REPORT of STSM ACTIVITY

STSM Topic: Assess the saponins effects on rumen metabolism of peri-partum dairy goats, by metabonomic approach
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The purpose of the STSM

In having a sustainable farming it is important to limit or anticipate nutritional and metabolic imbalances of the animals related to their physiological disorders. The time of peri-parturition for dairy ruminants is a critical physiological period. During early lactation, these animals experience an enormous energy and nutrient demand but they cannot consume enough feed to meet nutrient requirements. As a consequence, animals drop into negative energy balance and mobilize body reserves including muscle protein and glycogen for milk production, direct oxidation, and hepatic gluconeogenesis (Kuhla, 2011) and the risk of pregnancy toxemia in late gestation is high due to the very important foetal glucose requirements. The onset of milk production initiate the mobilization of body fat resulting in an increased hepatic oxidative metabolism, which has been suggested to signal for depressing hunger and ketosis after calving (Schäff, 2012). For avoiding these severe health consequences, the dietary additives are used and the benefits may be improving both the health and welfare of dairy animals and also minimizing the negative impact of animal production on the environment. In present, chemical origin additive like propylene glycol (PG) are usually used by farmers in preventing ketosis as a glucose precursor. Recently, saponins have received some attention as natural feed additives. They are extracted mainly from *Yucca sp.* plants and have been studied for their effects on reducing methanogenesis. But saponins can also increase the ruminal propionate concentrations and may help alleviate the glucose needs by supplying more precursors for gluconeogenesis. This beneficial effect on energetic metabolism during the stage of metabolic imbalance like peri-parturition needs to be checked. This study is a dynamic prospecting view about the metabolic disturbances during peri-partum period in dairy goats and the putative effect of a plant additive by

using a metabonomic approach. Metabonomics can be defined as a quantitative study of the metabolites (molecules of less than 1500 daltons) in a biological system, and of changes in their concentrations or flows in connection with the physiological needs, genetic disorders or the environmental influences (Dunn, 2011). Here, the ^{1}H-NMR spectroscopy technique was used for achieving the metabolic fingerprint or "fingerprinting" and then for comparing the two profiles of metabolites of the dairy goat (period of equilibrium in middle/late lactation) and of the peri-partum goats (period of physiological disorder). Looking for the discriminating metabolites that can be found in the disturbed metabolic period it can be possible to finely detect metabolic imbalances (before detection by conventional measures or before the appearance of clinical signs) and simultaneously, to test the putative beneficial effect of plant additive. The purpose and the originality of this study is to integrate different types of data that will be obtained (metabolic, biochemical and animal husbandry) in an overall index for the prediction of ruminal and systemic metabolic disturbances in the dairy goat model.

Description of the work carried out

In idea of having two metabolic fingerprints of dairy goats, two experiments were provided. The first experiment (named 1503_CD and fulfilled in May-July of 2015) was designed for studying the effects of the plant additive on the metabolism (rumen + systemic blood circulation) of dairy goat during the period of middle / late lactation. The second experiment (named 1505_CD and fulfilled in November 2015 – January 2016) evaluated the same additive from the late gestation until the beginning of lactation period when an increased energy need is recorded.

Experiment 1 – lactating goats
Experiment 2 – peri-partum goats

Experimental design

The Experiment 1 was carried out using 20 Alpine and Saanen dairy goats in the middle lactation period. They were divided in 2 balanced groups of 10 animals each:
- (1) group with plant additive in the standard diet (25 g/animal/day)
- (2) control group: without additive, only standard diet.

Goats have been housed in individual cages (1,20m x 0,75m, equipped with devices for continuously measuring their intake of feed and water) enabling tactile contact with the neighboring counterparts. They have been observed daily in their behavior, and the amounts of feed and water intake for each goat was measured and recorded in order to calculate refusals (about 10 to 15% of administered quantity) and intake kinetics. Other measurements were recorded every 2 hours for the temperature and humidity in the farm building and daily for the milk production and the body weight of goats. The variability of responses of these kinetic data must be taken in account for multivariate statistical analysis and for the implementation of the overall index.

The standard diet ration of the goats included: 28% Rumiluz (dehydrated long-strand alfalfa), 15% concentrate Fluvia (Sanders company), 5% concentrated defatted rape, 24% prairie hay and 28% overpressed beet pulp (20%) and was distributed twice times during the day (1/3 of the amount in the morning, and 2/3 in the afternoon). A sampling of the ration and also a sampling of the refusals (24 hours after the distribution of the evening

ratio) was done weekly. A sampling of different raw materials of the ratio was made at the beginning and end of the experiment. The water was available *ad libitum*. The laboratory tests of dry matter (DM), crude protein/nitrogen, starch, ash and NDF/ADF/ADL (neutral detergent fiber/acid detergent fiber and lignine by Van Soest method) were done for rations and only NDF/ADF/ADL for the refusals. Also, for each goat at each milking, the measurement of milk production and the measurement of body weight were recorded.

The procedure of experiment 1 lasted 6 weeks including:
- 1 week before starting the experiment in which goats had to get used to individual cages and received the standard diet.
- 4 weeks of regime (with or without plant additive) to view the adaptation and the specific metabolic pathways orientations after this dietary change. The additive administrations were dusted in the form of powder in individual bowls to ensure the intake by each animal.
- 1 last week when all the goats have returned to the standard diet, in order to visualize or not a possible resilience of the metabolism.

Weekly sampling of blood, rumen liquid and milk have been done. 10 ml were collected in the jugular vein per animal on heparin tube - (before (Time T0) and 2 hours (Time T2) after distribution of feed) then centrifuged in order to get plasma (2 ml stored at -80°C for the subsequent analysis: ^{1}H-NMR spectroscopy and the rest stored at -20°C for biochemical classical analysis (glucose, urea, non-esterified fatty acids or NEFA, beta-hydroxy-butyrate or BHB),immunoglobulins G and haptoglobin analysis.

Around 50 ml of rumen liquid was collected by stomach tube, concomitantly with blood samples (time T0 and time T2, Table 1) and conserved for subsequent analysis of the rumen metabolism: ^{1} H-NMR spectroscopy (2 ml stored at -80°C), the protozoa count and the composition of volatile fatty acids (VFA). In the tubes for VFA, 25% H_3PO_4 was added and samples were stored at -20°C. The pH and ammonia were immediately measured after collecting (table 3). Taking in consideration the above mentions, six sampling of rumen liquid have been done for each goat, and for each of them, 2 times of collecting (table 1).

Table 1. Sampling of ruminal liquid and blood in experiment 1

week	days	time
Sampling W1	2015.05.20	T0 and T2
Sampling W2	2015.05.(26 and 27)	T0 and T2
Sampling W3	2015.06.(03 and 02)	T0 and T2
Sampling W4	2015.06.(11 and 12)	T0 and T2
Sampling W5	2015.06.(17 and 18)	T0 and T2
Sampling W6	2015.07.02	T0 and T2
TOTAL		240 samples of rumen liquid 240 samples of plasma

T0=before feeding, T2=2 h after feeding

The milk samples were obtained from two successive milkings in every week. 10 ml of milk were separated into 2 aliquots (one stored at -20°C for measurement of protein and butterfat content, and the other (1-2ml) stored at -80°C for subsequent analysis by ^{1}H-NMR spectroscopy for metabolic profile.

In Experiment 2, a total of 24 goats in gestation have been allocated into 2 balanced groups of 12 goats, according to body weight, race, age, average number of kids, and randomly assigned to the standard diet with plant supplement and to standard diet, respectively. A blood test was performed at day 90 of gestation for each goat to measure PSPB (Pregnancy Specific Protein B) or PAG (Pregnancy Associated Glycoprotein) and so to estimate the number of foetus. Among these 24 goats, were goats in gestation which have undergone previous experiment on plant additive in June 2015 with the same distribution of animals in treated/control groups.
Goats have been housed together because of ethically reasons during gestation but housed in individual pens during samplings.

The experimental design of experiment 2 was similar with experiment 1.The proceed thus lasted 9 weeks including:
- 1 week before starting the experiment in which all the goats received only the basic diet
- a first period of 5-6 week (in fact, until delivery of each goat) antepartum when the groups were fed with or without plant additive to view the adaptation and specific metabolic pathways
- around 2 weeks after delivery when all the goats received only standard diet to visualize or not a possible resilience of metabolism.

Both diets have been offered *ad libitum* twice daily with a free access to water, microminerals and vitamin blocks. A total of 7 samplings were done: sampling 1–5: before parturition, and sampling 6-7: after parturition (no additive for both groups). Similar weekly sampling of blood and rumen liquid and similar tests have been done as in experiment 1 (table 2 and 3). After delivery, the first colostrum was collected from each goat and also, a sample of colostrum mixture (after inactivation 1h at 56°C) was realised for biochemical, immunological tests and ^{1}H-NMR test.

The new-born kids were separated from their mothers soon after birth, weighed and identified. A blood test has been performed before the first suckling. Another blood sample was collected until 24h of age for biochemical, immunological tests and ^{1}H-NMR tests. Over the experiment measurements of the live weight of goats once a week and measurements of body weight of the kids at delivery and about 24 hours after delivery were carried out.

Table 2. Sampling of ruminal liquid and blood in experiment 2

Week	days	time
Sampling W1	2015.11.30	T0 and T2
Sampling W2	2015.12.(07 and 08)	T0 and T2
Sampling W3	2015.12.(15 and 16)	T0 and T2
Sampling W4	2015.12.(22 and 23)	T0 and T2
Sampling W5	2015.12.(29 and 30)	T0 and T2
Sampling W6	2016.01.(14 and 15)	T0 and T2
Sampling W7	2016.01.(22 and 21)	T0 and T2
TOTAL		336 samples of rumen liquid 336 samples of plasma

T0=before feeding, T2=2 h after feeding

Table 3. The planned analyses for each sampling in experiment 1 and 2

experiment 1	**RUMINAL LIQUID**	**PLASMA**	**MILK**	
	pH	glucose	TP	
	ammonia	urea	TB	
	VFA	BHB		
	protozoa number	NEFA		
	^{1}H-RMN	^{1}H-RMN	^{1}H-RMN	
experiment 2	**RUMINAL LIQUID**	**PLASMA**	**COLOSTRUM**	**PLASMA of new-born kids**
	pH	glucose		glucose
	ammonia	urea		urea
	VFA	BHB		BHB
	protozoa number	NEFA		NEFA
		IgG	IgG	IgG
		Cholesterol		
		Bilirubin		
		HDL		
		Magnesium		
		Calcium		
		GGT		
		ALP/ (alkaline phosphatase)		
		AST/GOT (aspartate aminotransferase)		
		Haptoglobin		
		pregnancy test (PSPB) in day 90 of gestation		
	^{1}H-RMN	^{1}H-RMN	^{1}H-RMN	^{1}H-RMN

^{1}H-NMR spectroscopy measurement

The NMR measurements were performed at 298 K on a Bruker Avance III HD 600 NMR Spectrometer (Bruker BioSpin, Germany), operating at a ^{1}H frequency of 600.19 MHz, and equipped with a standard 5-mm ^{1}H TCI CryoProbe. Prior to the measurements, plasma samples were thawed. The skim milk samples and also, the ruminal liquid samples must thawed and thoroughly shaken to be homogenized. Subsequently, the samples must be filtered to remove residual lipids and proteins using a Amicon filter 10-kDa cut-off. For analysis 150 µl aliquots of plasma were mixed with 450 µl D_2O.

Standard 1-dimensional spectra were acquired using a single 90^{O} pulse experiment with a relaxation delay D1 of 2.5 s. Water suppression was achieved by irradiating the water peak during the relaxation delay, and a total of 256 scans were collected into 32768 data points spanning a spectral width of 11 ppm. The whole parameters were:

-tilt angle = 90^{O}
-number of data points (TD) = 32768
-number of scans (NS) = 256
-spectral width (SW) = 11 ppm
-acquisition time (AQ) = 2.45 s
-relaxation delay (D1) = 2.5 s
-receiver gain (RG) = 362
-centre of window (OSP) = 4.709 ppm

Prior to Fourier transformation, the data were multiplied by a 0.3 Hz line-broadening function. The ^{1}H-NMR spectra were phase and baseline corrected manually using Topspin 3.2.1 software (Bruker BioSpin).

A short overview of processing data in ^{1}H-NMR spectroscopy

The large amount of data obtained by this technique requires a flow of data processing procedures (conversion, alignment, bucketing and normalizations, etc) in order to create matrix data for statistical analysis and interpretation. Untargeted metabolomic studies like this one, are characterized by simultaneous measurements of metabolites from blood or other biofluids, as a top-down strategy, without considering a particular set of metabolites, but focusing on a global metabolomic profile (fingerprint). Such studies generate "big data", in terms of not only their volume, but also their complexity, implying a need for high performance bioinformatics tools (Lazar, 2015).
RAW DATA ACQUISITION is made by analytical techniques like ^{1}H-NMR spectroscopy using the software of the spectrometer.
DATA PROCESSING. It is necessary to guarantee the baseline correction, alignment of retention times, the elimination of noise and outliers, peak detection, identification and then normalization against specific molecules (or internal standards), creating specific matrices (buckets) to be processed statistically. Data conversion is the first step in data processing binary format files, using different algorithms. There are several softwares which can be utilized for pre-processing, such as Chenomx NMR Suite which is the current state of art in metabolomics and is used also for statistical evaluation of data. Peak detection step means to identify and quantify the features present in the spectra and spectral alignment step means to position the peaks corresponding to the same metabolic

feature which may be affected by non-linear shifts when analyzing multiple spectra. It is a time consuming manual process and also, requiring manual validation. The 1-D methods are used (e.g. NOESY) but 2-D (e.g. COSY), 3-D, 4-D methods are continuously developed. The result is the metabolite profiling for the samples in concordance with metabolic pathways of aminoacids, carbohydrates, lipids, energy, cofactors and vitamins, nucleotides, and for tricarboxylic acids cycle.

DATA ANALYSIS by CHEMOMETRICS or STATISTICAL EVALUATION (Lazar, 2015).

In **unsupervised methods**, the similarity patterns within the data are identified without taking into account the type or class of the metabolite. These are often applied to summarize the complex metabolomic data. They provide an effective way to detect data patterns to be correlated with experimental and/or biological variables. Principal component analysis (PCA) is the most commonly used unsupervised method; PCA is based on the linear transformation of the metabolic features into a set of linearly uncorrelated (i.e., orthogonal) variables known as principal components. This decomposition method maximizes the variance explained by the first component, while the subsequent components explain increasingly reduced amounts of variance. PCA makes clusters of samples (scores) and identifies metabolites responsible for the group clustering (loadings). At the same time, PCA minimizes the covariance between these components.

Supervised methods are used to identify metabolic patterns that are correlated with the phenotypic variable of interest while suppressing other sources of variance. Partial least squares (PLS) is one of the most widely used supervised method in metabolomics, being used either as a regression analysis (i.e., quantitative variable of interest) or as a binary classifier (PLS-DA). Unlike PCA, PLS components do not maximize the explained dataset variance but the covariance between the variable of interest and the metabolomic data.

However, one weakness of PLS is that some metabolic features that are not correlated with the variable of interest can influence the results. To surpass this problem, orthogonal PLS (O-PLS) was developed, as a model which factorizes the data variance into two components: a first component correlated with the variable of interest, and a second uncorrelated component (i.e., orthogonal). Classification of metabolomics samples is commonly performed by fitting the discriminant analysis versions of PLS and O-PLS models, named PLS-DA, O-PLS-DA. PLS/OPLS evaluates repeated variables between groups and highlights differences between groups.

In metabolomics, many classical statistical tools (t-test, ANOVA) such as correlation analysis and simple linear regression also utilize pairs of variables, and these methods can be extended to manage hundreds or thousands of variables simultaneously.

DATA BASES and METABOLITES IDENTIFICATION. The common metabolite identification approach is based on querying metabolomic databases for their chemical shift or for their neutral molecular mass values (in MS techniques) of the identified peaks using a tolerance window. **The pathway and network analysis** amplifies the information generated by metabolomic studies useful for systems biology. Both approaches exploit the relational properties found in metabolomic data. Pathway analysis uses the preliminary biological knowledge to analyse metabolite patterns from an integrative point of view.

INTEGRATION of OMICS DATA. Identification of biomarkers is accessible by comparison with free Biological databases such as Kyoto Encyclopedia of Genes and Genomes (KEGG) which help the elucidation of metabolites and specific pathways.

A novel statistical model of data processing for 1D NMR spectra is the Bayesian automated metabolite analyzer for NMR (BATMAN) R package (Hao, 2012). BATMAN models resonances on the basis of a user-controllable set of templates, each of which specifies the chemical shifts, J-couplings and relative peak intensities for a single metabolite. Bayesian statistics allows the incorporation of prior knowledge about resonance positions, shapes, coupling for known molecules, peak shift and concentration, wavelets. It can construct a template spectrum for each metabolite using Lorentzian peaks and information from metabolome data bases. A soft truncation is made allowing small negative deviations for peaks and obtaining an improved residual spectrum. BATMAN is a regression model using a vector of spectral intensities and a matrix, each column of which is a spectral template corresponding to a metabolite. Parameters which determine the shape of the metabolite template curves are also, included:
– the number of multiplets for each metabolite
– the form of each multiplet (e.g. singlet, doublet etc.)
– the position (chemical shift) of each multiplet
– the relative area under each multiplet
– the peak width.
The BATMAN model includes more: a vector of relative concentrations for the metabolites, a matrix of wavelets, a vector of wavelet coefficients, a vector of Gaussian errors. The BATMAN R package become widely used because it enhances the robustness of the biomarker/metabolite selection process.

Description of the main results obtained

For experiment 1, the results of pH and ammonia in ruminal liquid are presented in Table 4 and 5. Is no difference between groups for each time T0 and T2. At time T2, the pH normally decreases in both groups, and ammonia has a normal increase also in both groups. Other authors had been shown that saponins do not modify the rumen pH value in dairy cows (Hristov, 1999) and ammonia level on steers (Hussain, 1995). For goats, Zhou (2012) presented that ruminal pH, ammonia and volatile fatty acid concentrations were not affected by addition of tea saponins. Santoso (2007) presented that ruminal pH increased linearly in goats fed saponins and ammonia decreased at increasing dose of saponin but the ruminal liquid collection was at the end of the day and doses of saponin were much higher than in this present study.

Table 4. Results of the pH values for ruminal liquid in Experiment 1

pH	**TREATED group**	**CONTROL group**
time T0 (average of all samplings)	6.75±0.26	6,78±0.18
time T2 (average of all samplings)	6.29±0.19	6.32±0.19

Table 5. Results of the ammonia concentration for ruminal liquid in Experiment 1

NH3 (mg/L)	**TREATED group**	**CONTROL group**
time T0 (average of all samplings)	67.65±29.24	70.78±30.44
time T2 (average of all samplings)	146.40±59.20	147.43±42.58

The protozoa number have no variation between groups at each time (Table 6) but only 2 exceptions of increases in treated group were registered: in sampling W5 (time T0) and in sampling W4 (time T2).

Table 6. Results of the ruminal liquid protozoa number ($x10^5$/ml) in Experiment 1 for each sampling (W1-W6)

	W1	W2	W3	W4	W5	W6
time T0						
TREATED GROUP	12.20±2.03	11.86±3.40	14.61±5.72	10.36±3.72	14.72±2.71	12.76±3.86
CONTROL GROUP	12.90±3.16	13.06±2.92	14.63±4.65	9.16±2.67	11.64±1.84	12.86±3.59
time T2						
TREATED GROUP	9.59±1.91	10.97±3.57	12.74±5.14	11.62±3.51	11.58±2.31	10.16±3.15
CONTROL GROUP	8.43±2.08	9.76±2.68	14.26±4.47	8.88±1.83	10.41±1.84	12.04±3.34

There is no statistical difference of the molar proportion concentrations of the 3 most important VFA (C2, C3, and C4) between the groups or between times at each sampling moment (Table 7) and the total VFA productions were the same. Only the ratio acetic:propionic acids (C2/C3) in Table 8 present a slight difference which will be statistically verified. The decrease of this ratio in time T2 comparing to time T0 is more visible in treated group than in control group. The effect of saponine is that of producing a small increase in propionic acid proportion but acetic acid is constant. A small decrease for butyric acid proportion is consequently recorded. Also, other authors (Hussain, 1995; Hristov, 1999) reported a inhibited protozoa number, a not affected total VFA production but an increased propionate production and consequently, a decrease in acetic:propionic acids ratio. Santoso (2007) presented a linear decrease in total VFA concentration with increased saponin intake which might be due to the decreased level of protozoa or to increased level of *Selenomonas ruminantium* protozoa that can produce propionate from succinate metabolism (Wolin, 1997). There exists a interdependency between these

saponin effects and others like increased passage rate for intake, decreased feed apparent digestibility (in dry matter, fiber NDF and crude protein), reduced urinary N excretion, and consequently increased microbial N supply and retained N as a proportion of N digested (Santoso, 2007).

Table 7. Comparative results of the volatile fatty acids in ruminal liquid (molar proportion %) in Experiment 1 for samplings W1 and W6 – average group value

		TREATED group		**CONTROL group**	
		W1	W6	W1	W6
time T0	C2%	66.5	65.3	67.6	67.6
	C3%	18.0	17.9	17.6	16.3
	C4%	12.0	12.1	11.4	12.5
time T2	C2%	66.0	65.3	67.1	65.5
	C3%	20.8	21.5	20.0	20.8
	C4%	10.5	10.7	10.2	11.1

C2=acetic acid; C3=propionic acid; C4=butyric acid

Table 8. Ratio acetic:propionic acids (C2/C3) in ruminal liquid in Experiment 1

C2/C3	**TREATED group**	**CONTROL group**
time T0 (average of all samplings)	3.57±0.10	3.75±0.22
time T2 (average of all samplings)	3.05±0.15	3.20±0.08

The data of biochemical tests of goat plasma are presented in fig.1. The glucose (fig.1a, 1b) and the urea (fig.1c, 1d) concentrations don't display any variations between groups or between times, that means no evident effect induced by saponine. The glucose values are normal (0.5-0.75 g/L - Cornell University College of Veterinary Medicine) thus indicating that no consequences after saponin administration on glucose metabolism are present. The lactating goats are in metabolic equilibrium. Urea values are greater than normal physiological values of 0.10-0.20 g/L (Merck Veterinary Manual).

The BHB values (fig.1e, 1f) are not different between groups but they are higher in time T2 than in time T0 (average 100 mg/L in time T2 compared to average 55 mg/L in time T0) but remain in a normal range of physiological values. BHB along with acetone and acetoacetate, is considered as a ketone body. Ketones are produced from the metabolism of NEFA and volatile fatty acids. Mainly butyrate (butyric acid) is converted to BHB in the rumen epithelium and the liver. The primary ketone produced by the liver from NEFAs is acetoacetate. This latter is then reduced to BHB within the mitochondria and spontaneously decarboxylated to acetone. The ketones are excreted into the circulation, taken up by other tissues (e.g. skeletal muscle, mammary gland), where they are oxidized to yield energy or, in the case of the mammary gland, incorporated into milk fat. An abnormal increase in ketones can mean ketosis. Increased BHB concentrations in blood indicate stimulation of lipolysis and of ketogenesis or excess absorption of butyrate in ruminants (called dietary ketosis). Lipolysis is stimulated by condition of late pregnancy or lactation and ketogenesis is a consequence of a shift in energy metabolism from

glucose to fat, of a decreasing conversion of dietary or endogenous triglycerides to stored fat, and of a decreasing fatty acid oxidation in hepatocyte mitochondria (thus more acetyl CoA is available to form ketones). This is a negative energy balance due to stresses of calving and lactation in peripartum cows/goats. The normal values are 20-50 mg/L for equine, 10 mg/L for Alpaca goats, 12-42 mg/L for close-up dry cows (Pennsilvania University) and BHB values>270 mg/L are considered compatible with clinical ketosis – Cornell University College of Veterinary Medicine. The values>120 mg/L means subclinical ketosis (brochure Nor-Feed Sud after Suthar, 2013, J. Dairy Sci., 96(5):2925-2938).

The NEFA values (fig.1g, 1h) don't' display any variation along the samplings in time T2 with an average value of 130 µmol/L for both animal groups. At time T0, the NEFA concentrations are much higher (average 250 µmol/L but is a normal value for goats like: 260 µmol/L for Alpaca goats – Cornell University College of Veterinary Medicine or 30-460 µmol/L for close-up dry cows - Pennsilvania University. NEFA is constant in both groups for W1-W5 samplings. Only in sampling W6 is a sudden considerable increase to an average value of 500 µmol/L in both groups and but this is not an effect of saponin.
NEFA and BHB are used to assess the degree of negative energy balance in periparturient period and tissue lipid mobilization. Late lactation cows usually have NEFA values less than 200 µmol/L, and concentrations begin to rise as cows approach calving reaching the highest values of 800 to 1200 µmol/L during the first week of lactation. Values return to less than 300 µmol/L by 30 days in milk. Similarly, BHB concentrations increase as calving approaches and start to decrease after 30 days in milk (Michigan Dairy Review). Changes in the concentration and composition of plasma NEFA directly affect white blood cell function, in fact the fatty acid composition of the cellular membrane. Therefore any change in the content of blood NEFA can be reflected directly in the immune function of animal body or inflammation. Plasma antioxidant parameters like triacylglycerols (TG), superoxidase dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in plasma were analyzed by Zhou (2012) and only TG concentration increased after tea saponins treatment. This confirms that tea saponins exert a protective action against free radicals.

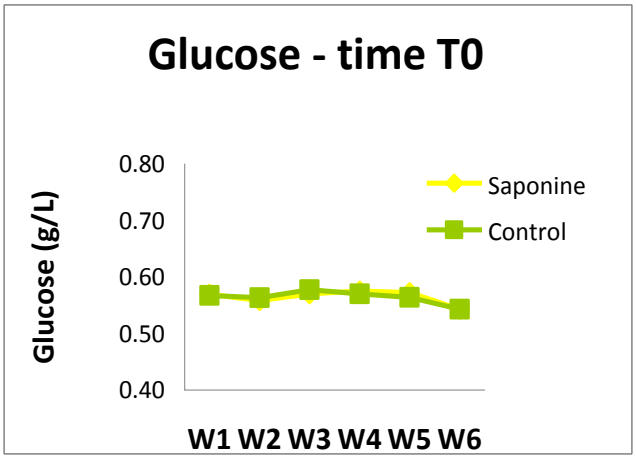

Fig. 1a. Glucose level at time T0

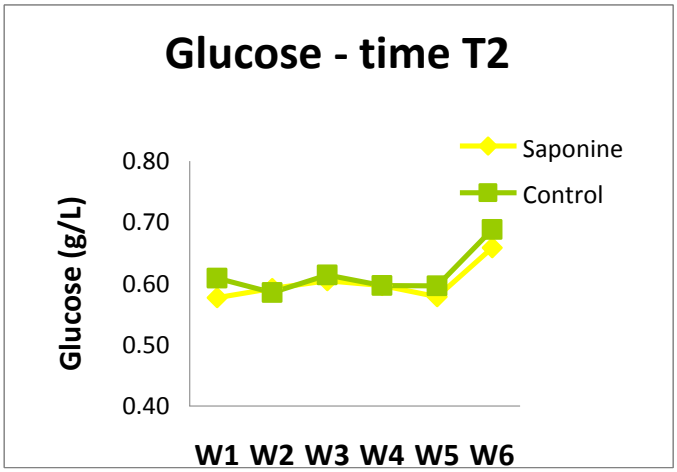

Fig. 1b. Glucose level at time T2

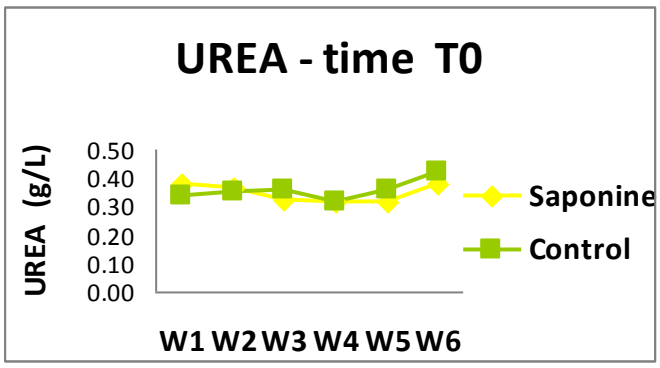

Fig. 1c. Urea level at time T0

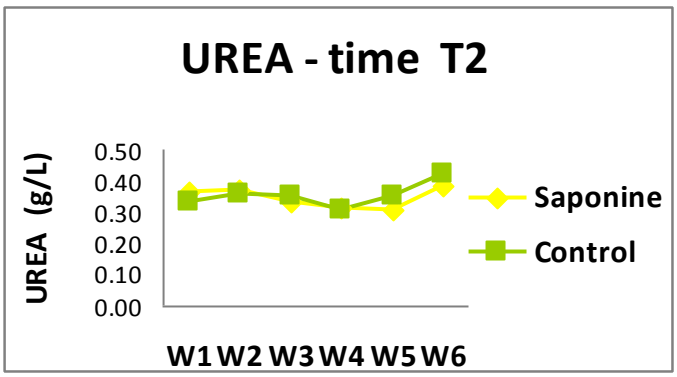

Fig. 1d. Urea level at time T2

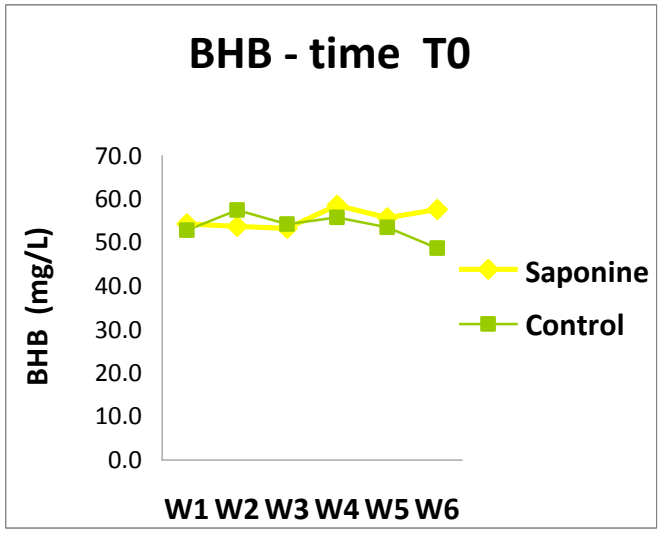

Fig. 1e. BHB (beta-hydroxybutyrate) level at time T0

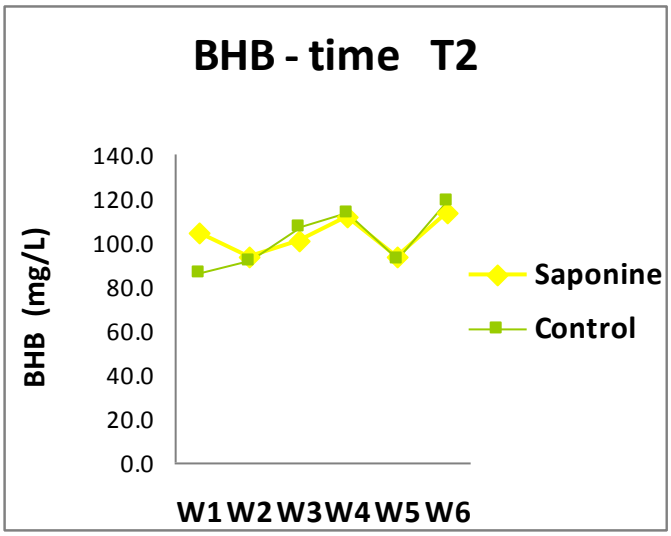

Fig. 1f. BHB (beta-hydroxybutyrate) level at time T2

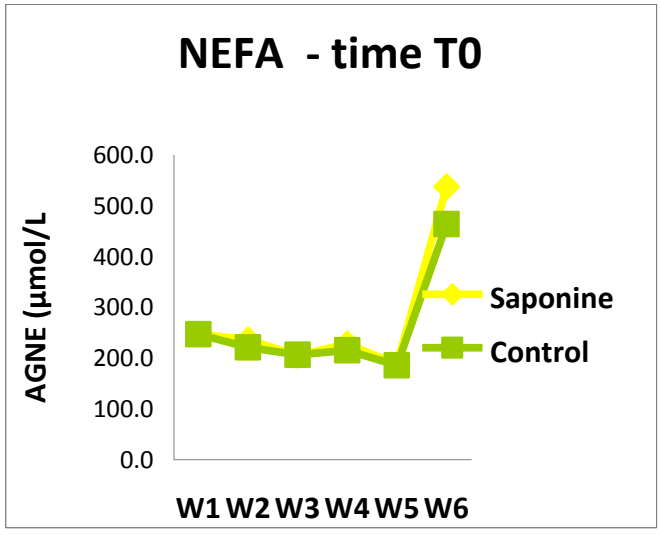

Fig. 1g. NEFA (non-esterified fatty acids) level at time T0

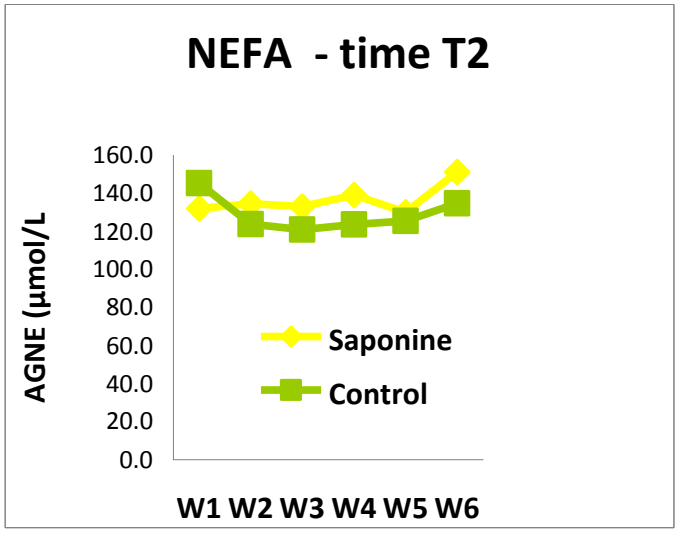

Fig. 1h. NEFA (non-esterified fatty acids) level at time T2

Fig. 1. Biochemical parameters for plasma goats in Experiment 1

Only a part of the experiment 1 samples have been tested by the ^{1}H-NMR spectroscopy. Profiling analysis will led to the identification of the most abundant metabolites. A complete list of metabolites was settled and the BATMAN R software package will be used to process the spectral data and to differentiate metabolic fingerprints of lactating and periparturient goats. This multidimensional new statistical analyse will be performed on data in order to reveal the main metabolites and metabolic pathways involved in the peripartum period and the putative effect of saponins on them.

An example of applying ^{1}H-NMR technique in goat metabolome study is the presentation of M. Palmera in The DairyCare Conference in Zadar, Croatia in 5 – 6 October 2015 for identifying the physiological markers candidate indicative of Seasonal Weight Loss tolerance for 2 breeds of goats. The characterization of the goat mammary gland and milk metabolomes using NMR concluded to significant differences between control and restricted groups in both breeds, related to variations in carbohydrates and aminoacids-related metabolites which demonstrate an adaptation to the low-energy diet. Among the list of metabolites, some of them permitted differentiation between groups like Acetyl-L-carnitine, Creatine, Fucose, Hippurate, Adenosine, Carnitine, Citrate, Dimethylglycine, Fumarate, N-acetylglucosamine, Succinate, TMAO, UDP-galactose, UDP-glucose, Alanine, Lactate, Methylmalonate.

Also, this present study will lead to a specific metabolomic signature associated with saponin presence in rumen and systemic blood circulation. Collected data will show if saponins may be used as a potential beneficial feed additive for improving both the health and welfare of dairy goats in some energy disturbances states and will complete the available information on the use of plant additives on health and performances of goats.

Foreseen publications resulting from the STSM (if applicable)

This STSM report is part of a research theme developed by the INRA/AgroParisTech, Unit UMR 0791 MOSAR (Systemic Modelling Applied to Ruminants) for studying the complex metabolism of dairy ruminants and their responses to variations due to different nutritional or physiological challenges. These results of the STSM stage completed with the further data will be presented in a DairyCare Cost Project plenary meeting.

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Approval by the host institution.

see appendix - Execution Approval Letter (from the host that confirms the successful execution of the STSM and approves the report)