is directly related to the nutritional status of the animal, especially in the case of protein and vitamin A levels, and liver function. This metabolism is inevitably influenced by energy status. The main plasma proteins are albumin, globulins and fibrinogen. They are involved in multiple functions such as maintaining osmotic pressure and blood viscosity, transporting nutrients, metabolites, hormones and excretory products, regulating blood pH and participating in blood coagulation (Sykes & Thompson, 1978; González & Scheffer, 2003).

A significant decrease in serum protein levels on day 145 of gestation, compared to pre-pregnancy and other stages of gestation, could be a consequence of an increase in the dam's basal metabolic rate, the maximal nutrient requirements of the placenta and the growing fetus, together with the transfer of serum albumin, immune globulins, and amino acids from the bloodstream to the mammary gland for colostrum synthesis. The fetus synthesizes all its proteins from the amino acids derived from the mother, and the growth of the fetus increases exponentially, reaching a maximum during late pregnancy (Jainudeen & Hafez 2000; El- Sherif & Assad, 2001; Balikci et al., 2007).

Studies showed a decrease in TP concentrations before parturition, increased globulins and decreased albumin in early lactation, which thereafter began to increase gradually when dietary protein intake became adequate. This decrease may be related to the amount of protein in the diet, an increase in the demand for amino acids for the synthesis of milk proteins, or a reduction in the liver synthesis capacity due to the fat accumulation (Contreras, 2000; Zambrano & Marques Júnior, 2009; Mohammadi et al., 2016).

Likewise this decrease in serum TP may be related to the fact that the fetus synthesizes all its proteins from the amino acids derived from the dam, and growth of the fetus increases exponentially, especially in muscle mass, during late pregnancy (Mohammadi et al., 2016). During lactation in goats, there is an increase in serum total protein level due to the catabolism of proteins, primarily muscle proteins, for milk synthesis (Krajnicakova et al., 2003).

2.1.4.2 Albumin

Albumin is the most abundant protein in plasma, accounting for about 50% of total protein. It is synthesized in the liver and contributes to 80% of the osmolarity of the blood. It is also an important protein reserve, as well as a transporter of elements such as FFA, amino acids, metals, calcium, hormones and bilirubin. Albumin also plays an important role in regulating blood pH by acting as an anion. The level of albumin may be indicative of the protein content of the diet, even though changes in its levels occur slowly. For detection of significant changes in serum albumin concentration a period of at least one month is required, due to the low rate of synthesis and degradation. Decreased albumin levels, together with decreased urea, indicate protein deficiency. Decreased albumin levels with normal or elevated urea levels accompanied by high enzyme levels are indicators of hepatic failure (González & Scheffer, 2003).

Serum TP and globulin levels were found to be lower in twin-bearing sheep than in single-bearing sheep on days 100 and 150 of pregnancy and could be related to the high production of globulins for colostrum production in ewes 3–4 weeks *pre-partum* (Davson & Segal, 1980). El-Sherif and Assad (2001) and Brozostowski et al. (1996) reported that blood proteins decreased during late pregnancy and gradually increased to reference values during lactation (Kaneko, 1989). Purohit et al. (1999) and Al-Dewachi (1999) recorded blood glucose and total protein levels at the end of the first, second, third and fourth month of pregnancy in ewes to be lower than in non-pregnant ewes. Similarly Gonzales-Montana et al. (1994) reported serum TP levels to increase during the first three months of pregnancy and twin pregnancies to produce significantly lower protein levels when compared to a single pregnancy. Shetaewi and Daghash (1994) demonstrated that lactation caused a decrease in the level of albumin and a trend for an increase in globulin when compared to pregnant ewes.

2.1.4.3 Globulin

Globulins are fractions of the proteins that are related to the immunological situation of the organism, in which high levels can be observed soon after the onset of health disorders (Payne & Payne, 1987). An increase in their values, in lactating ruminants is associated with an inflammatory process (González & Rocha, 1998). The concentration of this metabolite results from the difference between TP and albumin. The globulins can be divided into three types, alpha, beta and gamma, identified by electrophoresis. They have functions in the transport of metals, lipids and bilirubin, as well as a role in the immune system (gamma fraction). Moreover, globulins are also considered to be indicators of limited protein metabolism, but their importance is also significant as indicators of an inflammatory process (Thrall et al., 2006; González & Silva, 2008).

Globulins are responsible for variations in TP levels. Therefore a decrease in serum TP is due to a decrease in globulin, especially alpha 1 and gamma fractions. This is thought to be due to the production of globulin-rich colostrum. The ability to synthesize the constituents of milk appears in sheep three to four weeks before parturition. Capture of globulins by the mammary gland for colostrum synthesis may be considered to be the main factor in the reduction of serum TP (Mohammadi et al., 2016).

A study has reported that lower concentrations of globulins were observed in small ruminants within the clinical framework of pregnancy toxemia, associated with a renal and hepatic failure (Balikci et al., 2009).

2.1.4.4 Urea

The most studied blood metabolite in ruminants is urea and it is used to evaluate protein status due to its special link with protein digestion and the metabolism of ruminal microorganisms. The amount of ammonia converted to urea depends on the total amount of degraded protein and the rate of ammonia incorporation into microbial protein, thus high consumption of rumen degradable protein, results in elevated serum urea levels (Herdt, 2000).

Ammonia in excess of that incorporated into protein by ruminal bacteria is absorbed into the blood, transported to the liver, and converted into urea. Urea synthesized in the liver diffuses into milk, is extracted from the blood by the kidney and excreted into the urine, or recycled to the rumen. The amount of urea eliminated from the cow in milk and urine is positively correlated with the amount synthesized in the liver (Wilson et al., 1998).

Urea concentrations are not only determined by the rate of detoxification, but they are also influenced by the amount and rate of hepatic synthesis. Synthesis occurs from ammonia produced in the catabolism of amino acids and ammonia recycled of the rumen. Urea indexes are analyzed in relation to the protein content of the diet; in addition to evaluating renal function (Contreras, 2000; González & Scheffer, 2003).

This metabolite is a sensitive and immediate indicator of protein intake, while albumin indicates long-term protein status. However, high levels of urea are found when an energy deficit occurs due to the reduced ability of the ruminal microflora to utilize the nitrogen compounds for protein synthesis, therefore increasing the amount of ammonia absorbed from the rumen. A prolonged period of fasting may cause increased endogenous proteolysis which uses amino acids as an energy source. This causes an elevation in urea concentrations. Nitrogen balance in ruminants can be studied using urea levels in both the blood and milk (González & Scheffer, 2003).

The energy/protein balance in the ruminant diet is essential for the utilization of urea. Therefore, changes in diet, seasonal or even daily, influence urea blood levels and its efficient use by the animal. A reduction in energy intake acts inversely on the concentration of ruminal ammonia due to the decrease in microbial protein synthesis and raises blood urea concentrations (Wittwer et al., 1993; Wittwer, 2000).

As indicated by González (1997), increases in blood urea levels occur at the end of gestation; and these values decrease shortly before and after parturition, even in cows with adequate dietary protein intake. Previously studies have shown that plasma urea levels are lower at 150 days of pregnancy and 45 days *post-partum* than at 60 days of gestation in small ruminants. Plasma urea starts increasing in pregnant ewes from the 10th week of pregnancy, reaching a maximum level at parturition (El-Sherif & Assad, 2001; Balikci et al., 2007). Shetaewi and Daghash (1994) found the level of serum urea during pregnancy to slightly exceed that of early lactation. On the contrary, Brozostowski et al. (1996) observed an increase in urea level during early pregnancy. Sandabe et al. (2004) indicated unchanged urea concentrations between pregnant and non-pregnant goats. In ruminants, amino acids are not normally catabolized and are used for synthesis of milk proteins, and subsequently urea production in the body falls and plasma urea concentration decreases.

In addition, the simultaneous decrease in TP, globulin and urea concentrations in the last days before parturition may be associated with the decline in feed intake due to stress and hormonal changes during the kidding process (Cavestany et al., 2005; Sadjadian et al., 2013),

and to an uptake of globulin by the udder as mentioned by Kehrli et al. (1989), as the production of colostrum increases.

2.1.4.5 Creatinine

Creatinine is a small molecule produced by the degradation of creatine and creatine-phosphate, an energy-storing molecule mainly present in skeletal muscles. In other words, plasma creatinine is derived, practically solely from the catabolism of creatine present in muscle tissue. Creatine is a metabolite used to build energy in the muscle; in the form of phosphocreatine, and its degradation to creatinine occurs steadily at a rate of around 2% daily (González & Scheffer, 2003). Creatine is synthesized from the amino acids glycine, arginine, and methionine, the final step occurring in the liver (Kaneko et al., 2008).

Kidney function can be evaluated from the concentrations of analytes in plasma or urine and depend mainly on their elimination rate. Creatinine can be used as a marker. These indirect markers can be easily and rapidly measured, but their sensitivity is poor and generally remains unaltered until 75% of renal function has been lost and their concentrations may be modified by extra renal factors. Direct tests of kidney function are based on the elimination kinetics of markers of glomerular filtration, blood flow, or tubule reabsorption/secretion and are based on the clearance concept. Plasma creatinine is the test most often used to diagnose and monitor kidney disease in human and animal clinical pathology. Plasma urea is also used frequently but is subject to more numerous extra renal factors of variation. These molecules are almost totally eliminated by glomerular filtration, so in the case of kidney failure their plasma concentration increases. However, neither test is sensitive in the early diagnosis of kidney disease because of the large functional reserve of the kidneys (Kaneko et al., 2008).

Studies reported higher values of creatinine concentrations at 10 day before and at parturition when compared to other moments around parturition, although the results were below the normal limit for small ruminants. These findings are explained by muscle protein mobilization which aims to produce energy to meet the higher requirements during early lactation (Kaneko et al., 2008; Silva et al., 2013; Santos et al., 2012).

2.1.4.6 Haptoglobin (Hp)

Haptoglobin (Hp) is an acute phase protein (APP) and it was first described in 1939 by Polonovski and Jayle, who gave it this name due to its affinity with hemoglobin (Fagoonee

et al., 2005; Macedo, 2015). Hp belongs to the α -globulin fraction and is composed of two 20-kDa peptides (α chain) and two 35-kDa peptides (β chain) linked by disulfide bonds (Morimatsu et al., 1991; Ceciliani et al., 2012). Purified native Hp has a molecular mass of 1000-2000 kDa, the stable unit being a $\alpha_2\beta_2$ tetramer. Hp has a half-life of two to four days and it can be free in the bloodstream and in the form of an associated polymer with albumin (Godson et al., 1996; Murata et al., 2004).

Interestingly, most of the activities of Hp are related to the formation of a Hp-Hemoglobin (Hb) complex, and not to the pure form of Hp. Hp has long been identified as the principal scavenger of free hemoglobin in blood. Further biological activities, mostly derived from studies of Hp in non-ruminant species also include the regulation of innate immunity reactions in white blood cells, direct bacteriostatic effect and a chaperone activity (Ceciliani et al., 2012).

Hp has several biological functions such as to stimulate angiogenesis, to regulate lipid metabolism and to exert an immunomodulatory effect, however its main function is the maintenance of iron homeostasis, avoiding the loss of this mineral, preventing its renal excretion. Moreover, Hp also promotes a bacteriostatic action through the Hb-Hp complex, restricting its availability for bacterial growth where it is necessary (Thomas, 2000; Petersen et al., 2004; Eckersall, 2008).

During intravascular hemolysis, Hp forms complexes that cannot pass through the glomerular filter due to their large size. Each Hp molecule contains four binding sites, two for each Hb α - β dimer. Once formed, the complex is stable and is swiftly removed from the circulation by the monocyte/macrophage CD163 Hp-Hb receptor expressed on Kupffer cells in the liver. By binding Hb, Hp fulfills an additional anti-oxidant role by stabilizing iron, resulting in a reduction in oxidative damage to Hb, to albumin (since the free exchange of heme from Hb and albumin is inhibited), to lipids and eventually to the tissues, the kidney in particular (Yang et al., 2003; Cooray et al., 2007; Ceciliani et al., 2012).

This APP is secreted mainly by the liver, however Hp mRNA expression has also been described in the mammary gland and leukocytes of healthy animals. It is an inflammatory marker of great importance in ruminants, having very low or insignificant circulating values in healthy animals, up to 10 mg/dL, but rising to more than 100 times that when stimulated (Skinner et al., 1991; Chan et al., 2004; Hiss et al., 2004; Cooray et al., 2007). Hp is mainly present in plasma, but can also be observed in other fluids such as milk, urine and saliva (Hiss et al., 2004).

The Hp concentrations depend on the extent of tissue injury caused by the acute inflammatory process; when the injury is severe, its concentrations increase rapidly, usually 24-48 hours after tissue damage, shortly before the elevation in plasma fibrinogen in cows and sheep (Cole et al., 1997; Costa et al., 2010)

Previous studies in ruminants suggested a relationship between selected APPs and lipid mobilization. One study reported increased Hp concentrations in cows with fatty liver, compared with healthy cows. In addition, increased Hp levels in cows around parturition have been described. It has been suggested that it is related to NEB, since cows with high milk Hp also had high serum NEFA concentrations. A significant correlation has been also reported between Hp and β HB in lactating goats (Trevisi et al., 2005). Previous findings reveal a possible relationship between Hp and markers of negative energy status in ruminants (Hiss et al., 2009; González et al., 2011; Macedo, 2015).

In a study that was conducted by Bastos (2008) to establish the kinetics of some APPs in Santa Inês sheep, during pregnancy and the *post-partum* period. They showed higher levels of Hp in late pregnancy with highest concentrations in early lactation. However, a study has produced the opposite results in that Hp was not a good marker of subclinical pregnancy toxemia. Other markers such as plasma NEFA and ketone bodies in urine showed significant changes at 24hr (Skinner et al., 1991). While, previous related studies showed that increases in Hp appeared in fasted goats at the same time as the clinical signs, there is no correlation between the severity of clinical signs and Hp concentrations (González et al., 2011).

2.1.5 Enzyme profile

2.1.5.1 Aspartate Amino Transferase (AST)

Aspartate aminotransferase (AST) was previously known as glutamic oxaloacetic transaminase (GOT), it is an indicator of liver damage due to its high concentrations in the liver. It is generally considered to be a good diagnostic tool even though it is a non liver specific enzyme (Radostits et al., 2000).

AST activity is measured in production animals as a screening test and is relatively high and found in similar amounts in liver and in skeletal and cardiac muscle, but it varies between species. It is routinely used in equine and food animal medicine as a screening test for injury to both organs. Serum AST activity is readily available from the biochemical profile, it has a longer blood half-life than sorbitol dehydrogenase and creatine kinase. Increased serum AST

activity is observed with both reversible and irreversible injury to hepatocytes and can be seen following hepatocellular injury and cholestasis. Serum AST is increased following myocyte injury. In both cases, the definitive disease process cannot be identified, only that cellular injury in muscle or liver has occurred. Because serum AST activity cannot differentiate between hepatocellular or myocyte injury, further testing is often required using organ-specific enzymes such as sorbitol dehydrogenase or creatine kinase (Thrall, 2006; Kaneko et al., 2008).

The increase in AST concentrations around the parturition could be explained by the degradation of muscle cells due to mobilization of body reserves (Verheyen et al., 2007). Previous studies around parturition in ruminants showed an increase in AST concentrations during lactation when compared to pregnancy (Seifi et al., 2007; Taghipoor et al., 2011; Sadjadian et al., 2013; Lima et al., 2016).

The high proportion of dams with abnormal AST activity may be an indicator of fatty infiltration in the liver in the animals studied due to NEB during the *peri-partum* period. The results of the AST are in agreement with the increased proportion of dams with NEFA concentrations greater than the cutoff level. DMI and milk production were decreased when AST activity was high (Sadjadian et al., 2013). Increased fat mobilization and the increase in ketone bodies cause damage to the hepatocytes, and consequently extravasation of this enzyme to the bloodstream (Mundim et al., 2007).

2.1.5.2 Gamma Glutamyl Transferase (GGT)

The gamma glutamyl transferase (GGT) range has a high specificity and is almost invariably elevated in cases of chronic hepatic disease. It is detected mainly in the bile ducts, indicating biliary lesion (Pearson, 1993). The evaluation of this enzyme in the serum can be used with the cholestasis index, mainly in ruminants and equine species (Birgel et al., 1982).

Previous studies mentioned an increase in this enzyme in ruminants during the peri-parturient period, although it is generally associated with cholangitis perhaps its concentrations were within the normal range for ruminant species (Kaneko et al., 2008; Braun et al., 2010; Lima, 2013). The increase after parturition has been shown by Anwar et al. (2015), when determining the level of certain biochemical metabolites that reflected the energy metabolic status during early lactation in different breeds of goats.

Studies with small ruminants affected by pregnancy toxemia showed an increase in AST and GGT hepatic enzymes in sheep and goats that occurred due to liver damage, since AST, according to González and Silva (2006), is a good indicator of liver function in ruminants.

Therefore, studies have found a positive correlation between the severity of histological hepatic lesions and AST activity (Kabacki et al., 2003; Barakat et al., 2007; Cal et al., 2009; Santos et al., 2011; Souto, 2013).

2.1.5.3 Creatine Kinase (CK)

Creatine kinase (CK) catalyzes the exchange of a phosphate moiety between creatine phosphate and ATP. In myocardial and skeletal muscle, CK allows energy storage as creatine phosphate when demand is low, but when energy is needed for muscle contraction, CK catalyzes the transfer of the high-energy phosphate from creatine phosphate to ADP to form ATP. A small amount of CK activity is associated with the mitochondria, where it is responsible for transfer of high-energy phosphate to creatine, the cytosolic carrier. CK activity is at its highest in skeletal muscle, followed by heart muscle, the diaphragm and the smooth muscle, and then brain. The half-life of CK activity in blood is relatively short in all species (Kaneko et al., 2008).

In domestic species, increases in CK activity may occur mainly as a result of increased muscle activity and/or heart damage, such as an exercise, mechanical trauma, prolonged recumbency, and especially in the case of intramuscular injection. Indeed, its activity is used as a marker of skeletal muscle injury associated with trauma, nutritional myopathies, exercise-induced muscle injury, or congenital myopathies (Russell & Russell, 2007; Kaneko et al., 2008; Braun et al., 2010; Lima, 2013).

The kinetics of CK activity has been used to quantify muscle damage in experimental models. Although these results were not likely to be extrapolated to drug formulation, they confirm that the pharmacokinetic analysis of CK release provides a minimal invasive and quantitative mean evaluation for post injection muscle damage and is likely enable the pharmaceutical company to simultaneously investigate the effects of formulation-dependent factors, such as volume, local tolerance and drug bioavailability during initial stages of drug development in sheep (Ferre et al., 2006).

At parturition CK concentrations increase. This finding suggests an extensive muscular degeneration, which explains the observed pain during the labor (Morais et al., 2000). In other words, in cases of prolonged recumbency in ruminants, that is basically characterized by the animal's inability to rise after parturition. The cause of this problem is related to low levels of circulating calcium (hypocalcemia), especially in high-producing animals. The main complication of persistent decubitus is the ischemia of the hind limb musculature due to the

pressure of the weight of the decubitus animal. Causing progressive degeneration and muscular necrosis, a fact that can be evaluated biochemically through the measurement of serum levels of CK (Rodrigues & González, 2004; Garcia, 2007).

2.1.5.4 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is present in the cytoplasm of all epithelial and bony cells. It is excreted in bile. Its serum concentration varies with the age of the animal and with the affected organ. In cattle the individual variations are very large (Mullen, 1976). It is elevated in cases of intra- and extrahepatic cholestasis. Significant increases in this enzyme are seen in cases of hepatobiliary diseases due to overproduction (Kaneko et al., 2008).

ALP is a membrane-associated enzyme, located in several tissues. Although many tissues or cell types have some ALP activity such as cells from liver, bone, kidney, intestinal mucosa, and placenta, only two are important in diagnosis of a disease (the bone and hepatobiliary tissue). Serum ALP activity, however, is not generally a reflection of tissue concentration (Meyer et al., 1995; Kaneko et al., 2008). Due to the fact that this enzyme is not organ specific in large animals, increases in ALP activity should be interpreted in comparison with more organ-specific enzymes such as sorbitol dehydrogenase, AST and GGT. In addition, this enzyme is also released by osteoblasts from metabolically active bone. This could be the reason why fast-growing offspring usually have high serum ALP levels (Smith, 2006).

Studies showed the ALP activity increases in *pre-partum*, and decreases before and after parturition in ruminants. This elevated plasma activity in the goat *pre-partum* may be explained by the increased placental production of this enzyme, as has been reported for cows (Peter et al., 1987; Tharwat et al., 2013). On the other hand, a study observed higher concentrations of ALP in first lactation goats (Mundim et al., 2007). These findings may be explained by the fact that these animals were young growing goats that had high concentrations of bony isoforms and which would decrease with maturity. This result was confirmed by Sarma and Ray (1985) who showed that age and the number of parturitions exert a certain influence on ALP activity. In addition, cholestasis is the most common cause of significant increases in ALP concentrations in most species (Kaneko et al., 2008).

2.1.6 Mineral profile

Mineral elements exert an important influence on reproduction and production in small ruminants (Elnaggeb & Adelatif, 2010). However, clinical symptoms or signs of macro mineral deficiencies, imbalances or excesses are generally not apparent until shortly after parturition. In late pregnancy, the requirements for macro minerals are increased by both the dam and the offspring. Therefore, reserves will be mobilized in order to meet requirements, especially those of the fetus (Yildiz et al., 2005). Essential minerals for animals have vital functions such as in the structure of organs and tissues and in metabolism. They occur in body fluids and tissues as electrolytes, favoring the maintenance of osmotic pressure, acid-base balance, membrane permeability and transmission of nerve impulses (Suttle, 2010).

Pregnancy and lactation are phases of metabolic stress, associated with changes in the mineral profile which are dependent on the reproductive status of small ruminants. In addition, substantial mineral losses occur during these periods due to increased demand in these phases. Therefore, macro and trace-element concentrations in the bloodstream represent homeostatic mechanisms that are in close relation with the hormonal and neuro-humoral regulation and nutritional status (Krajnicakova et al., 2003; Elnaggeb & Adelatif, 2010; Moreira, 2013).

2.1.6.1 Total Calcium (Ca)

Total calcium is found in the bloodstream in its ionized form, which is biologically active and non-ionized forms. This mineral is always in equilibrium, and its final distribution depends on the pH, albumin concentration and acid-base ratio. According to González et al. (2000) it is closely linked to metabolism responsible for the transmission of nerve impulses, contraction of cardiac and skeletal muscles, blood coagulation, as a component of milk and enzymatic activity, as well as of skeletal tissues among other functions (NRC, 2001; Goff, 2004).

Low albumin levels cause a decrease in serum calcium (González et al., 2000). Its distribution by the organism is very unequal, with the majority (98%) located in the skeleton, providing structural strength and hardness to the bones. The other minor portion (2%) is primarily distributed in the extracellular fluid (NRC, 2001; Degaris & Lean, 2008). The extracellular calcium concentration also influences the secretion of substances through nerves and endocrine glands. An example is a hypocalcemia cow that is unable to secrete insulin from the pancreas and therefore becomes hyperglycemic (Goff, 2006).

Due to the need to maintain calcium concentrations within physiological limits, vertebrate animals have developed an elaborate system to maintain homeostasis, increasing calcium intake to the extracellular fluid whenever Ca levels are low (NRC, 2001). However, there are some delicate moments such as in the *peri-partum*, when a large amount of this mineral must be mobilized for colostrum and milk production. The quantity necessary is about nine times greater than the total amount of calcium present in the animal's plasma compartment. It is a situation that increases the adaptive challenge of the ruminant, putting it at risk from developing metabolic disorders (Rodrigues & González, 2004).

Several studies have observed that total calcium concentrations decrease in late gestation, the lowest values being seen at parturition and levels remain low throughout the first three weeks of lactation (Azab et al., 1999; Iriadam, 2007). In addition, Ca levels rise generally 10 days prior to parturition, and this rise is associated with the increase in albumin concentrations, and these two elements are strongly correlated. This could be because a fraction of the total pool of calcium is linked to albumin and thus depends partly on the latter's concentration (Goff, 2000; Cavestany et al., 2005).

A sudden decrease in feed intake or forced exertion of small ruminants can cause a marked depression in serum calcium levels. However, in early lactation, sheep are susceptible to hypocalcemia, as they are in NEB (Radostits et al., 2007). In other words, the high requirement of blood calcium by the mammary gland for colostrum and milk production and fetal skeletal formation, associated with NEB due to low dry matter intake during the *peri-partum* period, results in low serum levels of this mineral (Seifi et al., 2007).

2.1.6.2 Sodium (Na^+)

Sodium (Na^+) is the main ion present in the extracellular fluid (ECF) and an important component of the skeleton. The ECF volume contains approximately one-half to one third of the body's Na^+ (45%). Most of the remaining Na^+ is bound in skeletal bone (45%) and only 10% is intracellular, relatively little of which is rapidly exchangeable (Reece, 2006; Kaneko et al., 2008). Much of the volume of ECF and plasma osmolarity is determined by the concentration of Na^+ (González & Silva, 2006). The ECF volume thus contains essentially all of the body's readily available and exchangeable Na^+ . The exchangeable Na^+ content is the principal determinant of ECF volume, and a Na^+ deficit is the principal cause of decreased ECF volume (Kaneko et al., 2008). The level of Na^+ inside cells is kept low due to the cell membrane

being relatively impermeable to the entrance of Na^+ and the Na^+ and K^+ pump that returns it to the ECF (González & Silva, 2006).

The sodium ion is essential in the regulation of osmotic pressure, acid-base balance, transmitting nerve impulses, and in the absorption processes of monosaccharides, amino acids and bile salts. Increases in Na^+ result in an expansion in ECF volume, which may lead to the development of hypertension or edema formation (Kaneko et al., 2008). On the other hand, hyponatremia occurs in cases of gastrointestinal disturbances, hyperglycemia and hyperlipidemia. It is one of the most common deficiencies and also the easiest to correct by supplementing the diet with mineral salt in ruminants (Tokarnia et al., 1999).

The kidneys regulate the amount of Na^+ in the body, also controlling that of water, thus maintaining the plasma concentration of this ion within narrow limits, despite fluctuations due to daily ingestion. In other words, because monitoring daily electrolyte balance is difficult in most animal species, urinary fractional excretion or creatinine clearance ratios have been useful to provide an index of daily intake or potential deficits in Na^+ , K^+ , Cl^- , and other electrolytes. Normal values have been established for dogs, cats, horses and cattle (González & Silva, 2006; Kaneko et al., 2008). A reduction in these metabolites during the *peri-partum* is due to the mobilization of these ions for the formation of colostrum and milk (Silva et al. 2013).

2.1.6.3 Potassium (K^+)

Potassium is mainly an intracellular ion with over 98% of the exchangeable potassium located intracellularly, thus constituting the most abundant intracellular cation in the organism (Reece, 2006; González & Silva, 2006; Kaneko et al., 2008; Suttle, 2010). K^+ is necessary in vital functions such as osmotic, ionic, acid-base and water balances. Moreover, it acts as an intracellular ion besides being a cofactor in many enzymatic systems such as the transfer and utilization of energy, protein synthesis and carbohydrate metabolism (Carlson, 2006).

The distribution of K^+ is coupled with the active pumping of Na^+ from the cells, which is maintained by an energy-dependent Na^+ and K^+ pump at the cell membrane. K^+ distribution across the cell membrane plays a critical role in the maintenance of cardiac and neuromuscular excitability. Changes in K^+ concentration that alter the ratio of intracellular to extracellular K^+ alter membrane potential. In general, hypokalemia increases membrane potential, producing a hyperpolarization block resulting in weakness or paralysis, whereas hyperkalemia decreases membrane potential causing hyper excitability. These features depend on the state of total body K^+ content but also depend on the speed with which hypokalemia or hyperkalemia develops.

K⁺ homeostasis involves the regulation of an internal balance (i.e. the distribution of K⁺ between the ECF and intracellular fluid – ICF) as well as an external balance (i.e. the relationship between K⁺ input to output). Internal K⁺ balance is influenced by changes in acid-base status, glucose and insulin administration, exercise, and catecholamine release (Kaneko et al., 2008).

Some pathological conditions that interfere with the absorption and/or reabsorption of this electrolyte in the kidney or another situation involving loss of body fluids rich in K⁺ causes changes in its serum concentration (González & Silva, 2006). Previous studies have reported serum Na⁺ and K⁺ levels within the normal range in goats and sheep during pregnancy and lactation, although some fluctuations were observed (Althaus et al., 1995; Kadzere, et al., 1996; Krajnicakova et al., 2003; Dias et al., 2010; Waziri et al., 2010; Silva et al., 2013). A decrease in Na⁺ and K⁺ in late pregnancy; with recovery during lactation have been reported in sheep by Yildiz et al. (2005), attributing the loss of these ions to the formation of colostrum or due to the maintenance of the constant relationship between these ions in the ECF and ICF.

2.1.6.4 Magnesium (Mg³⁺)

Magnesium (Mg³⁺) is the main intracellular cation and an essential cofactor for many enzymatic reactions in metabolic pathways. Extremely important for normal conduction of nerve impulses, muscle function and bone mineral formation. Some enzymes such as ATPases, kinases and phosphatases require Mg³⁺ for their activation (Goff, 2004). It is also involved in some biochemical and physiological processes such as the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and proteins (Martens & Schweigel, 2000). It is worth noting that no hormone or vitamin is responsible for the homeostasis or metabolism of this mineral, and its plasma concentration is a reflection of the diet content, thus the pasture is the best source of Mg³⁺ (NRC, 2001; Goff, 2004). In addition, the bovine mammary gland requires about 120mg of this mineral for the production of one kg of milk, so the higher the milk yield the higher requirement (Castro et al., 2009).

Approximately 70% of Mg³⁺ is located in bones, 29% in soft tissues and 1% in body fluids. This mineral does not accumulate in animal tissues and should be supplied daily. Mg³⁺ milk secretion levels are low and therefore the offspring can suffer from hypomagnesemia disorders (González & Silva, 2006). Metabolic Mg³⁺ disorders usually occur in cattle and sheep (Carlson, 2006).

Low levels of Mg^{3+} present serious consequences for ruminants and can even lead to death. Tetany is a production disease usually caused by the low intake of this mineral in the diet. Hypomagnesemia is considered to occur in small ruminants at levels below 2.2 and 2.8 mg/dL for sheep and goats respectively (Kaneko et al., 2008), and generally the symptoms occur at concentrations below 1.0 mg/dL in cows. As a consequence, it can trigger hyperexcitability, retained placenta, as well as abnormal ruminal digestion and decreased production, and this also predisposes to milk fever in cows immediately after parturition. When Mg^{3+} levels are very low, the capacity to mobilize bone reserves is drastically reduced (González & Silva, 2008). Previous studies have shown that primiparous sheep have lower Mg^{3+} concentrations at different moments such as at parturition, at peak milk production and at the end of lactation when compared to those seen in pluriparous sheep. These periods are characterized by high energy demands (González et al., 2000; Cardoso et al., 2011).

2.1.6.5 Chloride (Cl^-)

Modest changes in hydration tend to produce roughly proportional changes in plasma Cl^- relative to Na^+ concentrations. Acid-based alterations are associated with disproportionate changes in plasma Cl^- concentrations. Cl^- concentrations increase in acidosis as the result of proportionately smaller losses of Cl^- than bicarbonate and enhanced renal Cl^- resorption in response to decreased bicarbonate. Cl^- depletion develops in these animals as a result of excessive loss or sequestration of fluids with high Cl^- content. Changes in water balance can result in modest alterations in the relative concentrations of plasma Na^+ and Cl^- (Kaneko et al., 2008).

2.1.6.6 Phosphorus (PO_4^{3-})

The majority of phosphorus (80%) is found in bones and teeth and the lowest concentration is in the body's cells. This mineral has several biological functions: it participates in molecules that transfer energy in almost all transactions involving the formation or breakdown of high energy bonds, that bind phosphate oxides to carbon or carbon/nitrogen compounds such as ATP. Indeed, PO_4^{3-} is involved in the acid-base buffer system of blood and other body fluids, in cell differentiation and is an important component of cell walls, phospholipids, phosphoproteins and nucleic acids (NRC, 2001; Goff, 2004).

The physiological levels of plasma PO_4^{3-} are between 1.62 and 2.36 mmol/L for sheep and 1.35 and 2.94 mmol/L for goats (Kaneko et al., 2008). The extracellular maintenance of this macro-element depends on dietary replenishment and bone resorption, replacing phosphorus lost in feces, urine, used for bone and muscle, and in milk production (Reinhardt et al., 1988). However, the production of colostrum and milk requires a great deal of phosphorus, causing an acute decrease in plasma concentrations (Sousa Júnior et al., 2011).

A study with sheep, analyzing the dynamics of PO_4^{3-} during the *peri-partum* showed that values were lower at parturition. The phosphorus concentrations are close to the lower limit for the species in late pregnancy and on day 30 of lactation (Brito et al., 2006). This could be explained by the rapid increase in fetal growth in the last third of pregnancy (Payne, 1983). Both calcium and PO_4^{3-} are vital for bone structure in the fetal skeleton and they combine in relatively constant proportions, although phosphorus is distributed differently.

Braun et al. (2010) reported that calcium and inorganic PO_4^{3-} concentrations were influenced by feed supply. They are also reported to be lower in twin than in single pregnant ewes, but no difference was observed between twin and triplet-bearing ewes. Ionized calcium has been poorly investigated in sheep, but, as in other species, it might be of considerable use in clinical diagnosis.

2.2 PREGNANCY TOXEMIA

Small ruminants are at risk of developing the metabolic condition called “ketosis” at two different stages: during late gestation called (pregnancy toxemia) and early lactation (lactation ketosis) (Smith & Sherman, 2009). Terms such as: twin lamb/kid disease, lambing/kidding sickness, lambing/kidding paralysis, and lambing/kidding ketosis are commonly used to describe the condition in sheep and goats (Rook, 2000).

Pregnancy Toxemia (PT) is one of the main peri-parturient metabolic diseases characterized by disorders in energy and protein metabolic profiles and the hormonal profile (Smith & Sherman, 2009; Santos et al., 2011; Souto et al., 2013). It occurs in small ruminants, during the last two to four weeks of pregnancy as a consequence of an energy metabolism disorder of fatty acids during the transition period when the liver is metabolically very active (Herd, 2000; Riet-Correa et al., 2007).

This metabolic disease causes high economic losses due to substantial mortality among the animals and it is widespread in certain regions such as the semi-arid region of Brazil. This disease can occur in animals; supplemented with concentrate, in a good and bad nutritional

state and it occurs in the more productive animal farms such as high genetic herds (Abdul-Aziz & Almujaalli, 2008; Carvalho, 2013).

PT was first described in Brazil by Ortolani and Benesi (1989), who detected both cases of pregnancy toxemia in sheep suffering from malnutrition and overfeeding as type I and II PT respectively. The first type of PT occurs in non-fatty animals with a body score of 2 or 3 and is characterized by undernutrition during pregnancy associated with the presence of multiple fetuses. It can be caused by a decrease of about 50% in the energy necessary for a pregnant female (Ortolani, 1985; Schild, 2007).

As also pointed out by Radostits et al. (2007) in the second type, the female is usually obese, which is a result of a very high energy diet offered throughout pregnancy and in animals with a higher body condition score, 3.5. The obese female suffers from a decrease in voluntary food intake due to a decrease in rumen volume and the pressure exerted by intra-abdominal fat and the developing fetuses.

Understanding the importance of nutrition, economic, and management factors in the progression of this disease is extremely important if practitioners and producers desire to develop practical flock or herd-based prevention programs. Producer-initiated feeding, management, and economic decisions involving a hypothetical flock may be helpful in briefly illustrating management's relationship to PT issues (Rook, 2000).

2.2.1 Etiology

Nowadays various determining factors for ketosis have been observed as well as the etiologic factors involved in the development of PT such as flaws in nutritional management, obesity, nutritional deficiency, and secondary diseases (Rook, 2000; Smith & Sherman, 2009).

However, the etiology of PT is not clearly defined, although it is known that it is not of an infectious disorder nor is it caused by a vitamin or mineral deficiency. The causes that predispose the onset of PT can be divided into three groups: the quantity and quality of feeding; occurrence of animal stress such as increased number of fetuses, body weight of kids, periods between parturitions, climate changes, transport, and sudden feed changes; animal factors such as pregnancy, decreased ruminal capacity, less physical activity, age, liver disorders, parasitic diseases, hormonal and genetic factors, and other secondary diseases (Ortolani, 1994; Prieto et al., 1994; Santana, 2001).

PT is usually limited to the last weeks before the parturition, although some studies have detected ketonuria in the third month of pregnancy. Its immediate causes can be called

crudely ‘starvation ketosis’; where the animal is not given access to enough nutrients, especially energy, to meet its basic vital needs and its multiple fetuses. Low quality roughage is a particular risk because not enough can be consumed to meet requirements when the volume of the rumen is reduced by the presence in the abdomen of a large uterus. Secondary ketosis is similar, except that other diseases temporarily interfere with feed consumption in an otherwise properly fed female (Smith & Sherman, 2009).

In over-nutrition (“state”) ketosis, the animal is overfed and its massive internal fat stores and the uterus occupy so much of the body cavity that dry matter intake is severely curtailed at a time when increased energy consumption is required. Leptin, a hormone produced by adipose cells, may also contribute to a reduction in feed intake by decreasing the obese animal’s appetite (Kolb & Kaskous, 2004).

Excessive grain feeding can lead to pregnancy toxemia because the goat that eats inadequate roughage is apt to go off feed at this critical time. When goats are fed large amounts of corn silage in late gestation they can become obese. Their level of ingestion then decreases dramatically before parturition. Rumen acidosis from energy-rich silage may contribute also to the development of PT (Smith & Sherman, 2009).

The development of this disease coincides with the unsuccessful attempt in females to meet energy demands, promoting the mobilization of body reserves (Radostits et al., 2007). Sheep and goats with multiple fetuses consume smaller volumes of dry matter compared to animals carrying a single fetus. This reduction in dry matter intake is due to the lower rumen volume and the increase in the size of the uterus; the production of heat by fetuses and changes in the concentrations of free fatty acids (Pugh, 2005). Furthermore, especially when there is a high body condition score, intra-abdominal fat also decreases food capacity. Thus, the pregnant female consumes less food when it needs it the most (Kronfeld, 1972; Côrrea et al., 2010, Henfnawy et al., 2011).

The mortality rate could approach 100% after the onset of clinical signs in the absence of treatment (Linzmeier & Avanza, 2009), and in some herds, the disease could be classed as an outbreak because the incidence is so high. Herds that are infected with PT have an above average neonatal lamb mortality rate and often have a decrease production (Radostits et al., 2007).

PT is typically more common than lactational ketosis, and occurs predominantly in ‘improved’ breeds with high prolificacy. It is not a disease expected to occur in native breeds carrying a single kid under extensive management conditions (Smith & Sherman, 2009).

2.2.2 Pathogenesis

In monogastric animals, most of the available glucose comes from intestinal digestion of starch which after hydrolysis gives glucose which is rapidly absorbed and transported in the bloodstream. In ruminants, carbohydrates (fibre and starch) are fermented in the rumen producing volatile fatty acids (VFA), (propionic, acetic and butyric acids). Part of the propionic acid is converted in glucose in the liver. This organ is responsible for 50% of the production of glucose. About 30% to 35% of glucose is derived from dietary amino acids (especially alanine and aspartate), which are incorporated into the gluconeogenic biochemical pathways. The other glucose precursors are derived from lactate metabolism in the liver and kidney and through the anaerobic metabolism of glycerol obtained from plasma triglycerides (Ortolani, 1994).

Seen from a biochemical point of view, glucose use as a major energy source in ruminants is limited to a small number of organs such as the nervous system, the liver, the kidney and the mammary gland. However, fetal tissues use it as the basic carbohydrate for its development. Thus, the higher the number of fetuses the larger the requirement for glucose especially at the end of pregnancy (Wastney et al., 1983; Kozloski, 2005).

The negative energy balance (NEB) represented by a decrease in blood glucose, probably triggers the signs of PT in sheep and goats, because there are cases in which hypoglycemia does not cause toxemia, due to the fact that the animals adapt to the NEB. It is important to know that some animals are susceptible to toxemia while others are resistant (Rook, 1993).

The nutritional requirements of pregnant goats and sheep are quite high in the final third of pregnancy. This picture is aggravated by pregnant small ruminants that have twins and triplets due to the fact that they require 180 to 240% more energy, respectively, than pregnant females with a single fetus. Therefore, the former pregnant sheep and goats are unable to consume enough food to respond to such high demands (Pugh, 2005; Smith & Sherman, 2009).

The developing fetuses depend on glucose (maternal hepatic gluconeogenesis) for their energy needs. Ketone bodies and free fatty acids do not cross the placenta in any substantial quantities (Reid, 1968). Insulin levels in the late pregnant female are decreased; this spares glucose for fetal needs since insulin-sensitive tissues cannot take up glucose without insulin. In addition, low insulin stimulates lipolysis and gluconeogenesis. Placental lactogen levels are greatly increased when multiple fetuses are present. Placental lactogen has growth hormone, as well as prolactin activity and is probably crucial to meet the metabolic needs of

the fetuses at the expense of the dam. Thus, the late pregnant ruminant is often subclinically ketotic (Smith & Sherman, 2009).

In an inappropriate feeding management system, the animals have a NEB when energy intake is below that which is needed for fetal development. Physiologically, the body tries to compensate for this energy deficit through mobilization of body reserves (lipolysis of fat tissue). However, the liver cannot metabolize all the free fatty acids (fat) mobilized by lipolysis, resulting in the formation of ketone bodies (Ortolani, 1994; Prieto et al, 1994; Van Saun, 2000; Rook, 2000; Santana, 2001).

In the liver, glycerol may be used for glucose production or may be recombined with the FA to synthesize triglycerides. The VFA can be degraded by oxidation and the two-carbon fatty acids are converted into acetyl-CoA. Then, Acetyl-CoA is combined with oxaloacetate in order to participate in the tricarboxylic acid cycle to produce energy. The tricarboxylic acid cycle is also responsible for gluconeogenesis by combining oxaloacetate with a glucose precursor molecule. If there is not enough oxaloacetate available, acetyl-CoA is converted to ketones which reduce food intake and result in NEB, its concentrations are high (Smith, 2006).

Low glucose production causes high levels of fatty mobilization from deposits, therefore mobilizing in this process large amounts of non-esterified fatty acids (NEFA), and producing hepatic steatosis and intense cetonemia and ketonuria. As a result ketone bodies such as β -hydroxybutyrate (β HB), acetoacetate and acetone are produced, that are primary compounds of fat metabolism. These products have a very acidic character and their accumulation causes intense metabolic acidosis, with a drop in blood pH and bicarbonate levels (Kaneko et al., 2008).

2.2.3 Diagnostic

Early diagnosis of PT is essential for better control of the disease (Abdul-Aziz & Al-Mujalli, 2008). Therefore, clinical diagnosis is based on epidemiology, clinical signs as well as anamnesis, including other signs such as abortion, diet and care of pregnant females (Marques, 1994). In addition, the laboratory assays for this disease should be considered for diagnosis such as glucose, β HB and the determination of ketone bodies in urine (Radostits et al., 2007).

2.2.3.1 Clinical signs

The early signs of pregnancy toxemia are vague. They probably originate from decreased glucose consumption by the animal's brain. This disorder is usually accompanied by nervous symptoms. The female may be slow to get up or may lie isolated in a corner of the pen, which is the opposite to what healthy herd-mates would do. It eats less and its eyes are dull. There is often a noticeable subcutaneous edema of the lower limbs. As the disease advances, sheep and goats may also show signs of drowsiness, listlessness, aimless walking, muscle twitching or tremors in fine muscles (facial and limbs), and teeth grinding. Generalized weakness is observed followed by more apparent neurologic abnormalities such as blindness, loss of threat response, opisthotonos (star gazing), nystagmus, ataxia, then coma. Fecal output is reduced to a few small, dry, mucus coated pellets (Pugh, 2005; Borges et al., 2009; Souto et al., 2013). Other signs are observed, such as salivation, abnormal posture with lateral deviations of head with uncoordinated movements and posture, in addition to acetone odor on the breath (Abdul-Aziz & Al-Mujalli, 2008; Hefnawy et al., 2011).

The symptomatology of PT develops in three different clinical phases: Phase 1 is the most basic and mildest due to the fact that it is characterized by the maintenance of appetite although it is lower; the absence of vision and hearing changes, this phase has also a good prognosis. The second phase is characterized by the lack of appetite and the position of the animal that remains stationed with legs spread wide to spread the weight of the animal. The clinical prognosis at this stage is reserved. The last phase is characterized by the maintenance of decubitus and the animal's incapacity to get up and it maintains its head folded back onto the thorax, this phase has a poor prognosis (Ortolani, 2013).

Hypoglycemia is the abnormality that is initially the most detected due to nutritional deficiency. It becomes inadequate for the development of the fetus (Scott et al., 1995; Andrews, 1997).

As metabolic acidosis develops, the animal may breathe more rapidly. Thus, advanced primary ketosis may be difficult to distinguish from a primary pneumonia which has caused the animal to go off feed and develop a secondary ketosis. A careful physical examination is also necessary to identify other problems such as parasites, lameness, and bad teeth that might have contributed to the animal's present state (Smith & Sherman, 2009).

In the terminal stages of PT, the animal becomes recumbent. Death of the fetuses at this stage releases toxins and hastens the demise of the animal and consequently a septicemia develops in the ewe or goat. Pulse and respiratory rates increase as endotoxic shock develops.

The course of the untreated disease varies from twelve hours to one week (Smith & Sherman, 2009).

Goats and sheep with PT that do not die tend to have dystocia and higher mortality at kidding. They are unable to provide milk as would a healthy ewe. Similar problems are noted in obese females, even when clinical PT does not occur; in addition, these animals are at high risk of developing lactational ketosis (Smith & Sherman, 2009).

2.2.3.2 Laboratory tests

There are three major ketone bodies that are produced in the course of this metabolic disease: β HB, acetoacetate and acetone. In the past, these were sometimes measured together in a poorly defined way and reported as total ketones. Currently, β HB, which is the most stable ketone in blood and accounts for approximately 85% of the total ketones in sheep with PT, has received the most attention in the laboratory (Smith & Sherman, 2009).

Some people can detect an odor of ketones on the breath of ketotic animals. Others must rely on simple diagnostic reagents or laboratory tests. In the early stages of PT, ketone bodies are easily detectable in the urine. The commonly used test strips and pills containing nitroprusside (Rothera reagent) turn purple in the presence of acetoacetate but react poorly with acetone and β HB. If the animal has only a trace of ketonuria (which is physiological when in late pregnancy with multiple fetuses), some other cause for this illness should be sought, but supportive treatment to prevent worsening of ketosis should be given. Late stages are usually accompanied by renal failure; marked proteinuria, epithelial casts, and ketonuria are present. (Ferris et al, 1969; Ortolani & Benesi, 1989; Bruere & West, 1993; Sargison et al., 1994; Andrews et al, 1996).

When urine is not available, plasma or serum can be checked for ketone bodies with ketone pills, powders or strips. Recently, with the advent of hand-held meters for testing human blood for β HB and glucose, precise on-farm β HB testing has become possible. The test strips are inexpensive and the results obtained rapidly. Unfortunately, specific β HB reference values have not been established for goats, but values established for sheep can be used by default. Thus, β HB values less than 1 mmol/L can be considered normal, values from 1.5 to 3.0 mmol/L can be considered indicative of severe undernutrition, and animals with PT often have a β HB concentration greater than 3.0 mmol/L. If the animal has died recently, aqueous humor or

cerebrospinal fluid can be tested instead of blood, but the accuracy of the hand-held meters using these fluids has not been established (Smith & Sherman, 2009).

When blood glucose levels are available, severe hypoglycemia or terminal hyperglycemia are both possible (normal range 50 to 75mg/dL) (Silva et al., 2010). This study working with serum biochemistry revealed hyperglycemia and hypoglycemia where blood glucose concentrations ranged from 90 to 132.4 mg/dL in 4 animals and was 20 mg/dL in one animal. In some natural cases of PT, hypoglycemia really seems to be the most important aspect and it results from hyperketonemia and inappetence, initially it was considered to be the cause of these changes (Andrews et al., 1996). Hyperglycemia which is a frequent finding may be explained by stress in the pregnant female. High levels of cortisol, result in gluconeogenesis, which becomes more pronounced when fetal death occurs (Ford et al., 1990).

High concentrations of NEFA are often seen in the clinical condition of the metabolic disorder and they are explained by lipolysis, thereby indicating that there is fat mobilization to meet an increased demand for energy on a part of the dam and the fetuses. The activities of the enzymes such as aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and creatinine kinase (CK) could be elevated. Increased levels of these enzymes indicate organ damage, for example, arising from fatty infiltration of liver, kidney or heart, or muscle damage or catabolic degeneration. When analyzing alkaline phosphatase (ALP) activity the concentrations are within the normal range for the species. Thus, when the three aforementioned enzymes are increased, they are good indicators, but it is possible for animals to already have severe liver steatosis while enzymes remain at low levels (Borges et al., 2009). Furthermore, high serum levels of urea and creatinine (azotemia) may indicate terminal renal failure (Andrews et al., 1996; Santos et al., 2011).

Other laboratory tests are not commonly performed in field cases of PT. Cortisol-induced changes in the hemogram (neutrophilia, lymphopenia, eosinopenia) and evidence of dehydration (elevated hematocrit and total protein) can be expected (Smith & Sherman, 2009).

2.2.3.3 Necropsy findings

A female that dies of PT usually has multiple fetuses in the uterus, unless these were removed just before death. The fetuses may be fresh or decomposed. The dam's liver is enlarged, with rounded edges and yellow because of infiltration with fat. The dam's adrenal glands are enlarged (Smith & Sherman, 2009).

The carcass appears dehydrated and has large quantities of fatty tissue in the abdominal cavity (in obese animals) and a small amount of fat (in underfed animals). Rumen content is usually dry, yellow and with an acetic acid odor. In addition, there is an increase in the size of mesenteric vessels and the lungs due to hypostatic congestion. If urine remains in the bladder, it shows a strong ketone reaction (Sanches, 1985; Ortolani, 1994; Smith & Sherman, 2009).

Histopathological findings have poorly defined lesions, which may cause cerebral and cerebellar neuronal necrosis and vacuolation, early structural maturity of the placenta and liver steatosis (Jeffrey & Higgins, 1992; Cal et al., 2009).

The liver can present a microvesicular fatty degeneration, affecting acinus regions of hepatocytes. The severity of liver damage was associated with increased activity of hepatic enzymes. It was suggested that fatty liver due to lipolysis interferes with hepatic gluconeogenic capacity, thus ketosis and fatty liver disease would play a central role in PT (Radostits et al., 2007; Schild, 2007; Cal et al., 2009).

According to Andrews (1997) and Radostits et al. (2007), the kidneys may have poorly defined lesions; however, Souto (2013) have observed vacuolation of hepatocytes and renal tubular cells.

The differential diagnosis should be performed with rabies, listeriosis and polioencephalomalacia. Diagnosis should also differentiate PT from hypocalcemia, which has similar clinical signs and can occur in the same period as PT and in conditions of stress. However hypocalcemia has a more rapid clinical course than PT and the animals respond quickly to treatment (Riet-Correa et al., 2007; Costa & Silva, 2011).

2.2.4 Treatment

Many different forms of treatment can be used, however, most of the animals do not respond to it. The treatment and prognosis depend on the stage and on a rapid and accurate diagnosis, if this is achieved then the chances of recovery are better but in advanced cases, recovery is rarely observed (Andrews, 1997; Radostits et al., 2007). Treatment of this disorder should be based on two general principles: administration of energy sources and removal of factors that increase energy requirements (Brozos et al., 2011).

The treatment of PT should be immediate and aggressive. Cesarean section in the early stages of the disease, possibly before the development of irreversible brain damage, may be a method of treatment by removing the fetal demand for glucose. If an animal is not too weak or

the livestock value does not justify surgery, labor can induced (Sargison et al., 1994; Andrews, 1997; Pugh, 2005; Brozos, 2011).

In the earliest clinical form, the goat easily eats offered grain. The diet should be improved to include better quality roughage and higher levels of concentrates. Propylene glycol can be given orally by dosing syringe, at the rate of 60 mL, two or three times daily as a glucose precursor. Although some authors suggest an amount of 175 mL to 250 mL of propylene glycol twice a day, this level seems excessive and is likely to overwhelm the ability of the liver to convert the propylene glycol into glucose. Overdoses of propylene glycol can be fatal, creating plasma hyperosmolality that impairs neurologic function. A commercial product that contains niacin as well as propylene glycol has been recommended. The goat can also be injected with enough mixed B vitamins to supply 1g of niacin/day. Calcium borogluconate (60mL of a 23% to 25% solution) is given subcutaneously to counteract any concurrent hypocalcemia. Approximately 20% of sheep with PT are also hypocalcemic. Some authors recommended the use of insulin, up to 40 units of protamine zinc insulin twice daily subcutaneously (Sanches, 1986; Smith & Sherman, 2009).

Previous studies have demonstrated that propylene glycol administration and cobalt associated with B12 vitamin do not affect the energy and protein metabolic profile, and there was no impairment of hepatic functionality of sheep in the peri-parturient period (Andrews et al., 1996; Santos et al., 2012).

If the goat is known to be within one week of its parturition date, hormonal induction of parturition with 10 mg of prostaglandin F2 alpha will end the energy drain caused by the fetuses. If the due date is uncertain and the owner desires to save the dam and offspring, 20 to 25mg dexamethasone may be preferred for its gluconeogenic effects and beneficial stimulus on appetite. If possible, transabdominal ultrasound examination of the uterus for evidence of fetal movement or heartbeat should be performed to verify that the fetuses are still alive. Dexamethasone treatment requires a live fetus, and slaughter or euthanasia may be more appropriate in commercial situations if the kids are already dead (Smith & Sherman, 2009).

If a valuable goat is down and very depressed or has failed to improve by the day after initiation of treatment, a cesarean section should be performed immediately. Severely toxemic animals do not kid rapidly or dependably after receiving hormones, but dexamethasone on the first visit may prepare the lungs of marginally immature kids and thereby increase their chances of surviving a cesarean operation that can be delayed for twenty-four hours. Even with surgery and extensive fluid therapy, prognosis is poor for survival of the recumbent goat in the late

stages of PT. The kids from these dams are also frequently delivered dead or die within a few hours of surgery (Smith & Sherman, 2009; Brozos et al., 2011).

Generally after removal of the fetus, the clinical status of the animal improves. However, it may often deteriorate, especially if the fetus is dead. In all cases, the intravenous administration of glucose in combination with electrolytes should be continued until complete recovery of the animal (Brozos et al., 2011).

Blood glucose levels in pregnant goats with PT can be a good indicator of the viability of the fetus, in a situation associated with hypoglycemia the cesarean section should be considered, knowing that the goat's condition may worsen rapidly and that performing rapidly the surgery will avoid suffering in the female and its offspring (Lima et al., 2012).

2.2.5 Prevention

Prevention is the best way to avoid the damage caused by PT. It should constantly analyze the herd's nutritional status; make adjustments to maintain the animal with a body condition score suitable for each physiological stage. Pastures should be well managed and supplemental feeding is essential for proper nutritional management during the dry period. Thus, the requirements to supplement dams with concentrate tend to be lower, contributing to a lower cost of production, less labor and consequently healthy animals (Azevedo, 2012).

If goats are already obese when the last trimester is reached, it is too late to propose a weight-reducing diet. Instead, animals must be fed high quality roughage and as much as 500g of concentrate daily. Anything that disturbs the comfort of the goat, such as lack of exercise, poor ventilation, or drafts, should be corrected. This means that the stall should be dry, with adequate litter, and uncrowded. The goats should be let loose for at least two to three hours per day. Timid dams and slow eaters should be housed separately from dominant, aggressive animals that might drive them away from the feeder. If fetal numbers have been determined by real-time ultrasonography, the goats can be grouped and fed according to litter size. Goats carrying three or more fetuses should receive the best quality roughage available in addition to adequate concentrate (Smith & Sherman, 2009).

In intensive farming systems, the improvement of the nutritional plan of animals should be made in the last half of pregnancy. The stressful situations should be avoided in the final third of gestation: shearing, anthelmintic treatments and transportation, as well as changes in the type of food (Radostits et al., 2007).

When one dam develops PT, the diet of the rest of the herd should be evaluated and corrected if necessary. Concentrates should be introduced gradually and under strict control, to avoid indigestion. It is unrealistic to expect a large herd of commercial goats to be totally free of PT. Routine monitoring of all late-pregnant dams for urinary ketones is also unrealistic. Healthy dams carrying large litters can be expected to excrete small quantities of ketones yet they do not need treatment. Drenching with prophylactic propylene glycol (60mL orally twice a day) is in itself a stress to a goat and should be reserved for those showing abnormal behavior or diminished appetite (Smith & Sherman, 2009).

2.3 NATURAL PRODUCTS: GLUCAN & SAPONIN

2.3.1 β -glucans: structure, properties and bioactivity

2.3.1.1 Introduction

Natural products, useful in treating and/or preventing several diseases, have been sought throughout the history of science. Most of these natural products are plagued with a common problem, *i.e.*, the fact that they often represent a complex mixture of individual ingredients, each of which can contribute to their biological activity. To a certain extent, this is the case of β 1,3-D-glucans (hereafter referred to as “glucans”). They form part of a group of natural biologically active materials generally called immunomodulators or biological response modifiers (BRMs). Generally, immunomodulators can act both positively (immunostimulators) or negatively (immunosuppressant) (Novak & Vetvicka, 2009). Using immunomodulators to modulate the immune function of animals is considered as a potential means to improve their performance and health status (Li et al., 2006).

Glucans are naturally occurring carbohydrates forming structural components of cell walls found in plants, yeast, fungi, mushrooms seaweed, cereals and some bacterial species. Generally, the term glucan is sometimes used as a chemical name of a glucose polymer and represents a group of chemically heterogeneous carbohydrates consisting of various numbers of glucose molecules bound together by various types of linkages (Mayell, 2001; Vetvicka & Vetvickova, 2015).

β -glucans have been known to scientists as a plant constituent for decades. For over twenty years, they have been studied because of their favorable biological effects on mammals such as a biological response modifier. They are recognized by the organism which triggers a

series of events in the immune response. It is common knowledge in the scientific community that β -glucan is a powerful molecule and can be used to treat several diseases (Akramienè et al., 2007; Vetvicka & Vetvickova, 2014).

Over the last decade glucans have received special attention due to their biological bioactivity, particularly as regards immunomodulation. Furthermore, a great many beneficial effects such as anticarcinogenic, antimutagenic, hypocholesterolemic and hypoglycemic have been ascribed to them. Recently, numerous studies have been performed to try to improve the extraction procedure of glucans from yeast cell walls. Moreover, there is a growing interest in the identification of structural and biological properties of glucan and its derivatives, including its immunostimulant potential (Magnani & Castro-Gómez, 2008; Malaczewska & Milewski, 2010).

In effective and nutritionally well-balanced animal diets, essential nutrients are often combined with feed additives and supplements that may contain microorganisms and their products. The results of numerous studies indicate that *Saccharomyces cerevisiae* dried brewer's yeast enhances animal productivity and delivers health benefits (Zabek et al., 2014). These positive effects can be attributed to the presence of mannans and glucose polymers in yeast cell walls. Yeasts are unable to colonize the gastrointestinal tract independently. By binding to the intestinal epithelium, they compete with pathogenic bacteria and stimulate the immune system of animals. β -glucans and mannan oligosaccharides (MOSs) found in yeast cell walls have immunomodulative properties (Li et al., 2006). MOSs are capable of neutralizing pathogenic bacteria and they support β -glucans in the process of stimulating defense mechanisms (Malaczewska & Milewski, 2010).

Accordingly, MOSs have several beneficial properties and they can be widely used in human and veterinary medicine, pharmaceutical, cosmetic and chemical industries as well as food and feed production. The beneficial properties of various β -glucans used in the food industry are discussed elsewhere (Petravic-Tominac et al., 2010).

2.3.1.2 Historical interest in β -glucans

The healing properties of mushrooms have been known for thousands years, with the first recorded report of their medicinal qualities dating from around 3000 BC. A number of fungal components have been implicated in these properties. The interest in polymers from mushrooms (like β -glucans) originated in the early 1900s, when the ability of yeast to inactivate serum complement was first described. This led to the development of an insoluble yeast cell

wall molecule, termed *Zymosan*, which was subsequently used to help define the pathway of alternative complement activation. Later studies showed that the direct intravenous injection of zymosan could activate the immune system, stimulating protective host responses. Although these particles were found to consist of a variety of components (including glucans, mannans, chitin, protein, and lipids), β -glucan was identified as the biologically active constituent. Since the discovery of its stimulatory activities, zymosan has been chosen for many studies of immune function both *in vivo* and *in vitro*, including inflammation, phagocytosis, arachidonate release and cell migration (Brown & Gordon, 2003).

It is likely that the first substance investigated with immunomodulating properties was called endotoxin lipopolysaccharide (LPS) from gram-negative microbes. A paper describing the endotoxin was published in 1865. LPS intensified phagocytosis with a potential protective effect for a host; however, its toxic effects were dominant. It was found that a saccharidic moiety of LPS, with prevailing glucose, galactose and mannose content, was non-toxic but bears immunomodulating activity. It was apparent that even polysaccharides themselves could act as immunomodulators, while their toxicity was negligible. The history of polysaccharides as immunomodulators goes back to the middle of the last century, when Shear and coworkers in 1943 described a substance called Shear's polysaccharide which caused necrosis of tumors (Novak & Vetvicka 2009).

Subsequently, other polysaccharidic immunomodulators were studied; among them were β -glucans. Investigation of β -glucans began in the 60s and 70s of the last century. Two lines can be traced in β -glucans history, based on different starting points, but generally converging. The first one took place chiefly in the U.S.A., the second one in Asia, specifically in Japan. Research about β -glucans in the euro-american milieu was based on the knowledge of the immunomodulatory effects of zymosan from a mixture of polysaccharides isolated from the cell walls of *S. cerevisiae* (Pillemer & Ecker, 1941). When zymosan was closely examined, β -glucan was identified as a primary effective component: it was subsequently isolated and its immunological effects were investigated (Di Luzio & Riggi, 1970; Williams et al., 1970).

In Asian medicine, the consummation of different medicinal mushrooms (shiitake, maitake, reishi *etc*) has been a long tradition. In detailed studies of the biological effects of these mushrooms, especially their anticancer action, β -glucans were again found to be responsible due to non-specific immunomodulation. This initial investigation was conducted by Goro Chihara at Teikyo University in Kawasaki, who isolated β -glucan, which he referred to lentinan, from the shiitake mushroom (*Lentinus edodes*, now *Lentinula edodes*) (Chihara et al., 1969).

The interesting activity as a polysaccharidic immunomodulator (β -glucans) was seen when purification was sufficient since they had very low toxicity. Conversely, the considerable heterogeneity of all natural β -glucans continues to be the cause of a series of contradicting conclusions. An attempt was then made to solve this problem using semi-synthetic and synthetic probes, suitable for accurate immunological research (Jamois et al., 2005). β -glucan extraction for therapeutic uses is generally performed using yeast, *S. cerevisiae*, and fungi, *Lentinula edodes*, and products have been marketed for therapeutic use in Japan and Brazil for a long time and they are used as adjuvants in antitumor therapy (Carreira et al., 2012).

2.3.1.3 Sources and structure

β -glucans are polysaccharides that make up the structure of the cell wall of yeast, fungi, mushrooms, and each differs from the others by the type of linkage between glucose units. These glucose polymers are produced also by a variety of plants, such as cereals (oat, barley) and seaweed. They are the constituents of the cell walls of certain bacteria (*Pneumocystis carinii*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Histoplasma capsulatum* and *Candida albicans*), however an important source of these polymers is *Saccharomyces cerevisiae* cell walls, also known as fermentation yeast (Table 01) (Akramienè et al., 2007; Mantovani et al., 2008; Magnani & Castro-Gómez, 2008).

The main components of the fungal cell wall are polysaccharides and glycoproteins. For example, yeast (*S. cerevisiae*) cell walls consist of three layers: an inner layer of insoluble β -glucan (30-35%), middle layer – of soluble β -glucan (20-22%) and external layer – of glycoprotein (30%). β -glucan has been purified from brewer's and baker's yeast, from oats and barley bran (Fig.01) (Akramienè et al., 2007).

Table 1 – Structure, origin and biological activities of β -glucan: Source: adapted from Mantovani et al. (2008)

Structure	Source	Effects
β (1 \rightarrow 3) (1 \rightarrow 6)	<i>Saccharomyces cerevisiae</i>	Antiparasitic
		Antibacterial
		Antiviral
		Antifungal
		Antimutagenic/antigenotoxic
		Antitumoral
		Hematopoietic stimulator
		Mitogenic
		Imunostimulating activity
		Antitumoral
		Cytokine induction
		Antimutagenic/antigenotoxic
		Inhibition of CYP450 isoenzymes
		Antitumor
β (1 \rightarrow 3) (1 \rightarrow 4)	<i>Candida albicans</i>	Inhibition of CYP450 isoenzymes
		Antitumor
	<i>Poria cocos</i>	Antitumor
		Antitumor
	<i>Agaricus blazei</i>	Antitumor
		Antitumor
	<i>Lentinus edodes</i>	Antitumor
		Antitumor
	<i>Schizophyllum commune</i>	Antitumor
		Antitumor
β (1 \rightarrow 3) (1 \rightarrow 4)	Oat	Antimicrobial
		Antiparasitic
		Hypocholesterolemic
		Anti-thrombotic
	Barley	Antimutagenic

S. cerevisiae is commonly called brewery and bakery yeast. It is a single-celled eukaryotic micro-organism which has different forms. Among its characteristics, it has the ability to adjust metabolically to the fermentation of sugars in the presence or absence of oxygen, producing alcohol and carbon dioxide (Tortora et al., 2000). In diverse environments, developing an outer protective layer is critical for the growth and survival of fungal cells (Durán & Nombela, 2004). The cell wall is a common structure in filamentous fungi and yeast, located at the interface between the microorganism and the environment. In recent decades, the cell-wall polysaccharide β -1,3-glucan, has been widely studied as a novel human antifungal drug (Firon et al., 2004).

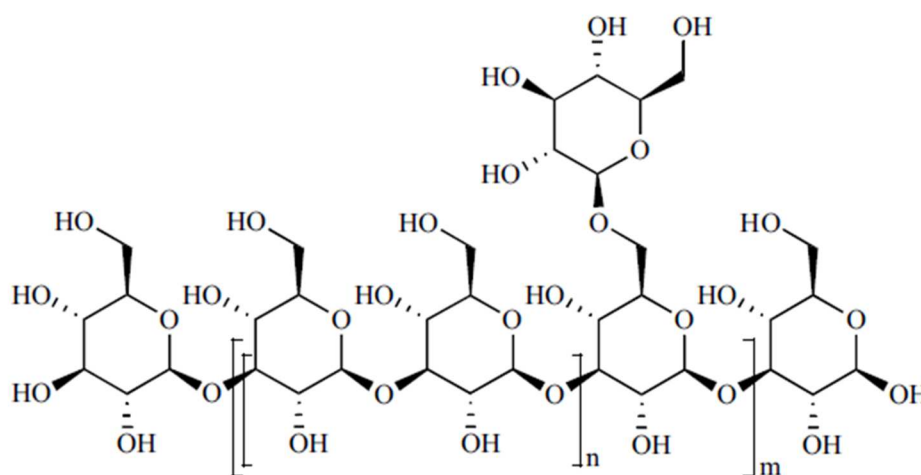


Figure 1. General structure of glucan (Novak & Vetvicka, 2009).

Glucans derived from different sources have some differences in their structure. They are a heterogeneous group of glucose polymers, consisting of a backbone of $\beta(1,3)$ -linked β -D-glucopyranosyl units with $\beta(1,6)$ -linked side chains of varying distribution and length. Oat and barley β -glucans are primarily linear with large regions of $\beta(1,4)$ linkages separating shorter stretches of $\beta(1,3)$ -linked branches coming off of the $\beta(1,3)$ backbone. Yeast β -glucans have $\beta(1,6)$ branches that are additionally ramified with extra $\beta(1,3)$ regions (Fig 02) (Chan et al., 2009).

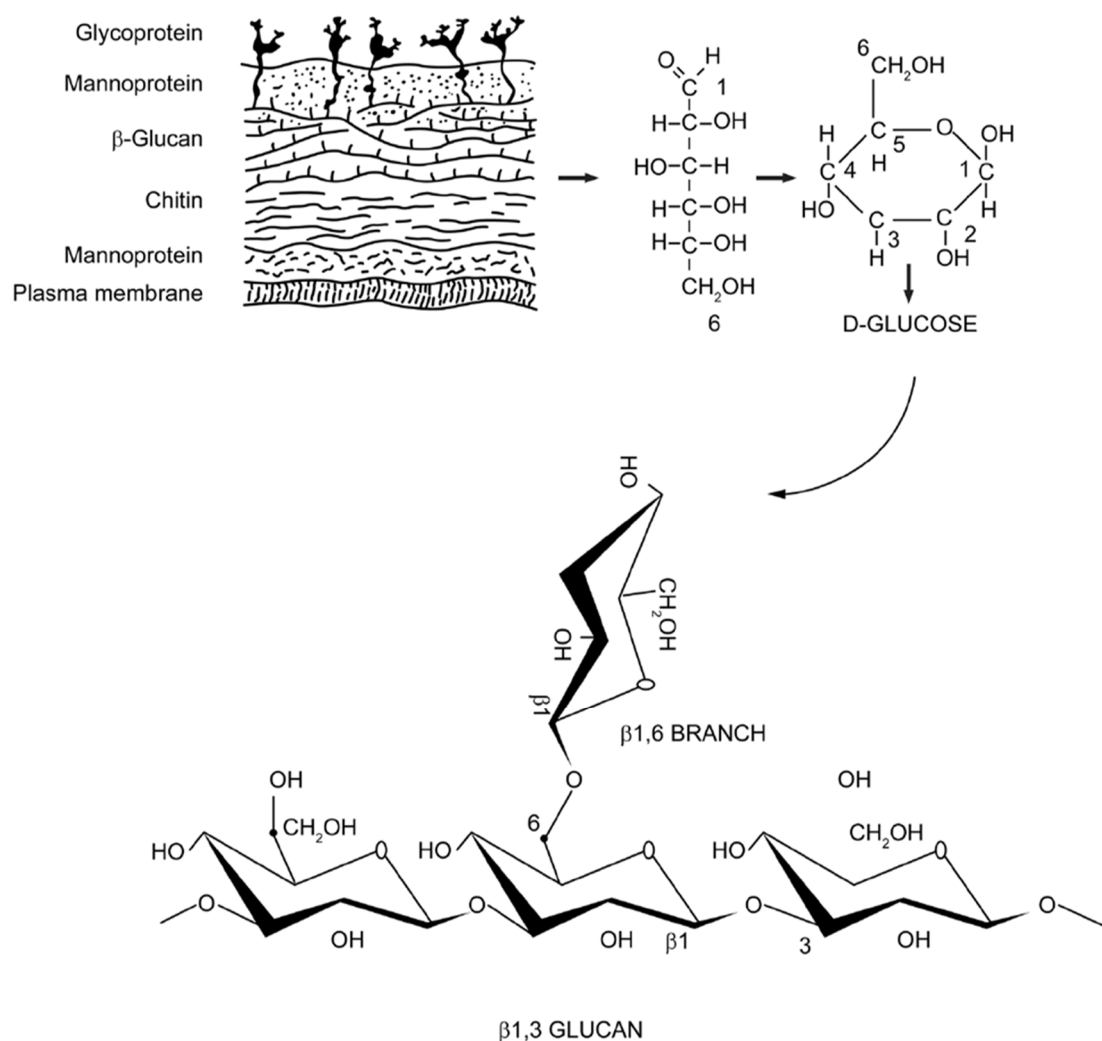


Figure 2. β -glucan is one of the key components of the fungal and yeast cell wall. The basic subunit of the fungal β -glucan is β -D-glucose linked to one another by 1 \rightarrow 3 glycosidic chain with 1 \rightarrow 6 glycosidic branches. The length and branches of the β -glucan from various fungi are widely different (Chan et al., 2009).

These structural differences can have large implications for the activity of the β -glucan. For example, differences in the length of the polysaccharide chain, extent of branching, and the length of the branches can result in differences between material extractable by hot water, such as mushroom β -glucans, and in different molecular weights. In general, *in vitro* studies have suggested that large molecular weight or particular β -glucans (such as *Zymosan*) can directly activate leukocytes, stimulating their phagocytic, cytotoxic, and antimicrobial activities, including the production of reactive oxygen and nitrogen intermediates. Intermediate or low molecular weight β -glucan (such as glucan phosphate) possesses biological activity *in vivo*, but their cellular effects are less clear. Very short β -glucans (<500-10 000 molecular weight; such as laminarin) are generally considered inactive. Several β -glucans have been investigated, they come from yeast β -glucan, since it is easily purified, and from mushroom β -

glucans, because there have been a lot of experiments performed in Japan, China, and Korea (Akramienè et al., 2007).

In *S. cerevisiae*, the cell wall may account for up to 30% of the total dry weight of the cell and its components are synthesized together and in synchrony with growth and cell division. It consists of three fractions, β -glucan, chitin and cell-wall proteins (mannan) that are interconnected. The structure of these components is depicted in Fig. (03). β -1,3-glucan is the most abundant component of the yeast cell wall (48-60%), which is a polymer of glucose β (1-3) and β (1-6) units forming a microfibrillar backbone to which other components are cross-linked. The outer layer being composed of mannoproteins and the inner layer of glucan and chitin in an interconnected structure by covalent bonds (Cid et al., 1995; Firon et al., 2004).

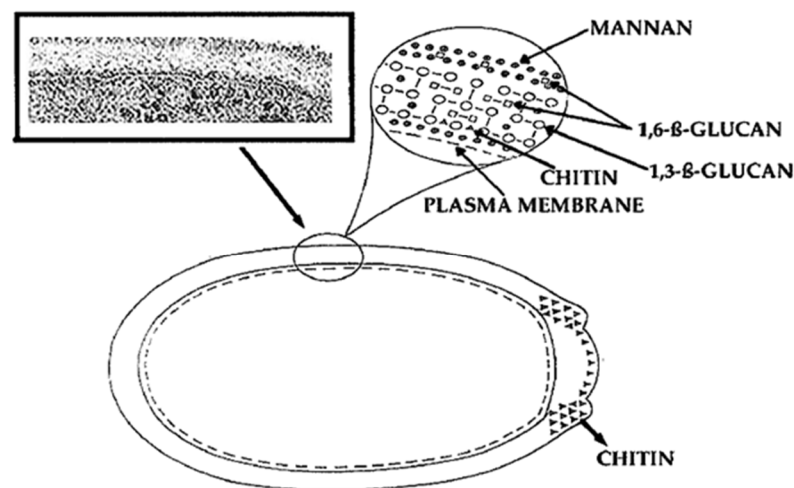


Figure 3. Model of cell wall structure in yeast. (Cid et al., 1995).

The wealth of mannoproteins that are incorporated into this structure and the functions that they perform are far from being established. Issues exist such as the genes involved in the biosynthesis of the polysaccharide components and the secretion of the enzyme complement required for this process to take place in an ordered manner. Such an array of proteins and structural polysaccharides maintains the integrity of the cell wall. Deficiencies in any of functions are rarely lethal but they are expressed in terms of alterations that lead to a defective wall which will make the cell more vulnerable (Durán & Nombela, 2004).

Morris et al. (1983) reported that the cell wall in *S. cerevisiae* is highly elastic, because the cells can rapidly decrease in volume in hypertonic solutions in response to osmotic pressure, and return to the original volume under favorable environmental conditions. The property of elasticity can be attributed to β -chains (1-3) glucan arranged in a kind of flexible spring. This

organization in the wall allows an increase or decrease in cell volume in response to external conditions and partly explains the increased permeability in living cells.

2.3.1.4 β -glucan immunostimulating activity

The most important quality of β -glucans and the reason why so much attention has been devoted to them is due to the physiological effects that they exercise. They are typical biological responses modifiers (BRMs) as immunostimulators. A large number of polysaccharides, which act only as immunostimulants, are known, but the most effective (and also most studied) are β -glucans. More than 6,000 papers describing the biological activities of glucans exist. Thus far, strong immunostimulating effects of β -glucans have been demonstrated in all animal species tested including earthworms (Beschlin et al., 1998; Kohlerova et al., 2004), shrimp (Chang et al., 2000), fish (Anderson, 1992), mice, rats (Feletti et al., 1992), sheep (Buddle et al., 1988; Milesweski & Sobiech, 2009; Zabek et al., 2014), pigs (Li et al., 2006; Bugni et al., 2008), cattle (Anantasook et al., 2014) and humans (Novak & Vetvicka, 2009).

Patients who suffer from systemic fungal infections including those by *Candida*, *Aspergillus* and *Cryptococcus* species have been described to possess high levels of circulation β -glucans in their plasma. It is possible that the β -glucans may have modulating effects on the immune system by activating macrophages, by phagocytosis of the pathogen and release of proinflammatory cytokines. It has been established that β -glucan has a key molecular pattern recognized by neutrophils (or polymorphonuclear leukocytes – PMNs) in response to *Candida albicans*, because of the production of antibodies specific for β -glucan, a major component of yeast cell walls (Lavigne et al., 2006). This mechanism, to recognize and respond to the structural components of fungi, particularly β -glucans, has evolved in mammals as defense against fungal pathogens (Akramienè et al., 2007).

Macrophages play a critical role in all phases of host defense be they innate or adaptive immune responses in reponse to infection. When a pathogen crosses an epithelial barrier, it is phagocytosed by macrophages and digested by lysosomal enzymes released by them. Lysosomal enzymes and phagocytic activity determine the macrophage function. The secretion of cytokines (interleukins and TNF- α) and inflammatory mediators (nitric oxide, NO and hydrogen peroxide, H₂O₂) are other effects of these cells. Therefore, the activation of macrophage function by β -glucans increases host immune defense. However, polysaccharides stimulate a dose-dependent increase in NO and TNF- α , but not in reactive oxygen intermediate in peritoneum macrophages (Kim et al., 2004). It was suggested that the ability of

polysaccharides in the up-regulation of these surface molecules involved in antigen-presenting processes may, by inference, activate T-cell-mediated immunity against malignant cells *in vivo*. Taken together, these results suggest that β -glucan acts as an effective immunomodulator and enhances the anti-tumoral activity of peritoneum macrophages (Fig 4) (Akramienè et al., 2007; Chan et al., 2009).

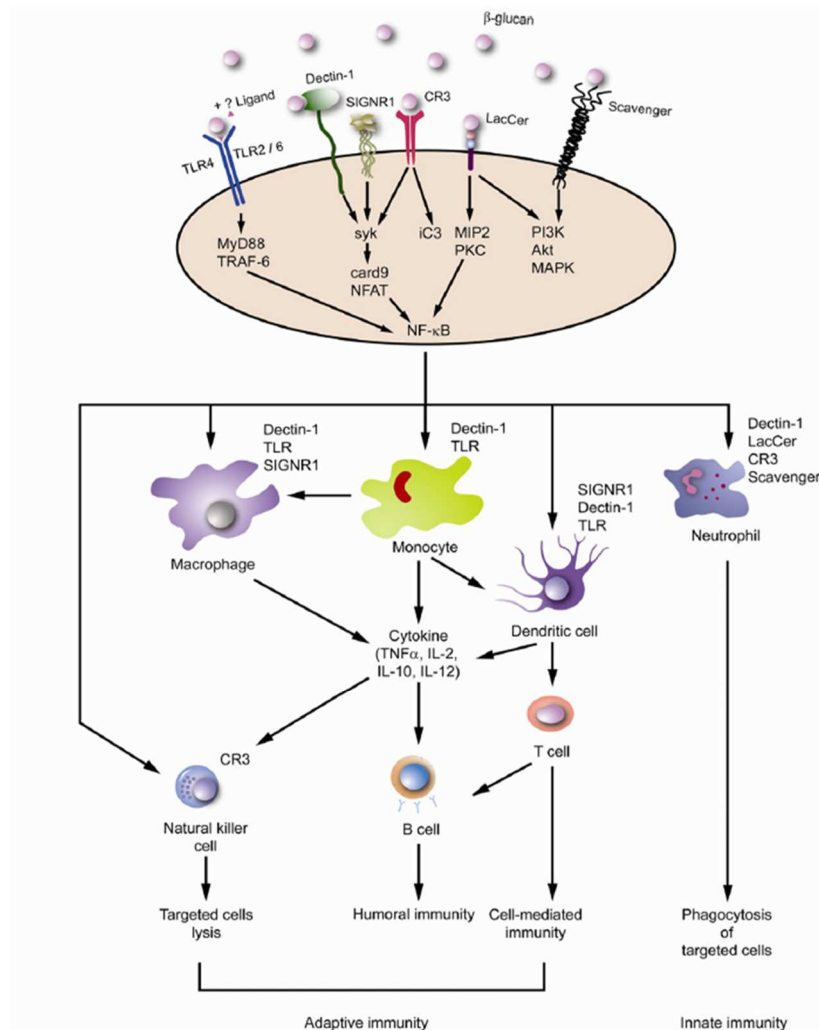


Figure 4. Immune activation induced by β -glucans. β -glucans can act on a variety of membrane receptors found on the immune cells. It may act singly or in combine with other ligands. Various signaling pathway are activated and their respective simplified downstream signaling molecules are shown. The reactors cells include monocytes, macrophages, dendritic cells, natural killer cells and neutrophils. Their corresponding surface receptors are listed. The immunomodulatory functions induced by β -glucans involve both innate and adaptive immune response. β -glucans also enhance opsonic and non-opsonic phagocytosis and trigger a cascade of cytokine release, such as tumor necrosis factor (TNF) α and various types of interleukins (ILs). (Chan et al., 2009).

In vitro studies have demonstrated enhanced microbial killing activity by monocytes and neutrophils in healthy volunteers after β -glucan administration. Besides activation of macrophages, T cells and natural killer (NK) cells, β -glucan activates complement by an alternative activation pathway. Pathogens that activate complement are first coated with the

C3b fragment of C3, which is rapidly proteolysed into the iC3b fragments which serve to promote a high-avidity attachment of the iC3b-opsonized pathogens to the iC3B receptors (CR3,CD11b/CD18) of phagocytic cells and NK cells, therefore stimulating phagocytosis and/or cytotoxic degranulation (Ross et al., 1999).

Studies provide clear evidence that the supplementation of diets fed to lactating ewes with *S. cerevisiae* dried brewer's yeast delivers health benefits. Yeast added to diets for pregnant ewes could prepare them better for lactation, but the resulting improvement in their milk performance was similar when yeast was administered in late pregnancy or only during lactation (Zabek et al., 2013; Zabek et al., 2014).

The productivity in small ruminant dairy farms is influenced by diseases such as mastitis. Milk from inflamed glands is characterized by higher albumin and globulin levels and lower lactose concentrations (Bianchi et al., 2004). Therefore, an immunostimulant such as glucan could be useful for elucidating the defense mechanism operating in the ruminant mammary gland (Buddle et al., 1988). Administration of *S. cerevisiae* dried brewer's yeast improves the parameters of nonspecific humoral and cellular immunity. The supplementation of diets fed to late-pregnant and lactating ewes with dried yeast increased gamma globulin concentrations, lysozyme and ceruloplasmin activity levels, and the indicators of nonspecific cellular immunity, the respiratory burst activity and potential killing activity of phagocytes (Malaczewska & Milewski, 2010).

2.3.1.5 Role of β -glucan on cholesterol and glucose levels

Chronic diseases in humans and animals can be reduced by dietary β -glucan. In a study on the biological activity of cell wall polysaccharides from *S. cerevisiae* fed to pigs, Kogan and Kocher (2007) showed the importance of the protective effect of β -glucan to the body by stimulating the immune system of common mucous membranes, which are areas permanently exposed to pathogens. β -glucan has a specific effect when considering the ingredients used in the production of functional foods (Mantovani et al., 2008). Fragments obtained from this macromolecule, oligosaccharides, may act as prebiotics selectively stimulating the growth of bacteria in the intestinal tract, and serving as an energy source for beneficial microflora (Przemyslaw & Piotr, 2003). Other positive aspects of the inclusion of β -glucan in the diet, such as reducing cholesterol and blood sugar, have also been shown in humans (Kim et al., 2006).

An active hypolipidemic component in oats, the soluble β -glucan fiber, has been concentrated in an oat fiber extract, this extract has been used to replace fat in food products. A

number of studies in humans have indicated that β -glucan has a major action in reducing cholesterol. When β -glucans are fed in a dose-dependent manner, significantly greater reductions in blood cholesterol concentrations are observed as β -glucan levels increase (Behall et al., 1997). Furthermore, treatment of oats with enzymes that destroy β -glucan results in the loss of the cholesterol-lowering potential of oats. Oats lower blood cholesterol levels *via* the formation of a viscous gel by the oat soluble fiber. This binds bile acids and increases their excretion in the feces. The production of bile acids from cholesterol of endogenous origin or the circulation is thus stimulated, reducing circulating blood cholesterol (Katz, 2001).

There may be additional ways in which oats lower cholesterol beyond the binding and removal of cholesterol-rich bile acids. Bacterial fermentation of the β -glucans increases the release of short chain fatty acids, which may inhibit cholesterol biosynthesis. Soluble fiber may also delay gastric emptying and reduce post-prandial insulin concentrations, which also inhibits cholesterol biosynthesis. And there has been some indication that soluble oat fiber may reduce the intestinal absorption of cholesterol (Katz, 2001).

Physically, the β -glucans are discrete, hollow microspheres that are highly miscible in most liquids and will not gel. β -glucan is composed of both soluble and insoluble fibers. Other soluble fibers such as pectin and psyllium tend to clump and gel when mixed with liquids. β -glucan from oat fiber has been shown to improve lipid profiles. In other words, β -glucans appear to be effective in lowering blood cholesterol concentrations, however, the mechanism by which this occurs is still unclear. There have been a few studies that have tested other fungal β -glucans for this activity in humans. They are associated with treatment of cardiovascular diseases due to elevated plasma cholesterol levels, in particular, LDL-cholesterol concentrations (Nicolosi et al., 1999).

Wilson et al. (2004) evaluated two fractions of barley β -glucan, low and high molecular weight, to reduce plasma cholesterol in a hypercholesterolemic diet using hamsters as an experimental model. The tests showed no differences in the activity of fractions, both of which promoted a reduction in cholesterol levels in serum. Similar observations were described by Matiazi (2005) in assessing the hypocholesterolemic effect of β -glucan extracted from *S. cerevisiae* administered to mice in a hypercholesterolemic diet.

Among the alternatives used in the control of glycemia, the β -glucans are outstanding. These polymers have been shown to be effective in the reduction of blood glucose concentrations, possibly because of their capacity to increase the viscosity of the intestinal content, forming a protective barrier that delays the absorption of glucose and lipids (Lobato et al., 2015)

Several dietary fungal β -glucans may reduce blood glucose concentrations possibly by delaying stomach emptying so that dietary glucose is absorbed more gradually (Chen & Serviour, 2007). A decrease in blood glucose levels by increasing insulin sensitivity has been observed in genetically diabetic mice fed with 20% whole mushroom maitake powder. Although β -glucans have been identified as the likely active constituents responsible for a beneficial effect on cancer and immunity, it is less clear which constituents may promote effects on blood sugar and blood lipids (Mayell, 2001).

2.3.1.6 Anticarcinogenic activity

Anticarcinogenic substances are able to prevent, reduce, and slow the appearance and development of cancer (Magnani & Castro-Gómez, 2008). The anti-tumor activity was first demonstrated nearly 50 years ago. Many animal experiments have demonstrated the remarkable effects of certain fungal β -glucans on a range of tumors (Vetvicka & Yvin, 2004). In this regard, the introduction of bio-modulators combined with chemotherapy significantly contributes to antineoplastic therapy. Among the known compounds as immunomodulators, β -glucans extracted from fungi and yeast cell wall have been evaluated for their antitumor activity (Miadokova et al., 2005).

The initial studies on the potential use of β -glucan against cancer were performed in the 70s. In 1979, Di Luzio and coworkers demonstrated that β -glucan preparations from *S. cerevisiae* significantly reduce the growth of breast carcinomas and melanomas. In the same study they found increased survival rates for mice with subcutaneous tumor implantation. Currently, it is known that the antitumor mechanism of action of β -glucans, including an effect in situations of metastases, acts through several immune functions in the host and enhances the host's survival. Research reported that the anti-tumor effects of β -glucan are based mainly on the ability to activate leukocytes by stimulation of their phagocytic activity and the production of TNF- α as (Xiao et al., 2004).

β -glucans have a backbone of $\beta(1,3)$ -linked β -D-glucopyranosyl units with side chains of different lengths. A variety of cell surface receptors bind β -glucan, including lectins, scavenger receptors, and integrins on monocytes/macrophages, neutrophils, and natural killer (NK) cells and various lymphocyte subpopulations (Brown & Gordon, 2003). β -glucan has anti-infective and antitumorigenic properties through leukocytes, phagocytic activity, production of inflammatory cytokines and chemokines, microbial killing and initiate the development of adaptive immunity of (Brown & Gordon, 2003; Ross et al., 1999).

2.3.1.7 Future perspectives

β -glucans have been shown to possess important biological properties regardless of their origin. This finding calls for future work in the domain of biotechnology using microorganisms such as *S. cerevisiae* to produce a pharmaceutical product. Studies could also involve the insertion of specific genes to control the production of a particular β -glucan in functional foods (transgenic food, Mantovani et al., 2008).

The identification of foods that produce high levels of these polysaccharides and where modifications of the β -glucan control gene are possible to improve absorption and efficacy; could be an approach in the production of functional foods with medicinal properties. Besides, the consumption of food with antimutagenic activity could contribute to a reduction in risk of cancer and of other degenerative diseases. However, further studies are needed involving epidemiologic evaluation of the public's intake of cereals such as oats and barley which are β -glucan-rich foods. For the moment no epidemiological study concerning the incidence of cancer and consumption of β -glucan is available, the only epidemiological study involving β -glucan relates to its consumption with the decrease of cholesterol levels (Mantovani et al., 2008).

2.3.2 Plant secondary metabolites and their use in ruminants

Ruminants have the most differentiated and complex stomach system of all mammals and they have a unique capability of converting a non-utilizable forms of energy, feed fiber (lignocellulosic agro-industrial by-products) to an utilizable form of energy i.e. volatile fatty acids, which the ruminants can use as a source of energy. This specialized digestive tract is well adapted to the digestion of structural plant polysaccharides, and enables ruminants to feed exclusively on forages. Most of the nutrients supplied to ruminants are produced in the rumen from microbial fermentation of carbohydrates and protein or non-protein nitrogen (Teferedegne, 2000; Jouany & Morgavi 2007; Kamra et al., 2012).

Four main targets have been identified concerning feed additives to optimize rumen function and performance: decreased methane production in favor of propionate to improve the energy balance of animals, reduced feed protein degradation to increase bioavailability of amino acids in the small intestine, reduced fermentation rate of rapidly fermentable carbohydrate (starch, sucrose) and therefore control of lactic acid production and improved fibre digestion. Ionophore antibiotics are capable of achieving these goals in the rumen. When added continuously at low concentrations in feeds (20-40 ppm), they slowed the rate of acid

production and prevented lactic acidosis (Osborne et al. 2004), mitigated methane production by redirecting metabolic hydrogen use towards propionate formation, decreased deamination of amino acids (Wallace et al., 1990), thus increasing peptide flow from the rumen into the small intestine (Gomez et al. 1991), and reduced foamy bloat in cattle grazing on legume pasture (Lowe et al. 1991). Post-ruminal effects of ionophores have also been reported. For example, they have been shown to be effective against coccidiosis, one of the most important and widespread parasitic diseases of ruminants (Stromberg et al., 1982). Hence, animals treated with feed antibiotics exhibited increased productivity (Jouany & Morgavi 2007).

There is increasing public and scientific concern about the use of antibiotics as feed additives in animal production. This concern is fueled by the emergence of antibiotic resistance in many human pathogenic bacteria, the release of contaminating residues into the environment (water, soil, etc.) and the risk that growth-promoting antibiotic residues occur in foods of animal origin (Yang & Carlson 2004; Manero et al. 2006; Parveen et al. 2006).

For all these reasons, the European Union (EU) decided that antibiotic used in livestock as production enhancers would be banned from 1st January 2006. (EU regulation N° 1831/2003 of the European Parliament and of the Council of 22 September 2003). This ban effectively ends nearly 50 years of antibiotic use for non-therapeutic purposes. It covers all classes of antibiotics, including ionophores, a group of substances extensively used as coccidiostats in poultry production and as growth promoters or production enhancers in ruminants. Ionophores improve animal performance in both dairy and meat ruminants (McDougall et al. 2004; Gallardo et al. 2005; Melendez et al., 2006). Consequently, there is nowadays a real demand among animal producers for alternative feed additives and among consumers for more natural and safe products in the human food supply chain (Jouany & Morgavi 2007).

One positive consequence of the ban is that hygiene standards in livestock production had to be raised to reduce the risk of pathogen infection. However, to maximize the efficiency of feed use and increase ruminant productivity through the manipulation of ruminal fermentation, alternative feeding strategies and new feed additives are needed (Jouany & Morgavi, 2007).

Many substances, such as probiotics, prebiotics, some organic acids involved in metabolic pathways, plant extracts, can offer some of the benefits that synthetic antibiotics provide (Jouany & Morgavi, 2007). Plants synthesize a broad range of compounds that are not directly involved in their growth, development or reproduction. These compounds are a complex group of substances, synthesized by the plants in some specific conditions in order to

defend and protect them. Most of these compounds were also used extensively as medicines before synthetic drugs and antibiotics were discovered. In addition, some of them have been extracted and concentrated for use in animal nutrition: such as flavouring agents in feeds, for their antimicrobial activity and their ability to influence the digestion of some ingredients (Elgayyar et al., 2001). Moreover, plants and their extracts have an important potential as manipulators of rumen fermentation for productivity and health benefits. They have specific effects on rumen microflora and fauna that can be beneficial to animal productivity and health (Wallace, 2004).

Studies on plant secondary metabolites (PSMs) have increased over the last 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals. Plant cell culture technologies were introduced at the end of the 1960s as a possible tool for both studying and producing PSMs. Different strategies, using *in vitro* systems, have been extensively studied with the objective of improving the production of secondary plant compounds (Bourgau et al., 2001).

Plants produce several organic compounds that are classified into primary and secondary metabolites. The primary metabolites are essential for their own growth, development and reproduction (Wallace, 2004; Kamra et al., 2006; Bodas et al., 2012). The secondary metabolites are used as a defense mechanism against the presence of pathogenic microorganisms and predation by insects and herbivores (Wallace, 2004). Moreover, the lack of plant secondary metabolites can negatively impact plant persistence and adaptability. In addition, they are recognized as being important in the nutrition and health of animals (Jensen et al., 2013).

PSMs play an important role in agro-ecosystems and they are a diverse group of molecules that are involved in the adaptation of plants to their environmental, but are not part of the primary biochemical pathways of cell growth and reproduction (Jensen et al., 2013; Ortiz et al., 2014). They are usually classified according to their biosynthetic pathways. Three large molecule families are generally considered: phenolics, terpenes, and alkaloids (Bourgau et al., 2001).

In general, the terms plant secondary metabolites, phytochemicals, antinutritional factors and plant xenobiotics have been used in the literature to refer to this group of compounds. This number does not include the oligomeric polyphenolic compounds (proanthocyanidins and hydrolysable tannins) that are now being more accurately described and will increase the number by several thousand. Some major PSMs or phytochemicals that occur in plants include protease inhibitors, lectins, alkaloids, non-protein amino acids,

cyanogenic glycosides, saponins and tannins. These compounds are involved in defense against herbivores and pathogens, regulation of symbiosis, control of seed germination, and chemical inhibition of competing plant species, and therefore are an integral part of the interactions of species in plant and animal communities and the adaptation of plants to their environment (Makka et al., 2007).

Much of the research on PSMs has concentrated on their toxic and antinutritional effects on livestock. Toxic PSMs are present in plants at low concentrations (generally less than 2% of the dry matter) and may have negative physiological effects when absorbed. Higher concentrations (>2% of dry matter) of these compounds are required to elicit negative effects, and the primary site of activity is in the digestive tract or the sensory organs associated with feeding behavior. They can induce neurological problems, reproductive failure, goiter, gangrene and even death. Examples are alkaloids, cyanogenic glycosides, toxic amino acids, saponins and many others.. These PSMs include tannins, proteases and amylase inhibitors. Compounds that have a structural role in the plant (e.g., lignin, biogenic silica, and cutin) lower the extent of microbial digestion of cell wall polysaccharides (Makka et al., 2007).

In addition, PSMs are also associated with improved nutritive value and may have beneficial effects on animal health for instance tannins may protect ruminants against helminthiasis. Growing interest in the potential health-promoting effects of plant secondary metabolites in human foods has prompted research on their potential to prevent or treat cancer, circulatory disease, and viral infection. The mechanism by which these substances have beneficial effects on health may also be related to their toxic effects, and the difference between toxicity and beneficial effects may depend on dose and molecular structure. However, the mechanisms of toxicity and health-promoting effects of most PSMs in human and animal diets are not well established (Makka et al., 2007).

Interest in PSMs has risen dramatically in recent years amongst plant molecular biologists and plant breeders because of their diverse effects, which, in addition to those mentioned above, include antioxidant, antiviral, antibacterial and anticancer effects. As pointed out by few recent studies, molecular biologists have made genetic modifications in biosynthesis in forage plants in order to improve the efficiency of conversion of plant protein into animal protein (increase rumen undegradable protein and thus increase protein availability postruminally), reduce greenhouse gases, and reduce gastrointestinal parasites (Haralampidis et al., 2002; Makka et al., 2007).

Most plants, especially in the tropical regions, contain PSMs, due to wide biodiversity and high temperature and humidity. The lack of information on the appropriate methods for

their determination has been the main bottleneck in understanding the enzymes and biochemical pathways in their synthesis, the genes responsible for controlling major biochemical processes, and the physiological significance of PSMs and in exploiting the beneficial effects of these phytochemicals (Diaz et al., 1993; Makka et al., 2007).

PSMs limit how much of a particular plant an herbivore can eat through feedback mechanism that prevent damage to animal cells, tissues, and metabolic processes. When plant diversity is high, herbivores can attenuate the negative impacts of these metabolites by eating a variety of forages with different PSMs, thereby spreading the load across many plant species. Increased intake of PSMs when eating a variety of plants can occur because diverse PSMs can be detoxified by different mechanisms, and they can inactivate one another through various chemical interactions, leading to greater intakes than when a single secondary metabolites-containing food is ingested. Thus, by eating a variety of secondary metabolites, herbivores can enhance nutrient intake while taking advantage of the beneficial properties of them (Jensen et al., 2013).

2.3.2.1 Saponins

The word ‘saponin’ is derived from the Latin word *sapo* (soap) and traditionally saponin-containing plants have been utilized for washing. Chemically, saponins are high-molecular weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety (Wallace, 2004).

Plant extracts containing saponin are of interest as potential feed additives for dairy and meat ruminants because they can influence productivity. Several authors have reported that some of the physical and chemical properties of these compounds have sparked research into their use in livestock production applications due to saponin improving ruminal organic matter digestion, increasing ruminal propionate concentrations, stimulating the growth of steers while not affecting animal performance (Goetsch & Owens 1985; Wu et al. 1994; Lila et al. 2005).

Several authors have reported that this substance enhances ruminal organic matter digestion because its mechanism of action affects the rumen microbial population directly, resulting in an improvement in rumen fermentation and decreasing methane production. The majority of research on saponins has focused on exploiting its characteristics in suppressing rumen ciliate protozoa and on its ability to selectively inhibit some bacteria, hence reducing the production of hydrogen ions which are emitted in the form of methane (Patra & Saxena, 2010b; Bodas et al., 2012; Bharathidhasan et al., 2013).

Saponins are known to have some distinctive features in plants due to their protective actions against bacteria, fungi and insects and as an additive in animal nutrition. In animal feeding, there is an increasing amount of research for nutraceutical and phyto-genic additives. Saponins are one of the important plant secondary metabolites which have been shown to selectively modulate the rumen microbial populations resulting in an improvement in rumen fermentation and to decrease methane production (Patra & Saxena 2010b; Bodas et al. 2012).

In ruminants that are fed on diets with saponins as an additive, there is a reduction in ammonia release in excrements, reducing odour and improving the stool consistency. Such characteristics are interesting for animal husbandry because they may improve air and bedding quality, ease of cleaning and others benefits that lead to improve production performance. Therefore these promising molecules are being used as additives in animal feeding. Several experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest (Francis et al. 2002).

2.3.2.1.1 Nature

Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups confer strong surface-active properties that are responsible for many of saponins' adverse and beneficial effects. The primary biological effect of saponins is its interactions with cellular and membrane components. For example, saponins haemolyse red blood cells by nonspecific interactions with membrane proteins, phospholipids, and cholesterol of erythrocytes (haemolytic activity) (Makka et al., 2007).

Saponins are natural surface-active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria. This easily observable character has attracted human interest from ancient times. Saponin consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenins) which may be triterpenoid or steroid in nature. The aglycone may contain one or more unsaturated C-C bonds. The oligosaccharide chain is normally attached at the C₃ position (monodesmosidic), but many saponins have as additional sugar moiety at the C₂₆ or C₂₈ position (bidesmosidic). The large complexity of saponin structure arises from the variability of the aglycone structure, the nature

of the side chains and the position of attachment of these moieties on the aglycone (Sen et al. 1998; Francis et al. 2002).

In general, very little is known about the enzymes and biochemical pathways involved in saponin biosynthesis. Triterpenoid saponins are synthesised *via* the isoprenoid pathway by cyclization of 2,3-oxidosqualene to give primarily oleanane (β amyrin) or dammarane triterpenoid skeletons. The genetic machinery required for the elaboration of this family of plant secondary metabolites is as yet largely uncharacterised, despite the considerable commercial interest in this group of natural products. This is likely to be due in part to the complexity of the molecules and the lack of commercially available pathway intermediates for biochemical studies. A review describes the advances made in the area of 2,3-oxidosqualene cyclisation, the genes that encode enzymes giving rise to the diverse array of plant triterpenoid skeletons, and the characterisation of saponin glucosyltransferases (Haralampidis et al., 2002).

2.3.2.1.2 Occurrence

Saponins occur constitutively in a large number of plant species, in both wild plants and cultivated crops. In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties. Triterpenoid saponins have been detected in many legumes such as soybeans, beans, peas, lucerne, etc. and also in alliums, tea, spinach, sugar beet, quinoa, liquorice, sunflower, horse chestnut and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. One example of an extensively studied group of triterpenoid saponins is produced from *Quillaja saponaria*, a tree native to the Andes Region. The bark can be peeled off and the saponin extracted with water by the indigenous peoples and used as a shampooing agent, and by the Shamans as an overall curing agent. *Yucca schidigera* is the most common commercial source of steroid saponins (Francis et al. 2002).

A number of factors, such as physiological age, environment and agronomic factors, have been shown to affect the saponin content of plants. Reports reviewed indicate that saponins increase on sprouting in some plants such as soyabeans, lucerne, mung beans and peas but decrease in others such as moth bean and that luminocity during germination has a profound stimulating effect on saponin content. Generally, immature plants have been found to have higher saponin content than more mature plants of the same species (Francis et al. 2002).

The second major sources of saponin are from desert plants: *Yucca schidigera* (steroid saponins called also sarsaponins) from Mexico and *Quillaja saponaria* (triterpenoid saponins) from Chile. Lucerne and soya beans are the main examples of saponin-rich plants that are extensively used in ruminant's diets (Sen et al. 1998).

Yucca saponins have a steroidal nucleus, whereas *Quillaja* saponins are triterpenoids. A recent *in vitro* study showed that liquid extracts of *Y. schidigera* and *Q. saponaria* added at 2 to 6 mL/L of buffered ruminal fluid decrease rumen protozoa and have the potential to alter ammonia-N and propionate concentrations and the acetate:propionate ratio. In the same study, *Y. schidigera* reduced the rate and extent of methane production in a dose-dependent manner by up to 42 and 32%, respectively, whereas *Q. saponaria* did not affect methane production (Pen et al., 2008).

2.3.2.1.3 Mechanism of Action

Saponins have been variously attributed with a diverse range of properties, some of which included both beneficial and detrimental effects on human health, piscicidal, insecticidal and molluscicidal activity, allelopathic action, antinutritional effects, sweetness and bitterness, and as phytoprotectants that defend plants against attack by microbes and herbivores (Haralampidis et al., 2002).

Saponins are also characterized by their haemolytic activity and foaming properties and are responsible for imparting a bitter taste and sharpness to plant materials containing high concentrations of saponins. Saponins are reported to affect the permeability of the small intestinal mucosal cells and thus have an effect on active nutrient transport. Saponins have also been shown to inhibit various digestive enzymes, including trypsin and chymotrypsin, and are also known to inhibit protein degradation by forming saponin-protein complexes. On the other hand, the positive nutritional effects of specific saponins such as its hypocholesterolemic effects and the improvement in growth in various animal species have also been reported. For instance, *Medicago sativa* (alfalfa or lucerne) contains numerous saponins as well as medicagenic acid that is unique to alfalfa. Alfalfa saponins may lower growth rate in chicks and egg production of hens when included in poultry diets above 5%.

A more detailed understanding of the biochemical pathways and enzymes involved in saponin biosynthesis will facilitate the development of plants with altered saponin content. In some cases, enhanced levels of saponins or the synthesis of novel saponins may be desirable

(for example, for drug production or improved disease resistance) while for other plants a reduction in the content of undesirable saponins would be beneficial (Haralampidis et al., 2002).

2.3.2.1.4 Biological effects

In ruminants and other domestic animals, dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of waste (Cheeke, 1996). In these species, dietary supplementation with saponins has been also claimed to improve growth, feed efficiency and health. These effects have been explained partly by the action of saponins on ruminal microbes, resulting in a decrease in rumen degradability of feed proteins and an increase in microbial protein synthesis in the rumen, which both increase the intestinal flow of amino acids (Mader & Brumm, 1987; Makkar & Becker, 1996).

The majority of research on saponin has been focused on exploiting the characteristic that it inhibits rumen protozoa and on its ability to selectively inhibit some bacteria and thus reduces the production of hydrogen ions needed for the methane emission. A number of studies have reported that plants rich in saponins decrease methane production in the rumen. Moreover, methane production in ruminants is negatively correlated with energy utilization; thus, a reduction of methane production through the use of feed additives and rechanneling hydrogen to short-chain fatty acids and microbial mass is desirable, provided that the additives do not adversely affect animal productivity. Therefore, reducing methanogenesis is beneficial from the standpoint of increasing energy efficiency of the ruminant and from an environmental perspective, because methane is a potent greenhouse gas according to the Intergovernmental Panel on Climate Change, 2007 (Lila et al., 2003; Hu et al., 2005; Patra & Saxena 2009a; Patra & Saxena, 2010a,b; Bodas et al., 2012).

Several studies have found no indication of any effects of saponin on microbial growth or protein breakdown *in vitro* (Van Nevel & Demeyer, 1990). In contrast others studies have reported that lucerne (*Medicago sativa*) saponins appear to inhibit fermentation in continuous culture (Lu et al., 1987). A subsequent *in vivo* investigation confirmed a general decrease in fermentation activity when lucerne saponins were supplied to the sheep rumen, seen as a decrease in volatile fatty acids and a decrease in cellulose digestion. Importantly, this investigation also noted large decreases in protozoal numbers in sheep receiving lucerne saponins (Lu & Jorgensen, 1987). Goetsch and Owens (1985) have concluded that the benefits of saponins would depend on the type of diet, increasing the digestion of sorghum silage and other fibrous feeds but apparently decreasing digestion of cereal and protein meals.

Removal of rumen ciliate protozoa or defaunation has been an objective of rumen microbiologists for a generation. There are many consequences for fermentation, and consequently for nutrition, that result from the removal of protozoa. A meta-analysis has demonstrated that the benefits of defaunation outweigh any disadvantages (Eugène et al., 2004). Antiprotozoal agents, such as surface-active agents, that have been investigated in attempts to apply defaunation at the farm level have been hampered by problems with toxicity to other rumen microorganisms. Lipids are toxic to protozoa and also to fibre digestion. Thus, there has been, until now, no reliable safe on-farm method available for inhibiting rumen protozoa (Wallace, 2004).

In the last decade, some tropical plants have been found to have the potential to be used as a safe means of inhibiting or eliminating protozoa from the rumen. These plants all share the same characteristic that they are rich in saponins. Thus, research on saponins and defaunating plants has to some extent converged (Diaz et al., 1993; Newbold et al., 1997; Odenyo et al., 1997; Wallace, 2004).

Natural plant products are a particular case among the additives reviewed. Owing to the diversity of bioactive components in these natural, non-purified preparations, their effective doses are difficult to determine and the effects on animals are not totally controlled (interaction, synergism and antagonism). Farmers and consumers alike generally perceive 'natural plant extracts' to be less toxic than synthetic antibiotics or other chemical products. However, this perception is unsound as there are many examples of dangerous natural toxins. The dose-response relationship must be precisely established from well-designed experiments for a specific claim in animal production, and risks associated with the use of these negative natural preparations must be robustly tested in carefully designed clinical studies (Wallace et al., 2002; Jouany and Morgavi, 2007).

2.3.2.1.4.1 Effects on rumen microorganism

Dietary saponins are poorly absorbed, so their biological effects occur in the digestive tract (Cheeke, 1996). Although antimicrobial effects of saponins and saponin-containing plants are to be expected, based on the wealth of information from other biological systems, the earliest observations relating to rumen microorganisms have come relatively recently from *in vivo* continuous-culture systems. Saponins, from *Yucca schidigera*, have been observed to decrease protozoal numbers but not bacterial numbers in a 22d semi-continuous system (Valdez et al., 1986). In the presence of lucerne saponins, the bacterial population is changed from a

morphologically-diverse one to one in which fewer types are present (Lu et al., 1987). Not only can the saponins have an effect on rumen micro-organism, but microorganisms can metabolize the saponins, thus introducing another factor to be considered in the use of saponins in ruminant nutrition (Wallace, 2004).

Saponins have antimicrobial properties, particularly in suppressing ciliate protozoa (Wallace et al., 1994; Hristov et al., 1999), peptidase producing bacteria (Wallace et al., 1994; Wang et al., 2000) and cellulolytic bacteria (Wang et al., 2000). Furthermore, methanogenic bacteria were metabolically co-related with ciliate protozoa (Stumm et al., 1982; Finlay et al., 1994; Newbold et al., 1995) and protozoa actively produce hydrogen, which is utilized by methanogenic bacteria (Stumm & Zwart, 1986).

Studies about the effect of saponins from tea seeds in sheep reported an unexpectedly low effect *in vivo* which may in part be due to adaptation of the rumen protozoa to saponins, or through degradation of these compounds by rumen bacteria, which adapt to become capable of degrading the saponin antiprotozoal component (Newbold et al., 1997).

Saponins can also act in the distal part of the digestive tract. They increase the permeability of intestinal cells through their ability to complex sterols in mucosal cell membranes (Johnson et al., 1986), which enables the uptake of non-absorbable substances (Gee et al., 1997). This property has been exploited to enhance absorption of orally administered drugs (Chao et al., 1998). However, saponins fed at levels as low as 0.15% in the diet can alter intestinal mucosa integrity, and affect the active transport of nutrients by lowering transmural potential differences across the brush border membrane of intestinal cells (Gee et al., 1989).

Optimal levels for a positive effect of saponins on rumen fermentation or ruminant production are difficult to assess since saponins are supplied either as extracts or as ground saponin-rich plants and levels are cited variously as per unit of animal live weight, per head, per unit of dietary DM weight, or per unit of liquid volume in *in vitro* experiments. Several saponin sources have been tested (*Yucca*, *Quillaja*, *S. saponaria*, *S. rarak*, alfalfa root, tea) on various species of animal in various physiological states. Also, Cardozo et al. (2005) demonstrated that the effect of yucca saponins on rumen fermentation was highly dependent on rumen pH. They showed that saponins were much more active in the rumen at pH 5.5, corresponding to high-starch diets, than at pH 7.0, corresponding to low-digestible forage-based diets.

This aspect has also to be considered in *in vitro* comparative tests, where the pH values are usually set between 6 and 7. Nevertheless, studies indicate that optimal levels are generally lower for steroidal saponins than for triterpenoid saponins. They range from 1.5-8 to 20-60g/cow

or heifer/day for yucca extracts, depending on the mode of extraction. A dose as low as 280 mg/sheep/day of yucca showed significant effects on rumen fermentation in adult sheep (Santoso et al., 2004), whereas a dose of 30g/sheep/day of a product called 'Yucca extract' had no effect (Eryavuz & Dehority, 2004). Optimal doses for sheep were set at 12 to 50g/day and 24g/day of saponins from *Saponaria sp.* and alfalfa root, respectively. *In vitro*, the NH₃ concentration, acetate/propionate ratio, methane production and protozoa population were dose-dependently decreased by sarsaponin (Lila et al., 2003). A negative effect on DM digestibility appeared at a dose of 1.8 g/L of sarsaponin in the liquid of fermenters.

Dietary supplementation with *Yucca* extracts has also been recommended for non-nutritional purposes. They are used to reduce environment pollution due to odoriferous manures from animal sheds by decreasing the release of NH₃ to the air through the mechanism already described for the rumen (Killeen et al., 1998). Also, the antiprotozoal property of saponins could be exploited in the treatment of protozoal infections in ruminants such as *Giardia* (McAllister et al., 2001) and *Plasmodium* (Traore et al., 2000).

Various plant-derived compounds such as probiotics, dicarboxylic acids and enzymes are able to act positively on rumen feed digestion and ruminant production. These additives are diverse and exert their action through different mechanisms. Despite their diversity, they all ultimately affect the fermentation metabolic pathways and/or the digestive microbial ecosystem. However, unlike synthetic antibiotics, which have a specific microbial target, these additives generally have multiple, subtler modes of action. Their efficacy is influenced by the type of feed and physiological status of the animal. The interactions that occur between additives, active principles in additives, feeds and host are complex, and more research is needed to improve our understanding of these processes. This knowledge will help us to predict when a given feed additive may be useful, thus reducing inconsistencies in response observed in the field (Jouany & Morgavi, 2007).

2.3.2.1.4.2 Rumen antiprotozoal activity

As noted earlier, N metabolism in the rumen affects both the efficiency of ruminant production and the environmental impact of excreta from ruminant livestock production. Inefficient N retention by rumen microorganisms is compensated in production terms by feeding excessive amounts of dietary protein to the animal to meet required production levels. This process leads directly to the excretion of N-rich wastes. Microbial protein turnover in the rumen may result in the net microbial protein outflow being less than half the total protein

synthesized. *In vitro* studies suggest that engulfment and digestion of bacteria by protozoa is by far the most important cause of microbial protein turnover in the rumen (Wallace & McPherson, 1987). Thus, it is apparent that removing ciliate protozoa from the rumen (defaunating) should prevent the recycling of N between bacteria and protozoa, and thereby increase the efficiency of N metabolism in the rumen and stimulate the flow of microbial protein from the rumen (Williams & Coleman, 1992).

Several methods have been tried experimentally to remove protozoa from the rumen ecosystem. Rumen protozoa ingest and digest bacteria and fungi, degrading their cellular protein to NH_3 . Results from *in vivo* experiments have clearly shown that duodenal flow of both undegraded dietary and bacterial protein is generally increased by defaunation. However, no practical method has been developed to eliminate protozoa. Antiprotozoal plants may be promising, safe, natural defaunating agents. Recently, there has been increased interest in saponin-containing plants as a possible means of inhibiting or eliminating protozoa in the rumen. Saponins are glycosides and are generally considered to be anti-nutritional factors. Anti-nutritional effects differ depending on the digestive process of the ingesting animal, because in ruminants, saponins are differentially toxic to rumen protozoa (Teferedegne, 2000).

Since increasing levels of saponin infused in the rumen have been shown to decrease the concentrations of protozoa (Lu & Jorgensen, 1987), numerous studies have been conducted to determine the effects of feeding ruminants with saponins-rich plants, such as alfalfa (Lu & Jorgensen, 1987; Klita et al., 1996), *Enterolobium cyclocarpum* (Navas-Camacho et al., 1993), *Spinadus saponaria* (Diaz et al., 1993), *Sapindus rarak* (Thalib et al., 1996), *Sesbania sesban* (Newbold et al., 1997; Odenyo et al., 1997; Teferedegne et al., 2000), *Quillaja saponaria* and *Acacia auriculoformis* (Makkar et al., 1998) and *Yucca schidigera* (Wallace et al., 1994; Hristov et al., 1999; Wang et al., 2009).

These results have indicated that saponins have strong antiprotozoal activity and may serve as an effective defaunating agent for ruminants. The detergent action of saponins is believed to be responsible for killing the rumen protozoa (Makkar et al., 1998). The general mode of action of saponins on microorganisms is their interaction with the sterol moiety, which is present in the membrane of protozoa (Williams & Coleman, 1997). In other words, the detrimental effect on protozoa is caused by the reaction of triterpenoid or steroid saponins with the sterols located in the ciliate cell membrane (Jouany & Morgavi, 2007). Although the toxicity of saponins towards protozoa seems to be widespread and non-specific (Sliwinski et al., 2002; Hristov et al., 2003), some authors found no effect on protozoa number, or even observed an increase (Abreu et al., 2004; Eryavuz & Dehority, 2004).

The authors suggest that physiological activities associated with eating, including saliva secretion, may play a role in neutralizing the protozoa inhibiting substances found in saponin-containing plant (Eryavuz & Dehority, 2004). In addition, Newbold et al. (1997) reported that adding the saponins to the diet could cause an increase in bacterial numbers, whose activity could possibly alter the toxic properties of saponins.

Thus, the antiprotozoal activity of saponins may be due to the destruction of protozoal cell membranes, causing leaking of cell contents. It has been reported that the main sterols from different sources of the same plant material may elicit different responses on rumen metabolism. Also the method to measure the saponins in the extract or plant may differ, which may be explained differences in response despite similar supplementation levels (Patra & Saxena, 2009a).

As mentioned previously, a major effect expected from a supplement of saponin is a decrease in the ruminal ciliate protozoa population. Many authors and studies have suggested that total and partial removal of protozoa from the rumen is favorable to improve the utilization of low quality tropical forages and to increase animal productivity (Teferedegne, 2000). This hypothesis is based mainly on the often-found increase in the net efficiency of microbial protein synthesis in the rumen due to the decrease in bacterial protein degradation by the protozoa, followed by an increased flow of total microbial protein to the duodenum (Williams & Coleman, 1992). Foliage and fruits of several tropical and subtropical multipurpose shrubs and trees have been reported to adversely affect ruminal ciliate protozoa. For instance, the protozoa-suppressing effect of *S. saponaria* fruits has been demonstrated *in vitro* (Hess et al., 2003a) and *in vivo* (Diaz et al., 1993; Navas-Camacho et al., 1994). However, Abreu et al., (2004) noted that the ciliate protozoa count was increased by supplementation with *Sesbania sesban*, a saponin-containing forage from an African multipurpose leguminous tree. Variable effects on ruminal protozoa have also been reported after the use of foliage from this plant (Newbold et al., 1997; Odenyo et al., 1997; Teferedegne et al., 1999).

S. sesban depressed protozoa counts in the ruminal fluid of sheep (Newbold et al., 1997; Teferedegne et al., 1999) and also depressed protozoa counts when administered directly into the rumen of sheep; however it had no effect on protozoa number when fed orally to sheep (Odenyo et al., 1997). Therefore, Teferedegne (2000) suggested that, in sheep adapted to *S. sesban*, saponins could be degraded in the saliva before reaching the rumen. This cannot explain the absence of antiprotozoal activity of *S. saponaria* in the study of Abreu et al. (2004), since the fruit were supplied intraruminally.

A possible adaptation in this study of Abreu et al. (2004) cannot be excluded due to the fact that sheep were freely browsing native shrubs and grazing native and improved pastures prior to the experiment and could have had access to saponin-containing feeds. Also, Wallace et al. (2002), reviewing current literature, suggested that adaptation of mixed microbial populations in the rumen was a factor contributing to the variability of the antiprotozoal activity of saponins or saponins-containing plants. Additionally, the results of Hess et al. (2003b) suggest that effects of *Sapindus saponaria* on ruminal protozoa could be diet-dependent, because protozoa numbers were decreased by *S. saponaria* fruit only when added to a diet supplemented with a high-quality leguminous feed (*Arachis pintoi*), but not with *Cratylia argentea* which is a poor quality forage.

Adaptation of ruminal microbial populations to saponins could be responsible for the absence of antiprotozoal activity of *S. saponaria* in the Abreu et al. (2004) study, but cannot explain the highly significant increase in protozoa counts. Besides fiber, total sugars were the most abundant nutrient fraction in *S. saponaria* fruit, representing over 200g/kg of DM, thus providing a unique extra supply of readily fermentable carbohydrates available for ruminal microbes. This could have enhanced protozoa populations (Williams & Coleman, 1992).

The detrimental effect on protozoa is caused by the reaction of triterpenoid or steroid saponins with the sterols located in the ciliate cell membrane (figure 5) (Assa et al., 1975; Pacheco-Soares & de Souza, 2000). Although the toxicity of saponins towards protozoa seems to be widespread and non-specific (Valdez et al., 1986; Diaz et al., 1993; Hristov et al., 2003), some authors found no effect on protozoal number (Sliwinski et al., 2002; Hristov et al., 2003), or even observed an increase (Abreu et al., 2004; Eryavuz & Dehority, 2004). This may be due to the capacity of some rumen bacteria to hydrolyze saponins into free glucosyl and sapogenins fractions (Newbold et al., 1997), thus removing their toxicity against protozoa. When incubated *in vitro* with non-adapted rumen micro-organism, no degradation of *Quillaja* saponins was observed during the first 6h and about 50% of saponins disappeared during the next 6h (Makkar & Becker, 1997). Saponins may also be degraded or 'inactivated' by some still unidentified components of salivary (Odenyo et al., 1997; Teferedegne, 2000). This explains why the effects of saponins are more pronounced when they are directly added to the rumen rather than mixed with the diet (Odenyo et al., 1997). To avoid adaptation of bacteria and maintain the activity of saponins in the rumen, Thalib et al. (1995) advocate administering them once every 3 days.

The mechanism of protozoal lysis is suggested to be due to a change in cell membrane permeability as saponins form complexes with cholesterol in the protozoal cell membrane and

result in cell lysis which in turn decreases hydrogen ion transfer and ultimately reduces methane production (Francis et al., 2002).

The strong bioactivity of saponins, associated with the possibility for rumen microbes to deglycosylate them, explains why their effect on animal performance is rather inconsistent, and generally low when animals have been treated over a long period of time (Mader & Brumm, 1987; Wu et al., 1994; Calsamiglia et al., 2005). In view of their foaming properties, dietary saponins have often been suspected of favouring rumen bloat in ruminants, but clear evidence is lacking (Cheeke, 1996).

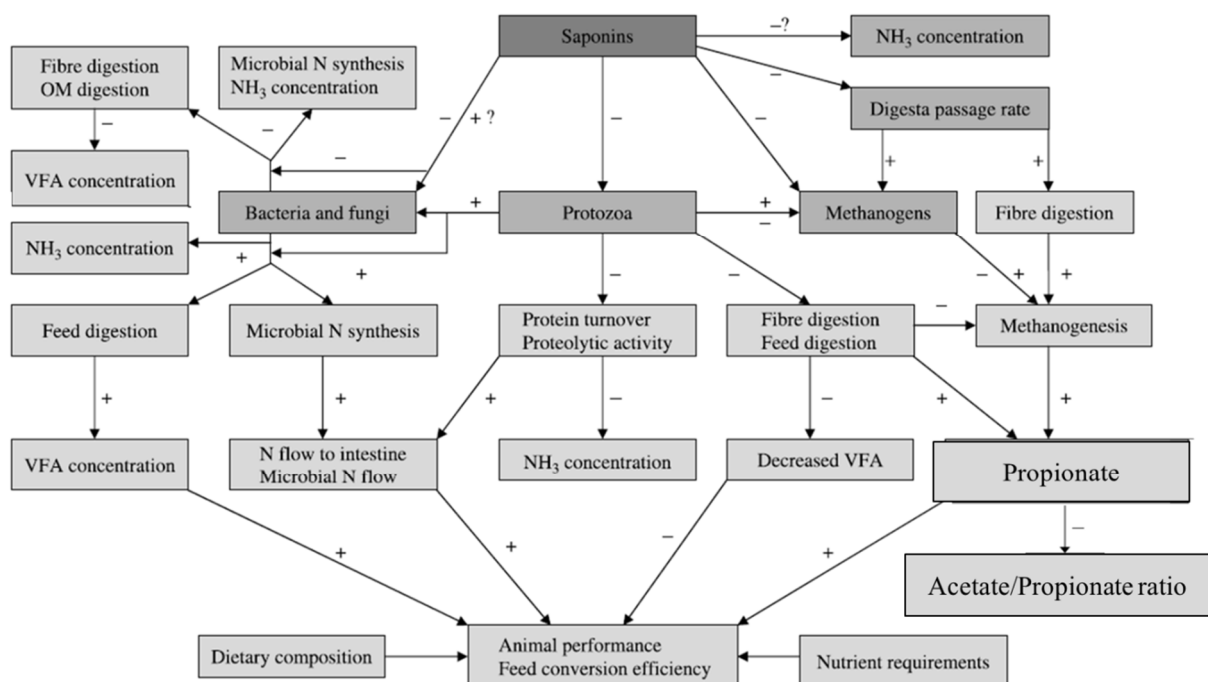


Figure 5 . A schematic presentation of the proposed effects of saponins on rumen microbes and fermentation. Primary effects modify the composition of rumen microbes and secondary effects modify the rumen fermentation. +, increase; -, decrease; OM, organic matter; VFA, volatile fatty acids; P, propionate (Adapted from Patra & Saxena, 2009a).

2.3.2.1.4.3 Effects on protein digestion

Saponins reduce protein digestibility probably by the formation of poorly digestible saponin-protein complexes. The heat stability of bovine serum albumin was increased by the addition of soyasaponin due to electrostatic and hydrophobic interactions. The digestibility of the bovine serum albumin-soyasaponin complex was much lower than that of free bovine serum albumin indicating that complexing with saponin obstructed digestion. A large number of foods and feed materials contain both saponins and proteins. The nature of the interactions between them could influence the nutritive value of a diet, and hence these interactions need to be studied to elucidate possible structure-activity relationships (Francis et al., 2002).

2.3.2.1.4.4 Hypoglycaemic activity

Saponins isolated from plants have been shown to have hypoglycaemic effects in mice. The hypoglycaemic action is due to suppression of the transfer of glucose from the stomach to the small intestine and inhibition of glucose transport across the brush border of the small intestine. The inhibitory activity is dependent on the level of serum glucose and mediated at least in part by the capsaicin-sensitive sensory nerves and the central nervous system. Petit et al. (1993) found chronically higher plasma insulin levels, probably caused by stimulation of the β -cells in male Wistar rats given 10 and 100 mg fenugreek extract/300g body weight mixed with food while Matsuda et al. (1999) did not find insulin-like or insulin-releasing activity in rats given oleanolic acid glycosides orally.

2.3.2.1.4.5 Effects on cholesterol metabolism

A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects. Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion when saponin-rich foods such as soyabean, Lucerne and chickpea are consumed. The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to decrease. Several dietary saponins have a hypocholesterolemic activity, since cholesterol binding takes place in the intestinal lumen and factors such as the quantity of saponins and cholesterol are important (Francis et al., 2002).

Saponins also have an antibacterial activity inhibiting peptidase producing bacteria and cellulolytic bacteria. They inhibit growth, particularly of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* bacteria (Wallace et al., 1994). It seems that saponins show a more marked antibacterial activity against gram positive than against gram negative bacteria (Patra & Saxena, 2009a).

Several studies have been shown the saponins have hypocholesterolemic, anticoagulant, anticarcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory and anti-oxidant activities in animals (Milgate & Roberts 1995; Hristov et al. 1999).

2.3.2.1.4.6 Methane

The observations that there was an interaction between defaunation and tea saponin in decreasing CH₄ production, and that tea saponin decreased CH₄ in both refaunated and defaunated sheep, indicates that reduced methanogenesis by tea saponin addition was not only due to inhibition of protozoa, but that another inhibition mechanism existed. Tea saponins may have a minor effect on other microbiota which results in a lower amount of H₂ being produced and thus less CH₄ in the refaunated and defaunated sheep (Zhou et al., 2011).

Using an *in vitro* fermentation technique, Goel et al. (2008a) observed that methane emission was decreased *via* a reduction in methanogen populations, by 78% using *Sesbania* saponins, by 22% with *Fenugreek* saponins and by 21% with *Kautia* saponins. Saponin extracts from *Yucca schidigera* and *Quillaja saponaria* have been demonstrated to reduce methanogenesis *in vitro* (Pen et al., 2008; Holtshausen et al., 2009). Thus, saponin appears to reduce methane emissions by inhibiting protozoa, which reduces the availability of hydrogen ions for methane production (Bharathidhasan et al., 2013).

2.3.2.1.4.7 Ammonia concentration

Ammonia concentrations in the rumen reflect a balance between degradation of feed protein and uptake of ammonia for synthesis of microbial protein. Ruminal ammonia-N concentrations are reduced with saponin supplementation. A number of studies with sheep fed a silage-based diet and *in vitro* ruminal culture fermentation noted that saponin from *Y. schidigera* reduced ruminal ammonia concentrations. Ammonia concentrations in the rumen are reduced when protozoa growth is inhibited, presumably as a result of depressed rumen degradation of feed protein or turnover of bacterial protein. Ammonia concentrations will also be altered by the binding of ammonia to compounds like saponin. Another possible explanation for the reduction in ruminal ammonia in saponin-supplemented goats may have been due to increased incorporation of ammonia, peptide or amino acids into microbial protein (Santoso et al., 2007).

Administration of saponins improve the assimilation of feed N by animals because less NH₃ is produced in the rumen and less urea is eliminated in urine (Santoso et al., 2004). Two mechanisms have been considered to explain the effect of saponins on N metabolism in the rumen: saponins extracted from leaves of *Sesbania sesban* or from lucerne roots have been shown to reduce significantly the numbers of protozoa (Lu and Jorgensen, 1987; Klita et al.,

1996; Newbold et al., 1997). They play a major role in ruminal feed protein degradation (Jouany, 1996) and the resulting NH_3 from microbial protein degradation, can be bound by saponins in a balanced chemical reaction regulated by NH_3 concentration (Headon et al., 1991). Thus, an adequate amount of NH_3 is continuously supplied for microbial protein synthesis in the rumen (Hussain & Cheeke, 1995).

Studies have shown that another type of saponin, sarsaponin can partially decrease methane and hydrogen *in vitro* (Lila et al., 2003). It also decreased the molar proportion of acetate, and increased propionate and butyrate with a corresponding decrease in the acetate:propionate ratio. Total volatile fatty acid (VFA) concentrations were increased and ammonia was decreased by sarsaponin. These changes in ruminal fermentation suggest that saponin behaves like ionophores, and the addition of saponin may improve the efficiency of ruminant production (Lila et al., 2005).

The effects of saponins on ruminal fermentation have been extensively studied *in vivo* and *in vitro*. They significantly decrease ruminal NH_3 concentrations and increase that of propionic acid at the expense of acetate and butyrate (Abreu et al., 2004; Santoso et al., 2004; Hristov et al., 1999). In agreement with the stoichiometry of ruminal fermentations, they significantly decrease methane production both *in vitro* (Lila et al., 2005; Hu et al., 2005) and *in vivo* (Santoso et al., 2004).

2.3.2.1.4.8 Bacteria and fungi

Saponins can alter the cell wall structure of Gram-positive bacteria such as *Ruminococcus flavefaciens* and *R. coccus albus*, and impair the digestion of filter paper by these bacteria, but had no effect on the cellulolytic activity of gram negative *Fibrobacter succinogenes* (Wina et al., 2006). Saponins inhibit the growth of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* bacteria (Wallace et al., 1994).

Some of the antimicrobial compounds have been identified as three butanol-extractable 5 β -spirostan-3 β -ol saponins (Killeen et al., 1998), which are thought to alter bacterial membranes by increasing their porosity (Schulman et al., 1955; Wang et al., 2000). The foaming property of saponins increases the surface tension of the bulk solution and accelerates lysis of microbial cells with weakened membranes. In addition, bacterial growth inhibition may be caused by complexation of essential minerals and steroids with saponins, thus limiting their bioavailability for bacterial metabolism (West et al., 1978; Simons et al., 2006).

The ruminal fungi *Neocallimastix frontalis* and *Piromyces rhizinflata* were totally inhibited by 2.25µg saponins per mL. Using the membrane hybridization technique with oligonucleotide probes, Wina et al. (2005) observed that a marked decrease in protozoal RNA offset an increase in bacterial RNA concentration when *S. rarak* saponins were added *in vitro* at 1mg/mL. Methanogen RNA concentration was also affected and was minimal at the saponin concentration of 4mg/mL. Because of the strong inhibiting effect of saponins on *S. cerevisiae* (Killeen et al., 1998), it is strongly recommended that saponins not be used in association with yeast-based probiotics.

3. MAIN OBJECTIVES AND APPROACH OF THE STUDY

3.1 GENERAL OBJECTIVE

The general objective was to study and better understand the effect of natural substances such as β -glucan and a saponin-based plant extract on the metabolism of small ruminants in the *peri-partum* period. This period is a particularly critical moment in the production cycle and nutritional and metabolic disorders can occur at this time.

3.2 SPECIFIC OBJECTIVES

- The glucan project was conducted to evaluate the influence of i.m. injection of β -glucan on the metabolism of peri-parturient Santa Inês ewes:
 - To assess energy and lipid profiles (glucose, beta-hydroxybutyrate - β HB, non-esterified fatty acids - NEFA, fructosamine, cholesterol and triglycerides), protein profile (total protein, albumin, urea and cholesterol), enzyme activities (aspartate amino transferase - AST, gamma glutamyl transferase - GGT and creatinine kinase - CK) and levels of minerals (total and ionized calcium, inorganic phosphorus, sodium, magnesium, potassium and chloride).
- The purpose of the saponin experiments was to assess the effects of a saponin additive on performance and systemic and ruminal metabolism of dairy goats:
 - During a period of metabolic balance (mid-lactation)
 - During a period of metabolic imbalance (late pregnancy and early lactation).
 - To assess energy profile (glucose, β HB, NEFA and urea) and pH and ammonia (NH_3) concentrations, and estimation of volatile fatty acids (VFA) in both periods.
 - During a period of metabolic imbalance the variables of lipid and protein profiles were total cholesterol (TC), total bilirubin (TB), total protein (TP), albumin, high-density lipoprotein (HDL) and haptoglobin (Hp). Concerning the enzyme profile the variables were AST, GGT and alkaline phosphatase (ALP). Magnesium, total calcium, phosphorus were also assessed in relation to mineral profile.

4. PAPERS

4.1 PAPER 1 – Submitted to *Revue de Médecine Vétérinaire*

Do intramuscular injections of β 1,3-glucan affect metabolic and enzymatic profiles in Santa Inês ewes during late gestation and early lactation?

Est-ce que des injections intramusculaires de β 1,3-glucane influencent les profils métaboliques et enzymatiques chez des brebis Santa Inês pendant la fin de la gestation et le début de la lactation ?

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Summary

The aim of this study was to investigate the influence of β1,3-glucan on the metabolic profile of Santa Inês ewes in the *peri-partum* period. Fourteen pregnant ewes were randomly divided into two groups: a glucan group (G) and a control group (C) 60d before expected lambing. Over eight weeks, between the -60 and -10 days in relation to lambing, each animal in the G group received weekly a 1mL intramuscular injection of β1,3-glucan. Nutritional requirements were covered. Blood samples were collected on the -30, -20, -10, 0, +10, +20, +30 and +60 day in relation to lambing. Glucose, L-lactate, non-esterified fatty acids (NEFA), β-hydroxybutyric acid (βHB), fructosamine, cholesterol, triglycerides, total protein, albumin, urea, creatinine, total and ionized calcium, chloride, sodium, potassium, inorganic phosphorous, magnesium, aspartate aminotransferase (AST), gamma glutamyl transferase and creatine kinase were measured. The results were analysed with a repeated measures model. The ewes were clinically healthy. Glucose, L-lactate, NEFA and βHB peaked at parturition ($p<0.05$). AST was significantly higher in C compared to G ($p<0.05$) and there was a similar trend for fructosamine and albumin ($p<0.10$). Urea concentrations tended to be higher in G compared to C ($p<0.10$). In conclusion, in this preliminary experiment, the changes with time were consistent with a change in physiological status (gestation/lactation). β1,3-glucan did not affect energy metabolism. β1,3-glucan appeared to protect muscle and liver because AST levels were lower than in controls. The use of β1,3-glucan did not negatively affect the animals.

Key-words: β1,3-glucan; Sheep; Blood; Metabolites; Minerals; Enzymes; *Peri-partum*.

Running title: β1,3-glucan and metabolism in the *peri-partum* sheep

Résumé

Le but de ce travail était d'étudier l'influence du β1,3-glucane sur le profil métabolique de brebis Santa Inés dans la période péri-partum. Quatorze brebis gestantes ont été réparties au hasard en deux groupes: un groupe glucane (G) et un groupe contrôle (C) 60 jours avant la date présumée de mise-bas. Pendant huit semaines entre -60 et -10 jours par rapport à l'agnelage, chaque animal du groupe G a reçu une injection intramusculaire hebdomadaire de 1ml de β1,3-glucane. Les besoins nutritionnels ont été couverts. Des échantillons de sang ont été prélevés à -30, -20, -10, 0, +10, +20, +30 et +60 jours par rapport à l'agnelage. Le glucose, le L-lactate, les acides gras non estérifiés (AGNE), le β-hydroxybutyrate (βHB), la fructosamine, le cholestérol, les triglycérides, les protéines totales, l'albumine, l'urée, la créatinine, le calcium total et ionisé, le chlorure, le sodium, le potassium, le phosphore inorganique, le magnésium, l'aspartate aminotransférase (AST), la gamma glutamyl transférase et la créatine-kinase ont été mesurés. Les résultats ont été analysés avec un modèle en mesures répétées. Les brebis étaient cliniquement saines. Le glucose, le L-lactate, les AGNE et le βHB ont atteint un pic à la parturition ($p<0,05$). L'AST était significativement plus élevée dans le groupe C par rapport au groupe G ($p<0,05$) et une tendance similaire était observée pour la fructosamine ($p=0,07$) et l'albumine ($p=0,0619$). Les concentrations en urée ont eu tendance à être plus élevées dans le groupe G par rapport au groupe C ($p=0,0987$). En conclusion, dans cette expérience préliminaire, les changements en fonction du temps étaient compatibles avec une modification de l'état physiologique (gestation/lactation). Le β1,3-glucane n'a pas affecté le métabolisme énergétique. Le β1,3-glucane a semblé protéger le muscle et le foie car le niveau d'AST était réduit dans le groupe G. L'utilisation de β1,3-glucane n'a pas eu d'incidence négative sur les animaux.

Mots-clés: β1,3-glucan; Brebis; Sang; Métabolites; Minéraux; Enzymes; Péri-partum.

Titre courant: β1,3-glucan et métabolisme dans le péri-partum chez la brebis

INTRODUCTION

The Northeast region of Brazil has a long tradition when it comes to small ruminant farming. Approximately 57% of the Brazilian sheep herd is reared in this region [21]. In this context, the state of Pernambuco occupies a prominent place in the Brazilian small ruminant industry [36]. Among the different breeds raised in Brazil, the Santa Inês breed is noted for being the hardiest and the most resistant when farmed under difficult conditions such as those seen in tropical and arid areas. Santa Inês sheep are generally raised using semi-intensive and intensive management systems that include a relatively rich diet in concentrate. Indeed, this breed of ewe has good growth rates and high reproductive rates throughout the year. This breed is an excellent option for crossbreeding, due to the Santa Inês' maternal ability, good prolificacy, high milk production, and rapid reproductive maturity [2, 5, 20, 34].

One of the goals of intensive farms is to predict and to limit nutritional and metabolic disturbances during critical physiological periods, such as the *peri-partum* period. This phase is particularly crucial because the energy needs of multi-foetus carrying pregnant animals are greatly increased and the ability to meet these demands is limited. The high requirements in productive systems and intensive management in modern farms have increased the risk and incidence of nutritional imbalances and metabolic disturbances in sheep during the *peri-partum* period. This period is characterised as a phase where changes occur in metabolism, due to the exponential increase in foetal growth and the need for colostrum production [11, 19].

Late pregnancy and early lactation are physiological states known to induce metabolic stress and can in some cases also lead to welfare problems and even mortality [15]. Metabolic disturbances in pregnant sheep can induce deleterious effects on foetal and newborn lambs by adversely affecting placental size, foetal growth, and deposition of foetal fat reserves for use after birth, maternal udder development and colostrum and milk production [33, 41].

A variety of nutritional methods can be used in the prevention and treatment of metabolic diseases such as pregnancy toxaemia. In addition to metabolic problems induced by underfeeding, the immune system has been reported to be depressed due to oxidative stress. Animals could benefit from having a stimulated immune system during critical stress periods such as the *peri-partum* period. Some studies have shown that β-glucan has immunostimulatory

properties including anti-tumour and anti-bacterial activities [1, 31, 43]. There is increasing interest in identifying its biological characteristics, in order to facilitate its use, and to take advantage of its potential as an immune-stimulant [28]. However, very little information is available concerning its possible effects on metabolism and it is necessary to ensure that immune-stimulation is not obtained to the detriment of the animal's metabolic adaptation to the *peri-partum* period.

Although extensive knowledge exists concerning metabolic disturbances in the *peri-partum* period, studies on the effects of treatment with β-glucan injections on metabolism are inexistent. Therefore, the current project was conducted to evaluate the influence of β-glucan administration on the metabolism of peri-parturient Santa Inês ewes.

MATERIAL AND METHODS

Animals and experimental protocol: The study was conducted in the northeast of Brazil at the Cattle Clinic of the Federal Rural University of Pernambuco in Garanhuns, for five months from September 2014 to February 2015. Fourteen clinically healthy pregnant Santa Inês ewes weighing between 40 and 50 kg were used in the experiment. The average body condition score of the ewes was 3.0-3.5 on a scale of 1-5 [35, 10]. The animals were raised under an intensive system, using the same hygiene, sanitary and nutritional management scheme, and assessed at the same metabolic stage (late gestation and early lactation).

On the 90th day of pregnancy, ewes were divided randomly into two groups of seven animals: glucan group, received β1,3-glucan (Imunoglucan® Glucana (β-1,3-D-glicopiranoze), Hebron Laboratory, Brazil) or control group. The β1,3-glucan was injected at the recommended dosage of 1 mL intramuscularly once a week, from the -60 day until -10 day in relation to parturition. All animals were vaccinated (Poli-Star®, Vallée S/A Veterinary products Ltd, Brazil), dewormed (Diantel®, Oral solution, Hipra Saúde Animal Ltda, Brazil) and submitted to a daily clinical observation to detect possible *peri-partum* diseases. Ultrasonography (Ultrassom GE, modelo Logic 100 pro, Brazil) was performed to diagnose and monitor gestation [14]. Gestation length for each ewe was calculated from mating and lambing records.

During the experiment, the animals were housed in an open wooden sheep barn, the two groups were fed a commercial concentrate, 150g/animal/day twice daily, plus elephant grass

(*Pennisetum purpureum*) and tifton (*Cynodon* sp). The diet was prepared to cover nutritional requirements. Fresh water and mineral salt were available *ad libitum*.

Sampling and measurement

Blood samples were collected on the 30th, 20th, 10th day ante-partum, at parturition and on the 10th, 20th, 30th and 60th day *post-partum* [12]. Blood samples were taken by jugular venipuncture, with 25x8mm needles, into sterile vacuum tubes. One tube contained sodium fluoride and oxalate as anticoagulants for glucose and lactate analyses, while the other tube did not include anticoagulant and was used for biochemical and mineral analyses. In the laboratory, samples containing anticoagulant were immediately centrifuged at 3000xg for five min, and the plasma was removed, placed in eppendorfs, and stored in an ultra-low temperature freezer at -80°C until required for analysis. The other samples were allowed to clot to produce serum, and they were then centrifuged and stored under the same conditions as the samples with anticoagulant.

Plasma samples were analysed for L-lactate (Biotécnica Ind. E Com. Ltda, Brazil) and glucose (Labtest Diagnóstica S.A., Brazil) and serum samples were analysed for beta-hydroxybutyrate (βHB) and non-esterified fatty acids (NEFA) (using kits from Rambot Randox Laboratories Ltd, UK) and fructosamine, cholesterol, triglycerides, urea and creatinine (Labtest Diagnóstica S.A., Brazil). Total protein and albumin (Labtest Diagnóstica S.A., Brazil) were measured by the biuret and bromocresol green methods respectively. Globulin concentration was calculated as the difference between total protein and albumin. Enzyme activities were determined in serum samples: aspartate amino transferase (AST), gamma glutamyl transferase (GGT) and creatine kinase (CK, Labtest Diagnóstica S.A., Brazil). All assays were performed using a semi-automatic analyser (Labquest, Labtest Diagnóstica S.A., Brazil) at 37°C.

Serum levels of minerals: total calcium, inorganic phosphorus, magnesium and chloride were analysed by a semi-automatic biochemical analyser with commercial kits (Labtest Diagnóstica S.A., Brazil). Ionized Ca²⁺, Na⁺ and K⁺ ion levels were determined by an electrolyte analyser (Mod.9180, Cobas, Roche Diagnostics, Brazil).

Statistical analysis:

Statistical analysis was performed using a linear mixed model with the MIXED procedure in SAS [37] for repeated measures, including a random female effect. The effects of experimental

treatment, time and their interaction were tested on plasma and serum measurements. The p values were adjusted for multiple comparisons between the treatment groups using F test. The significance level for all statistical tests was set at $\alpha = 0.05$, where p -Values < 0.05 were used to define statistical significance. All data are presented as Least Square (LS) means were subsequently compared with the Contrast statement in the MIXED procedure [27].

Ethical committee: the experimental protocol was approved by the Animal Ethics Committee (CEUA) of the *Universidade Federal Rural do Pernambuco* under license No. 004/2014 (protocol No. 23082.013890/2013-85) according to the Brazilian School for Animal Experimentation (COBEA -*Colégio Brasileiro de experimentação animal*) and the National Institute Guide for the Care and Use of Laboratory Animals.

RESULTS

Energy metabolism indicators

There was no change in BCS throughout the experiment. The BCS remained constant between 3.0 and 3.5 (data not shown). Glucose, L-lactate, NEFA and β HB were unaffected by treatment however, there was a significant effect of time on these parameters ($p < 0.01$, Table 1) where concentrations peaked on the day of parturition. Fructosamine tended to be higher in the Control compared to the glucan group ($p = 0.07$) and concentrations increased just before parturition and remained high thereafter ($p = 0.0002$, Figure 1). Cholesterol and triglycerides were unaffected by treatment (Table 1). However, their concentrations increased just before parturition and then declined with time ($p < 0.0001$).

Protein metabolism indicators

Total protein and globulin were not affected by treatment (Table 2). However, their concentrations decreased before parturition and then subsequently increased ($p < 0.0001$). There was a trend for albumin to be affected by treatment (control $>$ glucan, $p = 0.062$, Figure 2). A significant time effect was observed ($p < 0.001$) where the highest average value was observed at -10d compared to the other time points. Urea tended to be affected by treatment (glucan $>$ control, $p = 0.0987$, Figure 3).

There was also a significant effect of time on urea concentrations ($p=0.0110$), where urea peaked on the day of parturition. Creatinine was unaffected by treatment (Table 2) but concentrations peaked just before and at parturition ($p<0.0001$).

Enzyme profiles

AST activity was higher in the control compared to the glucan group ($p=0.035$, Figure 4). AST and GGT activity were higher in lactation than during the end of gestation ($p<0.05$, Figure 4 and Table 3). Creatine kinase was unaffected by treatment and time (Table 3).

Mineral metabolism indicators

Total and ionized calcium, magnesium, chloride, inorganic phosphorous, sodium and potassium concentrations were not affected by treatment (Table 4). Total and ionized calcium were unaffected time. Chloride increased after parturition ($p=0.002$). Inorganic phosphorous decreased up to parturition and then increased ($p<0.001$). Sodium and potassium peaked before parturition ($p<0.05$).

DISCUSSION

Metabolism

Body condition score (BCS) was constant throughout the present experiment. This is in contrast with other studies [37, 35] which showed that BCS decreased over the *peri-partum* period. It was concluded [42] that the change in BCS was due to a decrease in dry matter intake. The ewes in our experiment appeared to be able to maintain a neutral energy balance probably because our ewes produced singletons except one ewe which produced twins. Similar results have been obtained by others [13, 37, 3].

The blood concentrations of glucose, L-lactate, NEFA, β HB, cholesterol, triglycerides, total protein, albumin, AST and CK were within the normal ranges for sheep [23, 26].

Glucose, L-lactate, NEFA and β HB, fructosamine and potassium evolved with time, peaking just before or at parturition. This is a normal picture of the response in metabolism to the 'stress' of parturition due to increased cortisol concentrations seen at parturition. Total protein and globulin concentrations decreased significantly at parturition. This was probably due to the need

for globulins to make up the IgG component of colostrum. Total protein concentrations are highly dependent on blood globulin. Both AST and GGT were low prior to parturition and then increased after parturition. AST is an indicator of muscle damage and may indicate that there was mobilisation of muscle protein for lactation. GGT is an indicator of liver damage or insult as in the case of hepatic lipidosis [39], which is often encountered in early lactation. However, if hepatic lipidosis existed in the present experiment it was very mild since other parameters such as urea or albumin, which can also be used as indicators of hepatic lipidosis, were not affected in a way which was indicative of lipidosis.

There is a wealth of information concerning the decline in immune function in association with parturition and numerous mechanisms are involved. The most important appears to be an energy deficit which increases the release of NEFA from adipose tissue and the production of ketone bodies by the liver [17, 16]. High plasma NEFA contribute to the development of fatty liver syndrome which is a contributing factor to peri-parturient immunosuppression in the *post-partum* period [24, 22]. It was shown [44] that inflammatory immune genes were up-regulated in cows suffering from severe negative energy balance and that at the same time genes involved in the acquired immune response were down-regulated [29]. Elevated plasma NEFA signalling intense lipomobilization has been associated with reduced lymphocyte proliferation and their ability to secrete immunoglobulin M and interferon in response to polyclonal stimulation [25]. It has been proposed that plasma NEFA concentrations may be used as a diagnostic marker of impaired peri-parturient immunity and increased risk of infections [30, 32]. High concentrations of NEFA are associated with increased risk for displaced abomasum, clinical ketosis, retained placenta and metritis [32].

In this context treatment with glucan may possess the ability to up-regulate the immune system. Indeed, β1,3-glucan exerts potent effects on the immune system by stimulating anti-tumour and anti-microbial activity. They are thought to bind to receptors on macrophages and other white blood cells and hence activate them [8]. Indeed, glucan injection during lactation in sheep was able to reduce the severity of a *Staphylococcus haemolyticus* intra-mammary challenge [9]. However, it is necessary to evaluate any potential effects that glucan has on metabolism, particularly glucose, in the peri-parturient animal, since they face major metabolic

challenges during this period. Indeed, one of the main fuels for cells involved in the immune system is glucose [45].

In addition to glucose requirements for the immune system, the gravid uterus during late pregnancy and the lactating mammary glands also need glucose. Major adjustments in glucose production and utilization in maternal liver, adipose tissue, skeletal muscle etc... are required during this period. Ruminants rely principally on hepatic gluconeogenesis for their glucose supply since glucose absorption levels are low. Hepatic glucose synthesis increases to cover uterine or mammary demands even when glucose precursor supply is low. At the same time, glucose utilization by adipose tissue and muscle is reduced and this is mediated by reduced tissue insulin-sensitivity and responsiveness. Glucose transport into tissues by the GLUT4 transporter is also decreased. As lactation progresses peripheral tissues become more responsive to insulin [7].

Direct measurements of glucose concentrations indicate transient changes due to diurnal, dietary and individual factors. In human medicine, the measurement of products of blood protein glycation (HbA1c, fructosamine) has been established as an indicator of glycaemia over a longer period of time where the carbonyl group of a sugar binds to a free amino-group of a protein [4, 23]. Once formed, fructosamine is stable, until degraded during protein catabolism. The level of fructosamine depends on the average blood glucose concentration during the previous two weeks and the half-life of the blood proteins, and is not subject to changes due to transient hyperglycaemia [4].

There are several small indicators that glucan may have increased glucose use perhaps as a result of the immune system being stimulated. Blood albumin (and fructosamine) tended to be reduced and urea increased by Glucan compared to control. This could suggest that gluconeogenesis was higher in glucan animals because they used amino acids and labile proteins to produce glucose and urea concentrations increased due to increased levels of deamination during the process of gluconeogenesis. Indeed, in the present experiment the concentrations of fructosamine decreased in the transition period (parturition to +10d *post-partum*) and this was suggested to be linked to a decrease in blood albumin [18] and perhaps the increased requirements for lactation.

The activity of aspartate amino transferase was significantly lower in the blood of Glucan sheep compared to controls. AST is often used as an indicator of muscle damage [40]. However, creatine kinase, another enzyme used to judge muscle [6], was unaffected by treatment.

CONCLUSION

The weekly intramuscular administration of β1,3-glucan in the *peri-partum* period in Santa Inês ewes did not modify the metabolic profile. β1,3-glucan appeared to have a positive effect on muscle metabolism. Further research on its immunological activity in small ruminants would be interesting to conduct.

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Figure 1. Blood fructosamine concentrations in Santa Inês ewes during late pregnancy and early lactation in relation to seven intramuscular injections of β 1,3-glucan given during the injection period (-60 to -10 days in relation to lambing). Control, n = 7 and β 1,3-Glucan, n = 7. Values are LS means \pm standard error. a,b: different letters indicate a significant difference between average values at different time points.

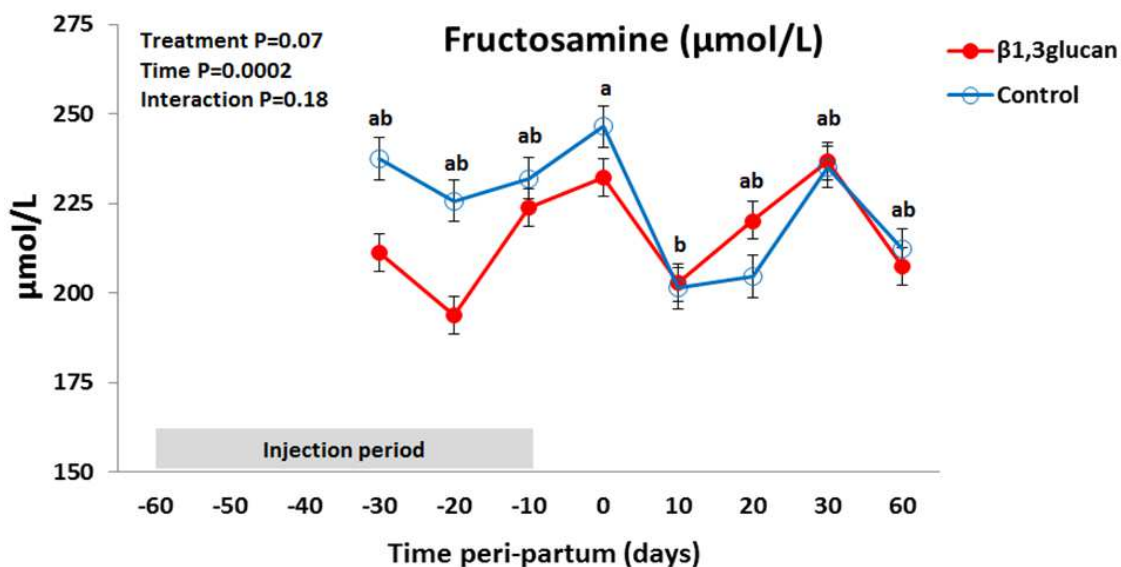


Figure 2. Blood albumin concentrations in Santa Inês ewes during late pregnancy and early lactation in relation to seven intramuscular injections of β 1,3-glucan given during the injection period (-60 to -10 days in relation to lambing). Control, n = 7 and β 1,3-Glucan, n = 7. Values are LS means \pm standard error. a,b: different letters indicate a significant difference between average values at different time points.

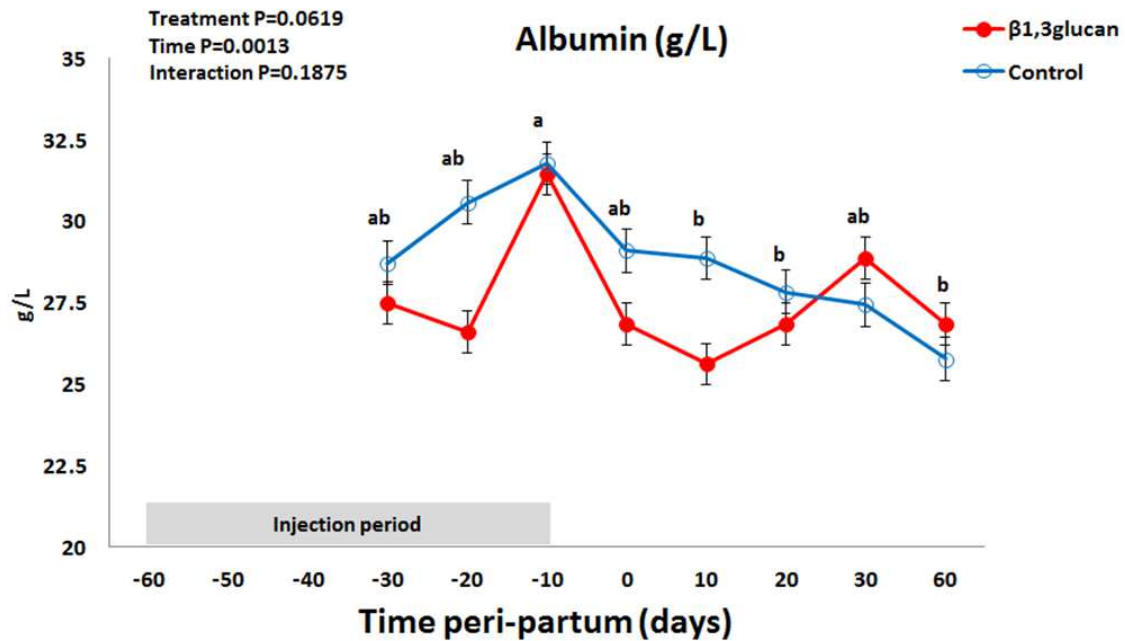


Figure 3. Blood urea concentrations in Santa Inês ewes during late pregnancy and early lactation in relation to seven intramuscular injections of β 1,3-glucan given during the injection period (-60 to -10 days in relation to lambing). Control, n = 7 and β 1,3-Glucan, n = 7. Values are LS means \pm standard error.

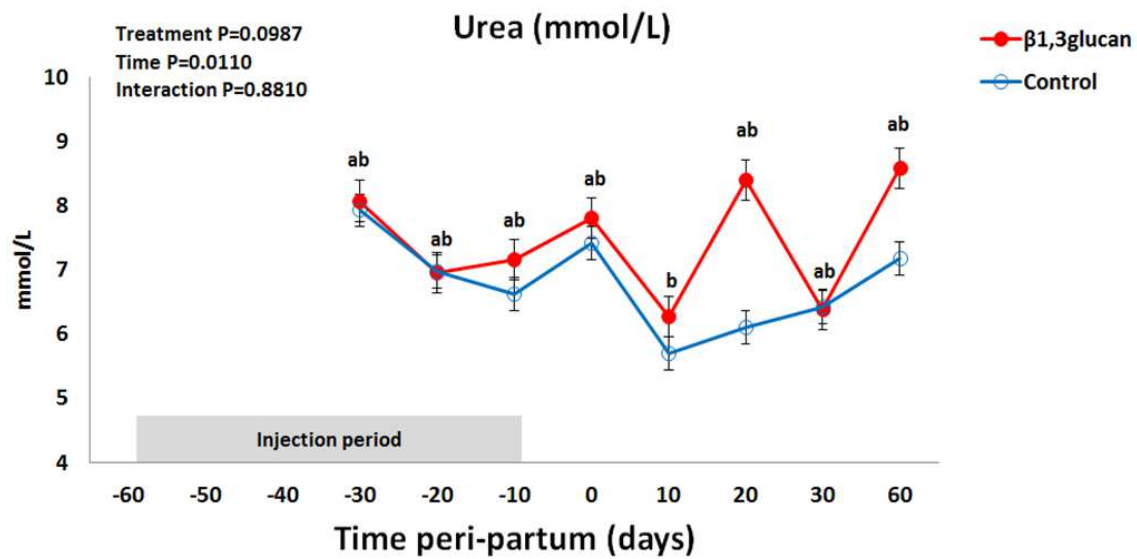


Figure 4. Blood aspartate amino transferase (AST) concentrations in Santa Inês ewes during late pregnancy and early lactation in relation to seven intramuscular injections of β 1,3-glucan given during the injection period (-60 to -10 days in relation to lambing). Control, n = 7 and β 1,3-Glucan, n = 7. Values are LS means \pm standard error.

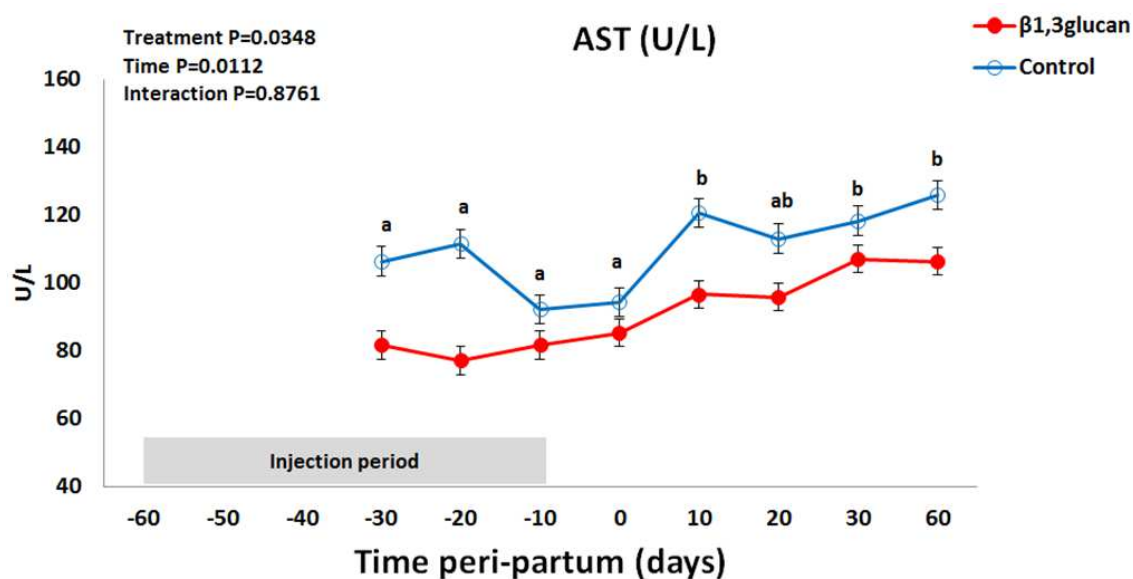


Table 1 - Effect of treatment (glucan, n = 7 and control, n = 7), sampling time and their interaction on blood energy metabolites in Santa Inês ewes during late pregnancy and early lactation. Values are LS means \pm standard error.

Metabolite	Group	Sampling time								GM	Variation factor (Pr>F)		
		-30d	-20d	-10d	Parturition	+10d	+20d	+30d	+60d		Treatment	time	Interact ion (T x t)
Glucose (mmol/L)	Glucan	2.8 \pm 0.22	3.1 \pm 0.61	3.1 \pm 0.48	7.2 \pm 3.20	3.0 \pm 0.34	2.9 \pm 0.37	3.2 \pm 0.29	3.0 \pm 0.26	3.5 ^A	0.2559	<0.0001	0.9936
	Control	2.8 \pm 0.25	2.8 \pm 0.37	2.5 \pm 0.23	6.6 \pm 2.44	2.8 \pm 0.36	2.6 \pm 0.37	3.1 \pm 0.19	3.0 \pm 0.16	3.3 ^A			
	GM	2.8 ^b	2.9 ^b	2.8 ^b	6.9 ^a	2.9 ^b	2.8 ^b	3.1 ^b	3.0 ^b				
L-lactate (mmol/L)	Glucan	1.1 \pm 0.47	1.5 \pm 0.59	1.2 \pm 0.89	2.4 \pm 1.12	0.7 \pm 0.31	0.5 \pm 0.14	0.8 \pm 0.30	0.5 \pm 0.13	1.1 ^A	0.713	<0.0001	0.7547
	Control	1.2 \pm 0.74	1.3 \pm 0.80	0.8 \pm 0.24	2.6 \pm 1.08	0.7 \pm 0.34	0.7 \pm 0.24	0.8 \pm 0.30	1.0 \pm 0.74	1.1 ^A			
	GM	1.1 ^{bd}	1.4 ^b	1.0 ^{bd}	2.5 ^a	0.7 ^{bd}	0.6 ^d	0.8 ^{bd}	0.8 ^{bd}				
NEFA (mmol/L)	Glucan	0.5 \pm 0.49	0.4 \pm 0.33	0.5 \pm 0.47	1.1 \pm 0.54	0.2 \pm 0.19	0.3 \pm 0.20	0.6 \pm 0.45	0.2 \pm 0.05	0.5 ^A	0.3598	<0.0001	0.227
	Control	0.6 \pm 0.54	0.2 \pm 0.16	0.4 \pm 0.20	1.1 \pm 0.54	0.8 \pm 0.28	0.5 \pm 0.36	0.4 \pm 0.49	0.5 \pm 0.52	0.6 ^A			
	GM	0.5 ^b	0.3 ^b	0.4 ^b	1.1 ^a	0.5 ^b	0.4 ^b	0.5 ^b	0.3 ^b				
β HB (mmol/L)	Glucan	0.3 \pm 0.09	0.4 \pm 0.06	0.3 \pm 0.07	0.5 \pm 0.13	0.4 \pm 0.08	0.3 \pm 0.09	0.4 \pm 0.15	0.3 \pm 0.08	0.4 ^A	0.2843	0.0087	0.0525
	Control	0.4 \pm 0.08	0.3 \pm 0.06	0.3 \pm 0.05	0.4 \pm 0.15	0.5 \pm 0.11	0.5 \pm 0.29	0.5 \pm 0.16	0.4 \pm 0.10	0.4 ^A			
	GM	0.3 ^b	0.3 ^b	0.3 ^b	0.5 ^a	0.4 ^{ab}	0.4 ^{ab}	0.4 ^{ab}	0.4 ^{ab}				
Fructosamine (μ mol/L)	Glucan	211.4 \pm 26.30	193.7 \pm 36.90	223.9 \pm 13.10	232.2 \pm 26.00	203.0 \pm 32.30	220.2 \pm 20.00	236.9 \pm 14.10	207.6 \pm 43.90	216.1 ^A	0.0722	0.0002	0.1865
	Control	237.5 \pm 16.80	225.8 \pm 20.00	232.0 \pm 9.10	246.5 \pm 24.10	201.4 \pm 11.90	204.7 \pm 12.80	235.1 \pm 7.30	212.3 \pm 29.40	224.4 ^B			
	GM	224.4 ^{ab}	209.7 ^{ab}	228.0 ^{ab}	239.4 ^a	202.2 ^b	212.4 ^{ab}	236.0 ^{ab}	209.9 ^{ab}				
Cholesterol (mmol/L)	Glucan	1.8 \pm 0.33	2.1 \pm 0.46	2.1 \pm 0.41	1.7 \pm 0.35	1.7 \pm 0.42	1.7 \pm 0.39	1.9 \pm 0.33	1.9 \pm 0.30	1.9 ^A	0.4945	<0.0001	0.9156
	Control	1.8 \pm 0.30	2.1 \pm 0.28	2.1 \pm 0.40	1.7 \pm 0.33	1.7 \pm 0.38	1.7 \pm 0.17	1.9 \pm 0.37	1.9 \pm 0.30	1.9 ^A			
	GM	1.8 ^{abc}	2.0 ^a	2.0 ^{ab}	1.6 ^{ab}	1.7 ^{ab}	1.6 ^{cd}	1.9 ^{ab}	1.9 ^a				
Triglycerides (mmol/L)	Glucan	0.2 \pm 0.05	0.3 \pm 0.07	0.3 \pm 0.10	0.2 \pm 0.06	0.2 \pm 0.07	0.2 \pm 0.05	0.1 \pm 0.03	0.2 \pm 0.09	0.2 ^A	0.2412	<0.0001	0.0885
	Control	0.2 \pm 0.05	0.3 \pm 0.08	0.2 \pm 0.06	0.2 \pm 0.05	0.2 \pm 0.06	0.1 \pm 0.03	0.1 \pm 0.02	0.1 \pm 0.02	0.2 ^A			
	GM	0.2 ^{bc}	0.3 ^{ab}	0.3 ^{ab}	0.2 ^{bc}	0.2 ^c	0.1 ^c	0.1 ^c	0.2 ^c				

GM: General mean; NEFA: non-esterified fatty acids; β HB: beta-hydroxybutyrate; a,b : different letters indicate a significant difference between a value and the next on the same line ($p < 0.05$). A,B : different letters indicate a trend for a difference between treatments ($p < 0.05$).

Table 2 - Effect of treatment (glucan, n = 7 and control, n = 7), sampling time and their interaction on blood protein metabolites in Santa Inês ewes during late pregnancy and early lactation. Values are LS means \pm standard error.

Metabolite	Group	Sampling time								GM	Variation factor (Pr>F)		
		-30d	-20d	-10d	Parturition	+10d	+20d	+30d	+60d		Treatment	time	Interaction (T x t)
TP (g/L)	Glucan	70.2 \pm 5.45	64.5 \pm 11.60	59.1 \pm 8.84	63.5 \pm 4.57	68.4 \pm 5.20	66.5 \pm 3.16	66.7 \pm 7.63	71.2 \pm 5.20	66.3 ^A	0.7996	<0.0001	0.6634
	Control	71.6 \pm 4.52	67.0 \pm 6.17	58.1 \pm 5.31	65.0 \pm 5.59	71.4 \pm 4.25	64.4 \pm 3.88	63.8 \pm 9.20	73.7 \pm 8.33	66.9 ^A			
	GM	70.9 ^a	65.8 ^a	58.6 ^b	64.3 ^b	69.9 ^a	65.4 ^b	65.3 ^b	72.4 ^a				
Albumin (g/L)	Glucan	27.5 \pm 2.60	26.6 \pm 3.00	31.4 \pm 6.00	26.8 \pm 3.10	25.6 \pm 2.00	26.8 \pm 2.40	28.9 \pm 3.20	26.80 \pm 2.40	27.6 ^A	0.0619	0.0013	0.1875
	Control	28.7 \pm 1.60	30.6 \pm 3.90	31.8 \pm 3.20	29.10 \pm 2.80	28.9 \pm 2.50	27.8 \pm 2.30	27.4 \pm 2.60	25.8 \pm 1.80	28.8 ^B			
	GM	28.1 ^{ab}	28.6 ^{ab}	31.6 ^a	28.0 ^{ab}	27.2 ^b	27.3 ^b	28.1 ^{ab}	26.3 ^b				
Globulin (g/L)	Glucan	42.7 \pm 4.83	37.9 \pm 11.23	27.6 \pm 14.47	36.7 \pm 5.31	42.8 \pm 3.72	39.6 \pm 3.63	37.9 \pm 7.82	44.4 \pm 5.56	38.7 ^A	0.8378	<0.0001	0.9103
	Control	42.9 \pm 5.50	36.4 \pm 5.94	26.3 \pm 5.74	36.0 \pm 6.39	42.5 \pm 5.90	36.6 \pm 5.00	36.4 \pm 10.71	47.9 \pm 9.41	38.1 ^A			
	GM	42.8 ^{bc}	37.2 ^c	27.0 ^d	36.3 ^c	42.7 ^{bc}	38.1 ^{bc}	37.1 ^{bc}	46.1 ^{ab}				
A/G	Glucan	0.7 \pm 0.10	0.8 \pm 0.23	1.9 \pm 1.77	0.8 \pm 0.16	0.6 \pm 0.05	0.7 \pm 0.11	0.8 \pm 0.28	0.6 \pm 0.10	0.8 ^A	0.8199	<0.0001	0.4644
	Control	0.7 \pm 0.12	0.9 \pm 0.20	1.3 \pm 0.34	0.8 \pm 0.22	0.7 \pm 0.13	0.8 \pm 0.14	0.8 \pm 0.34	0.6 \pm 0.13	0.8 ^A			
	GM	0.7 ^b	0.8 ^b	1.6 ^a	0.8 ^b	0.6 ^b	0.7 ^b	0.8 ^b	0.9 ^b				
Creatinine (μ mol/L)	Glucan	73.5 \pm 6.89	70.2 \pm 2.44	75.8 \pm 9.08	74.6 \pm 8.27	58.8 \pm 5.23	59.9 \pm 10.15	66.2 \pm 10.77	68.3 \pm 10.11	68.4 ^A	0.8128	<0.0001	0.1073
	Control	66.5 \pm 11.45	69.1 \pm 12.97	76.5 \pm 11.05	80.9 \pm 8.46	64.8 \pm 9.85	66.0 \pm 8.23	64.7 \pm 11.74	65.8 \pm 9.38	69.3 ^A			
	GM	70.9 ^{bc}	69.6 ^{bc}	76.2 ^{bc}	77.8 ^{ab}	61.8 ^d	63.0 ^d	65.4 ^d	67.1 ^c				
Urea (μ mol/L)	Glucan	8.1 \pm 1.59	6.9 \pm 1.60	7.2 \pm 1.32	7.8 \pm 2.03	6.3 \pm 1.03	8.4 \pm 1.81	6.4 \pm 1.52	8.6 \pm 1.19	7.4 ^A	0.0987	0.0110	0.8810
	Control	7.9 \pm 0.73	7.0 \pm 2.21	6.6 \pm 2.08	7.4 \pm 1.94	5.7 \pm 1.41	6.1 \pm 1.67	6.4 \pm 2.46	7.2 \pm 2.55	6.8 ^B			
	GM	8.0 ^{ab}	6.9 ^{ab}	6.9 ^{ab}	7.6 ^{ab}	6.0 ^b	7.2 ^{ab}	6.4 ^{ab}	7.9 ^b				

GM: General mean; TP: Total Protein; A/G: Albumin and globulin ratio; a,b : different letters indicate a significant difference between a value and the next on the same line ($p < 0.05$). A,B : different letters indicate a trend for a difference between treatments ($p < 0.05$).

Table 3 - Effect of treatment (glucan, n = 7 and control, n = 7), sampling time and their interaction on enzymes in Santa Inês ewes during late pregnancy and early lactation. Values are LS means \pm standard error.

Metabolite	Group	Sampling time								GM	Variation factor (Pr>F)		
		-30d	-20d	-10d	Parturition	+10d	+20d	+30d	+60d		Treatment	time	Interac tion (T x t)
AST (U/L)	Glucan	81.6 \pm 23.20	77.1 \pm 8.93	81.6 \pm 13.81	85.2 \pm 18.83	96.5 \pm 16.52	95.8 \pm 22.57	107.0 \pm 34.48	106.3 \pm 48.66	91.4 ^A	0.0348	0.0112	0.8761
	Control	106.3 \pm 21.97	111.5 \pm 23.19	92.0 \pm 15.38	94.3 \pm 11.32	120.5 \pm 24.93	113.0 \pm 22.41	118.2 \pm 26.85	125.8 \pm 63.86	110.2 ^B			
	GM	93.9 ^a	94.3 ^a	86.8 ^a	89.8 ^a	108.5 ^b	104.4 ^{ab}	112.6 ^b	116.0 ^b				
GGT (U/L)	Glucan	53.6 \pm 6.25	59.0 \pm 12.27	55.7 \pm 7.28	62.3 \pm 11.20	80.9 \pm 30.22	75.4 \pm 31.66	71.0 \pm 24.08	63.4 \pm 24.09	65.2 ^A	0.6106	<0.0001	0.5581
	Control	55.7 \pm 10.56	54.6 \pm 9.29	52.5 \pm 8.18	60.1 \pm 8.18	72.1 \pm 9.73	64.5 \pm 13.14	64.5 \pm 13.14	69.9 \pm 9.29	61.7 ^A			
	GM	54.6 ^b	56.8 ^b	54.1 ^b	61.2 ^b	76.5 ^a	69.9 ^b	67.8 ^b	66.7 ^b				
CK (U/L)	Glucan	121.4 \pm 48.58	131.8 \pm 52.21	118.0 \pm 29.51	121.4 \pm 28.00	149.2 \pm 25.98	121.4 \pm 34.35	142.2 \pm 69.29	138.8 \pm 23.11	130.5 ^A	0.9693	0.6098	0.6224
	Control	149.2 \pm 51.40	125.5 \pm 41.84	128.4 \pm 38.95	124.9 \pm 29.51	114.5 \pm 41.39	111.0 \pm 30.90	124.9 \pm 51.39	166.5 \pm 127.29	130.7 ^A			
	GM	135.3 ^a	128.9 ^a	123.2 ^a	123.2 ^a	131.8 ^a	116.2 ^a	133.6 ^a	152.6 ^a				

GM: General mean; AST: Aspartate amino transferase; GGT: Gamma glutamyl-transferase; CK: Creatine Kinase; a,b : different letters indicate a significant difference between a value and the next on the same line ($p < 0.05$). A,B : different letters indicate a trend for a difference between treatments ($p < 0.05$).

Table 4 – Effect of treatment (glucan, n = 7 and control, n = 7), sampling time and their interaction on blood ions in Santa Inês ewes during late pregnancy and early lactation. Values are LS means \pm standard error.

Ions	Group	Sampling time								GM	Variation factor (Pr>F)		
		-30d	-20d	-10d	Parturition	+10d	+20d	+30d	+60d		Treatment	time	Interac tion (T x t)
Total calcium (mmol/L)	Glucan	2.2 \pm 0.12	2.2 \pm 0.33	2.5 \pm 0.18	2.3 \pm 0.30	2.3 \pm 0.15	2.3 \pm 0.20	2.3 \pm 0.29	2.3 \pm 0.26	2.3 ^A	0.8167	0.4691	0.9811
	Control	2.3 \pm 0.21	2.4 \pm 0.16	2.3 \pm 0.26	2.2 \pm 0.17	2.4 \pm 0.11	2.2 \pm 0.14	2.3 \pm 0.14	2.3 \pm 0.24	2.3 ^A			
	GM	2.3 ^a	2.3 ^a	2.4 ^a	2.2 ^a	2.3 ^a	2.3 ^a	2.3 ^a	2.3 ^a				
Cl ⁻ (mmol/L)	Glucan	109.3 \pm 4.89	112.6 \pm 8.94	109.0 \pm 3.46	107.4 \pm 8.00	111.0 \pm 2.62	116.0 \pm 4.47	112.0 \pm 3.21	107.9 \pm 4.60	110.6 ^A	0.4718	0.0021	0.8261
	Control	107.6 \pm 5.44	115.1 \pm 9.70	111.3 \pm 2.93	111.0 \pm 5.39	110.9 \pm 2.98	114.4 \pm 3.36	113.6 \pm 4.28	109.0 \pm 4.97	111.6 ^A			
	GM	108.4 ^a	113.9 ^a	110.1 ^a	109.2 ^a	111.0 ^a	115.2 ^a	112.8 ^a	108.4 ^a				
PO ₄ ³⁻ (mmol/L)	Glucan	2.3 \pm 0.47	1.9 \pm 0.55	1.7 \pm 0.46	1.6 \pm 0.50	2.0 \pm 0.25	1.8 \pm 0.51	2.1 \pm 0.42	1.7 \pm 0.59	1.9 ^A	0.6794	0.0005	0.4076
	Control	1.9 \pm 0.52	1.6 \pm 0.36	1.5 \pm 0.57	1.4 \pm 0.60	2.1 \pm 0.50	2.0 \pm 0.49	2.0 \pm 0.60	1.9 \pm 0.58	1.8 ^A			
	GM	2.1 ^a	1.7 ^a	1.6 ^a	1.5 ^a	2.1 ^a	1.9 ^a	2.1 ^a	1.8 ^a				
Mg ³⁺ (mmol/L)	Glucan	1.1 \pm 0.23	1.1 \pm 0.15	1.1 \pm 0.14	1.1 \pm 0.32	1.0 \pm 0.16	1.1 \pm 0.34	1.1 \pm 0.17	1.0 \pm 0.15	1.1 ^A	0.2029	0.7137	0.4542
	Control	1.2 \pm 0.21	1.2 \pm 0.24	1.1 \pm 0.19	1.1 \pm 0.24	1.1 \pm 0.09	1.0 \pm 0.23	1.0 \pm 0.21	1.2 \pm 0.26	1.1 ^A			
	GM	1.2 ^a	1.1 ^a	1.1 ^a	1.1 ^a	1.0 ^a	1.0 ^a	1.0 ^a	1.1 ^a				
Na ⁺ (mmol/L)	Glucan	145.4 \pm 7.04	145.6 \pm 4.20	155.4 \pm 14.44	149.1 \pm 2.54	146.6 \pm 0.98	147.4 \pm 2.30	145.9 \pm 2.34	147 \pm 2.00	147.8 ^A	0.6545	0.0236	0.9559
	Control	150.0 \pm 18.97	146.0 \pm 3.37	153.6 \pm 10.34	150.6 \pm 1.90	146.3 \pm 1.89	146.6 \pm 1.90	147.9 \pm 1.95	146.6 \pm 1.62	148.4 ^A			
	GM	145.3 ^a	145.8 ^a	154.5 ^a	149.9 ^a	146.4 ^a	147.0 ^a	146.9 ^a	146.8 ^a				
K ⁺ (mmol/L)	Glucan	4.5 \pm 0.28	4.4 \pm 0.32	4.9 \pm 0.30	4.5 \pm 0.18	4.4 \pm 0.38	4.3 \pm 0.35	4.4 \pm 0.33	4.4 \pm 0.38	4.5 ^A	0.7699	0.0117	0.947
	Control	4.6 \pm 0.22	4.5 \pm 0.48	4.7 \pm 0.46	4.4 \pm 0.23	4.5 \pm 0.36	4.4 \pm 0.26	4.5 \pm 0.30	4.4 \pm 0.27	4.5 ^A			
	GM	4.6 ^a	4.5 ^a	4.8 ^a	4.5 ^a	4.5 ^a	4.4 ^a	4.5 ^a	4.4 ^a				
Ca ²⁺ (mmol/L)	Glucan	0.8 \pm 0.18	1.0 \pm 0.31	1.1 \pm 0.18	1.0 \pm 0.13	1.0 \pm 0.17	1.1 \pm 0.14	1.0 \pm 0.15	1.0 \pm 0.13	1.0 ^A	0.3672	0.1666	0.2193
	Control	1.0 \pm 0.32	1.2 \pm 0.15	1.1 \pm 0.21	1.0 \pm 0.10	1.0 \pm 0.13	1.0 \pm 0.15	1.0 \pm 0.15	1.0 \pm 0.14	1.0 ^A			
	GM	0.9 ^a	1.1 ^a	1.1 ^a	1.0 ^a	1.0 ^a	1.0 ^a	1.0 ^a	1.0 ^a				

GM: General mean. Cl⁻:Chlore; PO₄³⁻ : phosphate Mg³⁺: Magnesium; Na⁺: Sodium; K⁺: Potassium; Ca⁺⁺: Ionized calcium; a,b : different letters indicate a significant difference between a value and the next on the same line ($p < 0.05$). A,B : different letters indicate a significant difference between treatments ($p < 0.05$).

4.2 Paper 2

Effects of a Saponin-based Additive on Two Different Dairy Goat Metabolic Statuses

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ABSTRACT. - R. O. Rego; A. A. Ponter; C. Duvaux-Ponter; O. Dhumez; M. Taghipoor; J. A. B. Afonso; C. Domange. 2016. [Effects of a Saponin-based Additive on Two Different Dairy Goat Metabolic Statuses] 00(0):00-00. *E-mail: faelvet@yahoo.com.br.

The purpose of this paper was to evaluate the effects of saponin-based additive: during mid-lactation and peri-parturient period. Two experiments were carried out with 31 dairy Saanen and Alpine goats which were assigned to a control group (C group) or a group treated with saponin (S group). Experiment 1 consisted of 20 dairy goats (C group, n=10; S group, n=10) in lactation and experiment 2 had an overall number of 24 pregnant goats, including 13 goats from experience 1, around parturition (C group, n=12; S group, n=12). S group received the saponin additive powder blended with the total mixed ration at a dose of 25g/animal per day, whereas the C group was only given the standard diet. After one week of adaptation, the daily administration of saponin additive was conducted during four consecutive weeks followed by one or two weeks of wash-out. Blood and rumen fluid samples were taken weekly from both groups to perform a metabolic screening. The plasma samples were analyzed for: glucose, non-esterified fatty acids (NEFA), β -hydroxybutyric acid (β HB), total cholesterol (TC), high-density lipoprotein (HDL), total bilirubin (TB), total proteins (TP), albumin, haptoglobin (Hp), urea, aspartate-aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total calcium (Ca), magnesium (Mg^{3+}), phosphorus (PO_4^{3-}). Rumen sample analyses involved pH and ammonia nitrogen (NH_3) determinations, concentration of volatile fatty acids (VFA) and total protozoa counts. Feed samples, milk yield (MY) and body weight (BW) were measured on a weekly basis. Throughout both experimental periods, all the goats were clinically healthy. Statistical analyses were performed using the linear mixed model procedure of SAS. The results of the first experiment with lactating goats showed no saponin effect among the zootechnical measures, plasma biochemical metabolites (glucose, NEFA, β HB and urea) and ruminal fermentation parameters ($p>0.05$). However, there was a tendency for the acetate to propionate ratio ($S < C$, $p=0.0570$). The results showed an important time effect on BW, MY, glucose, NEFA and all ruminal fermentation parameters ($p<0.05$), with the exception of caproic acid ($p>0.05$). This time effect, which is mainly associated with the last week of the experiment, could be due to a high temperature during this week. In the second experiment there was an absence of saponin effect on almost all blood metabolites ($p>0.05$),

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except for plasma urea ($S > C$, $p=0.0544$). Concerning ruminal fermentation parameters, only total protozoa showed a tendency ($S > C$, $p=0.096$). A time effect was observed for most of zootechnical, plasmatic and ruminal variables around parturition because of the physiological imbalances during this transition period ($p<0.05$), with the exception of GGT, Mg^{3+} and total protozoa count ($p>0.05$). Hence, it was concluded that the daily administration of saponin additive during four consecutive weeks in mid-lactation and *peri-partum* in Saanen and Alpine dairy goats had not influenced plasma metabolites and ruminal fermentation parameters; neither did it influence the zootechnical animal performance. The observed tendencies for a saponin effect in relation to blood urea, total protozoa count and acetate/propionate ratio were relevant in this current research. The potential of saponin additive to improve efficiency of ruminal fermentation through increased microbial protein synthesis could be dependent on the dose, the period of administration and the type of basal diet feed.

Keywords: Metabolism; Rumen parameters; Feed additive; *Peri-partum*; Saponin; Goat.

RÉSUMÉ: Le but de cette étude est d'évaluer les effets de l'additif à base de saponine sur la performance et deux différents stades métaboliques chez les chèvres laitières. Deux expériences ont été réalisées avec 31 chèvres laitières, Saanen et Alpine. Elles ont été réparties en deux lots : un lot contrôle (lot C) et le lot saponine traité (Lot S), dans chacun des deux statuts métaboliques. L'expérience 1 a été réalisée avec 20 chèvres en lactation (Lot C, $n = 10$; Lot S, $n = 10$). La deuxième expérience a eu un nombre total de 24 chèvres gestantes (groupe C, $n = 12$; groupe S, $n = 12$), dont 13 chèvres de l'expérience 1. Le lot S a reçu la poudre d'additif saponine mélangée avec la ration complète à une dose de 25 g / animal par jour. Tandis que le lot C n'a reçu que le régime standard. Après une semaine d'adaptation, l'administration journalière d'additif saponine a été effectuée pendant quatre semaines consécutives, suivies d'une ou de deux semaines sans saponine. Des échantillons de sang et de jus de rumen ont été prélevés chaque semaine. Les échantillons de plasma ont été analysés pour: le glucose, les acides gras non-estérifiés (AGNE), le β -hydroxybutyrate (β HB), le cholestérol total (CT), la bilirubine totale (BT), les lipoprotéines de hautes densités (LHD), les protéines totales (PT), l'albumine, l'haptoglobine (Hp), l'urée, l'aspartate aminotransférase (AST), la gamma-glutamyl transférase (GGT), la phosphatase alcaline (PAL), le calcium total (Ca), le magnésium (Mg^{3+}), le phosphore (PO_4^{-3}). Les échantillons de jus de rumen ont servis pour mesurer le pH et l'ammoniaque (NH_3), les concentrations d'acides gras volatils (AGV) et le nombre total de protozoaires. Les échantillons de ration, la production laitière (PL) et les poids vifs (PV) ont été mesurés sur une base hebdomadaire. L'analyse statistique a été effectuée en utilisant le model linéaire mixte avec la procédure MIXED de SAS. Tout au long des deux phases expérimentales, toutes les chèvres étaient cliniquement en bonne santé. Les résultats de la première expérimentation avec des chèvres en lactation ont montré qu'aucun effet des saponines n'a été observé parmi les mesures zootechniques, les métabolites biochimiques plasmatiques (glucose, NEFA, β HB et urée) et les paramètres de fermentation ruminale ($p > 0,05$). Cependant, le rapport acétate/propionate a montré une forte tendance entre les groupes ($S < C$, $p = 0,0570$). De plus, un effet important du temps lié à la dernière semaine de l'expérience chez les chèvres en lactation a été observé pour les paramètres PV, PL, glucose, NEFA et tous les paramètres de fermentation ruminale ($p < 0,05$), à l'exception de l'acide caproïque ($p > 0,05$). Cet effet du temps pourrait être dû à une température ambiante élevée au cours de la dernière semaine expérimentale. Les résultats de l'expérience chez les chèvres gestantes ont montré de manière similaire une absence d'effet des saponines pour presque tous les métabolites sanguins ($p > 0,05$), sauf pour l'urée plasmatique qui présentait une forte tendance entre les groupes ($S > C$, $p = 0,054$). En ce qui concerne les paramètres de fermentation ruminale, seul le comptage de protozoaires a montré une tendance à un effet du traitement ($S > C$, $p = 0,096$) parmi les autres variables ruminales. La plupart des variables zootechniques, plasmatiques et ruminales

ont montré des effets du temps autour de la parturition en raison des déséquilibres physiologiques au cours de cette période de transition ($p < 0,05$), sauf la GGT, le Mg^{3+} et le nombre total de protozoaires ($p > 0,05$). Par conséquent, il a été conclu que l'administration journalière d'additif à base de saponines pendant quatre semaines consécutives en milieu de lactation et pendant la période du *péri-partum* chez les chèvres laitières Saanen et Alpine n'a pas influencé les métabolites plasmatiques et les paramètres de fermentation ruminale. Elle n'a pas non plus influencé les performances zootechniques des animaux. Les tendances observées de l'effet des saponines sur l'urée sanguine, le nombre de protozoaires et le rapport acétate/propionate étaient pertinents dans la présente recherche. Enfin, le potentiel de cet additif à base de saponines pour améliorer l'efficacité de la fermentation ruminale par l'augmentation de la synthèse des protéines microbiennes pourrait dépendre de la dose utilisée, de la période d'administration et du type du régime.

Mots-clés : Métabolisme; Paramètres ruminiaux; Additif alimentaire; *Péri-partum*; Saponines; Chèvre.

INTRODUCTION

In animal production, crucial consideration is given to rational feeding systems due to their effect on meat and dairy quality. Small ruminants are known for their excellent ability to digest fibrous feed; however the level of production is generally lower in these species than in other farm animals. Therefore, attempts are made to supply animal diets with feed additives that enhance productivity, increase milk production and reduce the stress of parturition (Milewski & Sobiech 2009).

High herd nutritional requirements influenced by genetic selection and intensive farming systems increase the risk and impact of nutritional imbalances and metabolic disorders (González et al., 2000; Campos et al., 2010). These disorders are the result of increased nutrient requirements during the transition period around parturition. This transition period in ruminants is marked by intense physiological, hormonal, metabolic and anatomical changes. These changes are associated with a decrease in feed intake, high demands for nutrients, high growth rates of the fetus(s), high milk yield, which all result in a negative energy balance (NEB) (Drackley et al., 2005). The intensity of NEB may lead to metabolic disorders such as pregnancy toxemia and hypocalcemia in small ruminants (González et al., 2000). These metabolic diseases result in decreased milk production, affect reproductive performance and increase herd culling rate, leading to economic losses for dairy farmers (Nielsen et al., 2005; Tharwat et al., 2013).

In general, pregnancy toxemia is a key metabolic disorder in small ruminant caused by NEB during the final stage of gestation. It is characterized by an increase in ketone bodies due to an imbalance in maternal metabolism that cannot meet fetal requirements at the end of gestation (Cal et al., 2009; Brozos et al., 2011, Hefnawy et al., 2011).

Nowadays, there is increasing interest in exploiting natural products as phytogetic feed additives for livestock, due to the fact that they represent an alternative feeding strategy in replacement of antibiotic growth promoters and other additives. As pointed out by Wallace et al. (2002), consumers and health authorities in developed countries, especially in the European Union, have banned or limited the use of chemical feed additives such as growth promoters, including antibiotics and ionophores in livestock feed since 2006 (Council Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition). Natural products when used as feed additives must be harmless to animals, leave no toxic residues in the carcass or in animal products, they must also be environmentally-friendly (Wallace et al., 2002; Santoso et al., 2007; Vrublova et al. 2010).

Some plants produce and store secondary compounds that are not directly involved in their growth, development or reproduction, but these compounds are a complex group of substances that defend and protect plants. They serve as chemical messengers between plants and the environment; they are responsible for attracting pollinator insects, and play a role in the protection against other plants, herbivores and abiotic stressor processes (Jouany & Morgavi 2007). Some of these compounds exert beneficial activities in human and animal organisms due to their antioxidant activity, their favourable effects on cardiovascular disease, inflammatory and tumour processes, on digestion and for their antiseptic and antimicrobial effects (Franz et al. 2005).

Saponins are a group of steroidal glycosides, which can be extracted from different plants such as *Yucca schidigera*. Several studies have shown an interesting role of saponins in animal nutrition because they improve the efficiency of ruminant production without having a detrimental effect on animal performance (Jouany & Morgavi, 2007; Patra & Saxena, 2009). Saponins have other properties such as being an antimicrobial agent (Chao et al., 1998), since they can inhibit populations of ciliate protozoa, peptidase producing bacteria and cellulolytic bacteria. Furthermore, a metabolic co-relationship exists between ciliate protozoa and methanogenic bacteria. In fact, protozoa produce hydrogen which is utilized by methanogenic bacteria. Indeed, saponins can partially decrease methane and hydrogen production *in vitro*, therefore, they are considered to be powerful inhibitors of rumen methanogens due to their effectiveness at low concentrations and they do not influence feed intake (Bodas et al., 2012). In addition, these substances also increase total volatile fatty acid (VFA) and decrease ammonia concentrations in the rumen. Moreover, saponins decrease the molar proportion of ruminal acetate, and increase that of propionate and butyrate with a corresponding decrease in the acetate/propionate ratio (Lila et al. 2005; Das et al., 2012; Wang et al., 2012).

The aim of the present study was to assess the effects of a saponin-based additive on dairy goat performance and metabolism during two different physiological states: in mid-lactation (which is a period of metabolic balance) and during late pregnancy and early lactation (which is a period of metabolic imbalance). The challenge was to better understand the underlying mechanisms of responses of animals during the dangerous transition period of *peri-partum*; and to validate the potential usefulness of the saponin additive by integrating several parameters using complementary analytical approaches. Criteria such as biochemical parameters and animal husbandry performance were determined.

MATERIALS AND METHODS

Animals and procedures: The present study was carried out according with the 3R guidelines and French legislation on animal experimentation (Rural code: articles R.214-87 to R.214-126) and in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 2010/63/UE). Furthermore, the experimental procedure was approved by the local ethic committee (*Comité d’Ethique en Matière d’Expérimentation Animale*) and received the authorization number 2015050418233758v2 (APAFIS#601).

This research was divided into two experiments, consisting of two different physiological statuses. The first experiment aimed at tackling the effects of a saponin additive on the systemic and ruminal metabolism of dairy goats, during a period of energetic equilibrium (mid-lactation). The second experiment evaluated the effect of the saponin additive during late pregnancy and early lactation on the systemic and ruminal metabolism, in order to study its importance in the *peri-partum* period where energy requirements are increased.

The experiments were conducted in two consecutive years from May to July 2015 (mid-lactation) and from December 2015 to January 2016 (*peri-parturient* period), at the Goat Research Unit of INRA/UMR 0791 MoSAR in Thiverval-Grignon, France.

Thirty-one high producing dairy goats (Saanen and Alpine, 2-3 years of age) were used in the two experiments. In each experiment, the goats were allocated to one of two groups of goats blocked by breed, age, body weight (BW), body condition score (BCS) and MY. The group treated with saponin (S) received the saponin additive powder⁸ from extracts of *Yucca schidigera*, blended with the total mixed ration (TMR), at a recommended level of 25g/animal per day by the manufacturer. The control group (C) was given the standard TMR. Voluntary

⁸ Norponin®K – Nor-Feed Sud – 3 Amedeo Avogadro street 49070, Beaucouzé, France

feed intake was estimated to minimize feed refusals to less than 5%. The TMR was pushed-up at least once during the day in the first experiment, and given in two meals in the second one.

The diets were formulated to cover the requirements: mid-lactation for experiment 1, and pregnancy and early lactation for experiment 2. The composition of the TMR for both experiments is presented in table 1. The animals had free access to water and mineral fortified salt licks.

Goats were weighed on a weekly basis before feeding. Daily average temperature ($T^{\circ}\text{C}$) and hygrometry (HR %) were measured during the experimental periods.

Experiment 1: lactating goats – mid-lactation (around 120th day)

The 1st experiment lasted six weeks and was performed on 20 dairy goats (S group, n=10 and C group, n =10), Saanen and Alpine (66.9 ± 7.12 kg of BW), around the 120th day of lactation (4.4 ± 0.72 kg/day of MY). During the experimental period, goats were always housed in individual pens (1.2m x 0.75m, L x W) with a slatted wooden floor. The individual pens allowed social interaction between goats and simplified sampling. The daily administration of the saponin additive was conducted during four consecutive weeks (W2, W3, W4 and W5). Saponin was not added in the first and last week (W1 and W6) of the experiment. Samples of blood and rumen fluid were taken every week throughout the experiment. Animals were automatically milked twice daily (7 am and 5 pm) in a stall autorotor milking⁹ parlor for goats with automatic measurement of milk production.

⁹ Fosomatic, Hillerød, Denmark

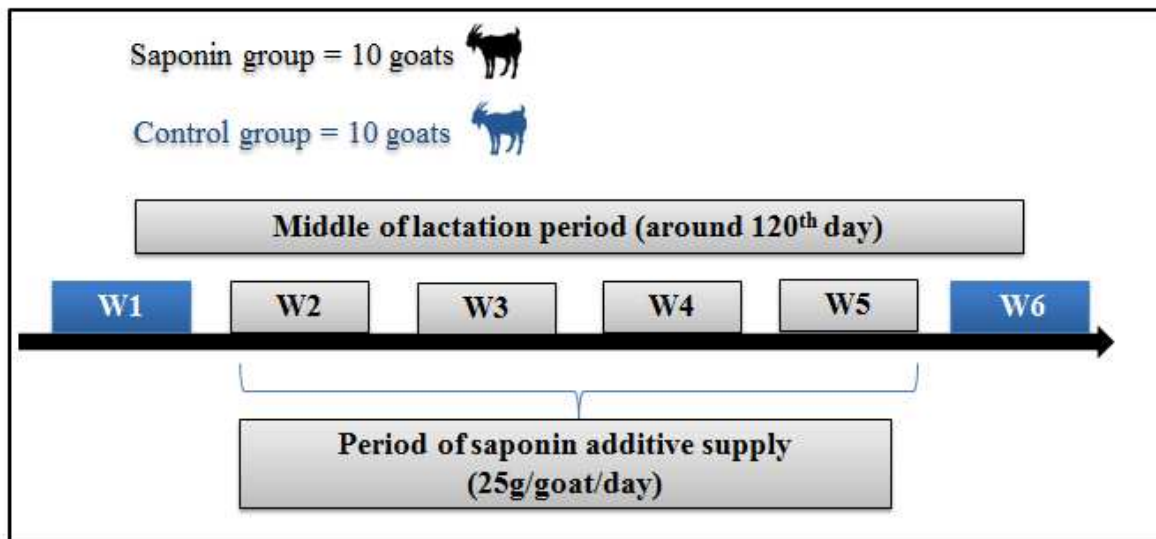


Figure 1. Diagram of experiment 1: lactating goats. This figure shows the experimental design during the middle of lactation (around day 120). This experiment lasted 6 weeks: 1 week of adaptation, 4 weeks of saponin supplementation (25g/goat/day; $n = 10$) or control ($n = 10$) followed by 1 week of wash-out.

Experiment 2: Pregnant goats – peri-parturient period

The 2nd experiment was conducted over seven weeks on 24 multiparous pregnant goats (S group, $n=12$ and C group, $n = 12$), Saanen and Alpine. We couldn't use all the same animals from the first experiment because all of these later didn't succeed in being pregnant. So, among the 24 goats of the 2nd experiment, only 13 of them were issued from the 1st experiment and kept in the same experimental group.

Before the start of the experiment, the animals were synchronized and inseminated in order to program parturition over a 10 day period. Roughly 60 days after insemination, pregnancy was confirmed by ultrasonography and approximately after 90 days of gestation the two groups were formed by blocking by breed, age (2-3 years of age), BW (75.3 ± 8.28 kg) and MY (1.02 ± 0.14 kg/day).

Five weeks before the expected parturition date, both groups received a standard TMR diet, and then during the four subsequent weeks a saponin supplement was given to half the goats until the day of parturition. After parturition, all the goats were given the standard TMR without saponin (Figure 2). The goats were housed in two separated straw bedded pens and were fed once daily at around 2 pm. To avoid a potential effect of pen position within the building on the results, the animals were switched once a week between the two pens. In order to take into account animal welfare at this stage of pregnancy the animals were placed in individual pens (1.2m x 0.75m, L x W) only for sampling during two hours once a week. In addition, blood and rumen fluid samples were taken weekly.

The goats were weighed before the beginning of the experiment and on a weekly basis thereafter, before feeding. The goats were also weighed 24h post-parturition.

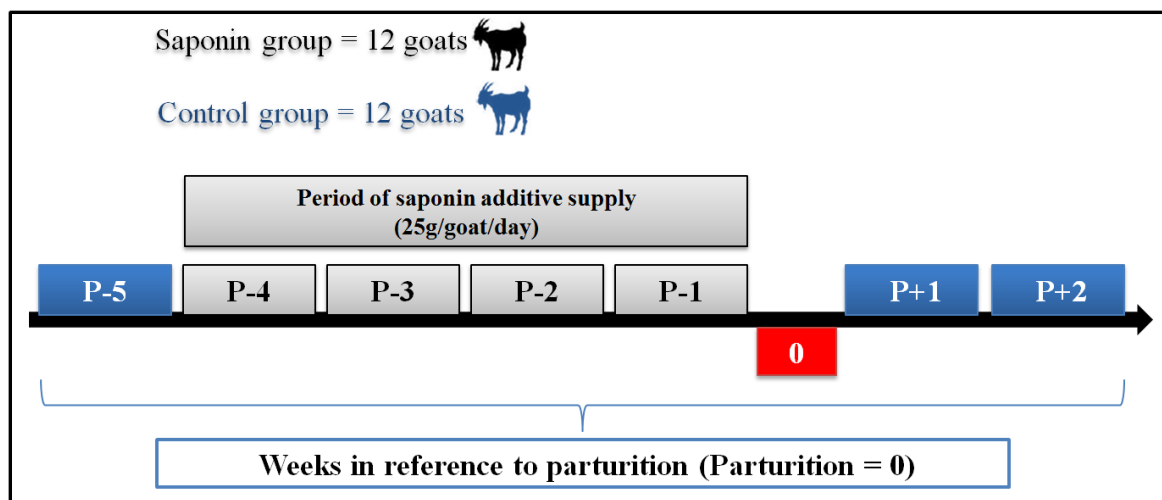


Figure 2. Diagram of experiment 2: pregnant goats. This figure shows the experimental design during the *peri-partum*. This experiment lasted 7 weeks: 1 week of adaptation, 4 weeks of saponin supplementation (25g/goat/day; n = 12) or control (n = 12) followed by 2 weeks of wash-out. The samples were taken weekly in reference to parturition (P=0) during late pregnancy (from P-5 to P-1) and early lactation (P+1 and P+2).

Sampling and measurements

Feed Samples: The amounts of feed offered and orts were measured daily, and 200g of feed for each group was sampled weekly for nutrient composition analysis. The samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL) and starch (Table 1) according to AFNOR (1997).

Table 1. Ingredients and proximate analysis of the total mixed ration (TMR) given to dairy goats during the experimental periods and adjusted according to the nutritional requirements of two different metabolic statuses (INRA, 2010).

Composition (%)	Mid-lactation	-10 to -5 week ¹	-4 to -1 week	-1 to +2 week
Sugar beet pulp	28	30	37.5	28
Meadow hay	24	30	37.5	28
Dried alfafa (Rumiluz® ¹⁰)	28	30	25	28
Protein concentrated Fluvialac® ¹¹	15	5	-	11
Defatted concentrated Colza® ¹¹	5	5	-	5
Chemical composition (g/kg dry matter)				
Organic matter	865	871	860	817
Neutral detergent fibre	460	467	473	481
Acid detergent fibre	263	276	279	278
Acid detergent lignin	44	50	54	43
Crude protein	119	115	110	128
Starch	41	21	24	27

¹ weeks in reference to parturition (parturition = 0).

¹⁰ Désialis, 27, 29 rue Chateaubriand, 75383 Paris Cedex 8, France

¹¹ Agralys aliment, Châteaudun, France

Blood Sampling: During the two experiments, 10 mL of blood were taken weekly by jugular venipuncture into sterile heparinized tubes (135mg of heparin)¹² in the morning. They were immediately centrifuged¹³ at 3000xg for 15 min at 4 °C, and the plasma was aliquoted and stored at -20°C until required for analysis. The plasma samples were then subjected to standard biochemical procedures to determine glucose¹⁴, non-esterified fatty acids (NEFA)¹⁵, β -hydroxybutyrate (β HB)¹⁶, urea¹⁷ in both experiments. In the 2nd experiment total cholesterol (TC), total bilirubin (TB), total protein (TP), albumin, high-density lipoprotein (HDL), magnesium (Mg^{3+}), total calcium (Ca), phosphorus (PO_4^{3-}), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate amino transferase (AST) and haptoglobin (Hp)¹⁸ were also determined. All these analyses were performed using an automatic spectrophotometer¹⁹ with commercial biochemical kits. β HB concentrations were analyzed from the enzymatic principle defined by Williamson and Mellanby (1974) and globulin content was calculated as the difference between TP and albumin. The biochemical analyses of plasma were performed at the MoSAR unit of AgroParisTech and the Alfort Veterinary School (ENVA). In-house control plasma was systematically used as a quality control.

Rumen fluid collection: All the analyses concerning rumen fluid were performed during the two experimental phases. Approximately 50 mL of rumen digest was collected using a flexible polyvinyl stomach-tube introduced *via* the oesophagus and a vacuum pump. The rumen sample was subsequently pressed and squeezed through nylon mesh tissue into an Erlenmeyer flask for pH and ammonia nitrogen (NH_3) determinations. Rumen pH was immediately measured after collection using a portable digital pH meter²⁰. The NH_3 concentration of rumen fluid was estimated by steam distillation as per gas micro-diffusion method (Broudiscou & Papon, 1994).

Estimation of Volatile Fatty Acids: Samples for determination of volatile fatty acids (VFA) were stabilized with a 25% H_3PO_4 solution (2 mL 25% H_3PO_4 /8 mL rumen fluid) and stored at -20°C until analysis. After thawing, the rumen fluid was centrifuged at 15000xg for 20 min at

¹² Venosafe, Terumo, Belgium – 135mg USP U lithium heparin.

¹³ Sigma 3K12 – 37507, Osterode am Harz, Germany.

¹⁴ Gluco-quant, glucose/HK, Roche.

¹⁵ NEFA-HR®, Wako Chemical GmbH, Neuss, Germany.

¹⁶ Beta-hydroxybutyrate, Roche.

¹⁷ Urea/BUN, Roche.

¹⁸ Haptoglobin kit, Phase™ Range, Tridelta Development Ltd. Maynooth, Co. Kildare

¹⁹ Cobas Mira-Analyser, Roche®, Mannheim, Germany.

²⁰ pH meter PHM220, Radiometer Analytical S.A., HACH LANGE, France.

4°C, and the VFA were analyzed with a gas chromatograph²¹ using a capillary column²² of phase (30 meter length x 0.32 micrometer diameter ID) (Hušek, 1998).

Total Protozoal count: The remaining rumen fluid was also preserved for counting of protozoa by combining 1:1 (1.500 µL of filtrate with 1.500 µL of methylgreen formalin-saline solution (MFS - 100 mL 35% formaldehyde, 900 mL distilled water, 0.6 g Methylgreen and 8g NaCl). Total Protozoa count was calculated as described previously (Ogimoto & Imai, 1981), with the aid of the Jessen counting chamber²³.

The enumeration of total protozoa count per mL effluent was calculated as shown below:

Dilution ratio: 1:16

Volume of Jessen chamber: $5 \times 1\text{mm}^2$ (area) \times 0.4 mm (depth) = 2.0 µL (mm³)

Total number of fields counted: 05

2.0 µL of diluted effluent contained: A protozoa

Conversion factor: 1000 to convert from µL to mL

Nº. of protozoa in 0.25 µL of effluent fluid = $A \times 5 \times 16 \times 1000 / 2 = x \times 10^5/\text{mL}$ of rumen fluid

Statistical Analysis: Statistical analysis was performed using a linear mixed model with the MIXED procedure of SAS²⁴. The parameters measured in this study were repeated measures over time on individual goats. This procedure was used to determine the effect of the saponin-based additive, time and the interaction between time and saponin treatment on plasma and rumen fluid measurements. The *p* values were adjusted for multiple comparisons between the treatment groups using *F test*. The significance level for all statistical tests was set at $\alpha = 0.05$, where *p-Values* < 0.05 were used to define statistical significance. All data are presented as Least square (LS) means were subsequently compared with the Contrast statement in the MIXED procedure. (Littell et al., 1998). For the statistical analysis of the second experiment, data related to ruminal fluid of one of the animals was removed because of difficulties obtaining esophageal samples.

²¹ Model CP 3800, Varian Chrompack, 2700 Mitchell Drive, Walnut Creek, CA-USA.

²² Restex Rtx®-502.2, 110 Benner Circle, Bellefonte, PA – U.S.

²³ Jessen, Marienfeld® GmbH and Co. KG, 97922, Lauda-Königshofen, Germany.

²⁴ SAS Version 9.1, SAS Institute, Cary, NC, USA.

RESULTS AND DISCUSSION

Environment

Temperature (T°C) and Hygrometry (HR %)

Experiment 1: The mean values and standard error of weekly temperature ranged from 15.9 ± 0.93 °C to 26.8 ± 1.84 °C. Temperature peaked in the last week of the experiment. Mean values and standard error of weekly hygrometry ranged from $58.4 \pm 6.95\%$ to $71.8 \pm 2.12\%$.

Experiment 2: Mean values and standard error of weekly temperature and hygrometry ranged from 11.1 ± 0.77 °C to 16.7 ± 1.44 °C and from $77.3 \pm 6.11\%$ to $89.4 \pm 6.02\%$ respectively.

Animal performance

Milk yield and body weight

Experiment 1: There was no effect of the saponin-based additive given during mid-lactation on BW and MY ($p > 0.05$, table 2).

Other studies have shown contradictory results concerning the effect of saponin-based additives on BW. Hu et al. (2006) reported that goats fed with a diet containing 3 g of tea saponin (TS) had higher daily gain and better feed conversion ratio than those on 0 and 6 g TS/day. Nasri et al. (2011) showed that the administration of 30, 60 or 90 mg of *Quillaja saponaria* per kg DMI had a ruminal defaunation effect but failed to improve feed digestibility, growth performance and meat quality in lambs. Indeed, from the information available on microbes and fermentation parameters, this variation in DMI could be attributed to increased efficiency of microbial protein synthesis and decreased degradability of feed protein because protozoa numbers were reduced (Wang et al., 2012).

Table 2 also shows that the effect of time was significant ($p < 0.0001$), mainly because of a decline in BW and MY in the last weeks of the experiment. This decrease was perhaps associated with the observed rise in temperature during the fifth week of the experiment.

Milk Yield steadily declines at the end of lactation when compared to the early- and mid-stages of lactation. In general, MY decreases at a rate of 18.4% in mid-lactation, compared with early lactation (El-Tarabany et al., 2016).

It has been reported that intake of tannins and saponin increased MY in sheep (Wang et al., 1996) and dairy cows (Turner et al., 2005; Anantasook et al., 2014). However, other studies showed that MY and milk composition were unchanged when ruminants were supplemented with plants containing tannin and saponin (Wilson et al., 1998; Benchaar et al., 2008; Holtshausen et al., 2009). This indicates that the concentration of the supplement and the saponin source could influence animal responses (Anantasook et al., 2014).

Experiment 2: In the second experiment, the animals were clinically healthy during the experimental period; no problems were detected during pregnancy and after parturition. There was a prolificacy of 2.04 ± 0.62 kid/goat. There were 24 saponin kids (17 females and 7 males) and 25 control kids (15 females and 10 males). Almost all the 12 dams in the saponin group carried twins but there were also two singletons and one triplet bearing goats, while in the control group there were four triplets, three twins and five singleton bearing females.

Body weight: There was no effect of saponin on BW ($p > 0.05$), however, there was a significant effect of time around parturition ($p < 0.0001$) (Table 6).

Some authors have reported that restricted feeding during pregnancy modifies fetal organ growth and overall foetal development (Koritnik et al., 1981; Greenwood et al., 1999). The differences observed in BW during the *pre-partum* period indicate that both groups were forced to mobilise body reserves because requirements were not covered. Sahlu et al. (1995) have produced similar results. They showed that dairy goats gained very little BW between 90 days of pregnancy and parturition. The metabolic environment at the end of gestation may result in the redirection of nutrients towards the conceptus and therefore an increase in BW at the end of pregnancy may be physiologically difficult (Laporte-Broux et al., 2011).

Blood biochemical metabolites

Experiment 1: Data summarizing the effect of a saponin-based additive during mid-lactation on goat blood biochemical metabolites (glucose, NEFA, β HB and urea) are presented in table 3.

No treatment effect was observed for these variables ($p > 0.005$). However, a significant time effect was observed for all variables ($p < 0.0001$), except for β HB. Glucose reached its lowest concentration in both groups in the last week of the experiment, differing

significantly from the previous weeks ($p < 0.0001$). NEFA was also affected by time ($p < 0.0001$) but with a peak in both groups at the end of the experiment.

The significant decrease in glucose observed in the last week of the first experiment was similarly reported by El-Tarabany et al (2016), suggesting an impact of the stage of lactation on biochemical parameters in dairy goats. On the contrary, other researchers stated that blood glucose levels were significantly higher in mid- and late-lactation compared to early-lactation (Slanina et al., 1992). Moreover, others have reported in goats no significant variation in blood glucose at different stages of lactation (Casamassima et al., 2007). Therefore, the time effect observed in our first experiment may be due to the higher temperatures observed in the last week of the experiment.

NEFA and β HB dynamics in the first experiment were within the normal range for small ruminants according to Bani Ismail et al. (2008), Rios et al. (2006) and Celi et al. (2008) (from 0.2 to 0.4 mmol/L for NEFA and ≤ 0.86 mmol/L for β HB).

Experiment 2: The effects of a saponin-based additive, sampling time and their interaction on blood energy and lipid metabolites in Saanen and Alpine goats during late pregnancy and early lactation are presented in table 7 (glucose, NEFA, β HB, HDL, TC and TB).

Glucose: There was no treatment effect ($p > 0.05$). Moreover, the plasma glucose concentrations of both groups were within the normal range for goats during our experiments (Kaneko et al., 2008) (2.78 to 4.16 mmol/L). However, a large time effect was observed ($p < 0.0001$) with an increase after parturition.

The increase in glucose concentrations from P-4 to P-1 and their decrease in P+2 were also reported by Sadjadian et al. (2013) and Manat et al. (2016). An increase in glucose concentrations on the day of parturition may be due to metabolic changes and hormonal changes at parturition, that promote gluconeogenesis and glycogenolysis (Herd, 2000).

According to Herd (2000), decreasing glucose concentrations during the first two weeks of lactation appears to be related to the high energy demands in high producing breeds of goats. A significant increase in glucocorticoid causes liver glycogenolysis and mobilization of amino acids for gluconeogenesis. In addition, the decrease in blood glucose as lactation advances may be attributed to the synthesis of lactose with increased milk production.

Another study has reported higher glucose concentrations in early-lactation in dairy goats which may be considered as compensatory mechanism to meet the high energy demand

for milk synthesis (Abdelrahman et al., 2002). Indeed, reduced energy supply during early-lactation leads to a greater risk of metabolic disorders (Bremmer et al., 2000).

Non-Esterified Fatty Acids (NEFA): No difference in plasma NEFA was observed between the two groups ($p>0.05$). However, there were significant effects of the time during the weeks after the parturition in both groups ($p<0.0001$). NEFA concentrations gradually increased from P-3 and reached peak levels in the two weeks after parturition (P+1 and P+2).

In this study, NEFA concentrations for both groups which presented no clinical problems ranged from 0.18 to 0.26 mmol/L in *pre-partum* and between 0.63-0.79 mmol/L *post-partum*. There are no published data of cut-off levels or reference range for NEFA in dairy goats; Kaneko et al. (2008) displayed a very wide range for cows (0.4-3.78 mmol/L), but none for sheep and goats. These findings are in agreement with other studies on variation in energy biochemical metabolites in sheep (Contreras et al., 2000) and peri-parturient Saanen goats (Rios et al., 2006; Laporte-Broux et al., 2011; Radin et al., 2015; Sadjadian et al., 2013) who found NEFA concentrations below <0.8 mmol/L in healthy animals.

The NEFA profile indicates an increase in lipid mobilization from adipose tissue or a reduction of its rate of utilization by other tissues. However, the former is probably the main effect due to the energy demands imposed by milk production, which are not covered by nutrient intake. Moreover, a reduction in DMI leads to a difference between the energy intake and expenditure which is very large and associated with different physiological functions resulting in a NEB (Sundrum, 2015).

β -Hydroxybutyric Acid (β HB): There was no effect of treatment ($P>0.05$). Plasma β HB concentrations of both groups increased gradually with the time during the experimental period. A time effect was observed at P+1 and P+2 ($P<0.0001$).

The mean β HB concentrations in both groups of pregnant goats were within the normal range for dairy goats (below 0.86 mmol/L) (Bani Ismail et al., 2008). This result confirms the absence of ketosis in the present experiment since β HB is one of the most important energy status indicators during the peri-parturient period (Bani Ismail et al., 2008; Kaneko et al., 2008). Normally, β HB concentrations increase from 15 days before parturition until 21 days post-parturition and they decrease thereafter (Sadjadian et al., 2013).

Harmeyer and Schlumbohm (2006) have previously shown that lipid mobilization causes an increase in β HB and that liver production of ketone bodies is greater than that of the rumen epithelium in restricted feeding. However, Laporte-Broux et al. (2011) found that β HB

concentrations were between 0.4 and 1.2 mmol/L in a group of healthy goats during the peri-parturient period.

Therefore, in this current study, β HB results were linked to normal physiological changes during the peri-parturient period, and the goats did not display any clinical and biochemical signs of ketosis.

Total Cholesterol (TC) and High Density Lipoproteins (HDL): No treatment effect was observed for TC and HDL concentrations ($p>0.05$). The TC and HDL levels of both groups decreased during the first three experimental weeks and then increased gradually until the last week of experiment ($p<0.0001$).

The mean values of TC in both groups during the peri-parturient period are within the normal range for goats, 2.07 to 3.37 mmol/L (Kaneko et al., 2008). Moreover, the increase in TC during the last weeks of pregnancy in sheep and goats appear to be related to the energy demand of the foetal-placental unit (Seifi et al., 2007; Sadjadian et al., 2013). However, decreased TC concentrations during the last weeks of the pregnant goat experiment could be due to increased requirements for foetal growth and synthesis of steroid hormones (Pysera & Opalka, 2000).

Concentrations of HDL in both groups during the whole duration of the second experiment were above the normal range for non-pregnant goats (from 1.1 to 1.18 mmol/L), pregnant goats (1.14 ± 0.29 mmol/L) and lactating goats on the 10th day *post-partum* (0.47 ± 0.16 mmol/L) (Nazifi et al., 2002b; Skotnicka et al., 2011). Significant increases in HDL observed during early lactation in the present study are different from the results of Skotnicka et al. (2011), who showed a decrease in their concentrations on day 10 *post-partum*.

TC and HDL concentrations gradually increased in the present experiment during P+1 and P+2 and this was also shown by Nazifi et al., (2002b). Therefore, the increase in TC during the lactation period could be explained by lipid mobilization that is stimulated by glucagon or by increased synthesis of plasma lipoproteins (Cavestany et al., 2005; Sadjadian et al., 2013). Different dynamics were found by Manat et al. (2016) in that there was a decrease in TC and triglycerides from parturition to day 45 *postpartum* indicating that the goats utilized the lipids for fat milk production.

This dynamic for TC and HDL after parturition is justified during the lactation period of sheep and goats because lipogenesis and esterification are reduced, and free fatty acid mobilization is stimulated by an increase in nor-epinephrine and epinephrine secretion. The activity of lipoprotein lipase is increased in the mammary gland and decreased in adipose tissue.

In addition, increased lipolysis around parturition is regulated by hormones and it is not only an expression of an energy deficit (Nazifi et al., 2002a; Nazifi et al., 2002b; Manat et al., 2016).

Total Bilirubin (TB): There was no treatment effect on TB ($p>0.05$). Plasma TB concentrations were higher in the *post-partum* period than during pregnancy ($p<0.0001$).

TB concentrations in both groups around parturition were higher than normal range for goats (1.71 mmol/L) (Kaneko et al., 2008). Higher plasma bilirubin levels were also recorded in pregnant small ruminants with an increase around the parturition period and at the beginning of lactation. This increase could be due to the degradation of fetal hemoglobin or due to inadequate glucuronic acid synthesis (Balikci et al., 2007). Generally, in hepatic disorders due to a fatty hepatic infiltration, an increase in plasma hepatic enzyme activity and total bilirubin is often seen (González, 2000).

Protein profile

Experiment 2: The effects of a saponin-based additive on goat blood protein profiles during late pregnancy and early lactation are presented in table 8 (TP, albumin, globulin, Hp and urea).

Total Protein (TP), albumin and globulin: No effect of treatment was observed on TP, albumin and globulin ($p>0.05$). However, an effect of time was observed for all the variables after parturition ($p<0.0001$). The plasma concentrations of these variables in the *pre-partum* period were lower than in the *post-partum* period. Albumin and TP were at their highest levels in the first and second week after parturition (41.9 and 81.9 g/L respectively). Both variables were at their lowest in P-3 while globulin concentrations peaked at P+2 (41.5 g/L).

The dynamics of TP, albumin and globulin concentrations in the present study are similar to those published by Tagipour et al. (2010) and Sadjadian et al. (2013) during the *peri-partum* period in goats and sheep, respectively.

The decrease in these variables may be due to protein synthesis by the fetus which comes from amino acids derived from its dam. The growth of the fetus increases exponentially reaching a maximum level during late pregnancy (Jainudee & Hafez, 1994). The low values could also be explained by the subsequent transfer to the colostrum which is rich in immunoglobulin and other proteins (Swenson & Reece, 1996; Kaneko et al., 2008). Therefore, uptake of proteins by the mammary gland for colostrum and milk synthesis may be considered to be the main factor which influences the evolution over time of these parameters. TP

concentrations were higher than normal values in goats (64 to 70 g/L, Kaneko et al., 2008), but similar values were found in healthy Saanen goats during the *peri-partum* period by Sadjadian et al. (2013) and in multiparous goats by Cavestany et al. (2005). Consequently, this increase is not linked to dehydration in the animals.

No effect of the saponin additive on TP and albumin concentrations were observed in our study, while Hu et al. (2006) observed higher concentrations of TP and albumin in growing Boer goats receiving 3 g of tea saponins/day compared to controls.

Haptoglobin (Hp): There was no effect of saponin on Hp concentrations ($p>0.05$). Hp values were significantly different before and after parturition ($p<0.0001$). The highest values were observed after parturition in both groups.

Concentrations of Hp in both groups before parturition are within the normal range for small ruminants between 0.09 and 0.10 g/L (González et al., 2010; El-Deeb, 2012). The increase in Hp found in this study after parturition could be related to the changes in lipid metabolism that occur due to the energy demand imposed by milk production. Hp is synthesized by adipocytes and hepatocytes which confirms a relationship between Hp and lipids (González et al., 2010). Moreover, Hp is bound to high-density lipoproteins in ruminants (HDL) and NEFA (Kato & Nakagawa, 1999).

Acute phase proteins (APPs) have been proposed as sensitive and rapid indicators of inflammatory disturbances in ruminants. Previous studies have shown a relationship between some APPs and lipid mobilization which can be related to NEB, since cows with high milk Hp also had high plasma NEFA. Moreover, a significant correlation has been also reported between Hp and β HB in lactating goats (Trevisi et al., 2005; González et al., 2008; Hiss et al., 2009).

However, another study showed that Hp is not a good marker of subclinical pregnancy toxemia because other markers in urine (ketone bodies) showed earlier changes (Skinner et al., 1991). Another study also showed that significant increases in Hp appeared in fasted goats at the same time as the clinical pregnancy toxemia signs (González et al., 2011; Macedo et al., 2016).

Urea

Experiment 1: No treatment differences were observed ($p>0.05$). However, there was an effect of time with a decrease in urea concentrations until W4 followed by an increase back to the pre-

treatment value on the last week of the experiment (W6) ($p < 0.0001$). While an interaction between the treatment and time was observed in W5 (Table 03).

Experiment 2: A tendency was seen between the two groups of goats with a trend for a higher urea concentration in saponin treated goats (S, 4.3 mmol/L and C, 3.7 mmol/L, $p = 0.054$). In addition, a time effect was observed ($p < 0.0001$) with a decrease until P-3, followed by an increase back to pretreatment values on P+2 (Table 08).

The urea concentrations of both experiments are within the normal range for healthy goats (Kaneko et al., 2008) (3.57-7.14 mmol/L). The decrease in urea concentrations around the parturition could be associated with the decline in feed intake due to stress and hormonal changes during the kidding process. Thereafter, urea levels increase after parturition because of increasing feed intake consequently (Bauchar, 1993; Sadjadian et al., 2013; Manat et al., 2016). Similar dynamics have been observed by Laporte-Broux et al. (2011) in restricted fed goats which had low urea concentrations around parturition and by Manat et al. (2016) who observed an increase during lactation.

Hussain and Cheeke (1995) reported a trend for a reduction in urea in steers fed with a *Y. schidigera* extract. Similar reductions in urea were observed by Hu et al. (2006) evaluating the effect of tea saponins in growing Boer goat. The decrease in plasma urea could be due to more efficient utilization of protein and high urea concentrations reflect losses of N (Wilson et al., 1998).

However, another study with cows supplemented with a similar additive did not show differences in plasma urea. This finding could be associated with the poorly defined interaction between *Y. schidigera* and soluble dietary protein. This may be explained by differences in the size of the soluble protein pool in the rumen in relation to the amount of *Y. schidigera* fed to the cows. In addition, ruminal NH_3 concentrations did not decrease when *Y. schidigera* was fed with a low soluble protein diet (Wilson et al., 1998).

Many animal and nutritional factors may influence variability in plasma urea concentrations which include: BW, MY, DMI, dietary crude protein, degradable protein intake and dietary fermentable energy content/intake (Broderick & Clayton, 1997). Therefore, it has been suggested that degradation of protein in the rumen may lead to increased ruminal NH_3 concentrations and then plasma urea concentrations (Hammon et al., 2005).

The different results of treatment effect on urea concentrations in both experiments might be linked to the NH_3 concentrations. Hence, excess production of ammonia from the degradation of ingested protein by ruminal bacteria is absorbed into the blood, transported to

the liver, and converted to urea. Urea equilibrates rapidly throughout body fluids, including milk and urine and its concentration in these body fluids is positively correlated with the amount synthesized in the liver (Wilson et al., 1998). Hepatic synthesized urea can diffuse between the blood and mammary gland and be secreted in milk. It also can be extracted from blood by the kidney and excreted into the urine and it can be recycled to the rumen (Broderick & Clayton, 1997).

Hepatic enzymes: Table 9 summarizes the effects of a saponin-based additive, sampling time and their interaction on some goat enzymes during late pregnancy and early lactation (GGT, AST and ALP).

Gamma Glutamyl Transferase (GGT): No effect of treatment and time was observed regarding plasma GGT concentrations ($p>0.05$) (Table 9).

The plasma GGT concentrations were within the normal range for healthy goats according to Kaneko et al. (2008) (20-56U/L). There was a high individual variability in GGT responses (figure 3).

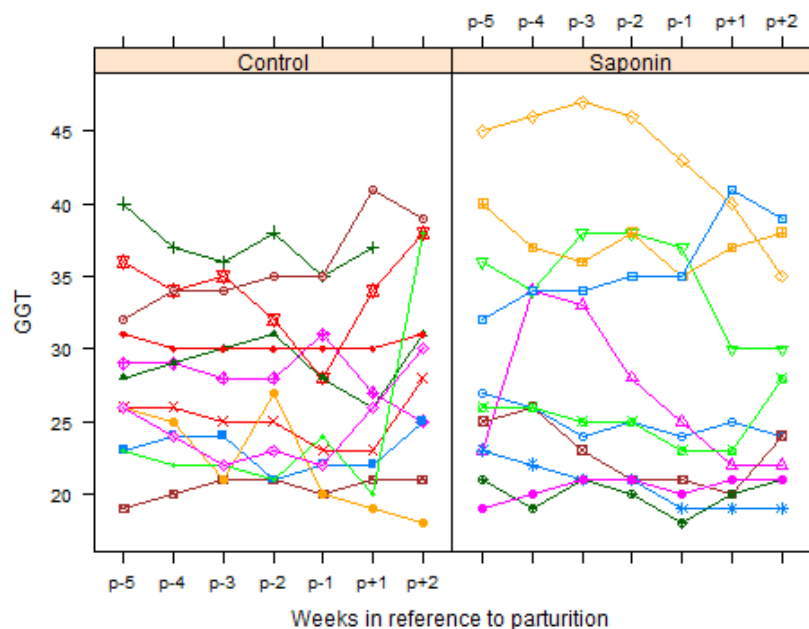


Figure 3. Individual variations in plasma gamma glutamyl transferase (GGT) concentrations in saponin supplemented and control goats during the peri-parturient period (parturition = p0).

Aspartate Amino Transferase (AST): No effect of treatment was observed on AST activity ($p>0.05$). A previous study reported that the concentrations of glucose and the activities of ALT

and AST were not affected by the addition of saponin, suggesting that this additive has no adverse effect on hepatic metabolism (Hu et al., 2006).

However, in comparison with the *pre-partum* period, the *post-partum* showed higher AST activities ($p < 0.0001$) (Table 9). The activity of AST increased from P-1 to P+2 in both groups (from 84.6 to 126.9 U/L). The effect of time observed before and after parturition could be used as an indicator of liver function in peri-parturient animals.

Numerous studies have shown higher AST activities during *post-partum* in ruminants (Seifi et al., 2007; Taghipour et al., 2011b; Sadjadian et al., 2013). DMI and milk production may be decreased with high levels of AST activity (Sadjadian et al., 2013).

In cattle, the sensitivity of AST is reported to be 94% to diagnose hepatic lipidosis (Kaneko et al. 2008). AST activity increases in cases of pregnancy toxemia in sheep and goats (Balikci et al. 2009; Barakat et al. 2007).

There were some animals from the saponin group which reacted excessively around P-4 and P+1 (Figure 4).

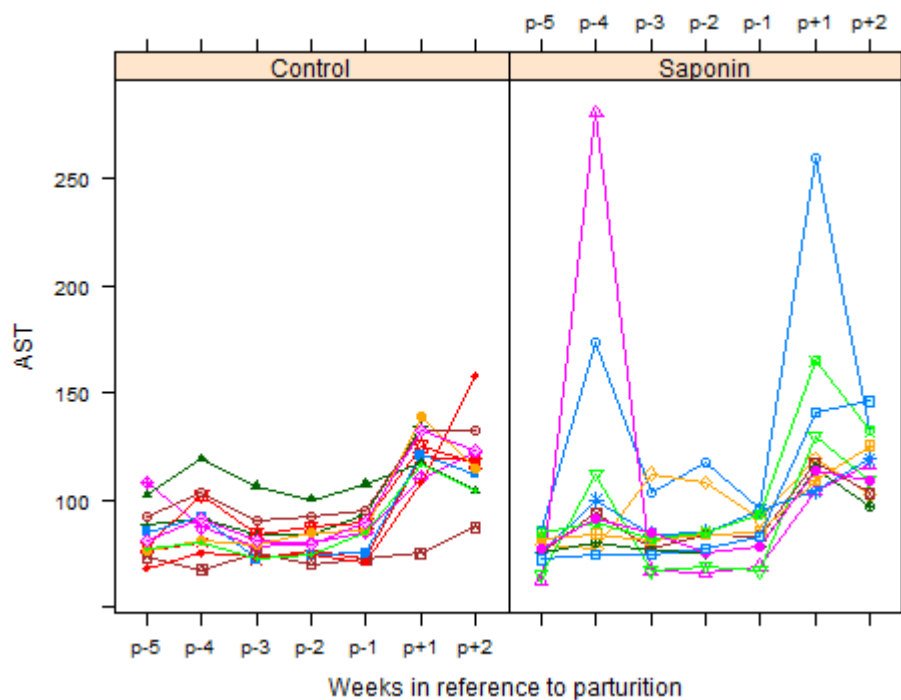


Figure 4. Individual variations in plasma aspartate amino transferase (AST) concentrations in saponin supplemented and control goats during the peri-parturient period (parturition = p0).

Alkaline Phosphatase (ALP): There was a large discrepancy between the groups which makes any interpretation difficult. The individual goat responses (Figure 5) highlight the large difference between the groups, even before the start of the experiment. It is due to a high

individual variability in the control group with three goats which had very high values compared to other animals.

An evolution over time was observed by Tharwat et al. (2013) with an increase pre-partum. This elevated activity *pre-partum* may be explained by an increased placental production of this enzyme in ruminants (Peter et al., 1987). ALP activity was in the normal range for goats (93 and 387 U/L, Kaneko et al., 2008). Higher concentrations of ALP were observed when tea saponin was given at a level of 3g/animal/d in Boer goats (Hu et al., 2006).

Mundim et al. (2007) showed higher ALP activities in goats in their first lactation compared to later lactations. This result is confirmed by Sarma and Ray (1985) showing that age and number of parturitions exert a certain influence on ALP activity. However, the number of parturitions was similar in the goats of the present experiment and cannot explain the high ALP concentration observed in three goats from the control group. Nevertheless, in most species the most common cause of increased ALP is cholestasis (Kaneko et al., 2008).

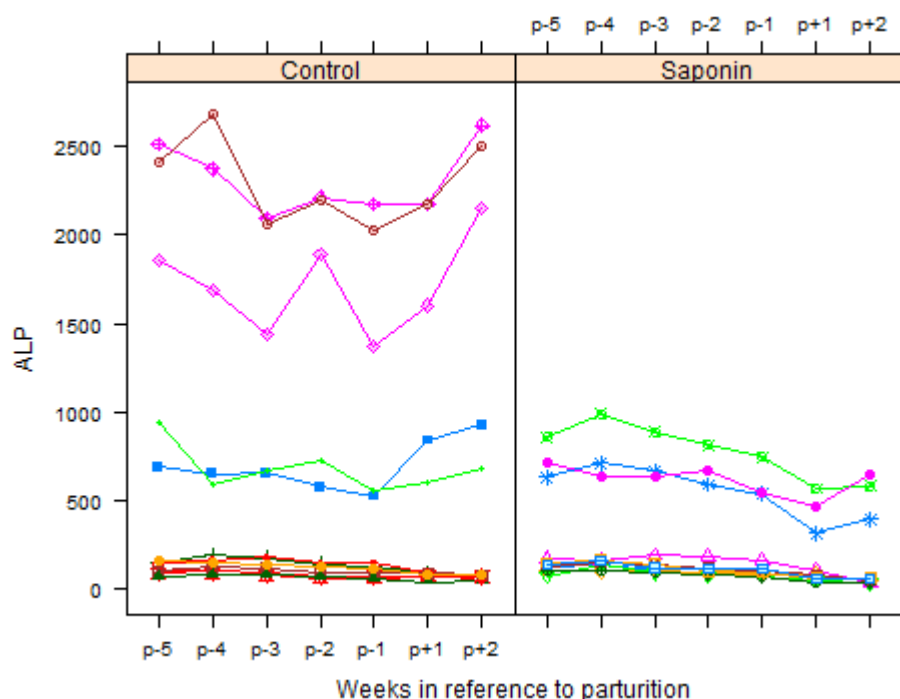


Figure 5. Individual variations in plasma alkaline phosphatase (ALP) concentrations in saponin supplemented and control goats during the peri-parturient period (parturition = p0).

Mineral profile

Total calcium (Ca), Phosphorus (PO_4^{3-}) and Magnesium (Mg^{3+}): There was no saponin effect on Ca, Mg^{3+} and PO_4^{3-} concentrations ($p > 0.05$). However, a time effect was observed after parturition for Ca and PO_4^{3-} with a reduction in these ions ($p = 0.0005$, Table 10).

Calcium, PO_4^{3-} and Mg^{3+} concentrations around the periparturition period were within the normal reference range for goats (Kaneko et al., 2008). Although we found no effect of saponin on these parameters an experiment with tea saponin in goats reported higher concentrations of serum Ca and PO_4^{3-} (Wang et al., 2012).

The Ca dynamics in the present study were in agreement with the results of Azab et al. (1999) and Iriadam (2007). They showed that total calcium concentrations decreased in late gestation, the lowest value was at parturition and the values remained low for the first three weeks of lactation. However, other studies showed a different dynamic for Ca (Goff, 2000; Cavestany et al., 2005). Ca levels rose generally 10 days prior to kidding, and this rise was associated with an increase in albumin. The two parameters were strongly correlated. This could be due to a fraction of the total calcium pool being linked to albumin and thus Ca partially depends on albumin concentrations. Initiation of lactation and the uptake of blood minerals into milk could be the cause of the observed decrease (Seifi et al., 2007).

The observed decrease in PO_4^{3-} during pregnancy could be explained by the rapid increase in fetal growth in the last third of pregnancy (Payne, 1983). Ca and PO_4^{3-} are also vital for fetal skeleton growth and are required in relatively constant proportions.

The dynamics of Ca and Mg^{3+} around parturition are due to several factors: a decrease in DMI when close to parturition, an increased requirement for these ions at parturition and in early-lactation and the level of Ca and Mg^{3+} in the diet (Goff & Horst, 1997; Degaris & Lean, 2009; Santos, 2011).

Ruminal fermentation parameters

Experiment 1: The effects of a saponin-based additive, sampling time and their interactions on different ruminal parameters (pH, NH_3 , total protozoa count and VFA) in goats during mid-lactation are presented in table 4 and 5.

Most of the ruminal parameters were not affected by adding saponin to the diet ($p > 0.05$). All the ruminal parameters were affected by time ($p < 0.05$) with the exception of caproic acid. This time effect was mainly observed when comparing the last week of the experiment with other weeks.

Experiment 2: Tables 11 and 12 present the effects of a saponin-based additive on goat ruminal parameters (pH, NH_3 , total protozoa count and VFA) during late pregnancy and early lactation.

Ruminal pH

Experiment 1: Ruminal pH concentrations were below 7.0 and remained almost constant in both groups during the experiment. Time effects were observed during the third and last weeks ($p=0.0098$).

The observed fluctuation in ruminal pH during the last weeks in the 1st experiment could be due to decreased feed consumption induced by high temperatures abovementioned. The physiological pH of the rumen fluid ranges from 5.5 to 7.4 (Dirksen, 1993). This was within the optimum pH for fibre digestion (Odenyo et al., 1997). Values of pH vary according to the quantity and different components in the diet (higher pH values, near neutral, with fibre-rich diets compared to lower pH values for starch-rich diets (Dirksen, 1993). The stability of ruminal pH could also be attributed to the stability in VFA concentrations in this current experiment which was also observed by Lima et al. (2016).

Experiment 2: Ruminal pH concentrations ranged from 6.6 to 7.1 and remained almost constant in both groups during the experiment. There was no treatment effect ($p>0.05$). Time effects were observed with a decrease in both groups in the first week after parturition ($p<0.0001$).

The observed fluctuation in ruminal pH during last weeks could be due to an increase in feed consumption. Effects of saponin supplementation on ruminal pH have been reported previously in different ruminant species with significant increase or decrease (Jouany & Morgavi, 2007; Patra & Saxena, 2009). Even though our experiments did not display a treatment effect, Eryavuz & Dehority (2004) have shown an effect at different concentrations of *Y. schidigera* extract in sheep. There was no effect on ruminal pH at 20g/animal/day but a significant increase at 30g/animal/day. Santoso et al. (2007) also reported an increased pH value in goats fed with saponins which could reflect a decrease in total VFA concentrations that could also be due to lower protozoal activity.

Ruminal NH₃

Experiment 1: Ruminal NH₃ concentrations increased during the last week of the experiment, compared to the previous weeks ($p<0.0001$). Saponin had no effect on NH₃ ($p>0.05$).

Experiment 2: Ruminal NH_3 concentrations fluctuated with time in the weeks before parturition ($p < 0.0001$), but they were not affected by saponin ($p > 0.05$).

The variations in NH_3 have been reported in *in vivo* and *in vitro* studies with different types and doses of saponins and in different ruminant species (Ivan et al., 2004; Jouany & Morgavi, 2007; Patra & Saxena, 2009) although no saponin effect was noted in these current experiments.

Ruminal NH_3 is a balance between degradation of feed protein and uptake of NH_3 for synthesis of microbial protein. In general, reductions in NH_3 concentrations associated with saponin have been observed in ruminants *in vivo* and *in vitro* (Wallace et al., 1994; Hussain and Cheeke, 1995), but *in vivo*, responses have been more variable and are apparently related to the levels used and time of sampling (Hristov et al., 1999).

Several studies have shown that ruminal NH_3 concentrations were reduced with saponin supplementation (Abreu et al., 2004; Ivan et al., 2004). This reduction might be due to a decrease in the digestibility of the diet, or a decrease in insoluble protein or to the less predatory activity of the protozoa on the rest of the microbial biomass or a combination of these factors (Diaz et al., 1993, Ivan et al., 2004).

A number of studies with sheep fed silage-based diets (Santoso et al., 2004) and *in vitro* ruminal culture fermentations (Wallace et al., 1994; Wang et al., 2000) noted that saponin of *Y. schidigera* reduced ruminal NH_3 . Ruminal NH_3 is reduced when protozoal growth is inhibited (Williams & Coleman, 1991), presumably as a result of depressed rumen degradation of feed protein or reduced turnover of bacterial protein. NH_3 concentrations may also be altered by NH_3 binding to compounds like saponin, as noted by Makkar et al. (1998) and Cheeke (1999). Another possible explanation for a reduction in ruminal NH_3 in saponin-supplemented goats may have been due to increased incorporation of ammonia, peptide or amino acids into microbial proteins (Santoso et al., 2007).

Similar results on sheep have been related by Diaz et al. (1993) who noted no significant effect of different levels of *Sapindus saponaria* on ruminal pH, NH_3 and VFA molar proportions.

Total protozoa count

Experiment 1: There was no effect of treatment on protozoa ($p > 0.05$). However, total protozoa counts were affected by time. The effect of time was mainly due to the value at week 4, when both groups had very low values ($10.4 \times 10^5/\text{mL}$, $p = 0.0106$).

Experiment 2: There was a trend for a treatment effect on protozoa ($p=0.0960$). Protozoa numbers tended to be higher in the S compared to the C group. There was no time effect ($p>0.05$).

In most previous studies, saponins have been found to have a detrimental effect on ruminal protozoa (Jouany & Morgavi, 2007; Patra & Saxena, 2009). In the present experiment, total protozoal count tended to be higher in the S compared to C group. Therefore, the effects of saponins on rumen protozoal population are variable and probably depend on numerous factors like the level of supplementation and the associated diet (Navas-Camacho et al., 1993; Abreu et al., 2004; Santoso et al., 2007).

Odenyo et al. (1997) tested the effect of *Sesbania sesban* containing saponins on the rumen of sheep. The numbers of ciliate protozoa remained higher in sheep that were allowed to eat the saponin supplement than in the sheep which received it through a cannula. They suggested that physiological activities associated with eating, including saliva secretion, could play a role in neutralizing the protozoal inhibiting substance(s) found in *S. sesban*. In addition, Newbold et al. (1997) suggested that adding the saponins to the diet could cause an increase in certain bacteria, whose activity could possibly alter the toxic properties of the saponins.

Similar results to our results were also found by Eryavuz and Dehority (2004). There was no effect on protozoal numbers in sheep fed *Y. schidigera* extracts when the level used was lower than 30g/animal/d. Navas-Camacho et al. (1993) observed an increase in rumen protozoal count by feeding *Enterelobium cyclocarpum* at a level of 100g/animal/day.

Thus, unchanged or increased rumen protozoal numbers in studies with *Y. schidigera* supplementation may possibly be attributed to: a) bacterial deactivation of saponins in *Y. schidigera* to nontoxic substances, b) physiological effects, such as an alteration in the rate of feed intake as seen in the periparturition period, c) an effect on ruminal turnover rate or increased salivary production; d) a difference in the biological effects of saponins from different sources; e) the presence of other secondary metabolites in saponin-rich plants f) the composition of the diet or g) feeding levels (Eryavuz & Dehority, 2004).

Newbold et al. (1997) found strong antiprotozoal properties associated with the saponin component of an African multipurpose tree (*S. sesban*) fed to sheep. After 10 days of saponin administration, the protozoal population in the rumen was back to initial value levels. The authors concluded that was not the protozoa that became resistant to the antiprotozoal compound and that another microbial population, probably the bacteria, adapted to become capable of degrading the antiprotozoal component. Then, it justifies that this adaptation could

be overcome by feeding the plant by intervals. However, Hristov et al. (1999) reported that this adaptation did not occur in their study.

Volatile Fatty Acids (VFA)

Experiment 1: Table 5 shows the effect of treatment, sampling time and their interaction on concentrations of total and individual VFA. Both S and C groups had similar mean values in total VFA and the proportions of individual fatty acids such as acetate, propionate, and butyrate ($p>0.05$). However, there was a strong trend for an effect of saponin on the ratio acetate to propionate ($S < C$, $p=0.0570$). While a significant interaction between the treatment and time was identified ($p=0.0213$). In addition, saponin had no effect on *iso*-butyrate, valerate and *iso*-valerate ($p>0.05$).

Experiment 2: Concentrations of total and individual VFA are presented in Table 12. Total VFA concentration varied between 71.3 and 97.2 mmol/L and were unaffected by treatment ($p>0.05$). There was an effect of time where levels rose up to parturition (P-4 and P+1, $p<0.0001$).

There was no effect of treatment on the main individual fatty acids (acetate, propionate and butyrate) (Table 12). However, these variables progressively increased during pregnancy until P-1, with a peak in concentrations in the first week after parturition (P+1) and a decrease in the next week (P+2) ($p<0.0001$). There was no saponin effect on *iso*-butyrate, valerate, *iso*-valerate and on the ratio of acetate/propionate ($p>0.05$).

As for the other ruminal parameters, differential effects of saponins or saponin-containing plants on *in vitro* and *in vivo* ruminal VFA have been reported by several studies (Jouany & Morgavi, 2007; Patra & Saxena, 2009). Other researchers have found similar results to our results such as, a non-significant decrease in total VFA and the unchanged proportion of individual fatty acids were observed by other research groups (Klita et al., 2006; Hristov et al., 1999; Santoso et al., 2004). Santoso et al. (2007) showed a linear decrease in total VFA concentrations with increasing saponin intake in goats due to decreased microbial VFA production by protozoa. In addition, Lu and Jorgensen (1987) noted a reduction in VFA concentrations when saponin was administered at 40g/kg of DM intake. Therefore, giving saponin could alter the pattern of ruminal fermentation (Hristov et al., 1999).

The short chain fatty acids produced by microbial fermentation of carbohydrates in the rumen represent the main source of metabolizable energy for ruminants and they are of utmost

importance in nutritional physiology. The total concentration of VFA is on average from 60 to 120 mmol/L of rumen fluid with acetic acid (50 to 65 mmol/%), propionic acid (20 to 25mmol/%) and butyric acid (10 to 20 mmol/%), formic, valeric, caproic acids and other fatty acids add up to 5 mmol/% (Dirksen, 1993; Benchaar et al., 2008). Wang et al. (2012) stated that the effects of different additives on VFA of rumen fluid were unpredictable, because variations in VFA, the proportion of individual fatty acids and the ratio of acetate to propionate in ruminants were not consistent.

Ruminal fermentation and products are dependent on diet, and the acetate to propionate ratio is also dependent the type of feed, being generally lower for cereal grains than for forages. The relationship between the acetate to propionate ratio and the diet has been explained by the metabolic characteristics of fiber-digesting and starch-digesting bacteria, but this explanation is not entirely convincing (Russell, 1998).

In summary, no clear effects of saponin supplementation on metabolic or ruminal parameters were observed, either in mid-lactation (1st experiment) or around parturition (2nd experiment). There was a trend for an increase in protozoa numbers in the 2nd experiment. This is contrary to the generally accepted effect that saponins decrease protozoa numbers (Santoso et al., 2007). However, the saponin levels used in previous studies were often high and some authors have also shown that low levels of saponins increase protozoa numbers (Navas-Camacho et al., 1993; Abreu et al., 2004). The effect of the saponin supplement in the present experiment on protozoa numbers might explain why there was an increase in ruminal NH₃ and in turn plasma urea concentrations. It is known that protozoa prey on bacteria in the rumen and that if bacteria are used as a food source by protozoa then NH₃ increases due to the catabolism of bacterial protein. The NH₃ so released is not recycled by the bacteria in the rumen because there are less of them due to predation by protozoa. Higher levels of NH₃ will be absorbed from the rumen and as a result be converted to urea by the liver, therefore increasing plasma urea concentrations. However, this trend was already detected in the first week of the experiment and therefore before the beginning of saponin supplementation. Although the two groups were balanced for production parameters, there were obviously not balanced for metabolic parameters like urea concentration.

Another possible confounding factor in this experiment was that the forage:concentrate ratio increased as the 2nd experiment progressed because of the need to try to develop the volume of the rumen for the future lactation. Saliva production is known to increase when there is more forage in the diet. Saliva is thought to neutralise part of the effect of saponins in the rumen. Indeed, Hess et al. (2003) showed that saponin had no effect on

protozoa numbers when forage levels were increased in the diet. Therefore, the increase in the forage:concentrate ratio may have acted to neutralise the saponin effect on protozoa via high saliva secretion.

In the 1st experiment, there was no effect of treatment on metabolic or ruminal parameters, but there was an interaction between treatment and time for urea and rumen acetate/propionate ratio. However, these interactions were mainly due to a difference between treatments in W5, the week during which a large increase in ambient temperature was observed. Moreover, the higher acetate/propionate ratio in the saponin treated group compared to control was already observed in W1, before the beginning of the treatment. Therefore, no clear effect of saponin was shown in these two experiments.

CONCLUSION

In conclusion, the daily administration of a saponin additive at a level 25g/goat over four consecutive weeks in two different physiological statuses (mid-lactation and *peri-partum*) did not influence plasma metabolites, ruminal fermentation parameters and animal performance studied. However, there were some small effects of saponin on: blood urea, total protozoa numbers and the acetate to propionate ratio. The potential of a saponin-based additive to improve the efficiency of ruminal fermentation through increased microbial protein synthesis probably depends on the supplementation level, the period of administration and the type of basal diet.

It is worth pointing out that the response of animals to any treatment depends to a large extent on environmental conditions. Indeed, our results from the first experiment highlight the possible influence of a spike in temperature during the last week of the experiment. In the second experiment, a significant time effect was observed mainly because of physiological imbalance due to parturition.

Further studies are required to better understand how saponin can be administered: level and method of supplementation, in order to investigate its ability to prevent metabolic diseases in small ruminants which reduce animal performance and consequently cause economic losses during the peri-parturient period.

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Table 2 - Effect of treatment (Saponin, n = 10 and Control, n = 10), sampling time and their interaction on goat BW, MY during mid-lactation (LSmeans \pm standard error). Saponin was given in the diet during weeks 2 to 5 (25g/animal/day).

Measure	Group	Weeks						M	Variation factor		
		W1	W2	W3	W4	W5	W6		Treatment	time	Interaction (T x t)
BW (kg)	Saponin	67.5 \pm 7.09	68.0 \pm 8.00	66.6 \pm 7.75	66.9 \pm 7.90	66.0 \pm 7.62	63.4 \pm 8.46	66.0 ^A	0.7882	<0.0001	NS
	Control	66.6 \pm 7.48	67.1 \pm 7.63	65.4 \pm 7.54	65.9 \pm 7.50	65.6 \pm 7.54	62.5 \pm 7.15	65.5 ^A			
	M	66.9 ^{ab}	67.5 ^a	66.0 ^b	66.9 ^{ab}	65.8 ^b	63.4 ^c				
MY (kg/day)	Saponin	4.41 \pm 0.78	4.73 \pm 0.88	4.52 \pm 0.88	4.83 \pm 0.86	4.11 \pm 0.68	3.65 \pm 0.85	4.32 ^A	0.8478	<0.0001	NS
	Control	4.48 \pm 0.74	4.63 \pm 0.62	4.44 \pm 0.70	4.40 \pm 0.55	3.97 \pm 1.10	3.64 \pm 0.52	4.22 ^A			
	M	4.45 ^a	4.68 ^a	4.48 ^a	4.62 ^{ab}	4.04 ^b	3.65 ^c				

M: Mean; BW: Body weight; MY: Milk Yield; kg/w: kilogram/week **a,b**: different letters indicate a significant difference between values within the same line ($p < 0.05$). **A,B**: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant

Table 3 - Effect of treatment (Saponin, n = 10 and Control, n = 10), sampling time and their interaction on blood biochemical metabolites in goats during lactation period (LSmeans \pm standard error). Saponin was given in the diet during weeks 2 to 5 (25g/animal/day).

Metabolite	Group	Weeks						M	Variation factor		
		W1	W2	W3	W4	W5	W6		Treatment	time	Interaction (T x t)
Glucose (mmol/L)	Saponin	3.16 \pm 0.27	3.12 \pm 0.17	3.16 \pm 0.18	3.19 \pm 0.27	3.18 \pm 0.22	3.01 \pm 0.22	3.14 ^A	0.9753	<0.0001	NS
	Control	3.15 \pm 0.22	3.10 \pm 0.26	3.20 \pm 0.19	3.16 \pm 0.16	3.13 \pm 0.22	3.01 \pm 0.14	3.13 ^A			
	M	3.15 ^a	3.11 ^{ab}	3.18 ^a	3.18 ^a	3.15 ^a	3.01 ^b				
NEFA (mmol/L)	Saponin	0.25 \pm 0.07	0.24 \pm 0.06	0.21 \pm 0.10	0.23 \pm 0.08	0.19 \pm 0.05	0.54 \pm 0.12	0.27 ^A	0.5024	<0.0001	NS
	Control	0.25 \pm 0.07	0.22 \pm 0.07	0.21 \pm 0.09	0.22 \pm 0.09	0.19 \pm 0.05	0.46 \pm 0.08	0.26 ^A			
	M	0.25 ^a	0.23 ^a	0.21 ^a	0.22 ^a	0.19 ^a	0.50 ^b				
β HB (mmol/L)	Saponin	0.52 \pm 0.10	0.52 \pm 0.10	0.51 \pm 0.16	0.56 \pm 0.15	0.53 \pm 0.09	0.47 \pm 0.20	0.53 ^A	0.6758	0.7255	NS
	Control	0.51 \pm 0.10	0.55 \pm 0.08	0.52 \pm 0.11	0.54 \pm 0.11	0.51 \pm 0.10	0.55 \pm 0.08	0.52 ^A			
	M	0.51 ^a	0.53 ^a	0.52 ^a	0.55 ^a	0.52 ^a	0.6 ^a				
Urea (mmol/L)	Saponin	6.31 \pm 1.55	6.15 \pm 0.96	5.47 \pm 0.68	5.34 \pm 0.81	5.24 \pm 0.99	6.34 \pm 0.73	5.80 ^A	0.6610	0.0001	0.0362
	Control	5.59 \pm 1.32	5.86 \pm 0.97	5.96 \pm 1.40	5.33 \pm 1.13	6.04 \pm 1.24	7.02 \pm 1.44	5.97 ^A			
	M	5.95 ^a	6.00 ^a	5.71 ^{ab}	5.33 ^b	5.64 ^{ab}	6.68 ^c				

NEFA: Nos-esterified fatty acid; β HB: β -hydroxybutyrate; M: Mean; **a,b**: different letters indicate a significant difference between values within the same ($p < 0.05$). **A,B**: different letters indicate a trend for a difference between treatments ($p < 0.05$). NS: Non-significant.

Table 4 - Effect of treatment (Saponin, n = 10 and Control, n = 10), sampling time and their interaction on ruminal fermentation parameters (pH, Ruminal NH₃ and Protozoa) in goats during lactation period (LSmeans \pm standard error). Saponin was given in the diet during weeks 2 to 5 (25g/animal/day).

Variable	Group	Weeks						M	Variation factor		
		W1	W2	W3	W4	W5	W6		Treatment	time	Interaction (T x t)
Ruminal pH	Saponin	6.87 \pm 0.29	6.67 \pm 0.27	6.36 \pm 0.75	6.69 \pm 0.31	6.78 \pm 0.17	6.89 \pm 0.18	6.71 ^A	0.2627	0.0098	0.0637
	Control	6.77 \pm 0.13	6.70 \pm 0.19	6.80 \pm 0.26	6.70 \pm 0.18	6.82 \pm 0.14	6.91 \pm 0.08	6.78 ^A			
	M	6.82 ^a	6.68 ^{ab}	6.6 ^b	6.69 ^{ab}	6.80 ^{ac}	6.90 ^c				
Ruminal NH ₃ (mg/dL)	Saponin	55.3 \pm 14.68	59.9 \pm 23.04	61.5 \pm 14.38	69.6 \pm 34.34	58.4 \pm 30.10	88.2 \pm 27.66	66.8 ^A	0.5235	<0.0001	NS
	Control	61.2 \pm 34.23	76.1 \pm 43.98	65.8 \pm 16.19	69.0 \pm 32.98	56.9 \pm 9.57	95.7 \pm 21.41	70.8 ^A			
	M	58.3 ^a	59.9 ^a	63.7 ^a	69.6 ^a	57.65 ^a	88.2 ^b				
Protozoa (x10 ⁵ /mL)	Saponin	12.2 \pm 2.03	11.9 \pm 3.40	13.2 \pm 6.99	10.4 \pm 3.72	14.9 \pm 3.03	12.8 \pm 3.86	12.6 ^A	0.8228	0.0106	NS
	Control	12.9 \pm 3.16	13.1 \pm 2.92	14.6 \pm 4.65	9.2 \pm 2.67	11.6 \pm 1.98	12.9 \pm 3.59	12.4 ^A			
	M	12.5 ^a	11.9 ^a	13.9 ^a	10.4 ^b	13.3 ^a	12.8 ^a				

NH₃: Ammonia-N; M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant

Table 5 - Effect of treatment (Saponin, n = 10 and Control, n = 10), sampling time and their interaction on ruminal fermentation parameters (total volatile fatty acids) in goats during lactation period (LSmeans \pm standard error). Saponin was given in the diet during weeks 2 to 5 (25g/animal/day).

Variable	Group	Weeks						M	Variation factor		
		W1	W2	W3	W4	W5	W6		Treatment	time	Interaction (T x t)
Total VFA (mmol/L)	Saponin	94.5 \pm 20.45	87.1 \pm 18.66	78.3 \pm 24.98	89.6 \pm 21.65	97.4 \pm 16.28	71.3 \pm 17.53	85.2 ^A	0.8548	<0.0001	NS
	Control	101.8 \pm 13.38	87.6 \pm 17.24	82.4 \pm 17.96	84.9 \pm 15.65	84.8 \pm 14.78	70.0 \pm 12.74	85.3 ^A			
	M	98.2 ^a	87.1 ^{ab}	80.4 ^b	89.6 ^{ab}	91.1 ^a	71.3 ^c				
Acetate (mmol/L)	Saponin	62.8 \pm 13.57	56.5 \pm 12.12	51.5 \pm 16.59	59.5 \pm 16.99	63.7 \pm 12.91	46.7 \pm 12.24	55.9 ^A	0.9661	<0.0001	NS
	Control	68.9 \pm 9.27	57.6 \pm 11.36	54.3 \pm 12.50	56.1 \pm 10.48	55.2 \pm 10.22	47.5 \pm 10.05	56.6 ^A			
	M	65.9 ^a	56.5 ^{ab}	52.9 ^b	59.5 ^a	59.4 ^a	46.7 ^b				
Propionate (mmol/L)	Saponin	17.1 \pm 4.23	16.2 \pm 3.85	14.6 \pm 4.88	16.5 \pm 3.68	18.5 \pm 2.77	12.9 \pm 4.74	15.8 ^A	0.5483	<0.0001	NS
	Control	18.0 \pm 3.26	16.1 \pm 3.88	15.0 \pm 3.80	15.5 \pm 3.55	15.5 \pm 2.94	11.4 \pm 2.13	15.3 ^A			
	M	17.6 ^a	16.2 ^a	14.8 ^{ab}	16.5 ^a	17.0 ^a	12.9 ^b				
Butyrate (mmol/L)	Saponin	11.3 \pm 2.54	10.8 \pm 2.45	9.7 \pm 3.20	11.0 \pm 2.00	12.0 \pm 1.61	8.5 \pm 1.63	10.4 ^A	0.8030	<0.0001	NS
	Control	11.6 \pm 1.90	10.5 \pm 2.07	10.3 \pm 1.90	10.5 \pm 1.80	10.7 \pm 1.83	8.7 \pm 1.30	10.4 ^A			
	M	11.4 ^a	10.8 ^a	10.0 ^{ab}	11.0 ^a	11.4 ^a	8.5 ^b				
Valerate (mmol/L)	Saponin	0.78 \pm 0.22	0.80 \pm 0.24	0.63 \pm 0.25	0.67 \pm 0.29	0.74 \pm 0.29	0.60 \pm 0.14	0.70 ^A	0.6583	<0.0001	NS
	Control	0.77 \pm 0.15	0.74 \pm 0.18	0.68 \pm 0.15	0.71 \pm 0.14	0.72 \pm 0.12	0.43 \pm 0.24	0.68 ^A			
	M	0.78 ^a	0.78 ^a	0.66 ^{ab}	0.69 ^{ab}	0.73 ^a	0.52 ^b				
Caproic acid (mmol/L)	Saponin	1.16 \pm 0.05	1.12 \pm 0.05	1.14 \pm 0.07	1.04 \pm 0.37	1.07 \pm 0.38	1.10 \pm 0.07	1.10 ^A	0.8575	0.1469	0.0913
	Control	1.15 \pm 0.05	1.13 \pm 0.08	1.11 \pm 0.09	1.18 \pm 0.07	1.18 \pm 0.07	0.87 \pm 0.46	1.11 ^A			
	M	1.16 ^a	1.12 ^a	1.13 ^a	1.11 ^a	1.12 ^a	0.99 ^a				
Iso-valerate (mmol/L)	Saponin	0.52 \pm 0.19	0.79 \pm 0.11	0.24 \pm 0.11	0.42 \pm 0.28	0.74 \pm 0.31	0.52 \pm 0.21	0.54 ^A	0.9432	<0.0001	NS
	Control	0.67 \pm 0.19	0.76 \pm 0.15	0.28 \pm 0.09	0.33 \pm 0.33	0.81 \pm 0.14	0.40 \pm 0.23	0.54 ^A			
	M	0.59 ^{ab}	0.78 ^a	0.26 ^b	0.38 ^b	0.78 ^a	0.46 ^b				
Iso-butyrate (mmol/L)	Saponin	0.79 \pm 0.09	0.74 \pm 0.12	0.63 \pm 0.18	0.55 \pm 0.31	0.68 \pm 0.28	0.90 \pm 0.14	0.72 ^A	0.9794	0.0048	NS
	Control	0.76 \pm 0.16	0.76 \pm 0.13	0.69 \pm 0.13	0.59 \pm 0.23	0.76 \pm 0.10	0.74 \pm 0.42	0.72 ^A			
	M	0.77 ^{ab}	0.75 ^{ab}	0.66 ^{ab}	0.57 ^a	0.72 ^{ab}	0.82 ^b				
Acetate/Propionate	Saponin	3.71 \pm 0.27	3.50 \pm 0.13	3.56 \pm 0.25	3.58 \pm 0.30	3.43 \pm 0.26	3.66 \pm 0.33	3.57 ^A	0.0570	<0.0001	0.0213
	Control	3.85 \pm 0.22	3.62 \pm 0.26	3.67 \pm 0.34	3.65 \pm 0.27	3.57 \pm 0.25	4.17 \pm 0.45	3.75 ^A			
	M	3.78 ^a	3.56 ^b	3.62 ^b	3.62 ^b	3.50 ^b	3.92 ^c				

VFA: Volatile fatty acids; M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant

Table 6 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on BW of goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Measure	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interacti on (T x t)
Body Weight (kg)	Saponin	79.93 \pm 7.01	80.27 \pm 6.75	83.94 \pm 6.75	86.11 \pm 7.12	87.51 \pm 7.06		77.44 \pm 9.08	72.11 \pm 7.87	81.0 ^A			
	Control	80.82 \pm 10.02	81.20 \pm 10.06	84.55 \pm 10.60	86.52 \pm 10.67	87.75 \pm 10.57		76.66 \pm 9.25	72.94 \pm 8.21	81.5 ^A	0.8953	<0.0001	NS
	M	80.4 ^a	80.7 ^a	84.2 ^b	86.3 ^{bc}	87.6 ^c		77.0 ^d	72.5 ^e				

M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant

Table 7 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on blood energy and lipid metabolites during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Metabolite	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
Glucose (mmol/L)	Saponin	2.86±0.25	2.50±0.32	2.60±0.31	2.57±0.26	2.60±0.29	2.65±0.49	2.73±0.31	2.64 ^A	0.4336	<0.0001	NS	
	Control	3.01±0.35	2.52±0.35	2.69±0.29	2.66±0.39	2.65±0.31	2.82±0.26	2.79±0.28	2.73 ^A				
	M	2.93 ^a	2.51 ^c	2.65 ^{bc}	2.62 ^{bc}	2.63 ^{bc}	2.73 ^b	2.76 ^{ab}					
NEFA (mmol/L)	Saponin	0.18±0.06	0.26±0.15	0.20±0.12	0.20±0.12	0.23±0.13	0.66±0.17	0.83±0.33	0.40 ^A	0.9625	<0.0001	NS	
	Control	0.19±0.05	0.30±0.14	0.21±0.10	0.24±0.13	0.30±0.15	0.61±0.30	0.74±0.37	0.40 ^A				
	M	0.18 ^a	0.28 ^a	0.20 ^a	0.22 ^a	0.26 ^a	0.63 ^b	0.79 ^b					
βHB (mmol/L)	Saponin	0.26±0.05	0.25±0.04	0.34±0.06	0.32±0.05	0.34±0.07	0.61±0.18	0.69±0.22	0.40 ^A	0.1676	<0.0001	NS	
	Control	0.27±0.05	0.23±0.05	0.30±0.07	0.29±0.07	0.31±0.07	0.57±0.21	0.55±0.20	0.36 ^A				
	M	0.27 ^a	0.24 ^a	0.32 ^a	0.31 ^a	0.32 ^a	0.59 ^b	0.62 ^b					
HDL (mmol/L)	Saponin	1.53±0.23	1.46±0.19	1.38±0.19	1.40±0.20	1.39±0.20	1.56±0.25	1.61±0.35	1.48 ^A	0.7097	<0.0001	NS	
	Control	1.54±0.22	1.50±0.24	1.37±0.18	1.43±0.24	1.38±0.17	1.57±0.18	1.76±0.37	1.51 ^A				
	M	1.54 ^a	1.48 ^{ab}	1.37 ^b	1.42 ^{ab}	1.39 ^{ab}	1.57 ^{ac}	1.69 ^{ac}					
TC (mmol/L)	Saponin	3.55±0.62	3.46±0.63	3.04±0.54	3.17±0.58	3.15±0.54	3.25±0.57	3.41±0.58	3.29 ^A	0.6277	<0.0001	NS	
	Control	3.48±0.49	3.28±0.59	2.95±0.45	2.98±0.43	3.03±0.40	3.12±0.57	3.51±0.75	3.19 ^A				
	M	3.51 ^a	3.37 ^{abc}	2.99 ^b	3.07 ^b	3.09 ^b	3.19 ^b	3.46 ^{ac}					
TB (μmol/L)	Saponin	1.78±0.36	2.04±0.56	1.92±0.49	1.95±0.52	1.98±0.50	2.66±0.78	2.67±0.84	2.14 ^A	0.7671	<0.0001	NS	
	Control	1.82±0.36	2.03±0.37	1.95±0.27	1.98±0.43	2.01±0.38	2.58±0.88	2.31±0.75	2.10 ^A				
	M	1.80 ^a	2.04 ^a	1.93 ^a	1.96 ^a	2.00 ^a	2.62 ^b	2.49 ^b					

NEFA: Nos-esterified fatty acid; β HB: Beta-hydroxybutyrate; HDL: high-density lipoprotein; TC: Total cholesterol; TB: Total bilirubin; M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant.

Table 8 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on blood protein metabolites in goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Metabolite	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
TP (g/L)	Saponin	77.9±3.71	78.5±5.66	75.1±5.23	74.7±5.00	74.7±4.58	80.6±7.47	80.5±7.58	77.4 ^A	0.697	<0.0001	NS	
	Control	78.2±4.81	77.2±4.80	76.4±4.89	75.2±5.28	75.5±5.07	80.6±6.62	83.5±6.34	78.0 ^A				
	M	78.0 ^a	77.8 ^{ab}	75.8 ^{ab}	75.0 ^{ab}	75.1 ^{ab}	80.6 ^c	81.9 ^c					
Albumin (g/L)	Saponin	39.5±3.09	39.3±3.52	37.8±3.16	38.3±2.86	38.0±3.25	40.9±2.91	40.1±2.27	39.1 ^A	0.3275	<0.0001	NS	
	Control	39.9±2.75	39.4±2.84	39.4±2.68	39.0±2.41	39.5±2.58	42.8±6.01	41.0±2.83	40.2 ^A				
	M	39.7 ^a	39.3 ^a	38.6 ^a	28.5 ^a	38.8 ^a	41.9 ^b	40.5 ^{ab}					
Globulin (g/L)	Saponin	38.4±5.52	39.3±6.85	37.3±7.07	36.4±6.89	36.7±5.79	39.6±8.27	40.4±8.41	38.3 ^A	0.3275	<0.0001	NS	
	Control	38.3±6.0	37.7±5.68	37.0±5.48	36.2±5.80	36.0±5.82	37.7±9.49	42.7±7.72	37.9 ^A				
	M	38.3 ^a	38.5 ^a	37.1 ^a	36.3 ^a	36.3 ^a	38.7 ^{ab}	41.5 ^b					
Hp (g/L)	Saponin	0.82±0.38	0.66±0.47	0.52±0.12	0.44±0.13	0.51±0.12	1.36±1.45	1.68±1.37	0.85 ^A	0.4023	<0.0001	NS	
	Control	0.71±0.24	0.52±0.10	0.51±0.11	0.45±0.11	0.55±0.11	0.99±0.42	1.43±1.79	0.74 ^A				
	M	0.76 ^a	0.59 ^a	0.51 ^a	0.45 ^a	0.53 ^a	1.17 ^{ab}	1.55 ^b					
Urea (mmol/L)	Saponin	4.92±0.78	4.86±0.73	3.71±0.79	4.49±0.92	3.90±0.85	3.61±1.21	4.57±1.68	4.29 ^A	0.0544	<0.0001	NS	
	Control	3.85±0.60	4.01±0.05	3.15±0.74	3.86±1.01	3.47±0.74	3.72±1.65	4.12±1.13	3.74 ^A				
	M	4.39 ^a	4.44 ^a	3.43 ^b	4.17 ^{ab}	3.68 ^{ab}	3.67 ^{ab}	4.34 ^a					

TP: Total proteins; Hp: Haptoglobin; M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant.

Table 9 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on blood enzymatic metabolites in goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Metabolite	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
GGT (U/L)	Saponin	27.9 \pm 8.56	28.5 \pm 8.54	28.0 \pm 9.06	26.5 \pm 8.64	26.5 \pm 8.64		26.3 \pm 8.49	26.8 \pm 7.17	27.5 ^A	0.9034	0.0928	NS
	Control	28.3 \pm 5.85	17.8 \pm 5.25	27.3 \pm 5.61	27.7 \pm 5.71	26.5 \pm 5.44		27.2 \pm 7.04	30.2 \pm 7.09	27.8 ^A			
	M	28.1 ^a	28.2 ^a	27.9 ^a	27.8 ^a	26.5 ^a		26.8 ^a	28.5 ^a				
AST (U/L)	Saponin	76.3 \pm 7.45	113.6 \pm 59.02	83.3 \pm 13.46	84.8 \pm 15.07	84.3 \pm 9.84		134.8 \pm 43.30	119.2 \pm 15.90	99.5 ^A	0.2880	<0.0001	NS
	Control	83.7 \pm 11.98	89.0 \pm 14.12	80.5 \pm 9.89	81.3 \pm 8.74	84.9 \pm 10.93		119.1 \pm 16.94	120.33 \pm 19.83	94.1 ^A			
	M	80.0 ^a	101.3 ^{ab}	81.9 ^{ab}	83.0 ^{ab}	84.6 ^{ab}		126.9 ^c	119.8 ^{abc}				

GGT: gamma glutamyl transferase; AST: aspartate amino transferase; M: Mean; **a,b**: different letters indicate a significant difference between values within the same ($p < 0.05$). **A,B**: different letters indicate a trend for a difference between treatments ($p < 0.05$). NS: Non-significant.

Table 10 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on blood mineral metabolites in goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Metabolite	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
Ca (mmol/L)	Saponin	2.28 \pm 0.15	2.30 \pm 0.13	2.34 \pm 0.15	2.32 \pm 0.15	2.35 \pm 0.12	2.30 \pm 0.17	2.25 \pm 0.19	2.31 ^A	2.31 ^A	0.8700	0.0005	NS
	Control	2.34 \pm 0.13	2.29 \pm 0.09	2.33 \pm 0.09	2.31 \pm 0.10	2.34 \pm 0.12	2.30 \pm 0.11	2.27 \pm 0.08	2.31 ^A				
	M	2.31 ^{ac}	2.29 ^{ac}	2.34 ^c	2.31 ^{ac}	2.35 ^{bc}	2.30 ^{abc}	2.26 ^a					
Mg ³⁺ (mmol/L)	Saponin	1.02 \pm 0.08	1.05 \pm 0.13	1.05 \pm 0.14	1.09 \pm 0.15	1.07 \pm 0.14	1.11 \pm 0.21	1.09 \pm 0.11	1.07 ^A	1.07 ^A	0.6483	0.1529	NS
	Control	1.06 \pm 0.14	1.02 \pm 0.14	1.03 \pm 0.14	1.05 \pm 0.15	1.05 \pm 0.16	1.01 \pm 0.17	1.09 \pm 0.14	1.04 ^A				
	M	1.04 ^a	1.04 ^a	1.04 ^a	1.07 ^a	1.06 ^a	1.06 ^a	1.09 ^a					
PO ₄ ³⁻ (mmol/L)	Saponin	2.00 \pm 0.60	1.32 \pm 0.43	2.00 \pm 0.45	1.81 \pm 0.43	1.87 \pm 0.55	1.60 \pm 0.44	1.51 \pm 0.37	1.73 ^A	1.75 ^A	0.8751	<0.0001	NS
	Control	2.01 \pm 0.45	1.36 \pm 0.41	1.89 \pm 0.52	1.81 \pm 0.25	1.77 \pm 0.45	1.80 \pm 0.37	1.64 \pm 0.38	1.75 ^A				
	M	2.01 ^a	1.34 ^b	1.94 ^{ac}	1.81 ^{ac}	1.82 ^{ac}	1.70 ^c	1.57 ^b					

Ca: Calcium; Mg³⁺: Magnesium; PO₄³⁻: Potassium; M: Mean; a,b: different letters indicate a significant difference between values within the same ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant.

Table 11 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on ruminal fermentation parameters (pH, Ruminal NH₃ and Protozoa) in goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Variable	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
Ruminal pH	Saponin	6.68±1.36	7.10±0.45	6.96±0.20	6.99±0.21	6.97±0.19	6.62±0.19	6.84±0.31	6.88 ^A	0.2123	<0.0001	NS	
	Control	6.93±0.10	7.16±0.30	6.84±0.17	6.84±0.18	6.87±0.12	6.67±0.14	6.90±0.29	6.89 ^A				
	M	6.80 ^{ab}	7.13 ^b	6.90 ^a	6.91 ^a	6.92 ^a	6.65 ^c	6.87 ^a					
Ruminal NH ₃ (mg/dL)	Saponin	102.6±31.90	103.1±15.65	59.0±27.06	72.6±22.34	63.1±14.78	63.7±24.64	76.3±33.44	77.6 ^A	0.3504	<0.0001	NS	
	Control	95.0±26.97	95.7±37.59	54.5±23.03	65.3±24.36	47.1±16.54	75.9±35.77	78.9±24.97	73.2 ^A				
	M	98.8 ^a	99.4 ^a	56.7 ^b	69.0 ^b	54.8 ^b	70.1 ^b	77.6 ^{ab}					
Protozoa (x10 ⁵ /mL)	Saponin	8.8±2.97	11.2±2.66	10.0±2.36	10.7±2.00	11.6±2.94	9.7±2.28	10.4±3.66	10.3 ^A	0.0960	0.1079	NS	
	Control	9.8±1.40	10.5±2.66	9.1±1.94	8.7±1.84	9.8±3.45	9.6±2.40	9.4±2.44	9.6 ^A				
	M	9.3 ^a	10.9 ^a	9.5 ^a	9.7 ^a	10.6 ^a	9.6 ^a	9.9 ^a					

NH₃: Ammonia-N; M: Mean; a,b: different letters indicate a significant difference between values within the same line ($p < 0.05$). A,B: different letters indicate a trend for a difference between treatments ($p < 0.05$); NS: Non-significant.

Table 12 - Effect of treatment (Saponin, n = 12 and Control, n = 12), sampling time and their interaction on ruminal fermentation parameters (total volatile fatty acids) in goats during late pregnancy and first weeks of lactation (LSmeans \pm standard error). Saponin was given in the diet from week P-4 to parturition (25g/animal/day).

Variable	Group	Weeks in reference to parturition								M	Variation factor		
		P-5	P-4	P-3	P-2	P-1	0	P+1	P+2		Treatment	time	Interaction (T x t)
Total VFA (mmol/L)	Saponin	69.1±24.09	66.7±17.63	78.7±12.58	83.0±9.88	81.6±8.06	99.5±12.84	85.0±14.94	80.2 ^A	0.1797	<0.0001	NS	
	Control	81.8±7.18	76.0±18.88	88.1±14.98	94.8±11.83	87.0±10.14	95.0±9.04	81.3±13.82	86.3 ^A				
	M	75.5 ^{ab}	71.3 ^a	83.4 ^{ab}	88.9 ^{abc}	84.4 ^{ab}	97.2 ^c	83.1 ^a					
Acetate (mmol/L)	Saponin	46.6±17.68	45.3±12.69	53.7±9.33	56.8±7.39	55.6±5.98	66.1±7.86	57.6±10.09	54.3 ^A	0.1484	<0.0001	NS	
	Control	55.2±5.55	52.2±14.25	60.1±11.20	66.1±9.15	60.3±8.25	63.8±6.10	55.5±10.75	59.0 ^A				
	M	50.9 ^a	48.7 ^a	56.9 ^{ab}	61.4 ^b	58.0 ^{ab}	64.9 ^b	56.5 ^{ab}					
Propionate (mmol/L)	Saponin	11.8±4.10	11.1±3.11	13.9±3.08	14.3±2.16	14.2±2.07	20.0±3.82	15.7±3.55	14.3 ^A	0.2589	<0.0001	0.0434	
	Control	14.4±1.33	12.9±3.39	15.8±2.90	16.6±2.73	15.6±1.88	18.4±2.53	14.6±2.65	15.5 ^A				
	M	13.1 ^a	12.0 ^a	14.8 ^b	15.5 ^b	14.9 ^b	19.2 ^c	15.1 ^b					
Butyrate (mmol/L)	Saponin	7.62±2.55	7.16±1.90	8.32±1.28	8.95±1.14	8.89±0.96	10.23±1.48	8.87±1.98	8.55 ^A	0.9300	<0.0001	NS	
	Control	9.13±1.11	7.68±1.81	9.20±1.40	9.22±1.16	8.31±0.83	9.55±1.19	8.43±1.23	8.79 ^A				
	M	8.37 ^a	7.42 ^a	8.76 ^{ab}	9.09 ^b	8.59 ^{ab}	9.87 ^c	8.64 ^{abc}					
Valerate (mmol/L)	Saponin	0.52±0.17	0.48±0.12	0.52±0.07	0.56±0.08	0.55±0.08	0.76±0.13	0.60±0.16	0.57 ^A	0.7259	<0.0001	NS	
	Control	0.61±0.09	0.50±0.07	0.60±0.12	0.57±0.08	0.52±0.06	0.75±0.15	0.57±0.10	0.59 ^A				
	M	0.56 ^a	0.49 ^a	0.56 ^a	0.56 ^a	0.54 ^a	0.75 ^b	0.58 ^a					
Caproic acid (mmol/L)	Saponin	1.1±0.07	1.1±0.05	1.1±0.07	1.1±0.06	1.1±0.06	1.1±0.09	0.9±0.05	1.08 ^A	0.1325	<0.0001	0.0254	
	Control	1.2±0.06	1.1±0.08	1.2±0.07	1.2±0.05	1.1±0.04	1.1±0.07	0.9±0.05	1.11 ^A				
	M	1.13 ^a	1.07 ^b	1.16 ^a	1.13 ^a	1.13 ^a	1.10 ^b	0.93 ^c					
Iso-valerate (mmol/L)	Saponin	0.58±0.32	0.66±0.22	0.48±0.16	0.50±0.17	0.50±0.15	0.61±0.33	0.56±0.29	0.56 ^A	0.4499	<0.0006	NS	
	Control	0.51±0.18	0.75±0.26	0.48±0.10	0.37±0.11	0.48±0.21	0.57±0.21	0.63±0.34	0.54 ^A				
	M	0.54 ^a	0.71 ^a	0.48 ^b	0.43 ^b	0.49 ^{ab}	0.59 ^{ab}	0.60 ^{ab}					
Iso-butyrate (mmol/L)	Saponin	0.92±0.32	0.93±0.20	0.68±0.21	0.78±0.13	0.73±0.11	0.73±0.19	0.69±0.19	0.78 ^A	0.4825	<0.0001	NS	
	Control	0.90±0.17	0.90±0.19	0.67±0.09	0.74±0.15	0.67±0.08	0.82±0.24	0.70±0.25	0.77 ^A				
	M	0.91 ^a	0.91 ^a	0.68 ^b	0.76 ^{ab}	0.70 ^b	0.78 ^{ab}	0.69 ^b					
Acetate/Propi onate	Saponin	3.92±0.23	4.09±0.34	3.90±0.30	3.99±0.33	3.95±0.35	3.34±0.23	3.71±0.38	3.85 ^A	0.8264	<0.0001	NS	
	Control	3.84±0.17	4.04±0.35	3.81±0.21	4.01±0.33	3.87±0.33	3.49±0.28	3.80±0.23	3.84 ^A				
	M	3.88 ^{ab}	4.06 ^a	3.85 ^{ab}	4.00 ^{ab}	3.91 ^{ab}	3.42 ^c	3.76 ^b					

VFA: Volatile fatty acids; M: Mean; a,b: different letters indicate a significant difference between values within the same (p<0.05). A,B: different letters indicate a trend for a difference between treatments (p<0.05); NS: Non-significant.

5 GENERAL DISCUSSION AND PERSPECTIVES

The objectives of this work were to gather information about the use of two natural substances (β -glucan and saponin) and their relationships with the metabolism and performance of small ruminants in different metabolic statuses. In relation to the economic and social importance of sheep and goat farming mainly in Northeastern Brazil and France, this work aimed to contribute to a better understanding of the underlying mechanism of female responses to the challenge of the physiological and/or metabolic imbalance such as NEB during the risky period around parturition.

The adoption of preventive practices in feeding management in small ruminants, especially at the beginning of gestation, promotes good body condition score, providing a better development of gestation, without compromising the health and productivity of the females (Lima, 2013). Therefore, another important aspect of these two studies was that for the first time the effects of these natural substances were evaluated, on the metabolism of pregnant sheep and goats, and the studies paved the way for future research in this field. This work also allowed the exchange of information between the research teams, in particular by studying the effects of these natural substances in two small ruminant species in order to access the differences in metabolic responses.

5.1 INTEREST IN THESE NATURAL SUBSTANCES (β -glucan and saponin)

β -glucan and saponin-based substances have been shown to possess a wide range of biological properties. These findings call for future perspectives into the diverse ruminant production systems using these natural substances, as well as their use in the prevention of metabolic disorders, especially during the peri-parturient period. Their utilization will depend on their potential purpose either as a pharmaceutical product or as a functional feed additive. Some studies have reported that these two natural substances have an important role on cholesterol and glucose modulation in human and animals, however the mechanism by which this occurs is still unclear (Milgate & Roberts 1995; Hristov et al. 1999; Magnani & Castro-Gómez, 2008). In animal production and in particular, in dairy ruminants, they may be promising alternative medicines in order to minimize disruptions in glucose metabolism around parturition.

Indeed, a common practice in small ruminant farms is to administrate gluconeogenic supplements in late pregnancy in order to prevent metabolic disturbances that usually happen

in this transition period. Among the precursors of glucose, propylene glycol stands out because it is responsible for increasing glucose and insulin concentrations, as well as for decreasing the release of non-esterified fatty acids (Castañeda-Gutiérrez et al., 2009; Chiofalo et al., 2009). In addition, the use of ionophores in the diet also plays an important role as a gluconeogenic additive by minimizing the NEB impact during the peri-parturient period (Lima et al., 2016). Ruminants supplied with these molecules showed higher proportions of propionate in the rumen, which is a glucose precursor (Nielsen & Ingvarsen, 2004; Chiofalo et al., 2009).

Over recent years, research on the use of ‘natural substances’ has grown due to the reduction and banning of ‘non-natural substances’ such as feed antibiotic additives. This concern is fueled by the emergence of antibiotic resistance in many human pathogenic bacteria, the release of contaminating residues into the environment (water, soil, etc.) and the risk that growth-promoting antibiotic residues will appear in foods of animal origin (Yang & Carlson 2004; Manero et al. 2006; Parveen et al. 2006). Consequently, there is nowadays a real demand among animal producers for alternative feed additives and among consumers for more natural and safe products in the human food supply chain (Jouany & Morgavi 2007).

5.2 EXPERIMENTAL DOSE

The dose of the saponin-based additive used in our study (25g/animal/day) and the dose of β -glucan (1ml/animal/day) correspond to the recommendations of the manufacturers.

The pharmaceutical and feeding industries have investigated dosage levels compatible with animal welfare and valid science. In the experimental phases of the safety assessment of new drugs and feed additives, normal practice of using multiples of the ‘effective doses’ is important in order to establish the necessary safety margins (Diehl et al., 2001; Damy et al., 2010). It is necessary to take into account different characteristics of the products such as toxicity, physical properties, palatability, cost and regulatory requirements. For example, the saponin-based additive was not consumed by the goats when incorporated into a concentrate and had to be mixed in the TMR as shown by Oleszek et al. (1994). These negative effects have been ascribed to several properties by the astringent and irritating taste of saponins (Oleszek et al., 1994). It was therefore not possible to measure individual saponin intake.

In the case of natural products such as the β -glucan and saponin, it is difficult to understand all their effects. Indeed, owing to the diversity of bioactive components in these natural product, drug or feed additive, their effective doses on specific animal species are difficult to determine and the effects (direct and indirect) on animals are not totally controlled.

Farmers and consumers alike generally perceive ‘natural substances’ to be less toxic than synthetic antibiotics or other chemical products. However, this perception is unsound as there are many examples of dangerous natural toxins. The use of natural substances must therefore obey the same general rules as non-natural products, e.g. they must be safe for both the animal and the handler of the product, they must not be found as residues in animal products, and they must not be a hazard to the environment. The dose-response relationship must be precisely established from well-designed experiments when a specific claim is made in animal production, and risks associated with the use of these natural preparations must be robustly tested in carefully designed clinical studies in animals (Jouany & Morgavi, 2007). Therefore, research should continue to investigate the safe dose of these substances in animals.

The literature shows that more studies have been conducted *in vitro* than *in vivo* to understand the effects of saponins. All these studies have tested the effects of saponins with different doses, associated diets, saponin sources, routes of administration and animal species (Jouany & Morgavi, 2007; Patra & Saxena, 2009a; Patra & Saxena, 2010; Bayourthe & Ali-Haimoud-Lekhal, 2014). In other words, they were performed with different sources from saponin-containing plants such as *Quillaja saponaria* in sheep (Pen et al., 2008; Holtshausen et al., 2009), *Sapindus saponaria* *in vitro* and in sheep (Diaz et al., 1993; Hess et al., 2003a; Hess et al., 2004), *Yucca Schidigera* in cows and sheep (Hussain & Cheeke, 1995; Santoso et al., 2004), *Biophytum petersianum* in goats (Santoso et al., 2007) and *Camellia sinensis* in steers or goats (Hristov et al., 1999; Hu et al., 2005; Hu et al., 2006). In addition to these various origins, saponins were studied by administering the whole plant (Holtshausen et al., 2009), or using different galenics such as total plant extract (Wu et al., 1994; Eryavuz & Dehority, 2004; Wang et al., 2009), or using specific parts of the plants like fruits (Hess et al., 2003b; Abreu et al., 2004), seeds and leave extracts (Goel et al., 2008b) or root (Klita et al., 1996), and as a vaccine adjuvant (Kensil, 1996).

The major problems in saponin chemistry lie in the enormous diversity of naturally occurring saponins. It is clear from the very different properties of a crude mixture of saponins isolated from different plants that subtle variations in structure give rise to substantial variations in physical, chemical and biological properties. It is therefore difficult to extrapolate the results of one study to other studies using different extracts. Isolation of pure compounds from a crude extract is very difficult, making it almost impossible to obtain pure compounds in sufficient quantity to investigate their biological properties (Oakenfull, 1981).

Overall, the oral dose of 25g/goat/day of saponin powder from a *Y. schidigera* extract used in our experiments showed some effects on metabolic and ruminal parameters. In the 1st

experiment, there was no saponin effect, but there was an interaction between treatment and time for urea and rumen acetate/propionate ratio (Figure 6). In the 2nd experiment, a trend for an increase in protozoa number was observed (Figure 7). Contrary to our results, Diaz et al. (1993) observed a decrease in protozoal population in sheep with a similar dose of 25g/animal/day of *Sapindus saponaria*. However, in their experiment, saponins were administered by a cannula directly into the rumen. These different observations show that the effects of saponins are variable and often contradictory, because of multiple factors. However, one may wonder whether a higher dose than 25g/animal/day *per se* would have led to significant effects. It would also be interesting to test at least two different doses to confirm and describe the dose-response and to avoid a potential experimental bias due to the fact that there was only one treatment group. Moreover, in order to be sure that saponins can act on ruminal bacteria, it would be interesting to test ruminal juice for saponin at different times after supplementation.

Another possible factor which could explain the lack of a clear effect of saponins in our experiments is the fact that our diets were adapted to requirements, while Bayourthe & Ali-Haimoud-Lekhal (2014) showed that saponins could be more effective in a protein deficient diet.

Moreover, the forage:concentrate ratio increased as the 2nd experiment progressed because of the need to try to develop the volume of the rumen for the future lactation. Saliva production is known to increase when there is more forage in the diet. Saliva is thought to neutralise part of the effect of saponins in the rumen. Indeed, Hess et al. (2003a) showed that saponin had no effect on protozoa numbers when forage levels were increased in the diet. Therefore, the increase in the forage:concentrate ratio may have acted to neutralise the saponin effect on protozoa via high saliva secretion. One could also wonder if the bitterness of saponins could have induced higher rates of salivation.

The lack of effect of saponins might also be due to the administration of levels of saponins which are too low and therefore inefficient to elicit a rumen response. It is necessary to identify the feeding conditions that would allow a beneficial response to saponins in ruminant production (Patra & Saxena, 2009a) and to target the most appropriate physiological statuses to use saponins. Therefore, complementary trials *in vivo* and *in vitro* in the short- and long-term remain essential to test the effects under varied conditions of use, but also to ensure the absence of residues in the animals and their safety for the consumer.

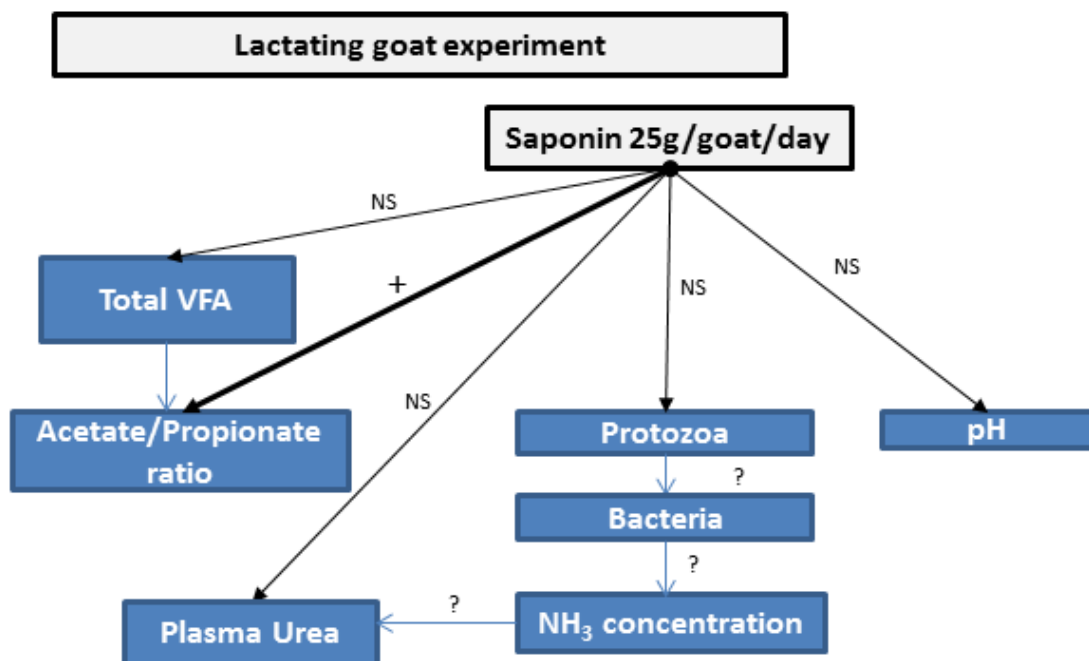


Figure 6. This diagram shows the main results of the 1st experiment with lactating goats. The arrow in bold signals a trend for a change in the acetate to propionate ratio (+); NS: represents non-significant effects; (?): represents the indirect effect of protozoa and bacteria on NH₃ concentration and therefore on plasma urea.

5.3 JUSTIFYING THE EXPERIMENTAL NUMBERS OF ANIMALS

The use of animals in scientific research is a dilemma that causes some of the greatest conflict in the bioethics debate proposed by Russell and Burch, (1992). They proposed the 3R principle: i) **reduction** of the number of animals used in each experiment; ii) **refinement** of experimental techniques in order to avoid unnecessary pain and suffering; iii) **replacement** with alternative methods as an imperative.

Reducing the number of animals in an experimental research should not impair the detection of biological effects and should not lead to a repetition of an experiment in order to obtain significant results. Therefore, the study design and sample size calculation, the control of variation, the statistical hypothesis being tested, the choice of statistical test used for data analysis and the interpretation of the results, all contribute towards refinement. Therefore, an experiment is likely to obtain more information without increasing the number of animals used (Festing & Altman, 2002).

According to previous studies, the number of animals needed to obtain statistically significant results at $p < 0.05$ for a range of differences between control and treatment groups and with coefficients of variation (CV) of 15% or 20% is from 5 to 8 animals (Elckelman et al.,

2007; Scheibe, 2008; Damy et al., 2010; Doke & Dhawale, 2015). However, the fact of working with farm animals compared to laboratory animals implies a higher variability. Moreover, at the present time due to the lack of experiments already done on the subject it was difficult to estimate beforehand the CV and therefore the minimum number of animals necessary. The slight effects observed in the current studies could have been clearer with a higher number of animals.

Finally, although we tried to use the same animals in both saponin experiments in order to compare the two metabolic statuses, we did not succeed because of reproduction problems and the impossibility to obtain sufficient synchronization of parturition dates. We were forced to choose additional animals to make up the experimental groups.

5.4 DIFFERENT METABOLIC STATUSES

Our objectives were to test the effect of saponins around parturition. In order to avoid a possible confounding effect of the metabolic imbalance, a first experiment was performed in a stable metabolic status (mid-lactation). However, no effects of saponins were observed in the 1st experiment and only trends in the second. More subtle modifications could be revealed by using more sensitive and global new approaches such as NMR spectroscopy (see section title 5.8 metabolomic by RMN spectroscopy). Moreover, concerning the β -glucan experiment, it would have been interesting to also test this substance in a stable metabolic status.

Pregnancy and lactation represent two important physiological statuses in dairy ruminants. The different tissues undergo an extensive series of metabolic adaptations to support the main changes involved such as fetus development during pregnancy and mammary gland during lactation. If physiological adaptations are not sufficient to supply enough nutrients and fulfill the needs of tissues, then animal performance is affected and animal health is compromised. Several metabolic changes occur to adapt to these different physiological statuses (Chávez et al., 2009).

In general, the peculiarities of the ruminant digestive tract lead to marked differences in absorbed nutrients compared to monogastric species. Moreover, there is a lack of information about the manner in which natural substances could help the animal to cope with high nutrient requirements during these two different metabolism statuses. To our knowledge, our research is one of the first to focus on the dynamic effects of saponin in different metabolic statuses with weekly measurements.

5.5 MICROBIAL COMPOSITION AND ADAPTATION IN SHORT AND LONG TERM FEEDING OF SAPONINS

If these substances are used as additives in the diet, differences in protozoa composition in the rumen of sheep and goats have to be taken into account, as shown by Ruiz et al. (2004) and Santra et al. (1998). A study showed an apparent discrepancy in different trials with supplementation of saponins due to the differences in the composition of the protozoal population of sheep and goats (Wina et al., 2006). A higher proportion of certain protozoa were found in the rumen of goats compared to sheep. As goats are browsers, they are confronted with more secondary plant components than sheep, which mostly eat grass. Therefore, the rumen microorganism of goats could be more adaptable to these substances than those of sheep (Silanikove, 2000), leading to possible different metabolic effects.

Defaunation is difficult to achieve as there are no safe defaunation agents commercially available for use under practical conditions. However, in contrast to known benefits of defaunation, reduced fauna might also be beneficial as it has been shown to increase MY in ruminants (Ivan et al., 2004). One of the challenges of using saponins or saponin-containing plants is that the effect of saponins on protozoa activity is transient (Newbold et al., 1997). Several studies have reported different protozoa responses to the saponins administered for days or months (Newbold et al., 1997; Ivan et al., 2004; Jouany & Morgavi, 2007; Patra & Saxena, 2009a). The majority of previous studies reported a detrimental effect on ruminal protozoa after saponin administration *in vitro* (Wallace et al., 1994) and in small ruminants (Lu & Jorgensen, 1987; Diaz et al., 1993; Klita et al., 1996). However, Eryavuz and Dehority (2004) showed in sheep that rumen protozoal concentrations either did not change or increased as a result of *Yucca schidigera* supplementation for 3 weeks. This last result is consistent with the trend for an increase in protozoa count observed in our 2nd experiment around parturition (Figure 7).

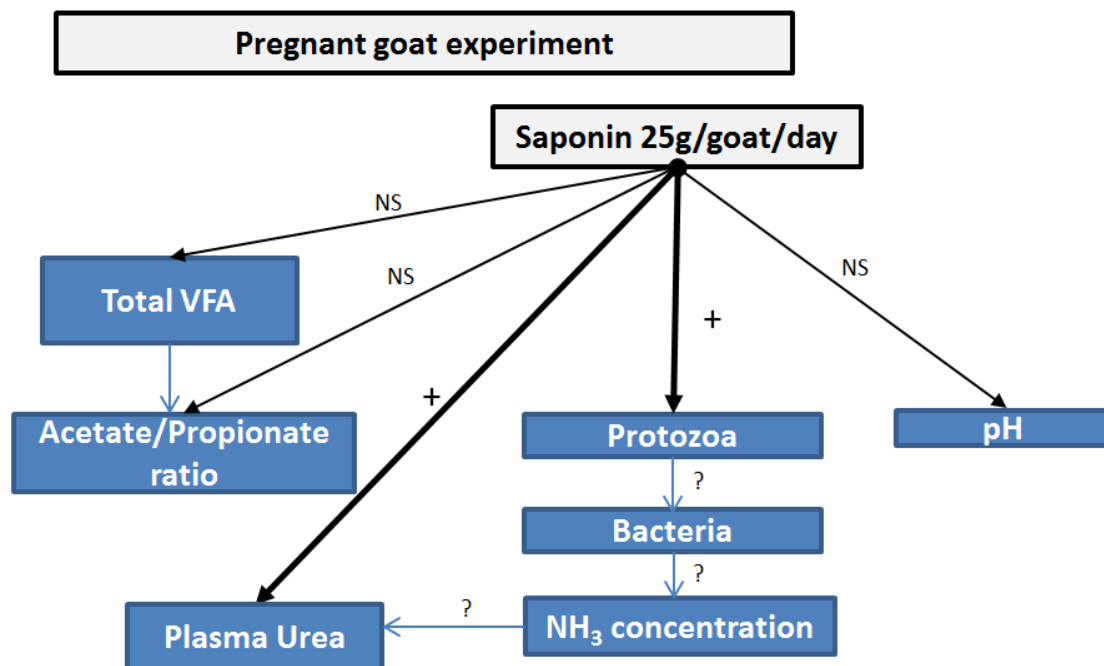


Figure 7. This diagram shows the main results of the 2nd experiment with pregnant goats. The arrow in bold signals a trend of an effect on protozoal count and plasma urea (+); NS: represents a non-significant effect; (?): represents the indirect effect of protozoa and bacteria on NH₃ concentration and therefore on plasma urea.

No effect of saponin was observed on microbial organisms in long-term saponin feeding by Wina et al. (2006), suggesting an adaptation of certain microorganisms to the saponins. Most studies on microbial adaptation were conducted *in vitro* and several mechanisms of adaptation to secondary plant components or plant cell wall substrates have been suggested (Wina et al., 2006). One possible mechanism of adaptation of protozoa and bacteria is probably due to an increase in wall thickness (Wang et al., 2000). This mechanism of adaptation could be via the production of extracellular polysaccharides around the microbial cells as a protective barrier. However, no information on the production of these substances in response to saponins is available (O'Donovan & Brooker, 2001).

Although no effect of saponin was found on microbial organisms in a long-term experiment, Wina et al. (2006) showed an increase in protozoa in a short-term one (after seven days of saponin) even if not in the same species. However, their conclusion on adaption of protozoa has to be taken with caution because they did not measure the protozoa in the first 7 days of the long-term experiment.

Nevertheless, Newbold et al. (1997) suggested that protozoa did not become resistant to these compounds, but rather that the rumen bacterial population degraded the saponins.

Therefore in the literature, either the protozoa become resistant to saponins, or the saponins are neutralized by bacteria. In our experiments, we were only able to count the protozoa and it was not possible to detect an eventual modification in their cell wall thickness.

Teferedegne (2000) suggested that saponin may be degraded in the saliva of sheep after prolonged feeding of saponin-containing plants. It is not known if the loss of anti-protozoal activity in the saliva of sheep fed *Sesbania sesban* for prolonged time is due to the binding of saponin to protein, or if a cleaving of saponin to sapogenin occurs in saliva as a detoxification process in the ruminal fluid. In other words, another adaptation to saponins might be due to a detoxification system that is induced by the saponins. Conversely, the anti-protozoal activity of saponin-containing plants might depend on their concentrations (Jouany & Morgavi, 2007; Patra & Saxena, 2009a). This factor warrants further investigations.

In both our experiments, the saponin and control groups were unfortunately different from the start in relation to the amount of total protozoa. At the beginning of the experiments, first of all, a strong individual variability was observed on rumen and plasma parameters, and secondly, the allocation of the goats to the two groups was not done on physiological or ruminal parameters but on production parameters like body weight, milk production, parity and breed. Therefore, this imbalance between the groups could explain part of our results.

5.6 EFFECT OF THESE NATURAL SUBSTANCES ON HORMONAL PROFILE

Despite extensive and intensive small ruminant livestock methods and the Brazilian semi-arid or French climate, nowadays these animals show good reproductive performance and have a better litter size at parturition than in the past. Nevertheless, there have been few studies on the hormonal profile and the influence that natural substances might have on it during the peri-parturient period.

Further studies could be planned to determine the effects of these natural substances on insulin, cortisol, glucagon, leptin and thyroid hormones and correlations between them and with some biochemical parameters, particularly parameters involved in basal metabolism.

5.7 IMPACT ON THE IMMUNE SYSTEM

The endocrine system generates coordinated metabolic changes to support pregnancy, parturition and lactation periods. These changes could involve several organs and systems: i) including the immune system, reducing the production of acute phase proteins and ii)

decreasing the leukocyte function, which can lead to other disorders in healthy ruminants (Cai et al., 1994; Shuster et al., 1996).

Therefore, β -glucans and saponins are thought to have an impact on the ability to potentiate and modulate immune response in several species (Pedroso, 1994; Rodriguez et al., 2003; Ilsley & Miller, 2005). Some studies have reported that oral and injectable administration of these substances might modulate the immune response, including enhancing antibody and cytokine production (Cainelli Gebera et al., 1995; Hoshi et al., 1999). Therefore, another area of interest is the impact of these natural substances on the immune system and in particular on the passive transfer of immunity from the dams to their offspring by the absorption of immunoglobulins found in colostrum. In addition, in this current work, data on the development or metabolism of the offspring have not yet been analysed.

Saponin and glucan based adjuvants have the ability to stimulate the cell-mediated immune system, as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity. The mechanism of immune-stimulating action of saponins and glucans has not been clearly understood, but many explanations have been put forward (Oda et al., 2000; Petrovsky & Aguilar, 2004).

Saponins reportedly induced production of cytokines, such as interleukins and interferons that might mediate their immunostimulant effects (Kensil, 1996; Petrovsky & Aguilar, 2004). There is evidence that saponins may increase the immune response by increasing the uptake of antigens from the gut and other membranes. Therefore, a saponin supplement added to colostrum may help the absorption of intact IgG molecules (Das et al., 2012).

On the one hand, Ilsley and Miller (2005) reported that a saponin supplement given to pregnant sows between 72-93 days of gestation did not enhance the antibody profile of sow colostrum or milk, nor did it alter the levels of antibodies found in sow serum during lactation. However, stillbirths were significantly reduced. The latter effect may be due to better oxygenation of the foeto-placental unit. On the other hand, another study has shown an increase in IgG levels in the serum of weaner pigs when given dietary saponin for 20 days post-weaning. This study indicated that dietary *Quillaja* saponins enhanced the immune function and also increased piglet feed intake. However, no benefit to growth was observed, and there was no improvement in feed efficiency (Ilsley et al., 2004).

One area of interest would be to determine if the administration of saponins to kids could increase the passive immunization response by facilitating absorption of maternal antibodies by the young animal.

5.8 METABOLOMIC BY NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

The objectives in this further research is to study the effect of saponin-based additive on goat performance in the mid-lactation and subsequently investigate its effect on goat responses to metabolic disorders related to the period of late gestation and early lactation, but using a novel global (or holistic) approach.

In this work, we analyzed the responses of goats at two different levels: (i) animal performance (macroscopic level) based on MY and BW and (ii) metabolites in the plasma and variables in the rumen fluid (or the microscopic level). The previous statistical analyses showed no significant effect of saponin. However, it is worth pointing out that the use saponin-based additives have increased in the last decade, and therefore its metabolic targets are not yet well known. Therefore, a good strategy would be to explore the effect of saponin, without *a priori*, on all the metabolic pathways for saponin and control groups by accessing all the metabolites (metabolome).

This work has been started and is still going on within our team. Fingerprinting of plasma and rumen metabolites was done by nuclear magnetic resonance (NMR) spectroscopy. The use of this sensitive method of analysis associated with an adequate statistical approach to study this high dimensional data will make possible to highlight the main metabolic pathways affected by saponin and, some putative metabolites as biomarkers of this metabolic disruption. Because of the intrinsic structure of the experimental plan (longitudinal follow-up through two physiological periods, first, during lactation with no feed additive perturbation, then, in late pregnancy combined with saponin supplementation or not, in a multivariate assessment), it is *a priori* complex and a canonical correlation analysis is chosen when multiple variables need to be processed at the same time.

The analyses of the metabolites in plasma of the 2nd experiment (pregnant goats) presented in figure 8 shows a slight difference between the two groups.

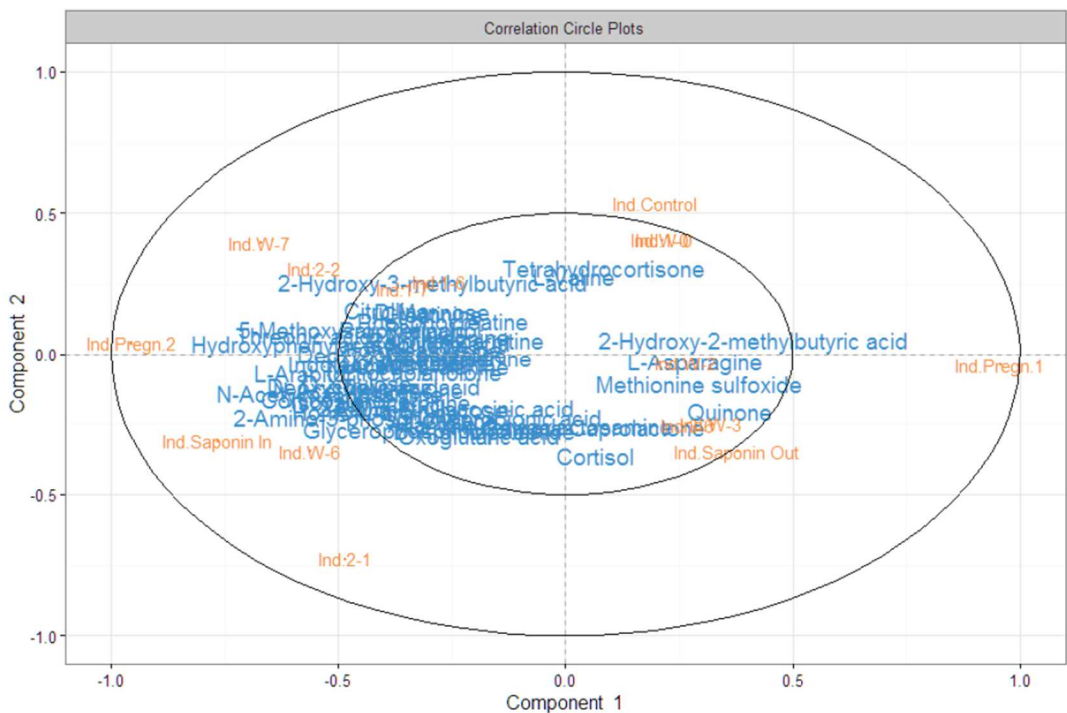


Figure 8. Biplot of the loadings explaining the canonical components 1 and 2 for both the set of metabolic variables (matrix X, variables in blue) and the set of dummy variables used to summarize the different factors with their respective levels (matrix Y, variables in orange). A cutoff fixed at 0.25 is used to discard no informative loadings which would be projected inside the central ellipse.

This figure 8 represents the most correlated metabolic variables to the two first canonical components. Because of the canonical nature of the statistical modeling, we have also access to corresponding projection of factor variables. Clearly, there is a clear opposition on the 1st component between the situation where goats were pregnant (Ind.preg.1) and that where goats were lactating (Ind.preg.2). There was also a clear opposition on the same component between the control (Ind. Saponin Out) and the treated group (Ind. Saponin In). A metabolic network gives the most correlated variables to this physiological situation with threonic acid, L-arabitol, deoxycholic acid, N-acetylmannosamine, corticosterone, 5-methoxysalicylic acid, and hydroxyphenylacetyl glycine being quantified at a higher value in the *post-partum* period (lactation) than in gestation (Figure 8 and table 2).

The effect of saponin can be mainly observed in component 2, in which the metabolic situation recorded for goats exposed to saponin in *pre-partum* period (noted "Ind.2.1" in figure 8) is contrasted with week 6 (first week after parturition; p+1) to the control group ("Ind.Control"); and this is no longer the case on week 7 (second week after parturition; p+2). For this 2nd component, a metabolic network gives the most interesting variables (Figure 8 and table 2) to the effect of saponin.

Table 2. Correlations matrix between metabolites and the controlled factors characterized by component 2.

Metabolites	Ind. Control	Ind.2-1
Cortisol	-0.19	0.26
Oxoglutaric acid	0	0.21
D-Arginine	0	0.20
Lipoamide	0	0.20
Gamma-Caprolactone	0	0.19
N-acetylgalactosamine	0	0.19
Glycerophosphocholine	0	0.19
2-Hydroxy-3-methylbutyric acid	0	-0.19
L-Valine	0	-0.20
Tetrahydrocortisone	0	-0.22

The following metabolites: cortisol, oxoglutaric acid, D-arginine, lipoamide, gamma-caprolactone, N-acetylgalactosamine, and glycerophosphocholine are positively correlated to controlled factors corresponding to the group of goats exposed to saponin in *pre-partum* period, while the 2-hydroxy-3-methylbutyric acid, L-valine and tetrahydrocortisone are negatively correlated to it (Table 2). These latter metabolites are present in plasma at a lower relative concentration in saponin-treated goats than in control ones on week 6. All these putative positively and negatively correlated metabolites are constitutive of the "memory" effect induced in lactating goats which were exposed *pre-partum* to saponin.

Altogether, these preliminary results suggest that more investigations on NMR data are required to highlight metabolic pathways influenced by a saponin-based additive, by using metabolomic data on milk, plasma and rumen fluid together with adequate statistical approaches.

5.9 CONCLUSION

More information was provided on the use of β -glucan and saponin natural substances in late pregnancy and early lactation in sheep and goats. A few effects were found but they were not strong enough to conclude on the efficiency of these natural substances to prevent the metabolic disturbances during the peri-parturient period. More research is needed on the different factors modulating these effects and on the underlying mechanism. In addition, the combination of different data from animal husbandry, biochemical and metabonomical

approaches may help to shed more light on the underlying metabolic pathways of small ruminants during this period of transition.

6. RÉSUMÉ SUBSTANTIEL

6.1 INTRODUCTION

Les petits ruminants sont connus pour leur excellente capacité à utiliser des aliments fibreux. Cependant, l'efficacité de la production est généralement plus faible chez ces espèces que chez d'autres animaux d'élevage. Par conséquent, des tentatives existent pour compléter les régimes avec des additifs alimentaires qui, en interagissant avec la flore ruminale, stimuleraient la productivité afin d'assurer des conditions optimales pour l'élevage de la progéniture (Milewski & Sobiech, 2009).

Les exigences productives requises par les éleveurs (sélection génétique et gestion de système d'élevage intensif) ont augmenté le risque et l'incidence de déséquilibre nutritionnel et de troubles métaboliques dans les troupeaux. Pendant la période du *péri-partum*, surviennent des modifications physiologiques qui peuvent prédisposer la femelle à des maladies métaboliques, comme par exemple la toxémie de gestation. Il faut ainsi bien connaître cette période de transition pour mettre en place des actions préventives afin d'éviter les pertes de production. En effet, ces périodes peuvent prédisposer à la morbidité voire à la mortalité chez les petits ruminants. Parmi les premiers signes se trouve alors une perte d'appétit, entraînant une réduction de l'ingestion de nutriments essentiels pendant une période durant laquelle les ruminants ont des besoins énergétiques importants (González et al., 2000; Campos et al., 2010).

Différentes stratégies nutritionnelles sont utilisées dans le but d'éviter ou minimiser le bilan énergétique négatif (BEN) et ses conséquences. Des alternatives aux médicaments allopathiques pour la prévention et le traitement de diverses maladies peuvent permettre d'améliorer l'apport en matière sèche et les performances de production. Le propylène glycol, un produit de synthèse, est le seul composé régulièrement utilisé par les éleveurs, comme précurseur de glucose, afin d'éviter les maladies métaboliques (Das et al., 2012).

Au cours des dernières années, un intérêt particulier a été montré dans la recherche d'un composé d'origine naturelle avec des effets similaires. Ainsi, il existe une recherche et un attrait croissants de produits d'origine naturelle, tels que les additifs phyto-gènes utilisés en élevage comme alternative aux promoteurs de croissance comme certains antibiotiques et hormones.

A l'heure actuelle, les autorités sanitaires des pays développés, en particulier ceux de l'Union Européenne, ont interdit ou limité l'utilisation des antibiotiques de synthèse en tant que promoteurs de croissance, dans les aliments pour animaux (Wallace et al., 2002). Les principes actifs, utilisés comme additifs alimentaires ou agents médicamenteux, doivent être inoffensifs pour les animaux, ne laisser aucun résidu toxique dans la carcasse ou dans les produits animaux (Wallace et al., 2002; Franz et al., 2005; Santoso et al., 2007; Vrublova et al. 2010).

Un stimulateur naturel, avec des propriétés pro- et prébiotiques, est la levure *Saccharomyces cerevisiae*, qui possède un large éventail d'activités. En effet, le β -glucane, produit à partir de *S. cerevisiae*, a comme principale caractéristique une action immunomodulatrice chez les humains et les animaux. Plusieurs études ont montré que ce composant présentait des propriétés immunostimulantes, y compris des activités antitumorales et antibactériennes. Il pourrait également réduire les teneurs de cholestérol et de triglycérides, normaliser les taux de glycémie, avoir des

propriétés anti-oxydantes (Akramienè et al., 2007; Novak & Vetvicka, 2009; Vetvicka & Vetvickova, 2010).

Cependant, très peu d'informations sont disponibles concernant ses effets possibles sur le métabolisme et il est nécessaire de s'assurer que la stimulation immunitaire n'est pas obtenue au détriment de l'adaptation métabolique de l'animal à la période *péri-partum*. Bien que des connaissances existent sur les troubles métaboliques au cours de cette période, les études sur les effets d'un traitement et/or prévention par des injections de β -glucane sur le métabolisme sont inexistantes.

De plus, actuellement dans les élevages, des stratégies alimentaires et aussi sanitaires tendent à favoriser la prévention en regard du curatif et à s'appuyer en première intention sur l'utilisation de substances naturelles, le plus souvent à base de plantes.

Certaines plantes, en raison de leurs compositions complexes, peuvent être utilisées comme additifs alimentaires avec de multiples activités biologiques. Elles produisent et stockent des composés secondaires qui ne sont pas directement impliqués dans leur croissance, leur développement ou leur reproduction, mais dans des processus divers de défense vis-à-vis d'agresseurs mécaniques ou biochimiques, contre d'autres plantes, les herbivores et les stress abiotiques. Ils peuvent également servir de messagers chimiques entre les plantes et l'environnement, ou attirer les insectes pollinisateurs (Jouany & Morgavi 2007).

Parmi ces composés secondaires, les saponines sont un groupe de glycosides stéroïdiens, qui peuvent être extraits de différentes plantes telles que le *Yucca schidigera* (L.). Plusieurs études ont montré le rôle bénéfique des saponines dans l'efficacité digestive des ruminants, les performances zootechniques et la limitation des productions de méthane (Jouany & Morgavi, 2007; Patra & Saxena, 2009). Ces bénéfices seraient dus à leurs propriétés antimicrobiennes sélectives, inhibant préférentiellement les populations de protozoaires ciliés, de bactéries cellulolytiques et à activité peptidasique (Franz et al., 2005).

Les saponines auraient des propriétés telles que la modification de la perméabilité à la membrane cellulaire des protozoaires, l'immunostimulation et des propriétés hypocholestérolémiques. Il a aussi été montré qu'elles auraient des effets sur la croissance et l'ingestion.

En outre, une co-relation métabolique existe entre les protozoaires ciliés et les bactéries méthanogènes. Les protozoaires produisent ainsi de l'hydrogène qui est utilisé par des bactéries méthanogènes. Les saponines peuvent réduire partiellement la production de méthane et d'hydrogène *in vitro*, et peuvent donc être considérées comme de puissants inhibiteurs des méthanogènes du rumen en raison de leur efficacité à de faibles concentrations. Enfin, ces substances augmenteraient également la production en acides gras volatils totaux (AGV) et diminueraient les concentrations d'ammoniac dans le rumen. Les saponines diminueraient la proportion molaire de l'acétate ruminal et augmenteraient celle du propionate et du butyrate avec une diminution correspondante du rapport acétate/propionate (Lila et al., 2005; Das et al., 2012; Wang et al., 2012). Ces composés affectent ainsi les animaux de manière positive ou négative.

6.1 PAPIER 1 – Soumis à la *Revue de Médecine Vétérinaire*

EST-CE QUE DES INJECTIONS INTRAMUSCULAIRES DE B1,3-GLUCANE INFLUENCENT LES PROFILS METABOLIQUES ET ENZYMATIQUES CHEZ DES BREBIS SANTA INES PENDANT LA FIN DE LA GESTATION ET LE DEBUT DE LA LACTATION ?

Introduction

La région Nord-Est du Brésil et surtout l'état du Pernambuco ont une longue tradition et occupent une place prépondérante dans l'élevage des petits ruminants. Environ 57% du cheptel de moutons du Brésil est élevé dans cette région. Dans ce contexte, la race Santa Inês se démarque des autres races de par une robustesse et résistance accrue lorsqu'elle est élevée dans des conditions difficiles, telles que celles observées dans les zones tropicales et arides, présentant de bons taux de croissance et une efficacité de la reproduction élevée tout au long de l'année.

Les exigences élevées dans les systèmes de production et la gestion intensive dans les exploitations modernes ont augmenté le risque et l'incidence des déséquilibres nutritionnels et des perturbations métaboliques chez les brebis pendant le *péri-partum*. Cette période est caractérisée par des changements métaboliques, en raison de besoins énergétiques augmentés pour satisfaire la croissance fœtale puis la production de colostrum et lait. Or ces besoins accrus sont difficilement comblés du fait d'une ingestion limitée par le volume fœto-placentaire. En plus des problèmes métaboliques induits par l'insuffisance de l'apport énergétique, il a été souligné une déficience du système immunitaire en raison de stress oxydatifs accrus (Akramienè et al., 2007; Novak & Vetvicka, 2009; Vetvicka & Vetvickova, 2010).

Objectifs

Diverses démarches nutritionnelles peuvent être utilisées dans la prévention et le traitement des maladies métaboliques telles que la toxémie de gestation. Plusieurs études ont montré que le β -glucane possède des propriétés immunostimulatrices, y compris anti-tumorales et anti-bactériennes. Cependant, très peu d'informations sont disponibles concernant ses effets possibles sur le métabolisme et il est nécessaire de s'assurer que la stimulation immunitaire n'est pas obtenue au détriment de l'adaptation métabolique de l'animal en période *péri-partum*.

Bien que des connaissances approfondies existent sur les troubles métaboliques au cours de la période *péri-partum*, les études sur les effets du traitement par les injections de β -glucane sur le métabolisme sont inexistantes. Par conséquent, le projet actuel a été réalisé pour évaluer l'influence de l'administration de β -glucane sur le métabolisme des brebis Santa Inês péri-parturientes.

Matériels et méthodes

Quatorze brebis Santa Inês, cliniquement saines, pesant entre 40 et 50 kg et ayant une note d'état corporelle moyenne de 3,0-3,5 ont été utilisées dans une expérimentation au nord-est du Brésil à la clinique des bovins de l'Université Rurale Fédérale du Pernambuco à Garanhuns, pendant cinq mois, de septembre 2014 à février 2015. Les animaux ont été élevés en système intensif, en utilisant le

même protocole d'hygiène, sanitaire et nutritionnel, et ont été étudiés au même stade métabolique (fin de gestation et début de lactation).

Au 90^{ème} jour de gestation, les brebis ont été séparées aléatoirement en deux groupes de sept animaux chacun : le groupe « glucane », qui a reçu le β 1,3-glucane (Imunoglucan® Glucane (β -1,3-D-glicopiranosé)), en utilisant une dose hebdomadaire recommandée de 1 ml par voie intramusculaire, du 60^{ème} au 10^{ème} jour avant l'agnelage, en totalisant sept injections, et le groupe « contrôle », qui n'a pas reçu d'injections.

Pendant toute la phase expérimentale, les animaux ont été logés dans une bergerie ouverte et ont reçu une ration à base de concentrés à raison de 150g/animal, adaptée aux besoins nutritionnels de chaque période (*pré* et *post-partum*), distribués deux fois par jour, en plus d'herbe à éléphant (*Pennisetum purpureum*, Schumach.), de foin du Tifton 85 (hybride de *Cynodon dactylon* L.), de sels minéraux et d'eau *ad libitum*.

Des échantillons de sang ont été prélevés les 30^{ème}, 20^{ème} et 10^{ème} jours avant l'agnelage, à l'agnelage et les 10^{ème}, 20^{ème}, 30^{ème} et 60^{ème} jours après l'agnelage. Les échantillons de plasma ont été analysés pour le L-lactate et le glucose et les échantillons de sérum ont été analysés pour le beta-hydroxybutyrate (β HB) et les acides gras non estérifiés (AGNE), la fructosamine, le cholestérol, les triglycérides, l'urée et la créatinine. Les protéines totales et l'albumine ont été mesurées par les méthodes du biuret et au vert de bromocrésol, respectivement. La concentration de globulines a été calculée par différence entre les teneurs en protéines totales et en albumine. Les activités enzymatiques en aspartate aminotransférase (AST), gamma glutamyl-transférase (GGT) et créatine kinase (CK) ont été déterminées dans des échantillons de sérum. Tous les essais ont été effectués en utilisant des kits commerciaux avec un analyseur semi-automatique à 37°C. Les niveaux sériques des minéraux (calcium total, phosphore inorganique, magnésium et chlorure) ont été analysés par le même analyseur biochimique semi-automatique. Les teneurs en ions Ca^{2+} , Na^{+} et K^{+} ont été déterminées par un analyseur d'électrolytes.

L'analyse statistique a été réalisée en utilisant un modèle mixte linéaire avec la procédure MIXED dans SAS pour les mesures répétées. Les valeurs de p ont été ajustées pour des comparaisons multiples entre les groupes en utilisant le test F. Le niveau de significativité pour tous les tests statistiques a été fixé à 5%.

Résultats

Aucun changement dans la note d'état corporel n'a été observé pendant la phase expérimentale. La note d'état corporel est restée constante entre 3,0 et 3,5. Les variables glucose, L-lactate, AGNE et β HB n'ont pas été affectées par le traitement, mais un effet significatif du temps sur ces paramètres ($p < 0,01$) a été noté, leurs concentrations ayant atteint un pic le jour de l'agnelage. Les teneurs en fructosamine ont aussi présentées une tendance à être plus élevées dans le groupe contrôle, comparées au groupe glucane ($p = 0,07$) et sa concentration a augmenté juste avant l'agnelage et est restée élevée par la suite ($p = 0,0002$). Les teneurs en cholestérol et triglycérides n'ont pas été affectées par le traitement. Cependant, leurs concentrations ont augmenté juste avant l'agnelage, puis ont diminué avec le temps ($p < 0,0001$).

Les valeurs en protéines totales et en globulines n'ont pas été affectées par le traitement. Cependant, leurs concentrations ont diminué avant l'agnelage, puis ont augmenté ($p < 0,0001$). Une tendance a

été constatée pour l'albumine (contrôle > glucane, $p = 0,062$) ainsi qu'un effet temps significatif ($p < 0,001$), la valeur moyenne la plus élevée ayant été observée 10 jours avant l'agnelage. Les concentrations en urée ont eu tendance à être modifiées par le traitement (glucane > contrôle, $p = 0,0987$). Il y a également eu un effet significatif du temps sur les concentrations en urée ($p = 0,0110$), ces concentrations ayant culminé le jour de l'agnelage. La concentration en créatinine n'a pas été affectée par le traitement, mais elle a atteint un pic juste avant et à l'agnelage ($p < 0,0001$).

L'activité d'AST a été plus élevée dans le groupe contrôle comparée au groupe glucane ($p = 0,035$). Les activités des AST et GGT ont été plus élevées pendant la lactation qu'en fin de gestation ($p < 0,05$). L'activité de la CK n'a pas été affectée par le traitement ni par le temps.

Les concentrations totales des minéraux et de leurs ions (calcium, magnésium, chlorure, phosphore inorganique, sodium et potassium) n'ont pas été affectées par le traitement. Le calcium total et ionisé n'a également pas été affecté par le temps. Le chlorure a augmenté après l'agnelage ($p = 0,002$). Le phosphore inorganique a diminué jusqu'à l'agnelage et a ensuite augmenté ($p < 0,001$). Les teneurs en sodium et en potassium ont atteint un pic avant l'agnelage ($p < 0,05$).

Discussion et conclusion

Les concentrations de glucose, L-lactate, AGNE, β HB, cholestérol, triglycérides, protéines totales, albumine et les valeurs des activités en AST et CK se situaient dans les intervalles de référence pour les ovins. Les concentrations en glucose, L-lactate, AGNE, β HB, fructosamine et potassium ont évolué avec le temps, atteignant un pic juste avant ou lors de l'agnelage. Il s'agit d'une réponse physiologique en réaction au «stress» de l'agnelage, en raison de l'augmentation des concentrations de cortisol, observée à cette période. Les concentrations en protéines totales et en globulines ont diminué significativement à l'agnelage, ceci étant probablement lié à la production en IgG du colostrum.

Les activités des AST et GGT ont été toutes deux faibles avant l'agnelage, et ont ensuite augmenté. Une augmentation en activité de l'AST est un indicateur de lésion musculaire et peut indiquer une mobilisation des protéines musculaires pour la lactation. L'activité de la GGT est un indicateur des lésions hépatiques, comme dans le cas de la stéatose hépatique, qui est souvent rencontrée en début de lactation. Cependant, si un processus de stéatose hépatique était présent chez les animaux de cette étude, elle serait très faible puisque d'autres paramètres, tels que l'urée ou l'albumine, qui peuvent également être utilisés comme indicateurs de stéatose hépatique, n'ont pas été affectés dans ce sens.

Des concentrations plasmatiques élevées en AGNE, signant un début possible de stéatose hépatique pourraient être liées aussi au statut d'immunosuppression propre à la péri-parturiente en période de *post-partum*. Les concentrations plasmatiques élevées en AGNE, signalant aussi une lipogenèse intense, ont été associées à une prolifération lymphocytaire réduite et à leur capacité à sécréter de l'immunoglobuline M et de l'interféron- γ en réponse à une stimulation polyclonale. Il a été proposé que les concentrations plasmatiques d'AGNE puissent être utilisées comme marqueurs diagnostics d'une immunité altérée et d'un risque accru d'infections chez la péri-parturiente.

Dans ce contexte, le glucane peut posséder une capacité à réguler le système immunitaire, en stimulant les activités antitumorales et anti-microbiennes de l'animal. Cependant, il est nécessaire d'évaluer tous les effets potentiels que le glucane aurait sur le métabolisme, en particulier sur le glucose, chez les péri-parturientes, car ces animaux sont confrontés à des défis métaboliques majeurs

au cours de cette période. En effet, l'un des principaux carburants des cellules impliquées dans le système immunologique est le glucose.

Les ruminants dépendent principalement de la gluconéogenèse hépatique pour leur apport en glucose puisque les taux d'absorption du glucose sont faibles. La synthèse de glucose hépatique augmente pour couvrir les besoins fœto-placentaires en fin de gestation, des systèmes immunologique et mammaire, même lorsque l'approvisionnement en précurseurs de glucose est faible. Dans le même temps, l'utilisation du glucose par le tissu adipeux et le muscle est réduite, ceci étant permis par une sensibilité et une réponse tissulaire à l'insuline diminuée. Le transport de glucose dans les tissus par le transporteur GLUT₄ est également diminué. À mesure que la lactation progresse, les tissus périphériques deviennent plus sensibles à l'insuline.

Les activités d'AST et GGT étaient significativement plus faibles dans le sang des brebis du groupe glucane, comparées au groupe contrôle. L'activité des AST est un indicateur de dommages musculaires et peut indiquer qu'il y a eu une mobilisation et catabolisme des protéines musculaires pour la lactation. L'activité en GGT est un indicateur de lésions hépatiques comme dans le cas de la lipidose hépatique, qui est souvent rencontrée lors d'une lactation précoce. Cependant, si la lipidose hépatique existait dans la présente expérience, elle serait très faible, car d'autres paramètres tels que l'urée ou l'albumine, qui peuvent également être utilisés comme indicateurs de la lipidose hépatique, n'ont pas été affectés de façon concomitante et significative.

En conclusion, l'administration intramusculaire hebdomadaire de β 1,3-glucane pendant la période du péri-partum chez les brebis Santa Inês n'a pas modifié le profil métabolique. Le β 1,3-glucane semble avoir un effet positif sur le métabolisme musculaire, en limitant le catabolisme musculaire pour satisfaire en partie les besoins lors de la lactation.

6.3 PAPIER 2

EFFETS D'UN ADDITIF A BASE DE SAPONINE SUR DEUX DIFFERENTS STATUTS METABOLIQUES CHEZ LA CHEVRE LAITIERE

Introduction

Les exigences nutritionnelles élevées augmentent le risque et l'impact des déséquilibres nutritionnels et des troubles métaboliques dans les troupeaux de petits ruminants. Ces troubles sont le résultat d'une augmentation des besoins en nutriments autour de la mise-bas. Cette période est marquée par des changements physiologiques, hormonaux, métaboliques et anatomiques intenses, associés à une diminution de l'ingestion, à une forte demande de nutriments, à des taux de croissance élevés du fœtus, puis à une production laitière élevée, qui entraînent tous un bilan énergétique négatif (BEN). L'intensité du BEN peut entraîner des troubles métaboliques tels que la toxémie de gestation et l'hypocalcémie chez les petits ruminants.

Actuellement en élevage, les stratégies alimentaires et sanitaires tendent à favoriser la prévention par rapport au curatif et à s'appuyer en première intention sur l'utilisation de substances naturelles, le plus souvent à base de plantes.

En effet, la plupart des plantes produisent et stockent des composés secondaires qui ne sont pas directement impliqués dans leur croissance, développement ou reproduction, mais pour se défendre vis-à-vis de différents stress abiotiques ou non. Certains de ces composés exercent des activités bénéfiques sur les organismes humains et animaux en raison de leurs propriétés antioxydantes, anti-infectieuses, anti-inflammatoires ou antinéoplasiques, de leurs effets favorables sur les maladies cardiovasculaires ou sur les processus digestifs.

Parmi ces composés secondaires, les saponines sont un groupe de glycosides stéroïdiens, qui peuvent être extraits de différentes plantes telles que *Yucca schidigera* (L.). Plusieurs études ont montré le rôle bénéfique des saponines dans l'efficacité digestive des ruminants, les performances zootechniques et la limitation des productions de méthane. Ces bénéfices seraient dus à leurs propriétés antimicrobiennes sélectives, inhibant préférentiellement les populations de protozoaires ciliés, de bactéries cellulolytiques et à activité peptidasique.

Objectifs

Le but de cette étude a été d'évaluer les effets d'un additif à base de saponines sur les performances et le métabolisme de chèvres laitières lors de deux statuts physiologiques différents: en milieu de lactation (période d'équilibre métabolique), et en fin de gestation/début de lactation (période de déséquilibre métabolique). L'originalité a été d'utiliser simultanément différentes approches analytiques complémentaires, ceci, afin de mieux comprendre les mécanismes sous-jacents des réponses des animaux et de valider l'utilité potentielle des saponines.

Matériel et Méthodes

Trente et une chèvres laitières (de races Saanen et Alpine, âgées de 2-3 ans) ont été utilisées sur l'ensemble des deux expériences, séparées en deux groupes : un groupe traité (S) recevant l'additif à base de saponines (issues de *Yucca schidigera* (L.)), mélangé à la ration complète (Total Mixed

Ration, TMR), à une dose journalière recommandée par le fabricant de 25 g/animal, et un groupe témoin (T) ne recevant que la TMR. Les animaux ont été pesés chaque semaine, les paramètres d'ambiance (température ambiante moyenne et hygrométrie (HR%)) ont été mesurés sur toute la durée des périodes expérimentales.

La première expérimentation a duré six semaines et a été réalisée sur 20 chèvres (groupe S, n = 10 et groupe T, n = 10), dont les poids vifs (PV) moyens et les productions laitières (PL) moyennes étaient homogènes (PV : $66,9 \pm 7,12$ kg, autour du 120^{ème} jour de lactation, PL : $4,4 \pm 0,72$ kg/jour). L'administration journalière de saponines a été réalisée durant les quatre semaines consécutives centrales (S2, S3, S4 et S5), les premières et dernières semaines (S1 et S6) en étant exemptes.

La deuxième expérimentation a duré sept semaines sur 24 chèvres gestantes multipares (groupe S, n = 12 et groupe T, n = 12). Cinq semaines avant la date prévue de la parturition, tous les animaux ont commencé à recevoir la TMR, puis le même additif à base de saponines a été administré au groupe T durant 4 semaines minimum jusqu'au jour de la parturition (P). Après la parturition, toutes les chèvres ont reçu de nouveau la TMR.

Au cours des deux expérimentations, des échantillons de sang et de jus de rumen ont été collectés toutes les semaines. Les teneurs plasmatiques en glucose, acides gras non estérifiés (AGNE), β -hydroxybutyrate (β HB) et urée ont été dosées dans les deux expérimentations ; les concentrations en cholestérol total (CT), bilirubine totale (BT), protéines totales (PT), albumine, lipoprotéines de haute densité (HDL), magnésium (Mg^{3+}), calcium total (CT), phosphore (PO_4^{3-}), haptoglobine (Hp), les activités des gamma glutamyl-transférase (GGT), phosphatase alcaline (PAL), aspartate aminotransférase (AST) ont été dosées seulement sur les chèvres gestantes.

Les échantillons de jus de rumen ont été prélevés par sonde œsophagienne. Le pH et l'ammoniac (NH_3) ont été mesurés immédiatement après les prélèvements, et un comptage des protozoaires a été effectué ultérieurement. Des mesures des acides gras volatils (AGV) ont été réalisées par chromatographie en phase gazeuse.

Les analyses statistiques ont été réalisées en utilisant un modèle mixte linéaire avec la procédure MIXED sous SAS pour des mesures répétées. Les valeurs de p ont été ajustées pour des comparaisons multiples entre les groupes en utilisant le test F. Le niveau de significativité pour tous les tests statistiques a été fixé à $\alpha = 0,05$, soit 5%.

Résultats

Les valeurs moyennes de température et d'hygrométrie hebdomadaires de la 1^{ère} expérimentation ont varié de $15,9 \pm 0,93^\circ C$ à $26,8 \pm 1,84^\circ C$ et de $58,4 \pm 6,95\%$ à $71,8 \pm 2,12\%$, respectivement. La température a atteint un pic exceptionnel lors de la dernière semaine de cette expérimentation. Lors de la seconde expérimentation, les valeurs moyennes de température et d'hygrométrie hebdomadaires ont oscillé entre $11,1 \pm 0,77^\circ C$ et $16,7 \pm 1,44^\circ C$ et entre $77,3 \pm 6,11\%$ et $89,4 \pm 6,02\%$ respectivement.

Les animaux ont été cliniquement sains pendant l'ensemble des deux périodes expérimentales et aucun signe clinique de toxémie de gestation n'a été détecté pendant la gestation ou après la parturition.

Concernant les performances, aucun effet de l'additif à base de saponines n'a été observé chez les chèvres en milieu de lactation ($p > 0,05$ pour PV et PL), ni en *péri-partum* ($p > 0,05$ pour PV). Cependant, un effet significatif du temps autour de la parturition a pu être souligné ($p < 0,0001$).

Concernant les métabolites biochimiques plasmatiques, aucun effet du traitement n'a été observé ($p > 0,005$) dans la 1^{ère} expérimentation, contrairement à un effet temps significatif pour les paramètres glucose, AGNE, β HB et urée ($p < 0,0001$). Les valeurs de β HB n'ont pas été significativement affectées. Le glucose a atteint sa plus faible concentration dans les deux groupes au cours de la dernière semaine de l'expérimentation ($p < 0,0001$). Les AGNE ont également été affectés par le temps ($p < 0,0001$), mais avec un pic dans les deux groupes à la fin de l'expérimentation.

Concernant la 2^{ème} expérimentation sur les chèvres autour de la parturition, il n'y a pas eu d'effet traitement sur les paramètres suivants : glucose, AGNE, β HB, CT, HDL, BT ($p > 0,05$). Cependant, un effet temps important a été observé sur le glucose ($p < 0,0001$) avec une augmentation après la parturition. Des effets significatifs du temps sur les AGNE ont été observés pendant les semaines suivant la parturition (P+1, P+2) dans les deux groupes ($p < 0,0001$). Les concentrations d'AGNE ont augmenté graduellement à partir de P-3 et ont atteint des pics à P+1 et P+2.

Les concentrations plasmatiques de β HB des deux groupes ont augmenté graduellement au cours du temps pendant la 2^{ème} période expérimentale et un effet temps a été observé également à P+1 et P+2 ($p < 0,0001$).

Les taux de TC et de HDL des deux groupes ont diminué au cours des trois premières semaines expérimentales, puis ont progressivement augmenté jusqu'à la dernière semaine d'expérimentation ($p < 0,0001$). En même temps, les concentrations plasmatiques de BT ont été plus élevées en *post-partum* que pendant la gestation ($p < 0,0001$).

Aucun effet traitement n'a été observé sur les PT, l'albumine, les globulines et la Hp ($p > 0,05$). Cependant, un effet du temps a été observé pour toutes les variables du profil protéique après la parturition ($p < 0,0001$). Les concentrations plasmatiques en PT, albumine et globuline dans la période *pré-partum* étaient plus faibles que dans la période *post-partum*. L'albumine et les PT étaient à leur plus haut niveau en P+1 et P+2 (41,9 et 81,9 g/L, respectivement). Les deux variables (PT et albumine) étaient les plus faibles en P-3 alors que les concentrations de globulines atteignaient un pic à P+2 (41,5 g/L). Les valeurs de Hp étaient significativement différentes avant et après la parturition ($p < 0,0001$). Les valeurs les plus élevées ont été observées après la parturition dans les deux groupes.

Concernant les concentrations en urée, aucune différence de traitement n'a été observée ($p > 0,05$) dans la 1^{ère} expérimentation, mais un effet temps (diminution des concentrations jusqu'en S4, puis augmentation jusqu'en S6 ($p < 0,0001$)) a été souligné. En revanche, dans la 2^{ème} expérimentation, une tendance a été observée entre les deux groupes de chèvres, où la concentration d'urée a été plus élevée dans le groupe saponine (S : 4,3 mmol/L et T : 3,7 mmol/L, $p = 0,054$). En outre, un effet temps a été également observé ($p < 0,0001$) avec une diminution jusqu'à P-3, suivie d'une augmentation des valeurs de prétraitement jusque P+2.

Aucun effet du traitement et du temps n'a été observé en ce qui concerne les activités plasmatiques en GGT et PAL ($p > 0,05$). L'activité des AST n'a également pas été affectée par le traitement ($p > 0,05$), mais les activités étaient plus élevées en *post-partum* qu'en *pré-partum* ($p < 0,0001$).

Il n'y a eu aucun effet des saponines sur les concentrations en Ca total, Mg^{3+} et PO_4^{3-} ($p > 0,05$). Cependant, un effet temps a été observé après la parturition avec une diminution de Ca total et PO_4^{3-} ($p = 0,0005$).

Dans la 1^{ère} expérimentation, la plupart des paramètres ruminiaux (pH, NH_3 et AGV) n'ont pas été affectés par les saponines ($p > 0,05$), à l'exception d'une forte tendance sur le rapport acétate/propionate ($S < T$, $p = 0,0570$). Tous ces paramètres ruminiaux ont été affectés par le temps ($p < 0,05$), sauf l'acide caproïque. Cet effet du temps a été principalement observé lors de la comparaison de la dernière semaine de l'expérimentation avec les autres semaines.

Dans la 2^{ème} expérimentation, aucun effet traitement n'a été noté sur les variables pH, NH_3 et AGV ($p > 0,05$). Les effets temps ont été observés sur le pH avec une diminution dans les deux groupes en P+1 ($p < 0,0001$), bien que les concentrations en NH_3 aient fluctué avec le temps dans les semaines précédant la parturition ($p < 0,0001$). Les niveaux d'AGV sont demeurés élevés autour de la parturition ($p < 0,0001$).

Aucun effet du traitement n'a pu être décelé sur le nombre de protozoaires dans la 1^{ère} expérimentation ($p > 0,05$) mais un effet temps en S4 a souligné des valeurs très faibles ($p = 0,0106$) dans les deux groupes T et S. Dans la 2^{ème} expérimentation, une tendance à un effet traitement a été observée sur le nombre de protozoaires ($p = 0,0960$) (valeurs plus élevées dans le groupe S par rapport au groupe T). Il n'y avait pas d'effet du temps ($p > 0,05$).

Discussion et conclusion

Aucun effet clair de l'additif à base de saponines sur les paramètres métaboliques ou ruminiaux n'a été observé, ni à mi-lactation (1^{ère} expérimentation) ni autour de la parturition (2^{ème} expérimentation). En effet, les résultats de la 1^{ère} expérimentation ne mettent en évidence que l'effet d'un pic de chaleur sur le métabolisme des animaux en dernière semaine. Dans la 2^{ème} expérimentation, un effet temps a été observé principalement en raison du déséquilibre physiologique dû à la parturition.

Une tendance à une augmentation du nombre de protozoaires a été observée dans la 2^{ème} expérimentation. Ceci est contraire à l'effet, généralement admis, des saponines dans la défaunation. Cependant, les doses de saponines utilisées dans les études précédentes étaient souvent élevées et certains auteurs ont également montré que de faibles teneurs en saponines augmentaient le nombre de protozoaires. L'effet de l'additif à base de saponines dans la présente expérimentation sur le nombre de protozoaires pourrait expliquer l'augmentation en NH_3 ruminal et, à son tour, les concentrations plasmatiques en urée. Il est connu que les protozoaires, en se nourrissant des bactéries ruminales, génèrent une augmentation en NH_3 en raison du catabolisme des protéines bactériennes.

Cependant, cette tendance avait déjà été détectée dans la première semaine de l'expérience et donc, avant le début d'administration de l'additif à base de saponines. Bien que les deux groupes aient été équilibrés pour les paramètres de production, il n'y avait pas d'équilibre pour les paramètres métaboliques, comme la concentration en urée.

Un autre facteur de confusion possible dans cette expérimentation pourrait être que le rapport fourrage/concentré a augmenté à mesure que la 2^{ème} expérimentation a progressé, pour des raisons zootechniques (nécessité de développer le volume du rumen). Or, la production de salive augmente

lorsque la ration est plus riche en fourrage. Cette salive pourrait être responsable de la neutralisation d'une partie de l'effet des saponines dans le rumen.

Dans la 1^{ère} expérimentation, aucun effet traitement sur les paramètres métaboliques ou ruminiaux n'a été observé, mais il existait une interaction entre le traitement et le temps concernant les paramètres acétate/propionate, et urée. Cependant, ces interactions sont principalement attribuables à une différence entre les traitements en S5, la semaine présentant un pic de chaleur. En outre, le rapport plus élevé d'acétate/propionate dans le groupe traité S par rapport au T a déjà été observé dans S1, avant le début du traitement. Par conséquent, aucun effet clair de l'additif n'a été montré dans ces deux expérimentations.

En conclusion, l'administration d'un additif à base de saponines à la dose de 25g/chèvre/jour pendant quatre semaines consécutives lors de deux statuts physiologiques différents (mi-lactation et *péri-partum*) n'a pas influencé significativement les métabolites plasmatiques, les paramètres de fermentation ruminale et les performances animales étudiées. Pour autant, il a pu être souligné une tendance quant à un effet des saponines sur l'urée sanguine, le nombre total de protozoaires et le rapport acétate/propionate. Ceci nous amène à émettre l'hypothèse que le potentiel d'un additif à base de saponines sur l'efficacité de la fermentation ruminale *via* une synthèse accrue de protéines microbiennes, dépendrait probablement de différents facteurs tels que le niveau de supplémentation, la période d'administration et la composition du régime de base.

6.4 DISCUSSION GÉNÉRALE ET PERSPECTIVES

Les objectifs de ce travail ont été de tester l'utilisation de deux substances naturelles (β -glucane et saponine) leurs propriétés biologiques et leurs relations avec le métabolisme et la performance chez les petits ruminants sous différents statuts métaboliques. Le challenge était de mieux comprendre les mécanismes sous-jacents des réponses des femelles face à un déséquilibre physiologique et/ou métabolique au cours du *peri-partum*.

A l'heure actuelle, les filières animales s'orientent vers l'utilisation en première intention d'alternatives aux méthodes allopathiques et vers une réduction du nombre d'additifs alimentaires de synthèse chez les animaux d'élevage. Cette volonté découle de préoccupations sanitaires et environnementales, dont des problématiques d'antibiorésistance et d'augmentation de résidus et contaminants dans les produits alimentaires et l'environnement.

En production animale, et en particulier chez les ruminants laitiers, la parturition est une période critique durant laquelle l'organisme animal est très sollicité, ce qui est alors propice à des désordres métaboliques, notamment énergétiques. L'utilisation de β -glucane et de substances à base de saponines, de par leur action potentielle sur les voies de métabolisme énergétique, pourrait être valorisée en prévention afin de minimiser les perturbations du métabolisme énergétique autour de la parturition. En effet, ces deux substances d'origine naturelle peuvent agir sur les teneurs plasmatiques en cholestérol et glucose chez les humains et les animaux, mais *via* des mécanismes encore à clarifier (Milgate & Roberts 1995; Hristov et al., 1999; Magnani & Castro-Gómez, 2008).

Les doses utilisées pour ces additifs ont été de 25 g/animal/jour (voie orale) pour l'additif à base de saponines et de 1 ml/animal/jour pour le β -glucane (voie intramusculaire), en accord avec les recommandations des fabricants. Il est difficile d'appréhender tous les effets des produits d'origine naturelle du fait de leur nature même, en général, des matrices complexes pouvant contenir différents

principes actifs pouvant agir en synergie/interaction. De ce fait, les doses efficaces et adaptées en fonction de l'espèce animale, de son âge, poids et statut physiologique sont particulièrement délicates à déterminer et les effets (directs et indirects) sur les animaux ne sont pas totalement élucidés.

Des études *in vitro* et *in vivo* ont été menées pour comprendre les effets des saponines (Jouany & Morgavi, 2007; Patra & Saxena, 2009a; Patra & Saxena, 2010; Bayourthe & Ali-Haimoud-Lekhal, 2014). Toutes ces études ont testé les effets de saponines en faisant varier les doses, les régimes associés, les plantes à l'origine des saponines, les voies d'administration et les espèces animales étudiées. En plus de ces facteurs de variation, les saponines pouvaient provenir de différentes parties de la plante : la plante entière (ou *totum* de la plante), des extraits de plante entière, ou des parties spécifiques comme les fruits, les graines et les racines.

Cette grande diversité dans la nature des saponines est le problème majeur en recherche des phytocomposés d'intérêt. En effet, il est clair qu'un mélange de saponines issues de sources diverses, présentera des variations subtiles de leurs structures et donc tout naturellement, donnera lieu à des variations importantes dans les propriétés physiques, chimiques et biologiques obtenues.

Globalement, la dose d'extrait de saponine utilisée issue de *Yucca schidigera* dans nos expérimentations a montré certains effets sur les paramètres métaboliques et ruminiaux. Dans la 1^{ère} expérimentation, un effet des saponines a été souligné, mais l'interaction entre les facteurs traitement et temps, notamment pour les paramètres urée plasmatique et rapport acétate/propionate ruminal complique l'interprétation. Dans la 2^{ème} expérimentation, une tendance à l'augmentation du nombre de protozoaires a, quant à elle, pu être observée.

Contrairement à nos résultats, une autre étude a montré une diminution de la population des protozoaires chez le mouton avec une dose similaire de saponines mais d'une origine botanique différente (*Sapindus saponaria* L.) (Diaz et al., 1993) et, après analyse d'un échantillon de contenu ruminal obtenu directement par canule ruminale, contrairement à nos prélèvements par sonde œsophagienne, pouvant potentiellement être contaminés par un excès de salive. Ces différentes observations montrent que les effets rapportés des saponines peuvent être variables et, parfois même contradictoires, du fait des très nombreux facteurs à prendre en compte.

Dans les deux expérimentations sur les effets des saponines, les groupes des animaux traités et témoins présentaient déjà une variabilité initiale différente de par leur quantité totale en protozoaires. En effet, l'attribution des chèvres dans les deux groupes n'a pas été effectuée sur des considérations de paramètres biochimiques mais sur des paramètres zootechniques, comme le poids vif, la production laitière, la parité et la race. Par conséquent, ce biais initial entre les groupes pourrait expliquer qu'une partie de nos résultats soit difficilement interprétable.

Un autre facteur limitant serait le faible nombre d'animaux étudiés. Bien que les règles de bioéthique tendent à réduire le nombre d'animaux utilisés en recherche expérimentale, cette réduction ne doit pas nuire à la significativité des résultats. Par conséquent, le nombre d'animaux retenus procède d'un choix issu de la prise en compte des paramètres mesurés et du test statistique utilisé pour l'analyse des données et l'interprétation des résultats.

Dans notre étude, n'ont été pris en compte principalement que des paramètres biochimiques liés au métabolisme énergétique de l'animal. Or, le système endocrinien intervient également lors des changements de statuts physiologiques comme lors de la gestation, de la mise-bas et de lactation. Ces

changements pourraient impliquer plusieurs organes et systèmes : le système immunitaire, réduisant la production de protéines d'inflammation de la phase aiguë et la diminution des activités leucocytaires, ce qui peut potentiellement entraîner d'autres troubles chez les ruminants sains.

Or, des études montrent que le β -glucane et les saponines ont un impact sur la capacité de modulation de la réponse immunitaire chez plusieurs espèces. Ainsi, l'administration orale ou par voie intramusculaire de ces substances pourrait moduler la réponse immunitaire, y compris améliorer la production d'anticorps et de cytokines chez les animaux (Oda et al., 2000; Petrovsky & Aguilar, 2004). De ce fait, il serait intéressant de mieux comprendre les modes d'action de ces substances naturelles sur le système immunitaire et, par exemple, sur le transfert passif de l'immunité des mères à leurs jeunes par l'absorption des immunoglobulines du colostrum.

Un autre domaine de prospection serait l'étude des effets de ces substances naturelles sur le profil hormonal, notamment sur l'insuline, le cortisol, le glucagon, les hormones thyroïdiennes, la leptine et leurs potentielles corrélations avec certains paramètres biochimiques. En effet, des études de l'impact de ces substances naturelles sur le système hormonal tendraient à montrer leur influence en *péri-partum*.

Un des objectifs de cette thèse était d'étudier l'effet de l'additif à base de saponines sur les performances et les paramètres biochimiques des chèvres durant deux statuts physiologiques : en milieu de lactation puis en fin de gestation/début de la lactation. Outre l'étude des réponses des animaux en termes de performances zootechniques (poids vif, production laitière) et de paramètres biochimiques classiques (glucose, urée, acides gras non estérifiés, beta-hydroxybutyrate, acides gras volatils ruminiaux,...) ou de populations microbiennes (comptage des protozoaires ruminiaux), l'ensemble des métabolites (métabolome) propres aux matrices plasmatique et ruminale a été analysé *via* une approche par métabonomique.

Les empreintes des profils des métabolites du plasma et du rumen ont été réalisées par spectroscopie à résonance magnétique nucléaire (RMN) du proton. L'utilisation de cette technique analytique particulièrement sensible pour étudier ces données multidimensionnelles a pour but d'accéder à toute modification, si infime soit-elle, et de façon quasi exhaustive des profils métaboliques des individus puis, de potentiellement remonter aux principales voies métaboliques affectées par les saponines et, enfin, si possible, d'identifier certains biomarqueurs potentiels de ces disruptions métaboliques.

En raison de la structure intrinsèque du plan expérimental (suivi longitudinal à deux périodes physiologiques, i.e., pendant la lactation, puis, en fin de gestation, combinée, dans chaque cas, à une supplémentation en saponine ou non), les données ont été analysées *via* des statistiques multidimensionnelles comprenant une analyse de corrélation canonique.

Les analyses des métabolites dans le plasma de la 2^{ème} expérimentation avec les chèvres gestantes montrent une faible différence entre les deux groupes, traités et témoins. En raison de la nature canonique de la modélisation statistique, nous avons également accès à la projection correspondante des variables factorielles. La représentation graphique a permis de visualiser une opposition claire entre la situation où les chèvres sont gestantes et celle où elles sont en lactation, de même qu'entre le groupe témoin et le groupe traité.

Les variables les plus corrélées à la différence de statut physiologique sont apparues comme étant l'acide thréonique, le L-arabitol, l'acide désoxycholique, la N-acétylmannosamine, la corticostérone,

l'acide 5-méthoxysalicylique et l'hydroxyphénylacétylglycine avec des valeurs supérieures après mise-bas.

Les métabolites, quant à eux, impliqués dans le traitement avec les saponines sont : le cortisol, l'acide oxoglutarique, la D-arginine, le lipoamide, la gamma-caprolactone, la N-acétylgalactosamine et la glycérophosphospholine (corrélés positivement au groupe de chèvres exposées à la saponine en période de *pré-partum*), tandis que l'acide 2-hydroxy-3-méthylbutyrique, la L-valine et la tétrahydrocortisone sont corrélées négativement.

Globalement, ces résultats préliminaires suggèrent que les données RMN issues de fluides biologiques, traitées grâce à des méthodes statistiques multidimensionnelles adéquates, peuvent être un atout pour mettre en évidence les influences subtiles d'un additif à base de saponines sur les voies métaboliques.

En conclusion, l'utilisation de substances naturelles en fin de gestation et début de lactation chez les brebis et les chèvres a entraîné quelques effets, mais qui n'étaient pas suffisamment significatifs pour conclure quant à l'efficacité véritable de ces substances naturelles pour prévenir les perturbations métaboliques en *péri-partum*. Un approfondissement de l'étude des données issues des différentes approches (biochimiques et métabonomiques) pourrait aider à mieux comprendre les voies métaboliques sous-jacentes chez les petits ruminants majoritairement impliquées pendant cette période de transition.

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8. ANNEXES

8.1 NOTIFICATION OF AUTHORIZATION OF PROJECTS USING ANIMALS FOR SCIENTIFIC PURPOSES

8.1.1 Ethical committee of glucan project

I- FORMULÁRIO UNIFICADO PARA SOLICITAÇÃO DE AUTORIZAÇÃO PARA USO DE ANIMAIS EM EXPERIMENTAÇÃO E/OU ENSINO

PROTOCOLO PARA USO DE ANIMAIS

USO EXCLUSIVO DA COMISSÃO
PROTOCOLO Nº 23082.013890/2013
RECEBIDO EM: 29/08/2013

LICENÇA Nº 004/2014

D02

No campo "fármaco", deve-se informar o(s) nome(s) do(s) princípio(s) ativo(s) com suas respectivas Denominação Comum Brasileira (DCB) ou Denominação Comum Internacional (DCI).

Lista das DCBs disponível em:
http://www.anvisa.gov.br/medicamentos/dcb/lista_dcb_2007.pdf.

1. FINALIDADE

Ensino	<input type="checkbox"/>
Pesquisa	<input checked="" type="checkbox"/>
Treinamento	<input type="checkbox"/>

Início: ---/03/2013

Término: ---/03/2017

2. TÍTULO DO PROJETO/AULA PRÁTICA/TREINAMENTO

Efeito da administração da β 1,3-glucana sobre o perfil metabólico de ovelhas da raça Santa Inês no periparto e suas consequências sobre os perfis bioquímico e imunológico dos borregos

Área do conhecimento: Clínica médica de ruminantes

Lista das áreas do conhecimento disponível em:

<http://www.cnpq.br/areasconhecimento/index.htm>.

3. RESPONSÁVEL

Nome completo	José Augusto Bastos Afonso da Silva
Instituição	Universidade Federal Rural de Pernambuco



eletroforético das proteínas séricas das ovelhas (mães) e dos borregos recém-nascidos será realizado, segundo a técnica de eletroforese em gel de agarose, de acordo com o kit comercial. Amostras de urina serão obtidas por meio de micção espontânea. A determinação do pH e qualitativa dos corpos cetônicos na urina das ovelhas será realizada empregando-se fitas reagentes comerciais. As análises das amostras de leite se iniciarão após a parição, uma vez ao dia, no momento do parto, 10, 20, 30 e 60 dias pós-parto, nelas serão realizadas a determinação da densidade, lactose, gordura, proteína, extrato seco total e do pH.

14. TERMO DE RESPONSABILIDADE

(LEIA CUIDADOSAMENTE ANTES DE ASSINAR)

Eu, José Augusto Bastos Afonso (nome do responsável), certifico que:

- li o disposto na Lei Federal 11.794, de 8 de outubro de 2008, e as demais normas aplicáveis à utilização de animais para o ensino e pesquisa, especialmente as resoluções do Conselho Nacional de Controle de Experimentação Animal – CONCEA;
- este estudo não é desnecessariamente duplicativo, tem mérito científico e que a equipe participante deste projeto/aula foi treinada e é competente para executar os procedimentos descritos neste protocolo;
- não existe método substitutivo que possa ser utilizado como uma alternativa ao projeto.

Assinatura:

Data:

José Augusto B. Afonso
José Augusto B. Afonso
Médico Veterinário
CRMV - PE - 1418
Clínica de Bovinos - UFRPE

Encaminhar em 2 vias.

A critério da CEUA, poderá ser solicitado o projeto, respeitando confidencialidade e conflito de interesses.

Quando cabível, anexar o termo de consentimento livre e esclarecido do proprietário ou responsável pelo animal.

15. RESOLUÇÃO DA COMISSÃO

A Comissão de Ética no uso de animais, na sua reunião de 13/01/2014, APROVOU os procedimentos éticos apresentados neste Protocolo.

Assinatura:

Coordenador da Comissão



8.1.2 Ethical committee of saponin project



MINISTÈRE DE L'ÉDUCATION NATIONALE,
DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE

Paris, le mercredi 9 septembre 2015

Direction générale
de la recherche
et de l'innovation

Service de la performance,
du financement et de la
contractualisation avec les
organismes de recherche

Département de la culture
scientifique et des relations
avec la société

Expérimentation animale -
Autorisation de projet

Affaire suivie par
Didier HOFFSCHIR
Conseiller scientifique
auprès du DGR

Florence HERVATIN
Chargée de mission

Téléphone
01 55 55 84 05
Fax
01 55 55 99 59
Mél.
florence.hervatin-queney
@recherche.gouv.fr

1 rue Descartes
75231 Paris Cedex 05

Objet : Notification d'autorisation de projet utilisant des animaux à des fins scientifiques

Madame, Monsieur

En application des dispositions du code rural et de la pêche maritime, notamment ses articles R. 214-87 à R.214-126, le projet :

- référencé sous le numéro 2015050418233758 v2 (APAFIS#601)
- ayant pour titre : « Etude des effets d'un additif à base de saponines sur le métabolisme de la chèvre laitière. Construction d'un index global de prédiction de sensibilité à la cétose »,
- déposé par l'Établissement Utilisateur : *Installation Expérimentale INRA Jouy en Josas*, numéro d'agrément A78-615-1002, dont le responsable est *Monsieur Benoit MALPAUX*
- et dont le responsable de la mise en œuvre générale du projet et de sa conformité à l'autorisation est *Madame Céline DOMANGE*,

est autorisé.

L'autorisation de projet est accordée, sous réserve de la validité de l'agrément de l'Établissement Utilisateur, pour une durée de 3 ans à partir du 09/09/2015

Le projet précité a été évalué sur le plan éthique par le Comité d'éthique en expérimentation animale n°45 et a reçu un avis *favorable*.

Ce projet ne fera pas l'objet, à l'issue de sa réalisation, d'une appréciation rétrospective.

Dans les conditions définies par l'article R.214-113 du décret n°2013-118 du 1^{er} février 2013, la réutilisation de tout ou partie des animaux d'un projet antérieur est autorisée.

Pour la ministre et par délégation,
L'adjointe au chef du service de la performance,
du financement et de la contractualisation avec les
organismes de recherche

Christine COSTE

Monsieur Benoit MALPAUX
Installation Expérimentale INRA - Jouy en Josas.

8.2 INSTRUCTIONS TO AUTHORS FOR THE PUBLICATIONS

8.2.1 *Revue de Médecine Vétérinaire*



Website for the Revue de Médecine Vétérinaire: <http://www.revmedvet.com/>

General:

The *Revue de Médecine Vétérinaire* publishes four kinds of text:

- 1) Scientific reviews on subjects related to veterinary and comparative medicine. Suggested length: 10 to 30 typed pages.
- 2) Original reports on fundamental or applied research. Suggested length: 10 to 15 typed pages.
- 3) Continuous education articles that should be easily understandable by non-specialists. Suggested length: 10 to 15 typed pages.
- 4) Clinical reports. Suggested length: 5 to 15 typed pages.

The publication can be done in French language or English language.

For an article written in English by not English native speaker authors, the manuscript must be subjected by attesting that it was read again by an Anglophone scientist or a scientific translator.

The authors must certify that the manuscript was not published or subjected for publication to another review.

The manuscript must be accompanied by a sheet signed by all the joint authors indicating their agreement for the tender of the manuscript.

The publication is free but a financial participation could be required for the photographs color. An estimate will be sent to collect the agreement of the authors.

Sending of the manuscripts:

The manuscripts must be sent by email with the format Word PC to the following address: ed.rmv@envt.fr.

The authors must make sure that the definition of the scanned documents is sufficient to provide an impression of quality (scanner minimal resolution of 300dpi recorded to format JPEG with a compression ratio of 15%).

The Editor in Chief will acknowledge receipt of the manuscript which will then be peer-reviewed. Authors will be notified of any recommendations for amendments and final acceptance will depend on application of these recommended modifications. Any reason for their non-application should be clearly stated and justified.

If electronic submission is not possible the following address can be used (send an original and 2 copies):

Editor in chief of the Revue de Médecine Vétérinaire
National school Veterinary of Toulouse
23 Chemin des Capelles
31076 Toulouse Cedex 03
FRANCE

General presentation:

The text is typed in double space with margins of at least 3,5 cm on the sides.

All the pages and all the lines are numbered.

The presentation is done according to the following order:

1 - Title | summary and keywords:

Page 1 of the manuscript indicates the title (in French and English), the name of the authors, the complete address of the establishments where work was carried out.

The author ensuring the correspondence must be clearly indicated thus that his address email.

The summary (roughly 150-250 words, in French and English) must be descriptive and emphasize the projecting points of the manuscript. For an original article, it must emphasize the aims of the study, the number of animals used, the material and methods, the results and conclusions. The key words (in French and English) must be selected carefully and be limited to ten.

The running title (in French and English) must be limited to 5 to 8 words.

No modification of this page will be possible once the accepted article.

2 - Article itself:

The text of the article must start on page 3.

For a scientific review, the introduction will emphasize the interest of the development, the text will be detailed and complete and the bibliography will be exhaustive.

For a report of original research, the introduction will locate the problem clearly, will emphasize the aims of the study and will quote only the essential references. "Material and methods" will describe the experimental procedure and the statistical methods used of such manner that another scientist can reproduce the results. If the techniques used were already described in an indexed review, this chapter will be very concise and will return to the bibliographical reference. The results will be presented in a logical and concise way, possibly in the form of tables or figures which will not have to make double employment. The discussion will be strictly limited to the subject, will emphasize the significant points and will propose adequate openings.

For a clinical case, the introduction will emphasize the interest for an expert, the commemorative ones and the procedures will be sufficiently detailed, the results will be described with exactitude, and the discussion will be relevant and consider the differential diagnosis. Illustrations are necessary.

For an article of continuing education, the introduction will emphasize the interest of the development, the text will be easily comprehensible for a non-specialist and the bibliography will be limited to some significant references. As far as possible, the text must be accompanied by a significant but nonredundant illustration (tables, figures, photographs).

3 - Bibliographical references :

The list of the references is presented alphabetically according to names' of authors and by chronological order for a given author. The titles of the periodicals must be shortened according to standards' of BioSciences Information Service (Biosis).

The style and the punctuation of the references must be in conformity with the following examples:

-Article (to indicate all the authors):

1. - TRAN CONG T., MILON A., BOURY M., TASCA C. : Colicines et effets de barrière écologique dans l'entérocolite à *Escherichia coli* O 103 du lapin sevré. *Revue Méd. Vét.*, 1992, **143**, 655-665.

Book:

2. - EUZEBY J. : Les échinococcoses animales et leurs relations avec les échinococcoses de l'homme, 163 pages, Vigot frères Éditeurs, Paris, 1971.

- Chapter of book:

3. - REGNIER A., TOUTAIN P.L. : Ocular pharmacology and therapeutic modalities. *In* : K.N. GELATT (éd.) : Veterinary ophtalmology, Lea & Febiger, Philadelphia, 1991, 162- 194.

In the text, the references are called by their sequence number between square brackets: [5], [5-8, 11], [6, 45, 78].

4 - Illustrations (photographs, figures and tables):

The illustrations are separated from the text. They are numbered in Arab numerals.

All the illustrations must be indexed in the text by call of their number. The legends are gathered at the end of the text of the manuscript.

They must be sufficiently detailed to make the illustrations to be comprehensible independently of the text.

To avoid a possible reduction, it is advised to provide illustrations whose width corresponds to a column (85 mm) or to two columns (180 mm).

The photographs and figures must be provided to format JPEG with a compression ratio of 15%. If a scanner is used for digitalization, the resolution used must be of 300dpi.

The photographs must be of good quality and integrate all the symbols, characters or arrows which appear in the legend.

The figures must be carried out in two dimensions, without background color or gray. The size of the characters used must be sufficient to allow the reading after reduction with a width of 85 mm (1 column) or 180 mm (2 columns).

Tables (Word PC) must be presented separated from the text according to the model below (single spaced):

	V. scophthalmi	V. aestuarianus	V. splendidus		V. vulnificus
			V. splendidus I	V. splendidus II	
ADH	+	+	(+)	V	-
LDC	-	V	-	-	+
ODC	-	-	-	-	+

Color should be used only for the photographs, if it is necessary to the comprehension of the manuscript. The figures and tables in color are not accepted. If the authors wish to publish colored figures or tables an overcost of impression will be invoiced to them (cf Cost of publication and Reprints).

Proofs:

Printing proofs (format pdf) are sent by Email to the author ensuring the correspondence. The author is requested to type the corrections to be carried out on a separate textual file and to return it within 3 day. If no correction is necessary this must be specified.

Only the corrections of form are allowed, they must be limited to the typographical corrections.

In the absence of answer of the authors within 3 days, the manuscript could be published without correction or its publication could be deferred.

Cost of publication and Reprints:

The publication of the manuscripts in black and white is free.

One board of photographs in color is offered to the author if they are necessary to the comprehension of the manuscript (cf. Illustrations).

Any other color publication must be required by the authors and will be invoiced (an estimate will be communicated).

The authors will receive for free a pdf file of their manuscript after its publication for scientific diffusion on individual request. The AUTHORS DO NOT HAVE THE RIGHT TO PRINT AND DIFFUSE THEIR MANUSCRIPT IN CONFERENCES OR FOR COMMERCIAL PURPOSES WITHOUT AUTHORIZATION. This kind of diffusion must be carried out by using Reprints provided by the *Revue de Médecine Vétérinaire*.

The authors who wish to obtain reprints must make the request of them BEFORE PUBLICATION OF the MANUSCRIPT (an estimate will be communicated).

8.3 THE PACKAGE LEAFLET OF β -GLUCAN MEDICINE

ESTA BULA É CONSTANTEMENTE ATUALIZADA. LEIA-A ATENTAMENTE ANTES DA ADMINISTRAÇÃO DO MEDICAMENTO.

Imunoglucan®

Glucana (β -1,3-D-glicopirranose)

FORMA FARMACÊUTICA E APRESENTAÇÃO

Suspensão injetável subcutânea e intramuscular. Frasco-ampola âmbar contendo 5mL.

VIAS DE ADMINISTRAÇÃO: Subcutânea e intramuscular

USO PEDIÁTRICO OU ADULTO

COMPOSIÇÃO

Cada frasco-ampola da suspensão injetável, com 5 mL, contém:

Glucana (β -1,3-D-glicopirranose) 10,0 mg

Excipientes: Intralipide®, cloreto de sódio, fenol, água estéril.

*Intralipide é composto por: óleo de soja, lecitina, glicerina, água para injeção.

INFORMAÇÕES AO PACIENTE

- Como este medicamento funciona?

Este medicamento é baseado em glucana, funciona como imunomodulador, aumentando as defesas do organismo.

- Por que este medicamento foi indicado?

Este medicamento está indicado para aumentar as defesas imunológicas do organismo.

- Quando não devo usar este medicamento?

Este medicamento é contraindicado em pacientes transplantados; nos três primeiros meses de gestação e nos casos em que houver formação de nódulos e/ou abscessos nos locais de aplicação.

Evitar a administração do produto na vigência de febre (aplicar só após baixar a temperatura).

*Não deve ser utilizado durante a gravidez e a amamentação, exceto sob orientação médica. Informe ao seu médico ou cirurgião-dentista se ocorrer gravidez ou se iniciar amamentação durante o uso deste medicamento.

*Este medicamento é contraindicado na faixa etária abaixo de 5 anos.

*Informe ao médico ou cirurgião-dentista o aparecimento de reações indesejáveis.

*Informe ao seu médico ou cirurgião-dentista se você está fazendo uso de algum outro medicamento.

*"Não use medicamento sem o conhecimento do seu médico. Pode ser perigoso para a sua saúde."

- Como devo usar este medicamento?

Suspensão estéril, levemente leitosa.

Este medicamento deve ser usado por via subcutânea ou intramuscular, e deve ser aplicado por profissional habilitado.

Uso adulto – Via intramuscular

Injetar 1 mL, de 7 em 7 dias, até terminar o conteúdo do frasco, ou a critério médico.

Uso pediátrico – Via subcutânea

Em crianças acima de 5 anos, injetar 0,25 mL, por via subcutânea, de 7 em 7 dias, durante cinco semanas ou a critério médico.

*Siga a orientação de seu médico, respeitando sempre os horários, as doses e a duração do tratamento.

*Não interrompa o tratamento sem o conhecimento do seu médico.

*Não use o medicamento com o prazo de validade vencido. Antes de usar, observe o aspecto do medicamento.

- Quais os males que este medicamento pode causar?

Pode apresentar febre (raramente), moleza no corpo, discreta dor de cabeça e muscular até 2 horas após a aplicação, geralmente de duração limitada. No local da aplicação pode ocorrer vermelhidão e dor, entre o 1º e o 6º dia. Com a continuação do uso, pode surgir nódulo e mesmo formação de abscesso, no entanto, ambos não possuem bactérias em seu interior. Portanto, devem-se tomar algumas medidas corretivas, conforme descrição abaixo.

Em caso da ocorrência de nódulo e/ou abscesso:

- Suspender a aplicação imediata da medicação;

- Não fazer uso de antibióticos, pois não se trata de processo infeccioso;

- Em caso de nódulos, colocar compressas geladas sobre os mesmos, várias vezes ao dia;

- Em caso de abscesso, que se rompa espontaneamente, cobrir o local com compressas de gaze para não haver possibilidade de contaminação externa.

Em pacientes com dermatite de contato, o uso deste medicamento pode exacerbar o quadro.

- O que fazer se alguém usar uma grande quantidade deste medicamento de uma só vez?

Caso ocorra aplicação acidental com dose muito acima do prescrito, suspender o tratamento e comunicar imediatamente ao médico. Neste caso, poderá ocorrer reação local com vermelhidão e dor.

- Onde e como devo guardar este medicamento?

Este medicamento deve ser guardado dentro da embalagem original, à temperatura entre 15 e 30 °C, ao abrigo da luz e umidade. Nessas condições, o prazo de validade do medicamento é de 24 meses, a partir da data de fabricação. Ao adquirir o medicamento, confira sempre o prazo de validade impresso na embalagem do produto. Nunca tome medicamento com prazo de validade vencido.

TODO MEDICAMENTO DEVE SER MANTIDO FORA DO ALCANCE DAS CRIANÇAS.

*Informe seu médico sobre qualquer medicamento que esteja usando, antes do início ou durante o tratamento. O uso deste, junto com outros medicamentos (principalmente antibióticos) deve ser orientado pelo médico.

NÃO TOME REMÉDIO SEM O CONHECIMENTO DO SEU MÉDICO, PODE SER PERIGOSO PARA A SAÚDE.

INFORMAÇÕES TÉCNICAS AOS PROFISSIONAIS DE SAÚDE

Imunoglucan® contém glucana (β -1,3-D-glicopirranose), biopolímero com peso molecular em torno de 6.500 Dalton, extraída da parede celular do *Saccharomyces cerevisiae*, através de hidrólise e processos físicos. A glucana não possui ação tóxica nem atividade imunogênica e, como propriedade farmacológica, estimula as defesas do sistema mononuclear fagocítico contra infecções por vírus, bactérias, protozoários e fungos patogênicos. Aumenta a imunocompetência do sistema imune; amplia a atividade dos macrófagos na modulação da resposta imune, de modo inespecífico; reforça o poder imunogênico do antígeno, quando injetado associado; modula a resposta humoral e celular, e estimula as respostas primárias e secundárias contra inúmeros antígenos. Estimula o sistema timo-dependente e aumenta as células formadoras de colônias de macrófagos e granulócitos do baço e da medula óssea. Estas propriedades indicam que a glucana, usada como agente imunoterápico, pode estimular o crescimento do número de células efectoras viáveis; produzir necroses de células tumorais por meio de injeções intralésionais e, reduzir, de modo significativo, o infiltrado de monócitos. As pesquisas indicam que o uso de glucana faz desaparecer a susceptibilidade às infecções dos animais tratados com ciclofosfamida; estimula a produção de IL-1, que amplia a resposta imune pela estimulação do T-helper; estimula os macrófagos do fígado a formar estruturas granulomatosas, retarda o crescimento de vários tipos de tumores (leucemia mielogênica aguda, adenocarcinomas, melanomas por células B), quando injetada por via intravenosa ou intralésional. A regressão dos tumores promovida pela glucana é sempre acompanhada por necrose das células tumorais e de infiltrado de células mononucleares. A glucana exerce ação anti-tumoral por vários mecanismos: estimulação do sistema mononuclear fagocítico, estimulação dos linfócitos Th1; proliferação do tecido de granulação fibroblástico; diminuição do tamanho dos granulomas existentes na esquistossomose, modificando sua morfologia e composição. Agrava as reações nos enxertos xenogênicos e ativa as reações enxerto-hospedeiro.

INDICAÇÕES

Imunoglucan® está indicado, como imunomodulador nos casos de tumores (por infiltração local); nas viroses crônicas, como Hepatites, Herpes (HSV-1 e HSV-2), HIV, HPV; Parasitoses; Neoplasias com e sem metástases; Micose profundas, como: Paracoccidiodoses, Criptococoses e Aspergiloses.

CONTRAINDICAÇÕES

Em caso de pacientes transplantados; nos três primeiros meses de gestação, nos casos em que houver formação de nódulos e/ou abscessos nos locais de aplicação e em crianças na faixa etária abaixo de 5 anos.

POSOLOGIA

ESTE MEDICAMENTO DEVE SER APLICADO POR PROFISSIONAL HABILITADO	
<ul style="list-style-type: none"> Agitar energicamente o frasco por pelo menos 3 minutos. Logo após a agitação, aspirar o conteúdo do frasco, utilizando "agulha de insulina" e seringa de 3 mL. Mudar de agulha e aplicar por via subcutânea (crianças) ou via intramuscular (adultos). 	
Crianças acima de 5 anos	Adultos
Injetar: <ul style="list-style-type: none"> Via subcutânea 0,25 mL Intervalo de 7 dias entre as aplicações durante 5 semanas 	Injetar: <ul style="list-style-type: none"> Via intramuscular 1 mL Intervalo de 7 dias entre as aplicações até o término do conteúdo do frasco
Realizar rodízio dos locais de aplicação. Não injetar no mesmo local de aplicações anteriores.	

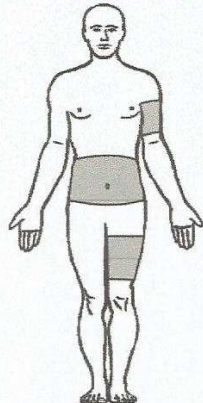
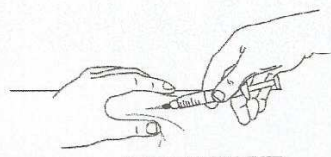
**AGITE ENERGICAMENTE O FRASCO
ANTES DA APLICAÇÃO DO PRODUTO.**

**O TEMPO RECOMENDADO DE
AGITAÇÃO DO FRASCO É DE NO
MÍNIMO 3 MINUTOS.**



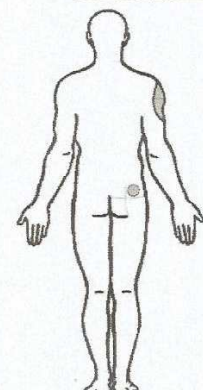
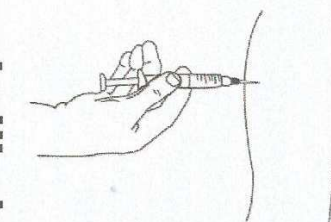
Aplicação Subcutânea

Locais de Aplicação



Aplicação Intramuscular

Locais de Aplicação



PACIENTES IDOSOS

Não existe restrição para o uso em pacientes idosos, na posologia recomendada.

INTERAÇÕES MEDICAMENTOSAS

A utilização concomitante de **Imunoglucan®** com outros medicamentos deve ser sempre feita com muita atenção. **Imunoglucan®** não deve ser misturado a outras drogas, na mesma seringa, durante a sua aplicação. Possíveis interações com fatores de crescimento hematopoiéticos não foram ainda investigados.

REAÇÕES ADVERSAS

Reações gerais: alguns pacientes podem apresentar febre e sensações de estado gripal, tais como: astenia, mialgia e cefaléias discretas, cerca de 2 horas após a aplicação, geralmente de duração limitada. A febre pode se fazer presente com baixa intensidade.

Reações cutâneas: pode ocorrer rubor e dor no local da aplicação, entre o 1º e o 6º dia. Isto se justifica, pois, com o uso prolongado do produto pode surgir, desde discreta hiperemia, passando a nodulação, podendo ocorrer até formação de abscesso. O **Imunoglucan®**, entre as suas várias ações, estimula os macrófagos a produzirem vários tipos de citocinas, algumas, localmente, podem levar à agressão, promovendo destruição tissular. Outras citocinas promovem migração dos leucócitos para o sítio estimulado, podendo favorecer reação local. Estas reações levam à formação de nódulo, que podem vir, inclusive, a romper para o exterior da pele, caracterizando abscesso. Ressalte-se que ambos - nódulo e abscesso - são estéreis (não possuem bactérias em seu interior). Portanto, devem ser tomadas algumas medidas corretivas conforme descrição abaixo:

CONDUTA EM CASO DE:	
NÓDULOS	ABSCESOS estéreis
<ul style="list-style-type: none"> Suspender a medicação de imediato; Colocar compressas <u>geladas</u> sobre a área do nódulo; Não fazer uso de antibióticos (não se trata de processo infeccioso). 	<ul style="list-style-type: none"> Suspender a medicação de imediato; Realizar drenagem cirúrgica dos abscessos que apresentem flutuação, deixando dreno laminar por 24 a 72 horas; Não fazer uso de antibióticos (não se trata de processo infeccioso); Cobrir o local abscedado com compressas de gaze, para não haver possibilidade de contaminação externa.

Em pacientes com AIDS, tem sido relatado o aparecimento de hiperqueratose palmar e plantar. Em portadores de dermatite de contato, o uso de **Imunoglucan®** pode exacerbar o quadro.

SUPERDOSAGEM

Caso ocorra uma superdosagem acidental, suspender o tratamento e comunicar imediatamente ao médico.

CUIDADOS DE ARMAZENAGEM

Este medicamento deve ser guardado dentro da embalagem original, à temperatura entre 15 e 30 °C, ao abrigo da luz e umidade.

CUIDADOS NA APLICAÇÃO

**ESTE MEDICAMENTO DEVE SER APLICADO POR
PROFISSIONAL HABILITADO**

- Agitar energicamente o frasco por pelo menos 3 minutos.
- Logo após a agitação, aspirar o conteúdo do frasco, utilizando "agulha de insulina" e seringa de 3 mL.
- Mudar de agulha e aplicar por via subcutânea (crianças) ou via intramuscular (adultos).

Via subcutânea	Via intramuscular
Aplicar: <ul style="list-style-type: none"> Face interna dos braços na região intermediária compreendida entre o 1/3 acima da dobra do cotovelo e o 1/3 abaixo da axila. Face interna das coxas na região intermediária compreendida entre o 1/3 acima do joelho e o 1/3 abaixo da virilha. Abdome. 	Aplicar: <ul style="list-style-type: none"> Região do deltóide (face superior externa do braço). Região dos glúteos (usar agulha mais longa, no quadrante superior externo).
Realizar rodízio dos locais de aplicação. Não aplicar no mesmo local de aplicações anteriores.	

PRECAUÇÕES E ADVERTÊNCIAS

Durante o estado febril, evitar a administração do produto. Na gravidez e lactação, o **Imunoglucan®** deve ser administrado somente sob orientação médica.

M.S.1.1557.0048.001-2

Farm. Resp.: Rosa Lúcia Carneiro da Silva - CRF-PE 1938

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Title: Improving the success of the critical peri-partum transition period in small ruminants: access to physiological and metabolic disturbances and prospection of effects of natural products to prevent them

Keywords: Metabolism; β 1,3-glucan; Saponin; Goat; Ewe; *Peri-partum*

Abstract:

The *peri-partum* period is a critical time for small ruminants because nutritional requirements are very high and feed intake is often limited. Nutritional diseases, such as pregnancy toxemia, can develop. Farmers try to limit these problems. β -glucan and saponin-based plant extracts have been shown to have favourable effects on animal production: immune-modulation for the former and reduced methane production and modification of rumen fermentation patterns for the latter. However, little is known about their effects on metabolism. Therefore, the general objective was to better understand the effects of 1) β -glucan injections and 2) dietary saponin, on metabolism and rumen fermentation in small ruminants during the transition period. The influence of β 1,3-glucan (i.m. injections, 1ml/animal/week for 60 days) on the metabolic profile of 14 Santa Inês ewes in the last weeks of gestation was studied. Two groups of ewes were formed in late gestation: a glucan group (G, n=7) and a control group (C, n=7). Nutritional requirements during the experiment were covered. Blood samples were collected throughout the study. Blood samples were analysed for metabolites, ions and enzymes. All ewes were clinically healthy. Glucose, L-lactate, non-esterified fatty acids and β -hydroxybutyrate peaked at parturition ($p<0.05$). Aspartate aminotransferase (AST) was higher in C vs. G ($p<0.05$) and there was a similar trend for fructosamine and albumin ($p<0.10$). Urea tended to be higher in G vs. C ($p<0.10$). In conclusion, the changes with time were consistent with a change in physiological status (gestation/lactation). β 1,3-glucan appeared to protect muscle and liver because AST levels were lower than in controls and it did not negatively affect metabolism. The influence of a dietary saponin-based additive (25g saponin/animal/day) on the metabolic and rumen fermentation profile of Saanen and Alpine dairy goats during two different metabolic situations: metabolic neutrality (mid-lactation) and metabolic imbalance (pregnancy/lactation) was studied in two experiments. Experiment 1, used mid-lactating goats (Saponin group (S), n=10; Control group (C), n=10). The 6 week experiment was divided into: 1 week of C diet, 4 weeks either S or C and 1 week C. Experiment 2, used peri-parturient goats (Saponin group (S), n=12; Control group (C), n=12). The 7 week experiment was divided into: 1 week C diet, 4 weeks either S or C (stopping at parturition) and 2 weeks C. Nutritional requirements during the experiments were covered. Blood samples and rumen fluid were collected throughout both experiments. Blood samples were analysed for metabolites, ions and enzymes. All the goats were clinically healthy. There was no effect of saponin in experiment 1 on animal husbandry measures, plasma metabolites and ruminal fermentation ($p>0.05$). The ruminal acetate to propionate ratio tended to be affected by treatment ($S < C$; $p=0.057$). There was no effect of saponin in experiment 2 on blood metabolites ($p>0.05$), except for plasma urea ($S > C$; $p=0.054$). Total protozoa numbers tended to be affected by treatment ($S > C$; $p<0.10$). Most of the animal husbandry, plasma and ruminal variables showed a time effect around parturition ($p<0.05$). In conclusion, dietary saponin during mid-lactation or the *peri-partum* period had little effect on metabolism and ruminal fermentation. The trends observed in blood urea, total protozoa count and acetate/propionate ratio could be potentially beneficial. Lastly, the potential for saponin to influence ruminal fermentation may depend on the level, the period of administration and the type of diet. β -glucan and saponin did not have a negative effect on metabolism and it would be interesting to conduct further work in animals suffering from pregnancy toxemia. Studies could also be conducted on the possible immune actions of β -glucan and saponin.

Titre : Améliorer le succès de la période critique de transition du peri-partum chez les petits ruminants: perturbations physiologiques et métaboliques et prospection des effets de produits naturels pour les éviter

Mots-clés : Métabolisme; β 1,3-glucane; Saponine; Chèvre; Brebis; Péri-partum

Résumé :

Le péri-partum est une période critique chez les petits ruminants car les besoins nutritionnels sont élevés et l'apport alimentaire souvent limité. Des maladies nutritionnelles comme la toxémie de gestation peuvent survenir et doivent être évitées. Des produits d'origine naturelle comme le β -glucane ou des extraits de plantes à base de saponine peuvent favoriser sur la production : modulation immunitaire dans le 1^{er} cas, réduction des émissions de méthane et modifications du profil fermentaire dans le 2nd. Cependant, leurs effets sur le métabolisme sont mal connus. L'objectif était de mieux comprendre les effets 1) d'injections de β -glucane et 2) de saponine alimentaire, sur le métabolisme et la fermentation ruminale de petits ruminants pendant la transition gestation/lactation. L'influence du β 1,3-glucane (1ml IM/animal/semaine pendant 60 jours) sur le profil métabolique de brebis Santa Inês a été étudiée en fin de gestation (groupes Glucane (G, n = 7) et Contrôle (C, n = 7)). Les besoins nutritionnels étaient couverts. Les métabolites, électrolytes et enzymes plasmatiques ont été dosés. Toutes les brebis étaient cliniquement en bonne santé. Le glucose, le L-lactate, les acides gras non-estérifiés et le β -hydroxy-butyrat ont atteint un pic à la parturition ($p < 0,05$). L'aspartate aminotransférase était plus élevée dans le groupe C vs G ($p < 0,05$) avec une tendance similaire pour la fructosamine et l'albumine ($p < 0,10$). L'urémie tendait à être plus élevée dans le groupe G vs C ($p < 0,10$). En conclusion, l'évolution dans le temps était cohérente avec une modification du statut physiologique (gestation/lactation). Le β 1,3-glucane a semblé protéger le muscle et le foie, les taux d'AST étant inférieurs à ceux des contrôles. Il n'y a pas eu d'effet négatif sur le métabolisme. L'influence d'un additif alimentaire à base de saponine (25g/animal/jour) sur le profil métabolique et de fermentation ruminale de chèvres Saanen et alpines a été étudiée dans deux expérimentations : équilibre (mi-lactation) ou déséquilibre (gestation/lactation) métabolique. Dans la 1^{ère}, des chèvres en mi-lactation ont été utilisées (groupes Saponine (S, n=10) et Contrôle (C, n = 10)). Les 6 semaines ont été divisées en 1 semaine de régime C, 4 semaines soit S soit C et 1 semaine C. Dans la 2^{nde}, des chèvres péri-parturientes ont été utilisées (groupes Saponine (S, n = 12) et Contrôle (C, n = 12)). Les 7 semaines ont été divisées en 1 semaine de régime C, 4 semaines soit S soit C (arrêt à la parturition) et 2 semaines C. Les besoins nutritionnels étaient couverts. Du sang et du contenu ruminal ont été prélevés dans les deux cas. Les métabolites, les ions et les enzymes plasmatiques ont été dosés. Toutes les chèvres étaient cliniquement saines. Dans l'expérience 1, il n'y a eu aucun effet de la saponine sur les paramètres de production, les métabolites plasmatiques et la fermentation ruminale ($p > 0,05$). Le rapport acétate/propionate du rumen a eu tendance à être réduit ($S < C$; $p=0,057$). Dans l'expérience 2, il n'y a pas eu d'effet de la saponine sur les métabolites sanguins ($p > 0,05$), sauf pour l'urémie ($S > C$; $p=0,054$). Le nombre de protozoaires a eu tendance à être augmenté ($S > C$, $p < 0,10$). La plupart des variables de production, plasmatiques et ruminales ont présenté un effet temps autour de la parturition ($p < 0,05$). En conclusion, la saponine alimentaire en mi-lactation ou en péri-partum a eu peu d'effets sur le métabolisme et la fermentation ruminale. Les tendances observées pour l'urémie, le nombre de protozoaires et le rapport acétate/propionate pourraient être potentiellement bénéfiques. L'effet de la saponine sur la fermentation ruminale pourrait dépendre de la dose, de la période d'administration et du type de régime. Le β -glucane et les saponines n'ont pas eu d'effets négatifs sur le métabolisme. Il serait intéressant de réaliser d'autres études chez des animaux atteints de toxémie de gestation ou d'évaluer les actions immunitaires des deux produits.