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Changes in maternal body composition and metabolism of dairy goats during pregnancy

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ABSTRACT - The objective of this study was to evaluate the mobilization of nutrients in goats of different gestation types and pregnancy stages. Forty-four Saanen and Oberhasli goats were studied. The goats of each breed and gestation type (single or twin) were slaughtered at different gestational ages (80, 110, and 140 days of pregnancy), forming a completely randomized design in a 2 × 3 × 2 factorial arrangement (two breeds, three gestational ages, and two types of pregnancy). The slaughter procedure involved separating the empty body, mammary glands, uterus with membranes and fetal fluid, and fetus(es). For the females slaughtered at 140 days of pregnancy, blood was collected to analyze metabolites and hormones every 15 days during gestation. The dry matter (DM) intake was lower in goats with twin pregnancies. The relative daily retention rate of the nutrients in the body was positive at 100 days of pregnancy but became negative at 140 days (–0.18±0.25 g DM kg^{-1} of maternal body d^{-1}) and did not differ with breed or number of fetuses. Fetal growth in twin pregnancies was 66% higher than in single pregnancies. The highest levels of ß-hydroxybutyrate and non-esterified fatty acids were observed beginning at 100 days of gestation. Serum total protein and albumin levels decreased after 125 days of gestation. Serum urea levels were reduced after 80 days of gestation. Plasma 17ß-estradiol levels increased with the advance of pregnancy, and IGF-1 was highest between 60 and 80 days of gestation. The maternal metabolism throughout pregnancy does not vary with the type of pregnancy, and pregnant goats need greater nutritional intake during the final third of the gestational period regardless of the breed or type of pregnancy.

Key Words: energy metabolism, hormone profile, metabolic and nutritional profile, pregnancy outcomes, type of pregnancy

Introduction

Profound metabolic changes take place in pregnant females, and the physiological mechanisms involved in these changes have primarily been studied for the last six weeks of gestation because this is the period during which approximately 70% of fetal growth and the majority of the development of the glandular and mammary tissues occur, contributing to an increase in energy requirements (Conway et al., 1996; NRC, 2007). At the same time, there is a decrease in dry matter intake caused by the compression of the rumen by the fetus and elevated estrogen concentration (Forbes, 2007).

This scenario may lead to increased efficiency in nutrient use in the final stage of pregnancy (Bauman and Currie, 1980; Bonnet et al., 2002; Duarte et al., 2013), which may not be sufficient to compensate for the decreased dry matter intake, so the pregnant goat may have a negative energy balance (Bell, 1995). As a consequence, the female starts to mobilize body reserves to meet its energy requirements as well as use alternative substrates to produce propionate for glucose synthesis in the liver (Prezotto et al., 2013). Although the negative energy balance has already been explored in studies of dairy cows, there are currently no studies addressing this physiological process in the goat, which may suffer from an even more marked energy deficit due to its high prolificacy and high incidence of twin births (Amoah et al., 1996).

Moreover, no information exists in the literature to indicate whether goat breeds with the same productive aptitude utilize the same metabolic mechanisms under a negative energy balance. Studies show that there are differences in productive and reproductive traits among dairy goat breeds; Saanen animals show higher precocity, milk yield and lactation longevity, whereas Oberhasli goats display a higher concentration of milk protein and fat (Boichard et al., 1989). These differences among genotypes may indicate different physiological adaptation mechanisms under the same nutritional conditions, especially during pregnancy (Silanikove, 2000; Macciotta et al., 2011).

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Another noteworthy factor is that many of the studies focus on the final stage of pregnancy or on the transition period (end of gestation and beginning of lactation), so it is possible that the adaptive mechanisms are activated before the final weeks, especially in twin pregnancies (Bauman and Currie, 1980; Bell, 1995). Therefore, it is important to understand the mobilization of nutrients in the female body and their transfer to other products of gestation. Thus, the objective of this study was to evaluate changes in the maternal body and the metabolic profiles of Saanen and Oberhasli goats with single or twin pregnancies over the entire gestational period.

Material and Methods

Forty-four non-pregnant and non-lactating multiparous goats, 22 of the Saanen breed and 22 of the Oberhasli breed, with an initial average body weight of 49.5±7.6 kg and an average body condition score of 2.75, were studied. The animals were stabled in individual stalls (1.2 m × 0.5 m) in a shed that was protected from wind and rain by a metal roof. All of the procedures adopted in this experiment were approved by the Committee of Ethics in Animal Use of UNESP, protocol no. 026167-07.

After showing estrus (natural or induced), the goats were naturally mounted by a sire of the same breed. During the seasonal estrous period, the females were subjected to a heat-induction protocol using a hormone treatment recommended by Westhuysen (1979) and Ritar et al. (1984). Thirty-five days after mounting, an ultrasound examination was performed to diagnose pregnancy and count the number of fetuses.

The diet (Table 1), formulated according to the NRC (2007), was supplied *ad libitum*, twice daily, at 08.00 h (60% of the daily total) and 17.00 h (40% of the daily total). The daily control of dry matter (DM) and nutrients was achieved by weighing the amount supplied and refused by the animals, and the amount to be supplied was adjusted to keep orts at 15% of the total supply. Animals had free access to water.

Eight animals (four of each breed) were initially slaughtered to estimate the body composition of non-pregnant and non-lactating goats.

After the diagnosis of pregnancy, the goats of each breed were divided into two groups according to pregnancy type (single or twin), and these groups were divided randomly according to the pregnancy ages for slaughter (80, 110, and 140 days after mating).

For the animals randomly chosen for slaughter at 140 days, blood was collected at 1, 35, 50, 65, 80, 95, 110, 125, and 140 days of pregnancy to evaluate the variation in the concentrations of metabolites and hormones throughout pregnancy in the same animal and thereby reduce a source of variation among the animals.

Upon reaching the pre-defined dates for slaughter, goats were weighed without a prior period of feed deprivation, and this was considered the body weight at slaughter. After being stunned with a pneumatic gun, the goats were slaughtered by sectioning the jugular vein and carotid artery. The total amount of blood was collected, weighed, and stored for subsequent reincorporation into the animal body. After death was confirmed, the gravid uterus and mammary glands were removed. The gravid uterus was weighed and then separated into three parts: empty uterus (uterus, placenta, and placentomes), fetus(es), and fetal fluid, which were identified, weighed, and stored individually and then frozen in a cold chamber at −15 ºC.

The components of the digestive tract were weighed full and empty to determine empty body weight (EBW), which was calculated by subtracting the contents of the gastrointestinal tract, urinary bladder, and gallbladder from the body weight at slaughter. The maternal body (MB) was

Table 1 - Composition of the ingredients of the experimental diet

Ingredients	g kg^{-1} as fed	DM	ME[1]	CP	EE	NDF
		g kg^{-1}	MJ kg^{-1} of DM	g kg^{-1} of DM		
Tifton hay[2]	100.0	915.0	5.10	69.0	9.4	765.4
Corn plant hay[3]	349.4	916.0	9.10	46.0	17.0	537.5
Corn	421.0	901.0	12.38	87.0	29.0	169.0
Soybean meal	119.3	910.0	12.09	473.0	19.0	237.1
Premix	3.7	990.0	-	-	-	-
Salt	0.8	980.0	-	-	-	-
Limestone	5.8	950.0	-	-	-	-
Total	1000	908.0	10.33	116.0	21.0	377.8

DM - dry matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber.
[1] ME - metabolizable energy = [kg TDN (according to Valadares Filho, 2002)] × [18.447 kJ] × [0.82]. The metabolizable energy of the corn plant hay was considered equal to the ME of the silage.
[2] Tifton 85 (*Cynodon* sp.)
[3] Whole corn plant without the roots that was cut when the grains reached 65% of the milk-line and then dried in the sun.

calculated by subtracting the sum of the weights of the gravid uterus and mammary glands from the EBW.

Four structures were considered for the calculation of total retention and relative daily nutrient retention rates: maternal body, fetuses, mammary glands, and empty uterus + fetal fluid (UFF).

The DM (AOAC, 1990; method no. 930.15) and mineral matter (MM) (AOAC, 1990; method no. 942.05) contents were measured in the samples of the ingredients of the diets, orts, maternal body, empty uterus, fetal fluid, fetuses, and mammary glands. Ether extract (EE) was determined using a Sohxlet extractor for 5 h, except for the samples of the maternal body and mammary glands, for which the extraction was performed for 8 h due to the high fat. The total nitrogen content of the samples was obtained by the Dumas combustion method using a LECO FP-528 LC analyzer following the procedure described by Etheridge et al. (1998) and then multiplied by 6.25 to obtain the total protein value. The neutral detergent fiber (NDF) contents of the ingredients were determined according to Robertson and Van Soest (1981) with the addition of thermostable α-amylase. The energy content of the amniotic fluid was determined using a Parr 6300 bomb calorimeter. The energy contents of the maternal body, fetuses, mammary glands and empty uterus were determined by summing the energy contribution of protein (23.4304 J g^{-1} protein) and ether extract (39.3296 J g^{-1} EE) according to ARC (1980). The ether extract values of the amniotic fluid were estimated by subtracting the protein energy contribution from the total energy content of this substance.

The retention of nutrients in the different structures (maternal body, fetuses, uterus and fetal fluid, and mammary gland) in the goats was calculated as the difference between the total amount of a certain nutrient on the day of slaughter and the total amount of the same nutrient at the onset of pregnancy.

The comparative slaughter method described by Lofgreen and Garret (1968) was used, and the composition at the beginning of pregnancy was estimated from the non-pregnant and non-lactating goats that were slaughtered at the onset of the experiment (4 Saanen and 4 Oberhasli). Regression equations were generated to estimate the total initial amounts of nutrients in the maternal body and in each component related to the pregnancy with the effect of breed taken into account when significant (Table 2).

The relative daily retention rate was calculated by equation (1):

$$\text{Relative daily retention rate} = TR\ W^{-1}\ DP^{-1} \quad [1]$$

in which TR = total retention of DM or protein or EE (g) and/or energy (kJ) in the maternal body in each pregnancy component; and W = average weight of the structure (kg). For the animals slaughtered at 80 days of pregnancy, the average weight from 0 to 80 days was used; for those slaughtered at 110 days of gestation, the average weight between 0 and 110 days was used, and for the goats slaughtered at 140 days, the average weight from 0 to 140 days was used. Finally, DP = days of pregnancy (80, 110, or 140 days).

Blood samples were collected by jugular venipuncture using Vacutainer® tubes without sodium heparin. The blood samples were placed in a refrigerated centrifuge at 4 ºC for 15 min at 1,370 ×*g* to separate the plasma and serum, which were stored separately in 15 mL Eppendorf tubes. The samples were frozen at −20 ºC until later analyses.

Table 2 - Intercept values (a), slope (b), probability values (P) and standard error of the mean (SEM) of the equations used to estimate the total nutrients in the maternal body (MBnp), mammary glands (MGnp) and uterus (Unp) according to the mass of the structure at the onset of pregnancy

Variable[4]	Parameters	MBnp (g)[1]			MGnp (g)[2]			Unp (g)[3]		
		Value	SEM	P-value	Value	SEM	P-value	Value	SEM	P-value
DM (g)	a	–7004	3055	**	–30.7	38.9	*	0.362	1.223	**
	b	0.648	0.063		0.533	0.092		0.176	0.009	
Protein (g)	a	2146	1215	**	21.7	8.86	*	0.003	0.719	**
	b	0.106	0.025		0.074	0.021		0.145	0.005	
EE (g)	a	–10348	4206	**	–53.6	35.3	*	–0.133	0.802	*
	b	0.514	0.087		0.441	0.084		0.017	0.006	
Energy (kJ)	a	–356	145	**	–1.60	1.41	**	–0.005	0.020	**
	b	0.023	0.003		0.019	0.003		0.004	0.001	

[1] Nutrients and energy in the MBnp = a + b * MBnp.
[2] Nutrients and energy in the MGnp = a + b * MGnp.
[3] Nutrients and energy in the Unp = a + b * Unp.
[4] DM - total amount of dry matter in each structure at the onset of pregnancy; Protein - total amount of protein in each structure at the onset of pregnancy; EE - total amount of ether extract in each structure at the onset of pregnancy; Energy - total amount of kilojoules in each structure at the onset of pregnancy.
* P<0.05; ** P<0.01.

Analyses of the energy and protein profiles were performed on the serum samples. The metabolic energy profile was evaluated as follows: the non-esterified fatty acids (NEFA) were analyzed using the commercial kit from Randox® (FA115) based on Elphick (1968); ß-hydroxybutyrate was analyzed using the Randox® commercial kit (FA 1007) based on Williamson et al. (1962). The metabolic protein profile was evaluated through urea analyses by the urease method (Labtest Diagnóstica S.A., Brazil); total proteins by the biuret method (Labtest Diagnóstica S.A., Brazil); and albumin by the bromocresol green method (Labtest Diagnóstica S.A., Brazil). A Labquest® semi-automatic device for biochemical measurements was used for reading.

Additionally, hormone measurements were performed on the plasma samples using commercial kits for immunoenzymatic (EIA) determination of estrogen (Catalog # ADI-901-174 - 17ß-Estradiol - Enzo Life Science), progesterone (Progesterone Test System 4825-300 AccuBind® - Monobind Inc.), and IGF-1 (Catalog # ADI-900-150 - Enzo Life Science). Measurements were read using the Multiskam MS from Labsystems® version 8.0 for EIA measurements.

The animals were distributed among experimental treatments according to a completely randomized, 2 × 2 × 3 factorial design (breeds × number of fetuses × days of pregnancy).

The total retention and relative daily nutrient retention rates of the maternal body, fetuses, empty uterus + fetal fluid, and mammary glands were analyzed as mixed models using the MIXED procedure of SAS (Statistical Analysis System, version 9.2). The models included random effects and the fixed effects of breed (Saanen or Oberhasli; 1 degree of freedom, DF), days of pregnancy (slaughter at 80, 110, or 140; 2 DF), number of fetuses (single or twin pregnancy; 1 DF), and their interactions. Distinct residual variances for the number of fetuses subclass and days of pregnancy were modeled using the GROUP option of the REPEATED command. When significant, means for the days of pregnancy were compared with Tukey's least significant difference test (PDIFF adjust=tukey option of the LSMEANS command). Significance was declared at $P<0.05$.

The intake was analyzed in a factorial arrangement with repeated measures over time using animals chosen at random to be slaughtered at 140 days of pregnancy. Mixed models with fixed effects of breed (Saanen or Oberhasli; 1 DF), number of fetuses (single or twin pregnancy; 1 DF), days of intake (139 DF), their interactions, and the random effect were analyzed using the MIXED procedure in SAS (Statistical Analysis System, version 9.2). The compound-symmetry covariance structure was adopted as it reaches the lowest convergence criterion (BIC). Significance was declared at $P<0.05$.

The blood metabolites were analyzed in a completely randomized design in a factorial arrangement with repeated measures over time. Mixed models with fixed effects of breed (Saanen or Oberhasli; 1 DF), number of fetuses (single or twin pregnancy; 1 DF), blood collection days (1, 35, 50, 65, 80, 95, 110, 125, and 140; 8 DF), their interactions, and the random error were analyzed using the MIXED procedure in SAS (Statistical Analysis System, version 9.2). The compound-symmetry covariance structure was adopted as it reaches the lowest convergence criterion (BIC). Significance was declared at $P<0.05$.

Results

There was no effect of breed on the calculations used to estimate empty body weight, maternal body, and mammary glands in non-pregnant goats, so the same equation was used for both breeds (Eqs. [2], [3] and [4]).

$$EBWnp = -7.77 + 1.03 * BWnp,\ SEM = 5.06,\ P<0.01,\ r^2 = 0.97 \quad [2]$$

$$MBnp = 0.302 + 0.981 * EBWnp,\ SEM = 0.075,\ P<0.01,\ r^2 = 0.99 \quad [3]$$

$$MGnp = -98.60 + 9.70 * MBnp,\ SEM = 143,\ P<0.05,\ r^2 = 0.44 \quad [4]$$

in which EBWnp = empty body weight at the beginning of pregnancy (kg); BWnp = body weight at the beginning of pregnancy (kg); MBnp = maternal body at the beginning of pregnancy (kg); and MGnp = mammary glands at the beginning of pregnancy (g).

The initial weight of the uterus (Unp) was estimated by difference, as described below (Eq. [5]):

$$Unp = EBWnp - MBnp - MGnp \quad [5]$$

The estimates of DM, EE, CP and energy from the maternal body, mammary glands and uterus at the beginning of pregnancy were different among breeds and were calculated from the equations (intercepts and slopes) shown in Table 2.

No effect of breed was observed on DM and protein intake. However, there was an effect of the number of fetuses on DM intake (g d^{-1}). In the goats with twin pregnancies, DM intake decreased at an increasing rate after the first third of the duration of pregnancy (quadratic, $P<0.05$; Figure 1). On the other hand, females with single pregnancies showed a constant DM intakes throughout pregnancy (1160±131 g DM d^{-1}). There was no effect of the number of fetuses on CP intake, which decreased at an increasing rate (quadratic, $P<0.05$) from the first third of pregnancy on (Figure 1). The variation in DM and CP intake over the gestation period

was similar regardless of the unit in which the intake was expressed (g d^{-1}; g kg^{-1} body weight d^{-1}; g kg^{-1} metabolic weight ($kg^{0.75}$) d^{-1}).

Overall, the variation in maternal body (MB) weight was 4.65 kg on average and was not affected by the number of fetuses. However, this variation in weight changed as pregnancy advanced. Up to 80 days, the rate of gain was 2.70±0.23 g kg^{-1} MB d^{-1}, but from 80 days on, MB weight gain dropped to 1.11±0.33 g kg^{-1} MB d^{-1} until 110 days into pregnancy, when it decreased to 0.02±0.34 g kg^{-1} MB d^{-1} until 140 days (P<0.01). Oberhasli goats showed a higher weight gain than the Saanen goats: 1.70±0.26 vs. 0.86±0.22, respectively.

The total retention and the relative daily nutrient retention rate in the maternal body were not affected by breed and number of fetuses (Table 3). The total retention of nutrients in the maternal body was higher until 110 days of pregnancy, whereas at 140 days of pregnancy, the total amount of nutrients decreased (P<0.05; Table 3). The relative retention rates of DM and protein (g kg^{-1} MB d^{-1}) in the maternal body declined as pregnancy advanced, while the relative daily retention rates of EE (g kg^{-1} MB d^{-1}) and energy (J kg^{-1} MB d^{-1}) only decreased at 140 days of pregnancy (P<0.05; Table 3).

There was no effect of breed on total retention and relative daily nutrient retention rate on the fetuses (P>0.05), except for fetal mass, for which the Oberhasli fetuses were 13% larger than the Saanen (P<0.05; Table 4). The total weight of the fetuses as well as the total retention of nutrients in them was higher (P<0.05) in twin pregnancies. However, the relative daily retention rates of nutrients and energy were the same for both single and twin pregnancies.

The total retention of nutrients in the fetus was higher at 140 days of pregnancy, and fetal growth between 80 and 140 days of pregnancy corresponded to 92.6% of the weight of the fetuses at 140 days of pregnancy (Table 4). The relative daily retention rates of EE and energy in the fetuses were higher (P<0.05) at 110 days of pregnancy (Table 4).

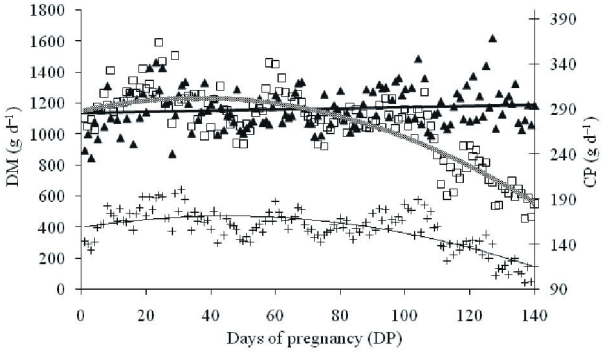
The greatest retentions of uterine mass and fetal fluid, DM, protein and energy in the uterus and fetal fluid were observed at 110 and 140 days of pregnancy (P<0.01) and were higher in Oberhasli goats (P<0.05). The highest relative daily retention rates of weight and these nutrients in the uterus and fetal fluid were at 80 days of pregnancy (P<0.05; Table 5) and were also higher for the Oberhasli goats (P<0.05). In addition, the twin pregnancies displayed higher retentions of uterine mass and fetal fluid, DM and protein (P<0.05). However, only the retention rates of uterine mass and fetal fluid were higher in the twin pregnancies (P<0.05).

Oberhasli goats had a higher retention of fat (EE) in the mammary glands (P<0.05; Table 6), and goats with a twin pregnancy showed greater retention of fat and energy in the mammary glands (P<0.05). The total retention of nutrients in the mammary glands increased as pregnancy advanced (P<0.05) but at a relatively constant daily rate (Table 6).

Up to the gestational age of 80 days, the products of gestation (fetuses, mammary glands, uterus, and fetal fluids) amounted to 209.1 g of retained protein (Tables 4, 5, and 6). After 80 days, protein retention increased exponentially until the end of pregnancy, with elevations of 262% and 522% at 110 and 140 days of gestation, respectively, which reflect the differences found in the protein retention rate in the maternal body as pregnancy progressed. The maternal body mobilized approximately 1,175 g of protein while it deposited 1,090 g of protein in the products of gestation. In contrast, the maternal body mobilized 4,436 g of fat, whereas that which accumulated in the products of gestation totaled 353 g.

The total amount of blood collected at the moment of slaughter increased linearly throughout pregnancy (P<0.01), showing an elevation of 34% at 140 days of gestation compared to that collected from non-pregnant females.

The highest levels of ß-hydroxybutyrate were observed from 110 days of pregnancy (Figure 2), and there was no effect of breed or type of pregnancy (P>0.05) on the serum levels of this metabolite. Likewise, the highest NEFA serum levels were observed in the last 30 days of gestation (Figures 4 and 5). However, Oberhasli goats showed higher (P<0.01) serum NEFA concentrations at 125 days compared



Equation to estimate the average daily DM intake in animals with a twin pregnancy: DMI (g d^{-1}) = 1149.2±42.84 + 4.61±2.60DP –0.063±0.04DP^2; SEM = 123.8; R^2 = 0.72.
Equation to estimate the average daily CP intake (g d^{-1}) = 158.51±4.92 + 0.548±0.15DP – 0.006±0.001DP^2; SEM = 65.12; R^2 = 0.53.
Breed had no effect on (P>0.05) DM and CP intake.

Figure 1 - Dry matter (DM; 1 fetus ▬ ▲ and 2 fetuses ▬ □) and crude protein (CP; — +) intake in dairy goats throughout pregnancy.

Table 3 - Total retention and daily relative retention rate of dry matter, nutrients and energy in the maternal body (±SEM) of Saanen and Oberhasli goats with single and twin pregnancies during gestation

	Treatment							P-value			
	Breed		Days of pregnancy			Number of fetuses					
	Oberhasli	Saanen	80	110	140	1	2	Breed	Days	Fetuses	Interaction
Total retention											
Dry matter (kg)	2.76±0.94	1.23±0.84	4.79±0.96A	2.51±0.95AB	–1.32±1.44B	1.93±1.02	2.05±0.82	ns	**	ns	ns
Protein (g)	392±217	63.8±194	717±256A	451±243AB	–458±266B	179±187	276±239	ns	**	ns	ns
Ether extract (g)	2416±767	1108±693	3897±856A	1927±743AB	–539±1163B	2018±928	1506±628	ns	*	ns	ns
Energy (kJ)	89.6±32.5	32.0±29.3	162.2±34.4A	68.2±32.0AB	–47.9±50.5B	65.2±38.0	56.4±27.1	ns	**	ns	ns
Relative daily retention rate											
Dry matter (g kg^{-1} MB d^{-1})	0.87±0.19	0.41±0.17	1.47±0.25A	0.61±0.19B	–0.16±0.23C	0.69±0.20	0.60±0.16	ns	**	ns	ns
Protein (g kg^{-1} MB d^{-1})	0.11±0.05	0.05±0.04	0.24±0.27A	0.09±0.05A	–0.08±0.05B	0,08±0.04	0.08±0.04	ns	**	ns	ns
Ether extract (g kg^{-1} MB d^{-1})	0.71±0.16	0.35±0.14	1.14±0.23A	0.49±0.16B	–0.03±0.18B	0.62±0.18	0.45±0.13	ns	**	ns	ns
Energy (J kg^{-1} MB d^{-1})	28.07±6.70	12.32±5.90	48.40±8.90A	17.76±6.51B	–5.57±7.96B	23.13±7.53	17.27±5.46	ns	**	ns	ns

ns - not significant (P>0.05); * P<0.05; ** P<0.01.
MB - maternal body.
A, B, C - means followed by the same letter do not differ statistically by Tukey's test (P>0.05).

Table 4 - Total retention and daily relative retention rate of dry matter, nutrients and energy in the fetuses (±SEM) from Saanen and Oberhasli goats with single and twin pregnancies during gestation

	Treatment							P-value			
	Breed		Days of pregnancy			Number of fetuses					
	Oberhasli	Saanen	80	110	140	1	2	Breed	Days	Fetuses	Interaction
Fetal mass (kg)	2.57±0.11	2.26±0.09	0.37±0.01C	1.88±0.10B	5.00±0.19A	1.8±0.09	2.99±0.112	*	**	**	ns
Total retention											
Dry matter (g)	381.5±24.5	346.3±21.2	38.5±2.8C	257.7±18.2B	795.4±45.0A	280.6±23.9	447.1±21.9	ns	**	**	ns
Protein (g)	265.7±16.9	241.8±14.6	25.7±1.9C	171.9±10.6B	563.7±31.8A	200.4±17.0	307.1±14.5	ns	**	**	ns
Ether extract (g)	35.0±2.7	29.4±2.3	2.4±0.2C	25.8±1.8B	68.5±5.1A	25.5±3.2	39.0±1.6	ns	**	**	ns
Energy (kJ)	7.60±0.47	6.81±0.41	0.69±0.05C	5.02±0.30B	15.90±0.89A	5.68±0.49	8.73±0.39	ns	**	**	ns
Relative daily retention rate											
Dry matter (g kg^{-1} Fetus d^{-1})	2.44±0.07	2.34±0.06	2.39±0.10	2.52±0.08	2.26±0.06	2.43±0.09	2.35±0.04	ns	ns	ns	ns
Protein (g kg^{-1} Fetus d^{-1})	1.71±0.06	1.61±0.05	1.69±0.09	1.69±0.05	1.61±0.04	1.67±0.06	1.65±0.05	ns	ns	ns	ns
Ether extract (g kg^{-1} Fetus d^{-1})	0.207±0.010	0.186±0.009	0.140±0.008C	0.257±0.013A	0.194±0.012B	0.206±0.012	0.188±0.006	ns	**	ns	ns
Energy (J kg^{-1} Fetus d^{-1})	46.79±1.32	44.93±1.18	42.93±1.77C	49.27±1.58A	45.37±1.20B	47.11±1.66	44.61±0.61	ns	*	ns	ns

ns - not significant (P>0.05); * P<0.05; ** P<0.01.
A, B, C - means followed by the same letter do not differ statistically by Tukey's test (P>0.05).

Table 5 - Total retention and daily relative retention rate of dry matter, nutrients and energy in the uterus and amniotic fluids (±SEM) of Saanen and Oberhasli goats with single and twin pregnancies during gestation

	Treatment							P-value			
	Breed		Days of pregnancy			Number of fetuses					
	Oberhasli	Saanen	80	110	140	1	2	Breed	Days	Fetuses	Interaction
Total retention											
UFF (kg)	3.68±0.22	3.10±0.20	2.30±0.17B	4.14±0.43A	3.72±0.20A	2.78±0.22	3.99±0.21	*	**	**	ns
Dry matter (g)	280.7±24.1	212.2±21.8	155.3±19.9B	308.7±49.2A	275.3±21.7A	211.3±23.2	281.6±25.1	*	**	*	ns
Protein (g)	226.3±20.9	172.9±18.9	121.6±17.5B	255.1±41.6A	222.1±18.8A	170.7±19.7	228.5±21.7	*	**	*	ns
Ether extract (g)	13.65±1.53	9.99±1.36	10.26±1.59	14.77±2.62	10.42±1.39	10.04±1.56	13.59±1.45	ns	ns	ns	ns
Energy (kJ)	5.55±0.43	4.36±0.40	2.89±0.32B	6.48±1.02A	5.51±0.48A	4.38±0.49	5.54±0.47	**	**	ns	ns
Relative daily retention rate											
UFF (g kg^{-1} UFF d^{-1})	17.35±0.20	16.47±0.18	21.64±0.32A	16.32±0.19B	12.78±0.19C	16.48±0.23	17.34±0.16	**	**	**	ns
Dry matter (g kg^{-1} UFF d^{-1})	1.37±0.05	1.02±0.05	1.40±0.08A	1.20±0.13AB	0.98±0.06B	1.15±0.07	1.23±0.08	**	**	ns	ns
Protein (g kg^{-1} UFF d^{-1})	1.05±0.06	0.86±0.05	1.09±0.08A	0.98±0.10AB	0.80±0.06B	0.93±0.06	0.99±0.07	**	*	ns	ns
Ether extract (g kg^{-1} UFF d^{-1})	0.13±0.02	0.10±0.01	0.13±0.02	0.13±0.02	0.08±0.02	0.09±0.02	0.14±0.02	ns	ns	ns	ns
Energy (J kg^{-1} UFF d^{-1})	27.27±1.56	22.51±1.38	29.31±2.04A	25.32±2.54AB	20.05±1.57B	24.27±1.60	25.51±1.86	*	**	ns	ns

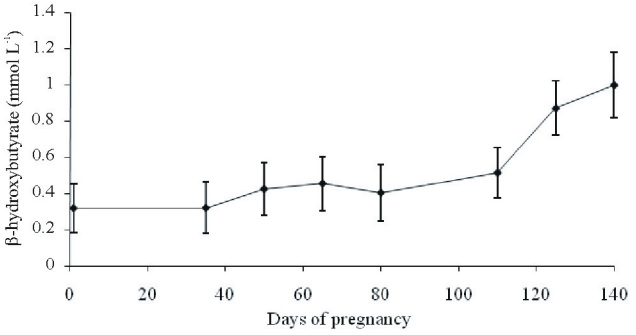
SEM - standard error of the mean.
ns - not significant (P>0.05); * P<0.05; ** P<0.01.
UFF - uterus and fetal fluids.
A, B, C - means followed by the same letter do not differ statistically by Tukey's test (P>0.05).

Table 6 - Total retention and daily relative retention rate of dry matter, nutrients and energy in the mammary glands (±SEM) of Saanen and Oberhasli goats with single and twin pregnancies during gestation

	Treatment							P-value			
	Breed		Days of pregnancy			Number of fetuses					
	Oberhasli	Saanen	80	110	140	1	2	Breed	Days	Fetuses	Interaction
Total retention											
MG (kg)	1.26±0.14	1.34±0.13	0.53±0.1C	0.97±0.14B	2.40±0.26A	1.21±0.14	1.39±0.13	ns	**	ns	ns
Dry matter (g)	376.2±37.5	379.2±33.2	146.4±32.9C	319±44.8B	667.3±56.8A	340.1±34.85	415.3±37.6	ns	**	ns	ns
Protein (g)	157.1±20.9	168.0±18.7	61.8±15.7B	120.5±24.2B	305.4±37.6A	159.6±20.4	165.5±20.1	ns	**	ns	ns
Ether extract (g)	185.5±14.2	145.6±12.2	58.6±13.0C	162.7±20.0B	275.2±16.9A	143.8±11.9	187.2±13.9	*	**	*	ns
Energy (kJ)	10.67±0.69	10.41±0.65	3.57±0.46C	9.41±1.09B	18.64±1.38A	9.48±0.65	11.6±0.85	ns	**	*	ns
Relative daily retention rate											
MG (g kg^{-1} MG d^{-1})	10.96±0.46	10.92±0.44	11.34±1.00	10.56±0.61	10.92±0.24	10.57±0.53	11.30±0.40	ns	ns	ns	ns
Dry matter (g kg^{-1} MG d^{-1})	3.18±0.20	3.22±0.19	3.13±0.31	3.36±0.34	3.11±0.14	3.09±0.22	3.31±0.19	ns	ns	ns	ns
Protein (g kg^{-1} MG d^{-1})	1.34±0.10	1.21±0.09	1.28±0.19	1.24±0.14	1.41±0.06	1.33±0.10	1.23±0.10	ns	ns	ns	ns
Ether extract (g kg^{-1} MG d^{-1})	1.52±0.21	1.72±0.19	1.8±0.36	1.57±0.29	1.47±0.15	1.54±0.26	1.69±0.17	ns	ns	ns	ns
Energy (J kg^{-1} MG d^{-1})	89.09±7.03	96.83±6.72	97.31±12.61	91.90±5.31	89.66±5.31	90.05±9.65	95.86±5.89	ns	ns	ns	ns

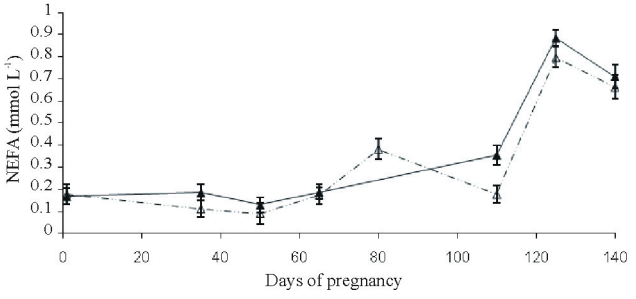
SEM - standard error of the mean.
ns - not significant (P>0.05); * P<0.05; ** P<0.01.
MG - mammary glands.
A, B, C - means followed by the same letter do not differ statistically by Tukey's test (P>0.05).

with the concentrations in Saanen goats (Figure 3). Moreover, animals with twin pregnancies showed higher concentrations of NEFA at 110 days compared with animals with single pregnancies (P<0.05; Figure 4).



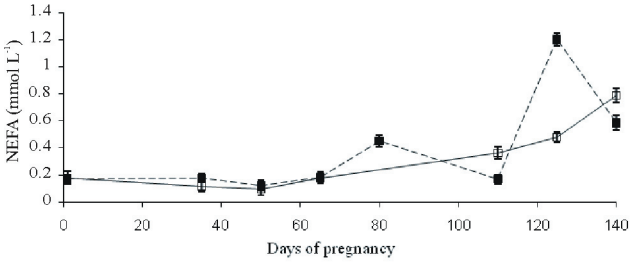
There was no effect of type of pregnancy or breed (P>0.05) on the levels of ß-hydroxybutyrate.

Figure 2 - ß-hydroxybutyrate serum concentrations in goats at different stages of pregnancy (±SEM).



Breed × days interaction (P<0.01).

Figure 3 - Serum concentrations of non-esterified fatty acids (NEFA) in Oberhasli (-△-) and Saanen (-▲-) goats at different stages of pregnancy (±SEM).



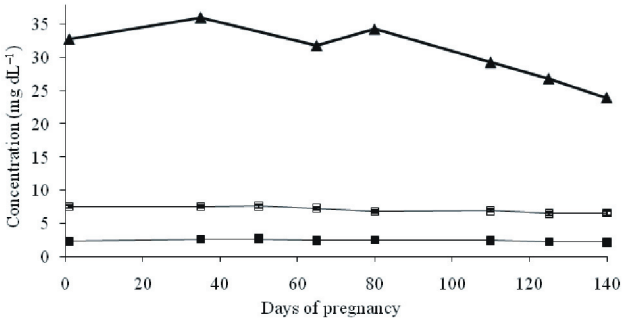
Fetuses × days interaction (P<0.05).

Figure 4 - Serum concentrations of non-esterified fatty acids (NEFA) in goats with single (-□-) and twin (-■-) pregnancies at different stages of pregnancy (±SEM).

The serum urea levels decreased (P<0.05) from 80 days of pregnancy (Figure 5) and were not affected by the number of fetuses or breed. Serum total protein and albumin (Figure 5) levels decreased after 135 days of pregnancy and were not affected by the type of pregnancy or breed.

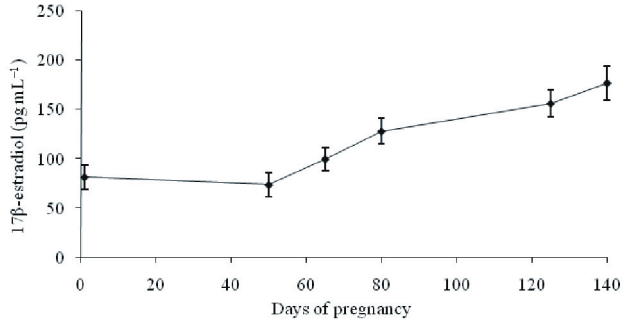
The plasma 17ß-estradiol levels increased with the advance of pregnancy (P<0.05; Figure 6). On the other hand, plasma IGF-1 levels were highest between 60 and 80 days of pregnancy (Figure 7), and there was no effect of breed or type of pregnancy.

Plasma progesterone concentrations were higher (P<0.01) in the goats with twin pregnancies, averaging 17.08±1.01 pg mL^{-1}, while single pregnancy goats showed an average of 13±1.08 pg mL^{-1}. Plasma insulin levels over the total gestation period remained constant, at approximately 10.43±0.36 µIU mL^{-1}.



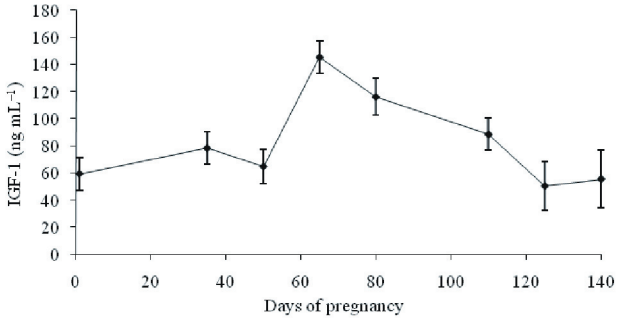
There was no effect of number of fetuses or breed (P>0.05) on the levels of this metabolite.

Figure 5 - Serum concentrations of urea (-▲-), total proteins (-□-), and albumin (-■-) in goats at different stages of pregnancy (±SEM).



There was no effect of type of pregnancy or breed (P>0.05) on 17ß-estradiol levels.

Figure 6 - Plasma 17ß-estradiol levels in goats at different stages of pregnancy (±SEM).



There was no effect of type of pregnancy or breed on plasma IGF-1 concentrations (P>0.05).

Figure 7 - Plasma IGF-1 concentrations in goats at different stages of pregnancy (±SEM).

Discussion

Our study sought to evaluate the effect of the type of pregnancy in different breeds of dairy goats throughout gestation. One of the most important findings was that the major changes in maternal metabolism as well as in nutrient retention during pregnancy were caused by the progression of pregnancy, regardless of the number of fetuses.

The total weight of the fetuses in a twin pregnancy was 66% higher than in a single pregnancy, which likely caused a compression of the rumen and lower DM intake in goats with twin pregnancies. The reduction of intake in pregnant females may not only be associated with the physical limitation but also with an increase in the levels of plasma estrogen released from the placenta (Grummer, 1990; Forbes, 2007). Additionally, it has been reported that lower estrogen:progesterone ratios interfere with intake (Grummer, 1995), which may also be related to the lower DM intake observed as the cows pregnant with twins showed a lower ratio of these hormones.

Although we observed a decrease in dry matter intake by animals with twin pregnancies at the end of gestation, this was not reflected in the mobilization of nutrients from the maternal body. This efficiency in maternal metabolism during twin pregnancy may be related to the increased digestibility of nutrients that occurs as reported by Macedo Junior et al. (2012). The energy expenditure associated with digestion can be influenced by factors such as the type of food and intake level (Lima et al., 2008; Labussière et al., 2011), and this may be directly reflected in the maintenance requirements. Thus, the goats with twin pregnancies might have reduced their maintenance requirements as they showed lower DM intake, but even with a lower intake, the input of nutrients to the goat was not impaired. Nevertheless, more studies related to changes in maintenance requirements during pregnancy are needed.

The order in which tissues are deposited in the body during growth starts with the nervous tissue followed by the bone, muscular, and, lastly, adipose tissues (Lawrence et al., 2012). In our study, this theory is supported because the proportion of protein deposited in the fetus during pregnancy was greater than fat. In addition, the protein in the maternal body was stored in larger quantities at the beginning of pregnancy (until 80 days) and began to decline with its advance due to the transfer of this nutrient to sustain protein deposition in the fetus(es).

The protein mobilized in the maternal body during pregnancy is not only intended for fetal development but is also the primary nutrient supplied to the uterine tissues. Because the quantity of protein is limited, the uterus cannot sustain normal fetal growth (Bell, 1995), and this was one of the causes of the high amounts of protein mobilized from the maternal body, especially from 110 to 140 days of pregnancy. Additionally, the lower crude protein intake observed during the final third of pregnancy period led to a decrease in serum urea levels. As a consequence, urea recycling via the ruminal epithelium and saliva may be more efficient and improve the uptake of nitrogen to the ruminal microorganisms (Brun-Bellut, 1997; Marini and Van Amburgh, 2003). This demonstrates that protein retention in the products of gestation cannot be achieved solely through the feed ingested by the goat; we observed that protein deposition in the products of gestation was 93% of the total that was mobilized in the maternal body.

The drop in the levels of serum total proteins and albumin with the advance of pregnancy may be related to the higher protein and energy requirements for gestation (Balikci et al., 2007), given that hepatic gluconeogenesis in ruminants is primarily performed with gluconeogenic amino acids (Kozloski et al., 2009). However, another aspect to be considered is that the reduction in the serum concentration of these metabolites may be related to the increased maternal blood volume during gestation (Azab and Abdel-Maksoud, 1999), which, in turn, is caused by an increase in serum estrogen levels (Dickson et al., 1969; Bell, 1975; Ford, 1995). An increase in blood volume was observed in this study, and although the amount of blood collected at slaughter does not directly represent the total amount of circulating blood, it can indicate an increase.

In contrast with protein, only 8% of the total amount of fat mobilized from the maternal body was proportionally retained in the products of gestation. Although the loss of fat due to the use of the body reserves is 80% (AAC-SCA, 1990), it is believed that the remaining energy mobilized

from the body reserves fulfills the maintenance energy requirements of the goat. As previously stated, we observed that this mobilization is concentrated in the final third of pregnancy (Khan and Lundri, 2002), which can be verified by the increasing levels of serum NEFA and ß-hydroxybutyrate during this phase.

Fetal growth between 80 and 140 days of pregnancy corresponded to 92.6% of the total weight of the fetus, which corroborates studies conducted by Ferrel (1992) and Lima (2011). The relative daily retention rates of DM and protein indicate that the composition of gain, proportional to the size of the fetuses, is the same throughout gestation. Unlike the fetuses, the increased development of the uterus and fetal fluid occurs over the first two thirds of pregnancy irrespective of the number of fetuses. This may be related to the higher plasma concentrations of the IGF-1 hormone, which is directly related to muscle tissue growth. In support of this hypothesis, we observed that the highest concentrations of IGF-1 occurred between 65 and 80 days of pregnancy, which was the phase when the uterus showed the highest daily growth rate.

The fetuses from the Oberhasli goats were larger, which could be due to the higher daily retention rates in the uterus and fetal fluid, whose masses are proportional to the size of the fetus (Bell et al., 2005). These relationships between the weight of the uterus and fetal fluid and the size of the fetus seem to be related to bone growth, since we did not find greater deposition of protein and fat in the Oberhasli goat fetuses. Comparative information for these breeds is still scarce, and further studies are needed to find differences in their production efficiency.

The results of the present study are of great importance to decision-making regarding feeding management for pregnant goats. Given that the mobilization of fat and protein in the maternal body as well as the deposition of the products of gestation are not affected by twin pregnancy, we emphasize the importance of maintaining the nutritional status of pregnant goats to prevent body mobilizations higher than what can be supported. In addition, goats may have triplet and quadruplet births, so more studies are needed to evaluate the effects of the gestation of three or four fetuses on maternal metabolism.

Conclusions

The maternal metabolism during pregnancy does vary according to the type of pregnancy (single or twin).

Changes in the metabolism of protein and energy occur mainly with the advance of pregnancy such that goats use their body fat reserves to meet their nutritional requirements of maintenance and gestation during the final third of pregnancy.

Acknowledgments

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