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Energy and protein requirements for maintenance of dairy goats during pregnancy and their efficiencies of use¹

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ABSTRACT: It has been suggested that maintenance requirements are similar among animals of different physiological stages; however, important physiological changes occur in the maternal body during pregnancy. Therefore, the aim of this study was to determine the energy and protein requirements for the maintenance of pregnant dairy goats and to estimate their efficiency of energy and protein utilization for maintenance and pregnancy. We used 66 multiparous pregnant goats having 49.0 ± 1.59 kg initial BW (around the third or fourth parturition) arranged in a randomized block design with a 3×3 factorial scheme including slaughter at different days of pregnancy (DOP; 80, 110, and 140 d) and feed restriction (0, 20, and 40% feed restriction). The comparative slaughter technique was used to estimate energy and protein maintenance requirements. Goats slaughtered at 140 DOP were subjected to digestibility trials at around 80, 110, and 140 DOP to estimate diet metabolizability and N balance (NBAL). Metabolizability decreased with feed restriction and was 63.3 ± 2.16 , 55.7 ± 2.35 , and $58.2 \pm 2.30\%$ at 0, 20, and 40% of feed restriction, respectively ($P < 0.01$). There was no effect of DOP on NE_m or the requirements of ME for

maintenance (ME_m), which were 197 and 315 kJ/kg empty body weight (EBW)^{0.75}, respectively, and the efficiency of ME utilization for maintenance (k_{mE}) was 0.63. Similarly, DOP did not affect the daily net protein requirements for maintenance (NP_m) estimated using the comparative slaughter technique (1.38 ± 0.512 g/kg EBW^{0.75}; $P = 0.003$) or the NP_m estimated using NBAL (2.49 ± 0.594 g/kg EBW^{0.75}; $P < 0.01$). The MP requirement for maintenance (MP_m) estimated using the comparative slaughter technique was not affected by DOP and was 3.22 g MP/kg EBW^{0.75} ($P < 0.01$). The efficiency of MP utilization for maintenance (k_{mP}) was 0.43. The efficiency of ME utilization for pregnancy (k_{pE}) increased with the progress of pregnancy and was 0.058, 0.10, and 0.19 at 80, 110, and 140 DOP, respectively. Similarly, the efficiency of MP utilization for pregnancy (k_{pP}) increased with DOP and was 0.12, 0.21, and 0.43 at 80, 110, and 140 DOP, respectively. There was no evidence that pregnancy affected NE_m , ME_m , NP_m , and MP_m or k_{mE} and k_{mP} , which were also unaffected by DOP. However, k_{pE} and k_{pP} increased with pregnancy progress as a response to the physiological changes that pregnant females are subjected to.

Key words: efficiency, metabolizable energy, metabolizable protein, nutritional demands

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doi:10.2527/jas2017.1490

INTRODUCTION

During pregnancy of ruminants, physiological adjustments occur in the maternal body to ensure fetal and mammary gland development (Bell and Ehrhardt, 2000; Castagnino et al., 2015). It is known that pregnancy induces adaptive responses in maternal hepatic synthesis and peripheral tissue utilization of glucose (Bell and Bauman, 1997); pregnant females increase

¹We thank the São Paulo Research Foundation (FAPESP) for providing financial support (grants numbers 2013/04758-5, 2009/10125-0, and 2007/58239-8). No conflict of interest was declared.

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Received February 20, 2017.

Accepted July 2, 2017.

basal metabolism because of the increase of blood volume, renal and respiratory work, adaptations in the cardiovascular system, greater tissue synthesis, and increase of fetal placental blood flow (U.S. Institute of Medicine, 1990; Mattison et al., 1991; Reynolds et al., 2006). Therefore, due to such physiological adjustments, our hypothesis is that energy and protein requirements for maintenance of goats may change during pregnancy.

At the beginning of pregnancy there are substantial hormonal changes to prepare the maternal body for pregnancy (Hafez and Hafez, 2000), which does not imply changes in body composition. The major changes in maternal body composition and DMI of goats have been observed only after 80 d of pregnancy (i.e., midterm pregnancy), specially with goats carrying twins (Castagnino et al., 2015). The decrease of DMI observed in the last third of pregnancy occurs simultaneously with the increase of energy and protein requirements (Härter et al., 2016). Furthermore, control of metabolism during pregnancy involves homeostasis and homeorhesis regulation (Bauman and Currie, 1980). Therefore, it is suggested that such physiological changes during pregnancy may increase the efficiencies of energy and protein use for maintenance as well as for pregnancy.

In the current feeding systems used worldwide for goats, energy and protein maintenance requirements are based on data obtained from adult and nonpregnant goats as well as from sheep data (AFRC, 1998; NRC, 2007). In addition, there is scarce information in the literature about the efficiency of ME and MP utilization in pregnant goats. Therefore, the objective of this study was to determine the energy and protein maintenance requirements of goats and to estimate the efficiencies of energy and protein utilization during pregnancy.

MATERIALS AND METHODS

The experiment was performed to determine the energy and protein requirements for maintenance of dairy goats (Saanen and Oberhasli breeds) during pregnancy. The comparative slaughter technique was used to estimate maintenance requirements. Humane animal care and handling procedures were performed according to guidelines set by the Committee on Ethical Animal Welfare (CBEA; São Paulo State University [UNESP]), School of Agricultural and Veterinarian Sciences (Jaboticabal, Brazil). The project was approved by CBEA under protocol number 026167-07.

Goats were mated either during natural estrus or after induced estrus (during the seasonal anestrus) using a hormone regimen recommended by Ritar et al. (1984) and Freitas et al. (1996). Once estrus was confirmed, females were individually exposed to a male for natural mating. Two males were used in this ex-

periment, 1 of each breed (i.e., Saanen and Oberhasli). Within breed, all females were mated to the same male. After mating, the goats were placed in individual stalls equipped with a feeder and automatic water supplier.

Between 35 and 45 d of pregnancy (DOP), ultrasonographies were performed to confirm pregnancy and determine litter size. As single pregnancies are less common in goats and a previous study showed that maternal body (MB; considered the pregnant goat body disregarding the gravid uterus and mammary gland) metabolism does not change with litter size (Castagnino et al., 2015), only does carrying twins were included in this study.

The study used a total of 66 multiparous (around the third or fourth parturition) pregnant goats weighing 49.0 ± 1.57 kg BW (at mating) carrying twins. Eight pregnant goats (baseline animals) fed ad libitum were slaughtered at 50 DOP (baseline slaughter date) to estimate initial body composition and to calculate energy and protein retention from 50 DOP to 80, 110, or 140 DOP. The remained 58 pregnant dairy goats weighing 49.8 ± 1.57 kg BW at mating were randomly distributed into 3 groups according to gestational length (80, 110, and 140 DOP). Each group was divided into blocks containing 3 pregnant does with similar breed and BW at mating, and each doe within a block was randomly subjected to a different feed restriction (0, 20, or 40%). Then, the experiment was performed in a randomized incomplete block design with a 3×3 factorial treatment structure, including DOP (80, 110, and 140 d) and feed restriction (0, 20, and 40% feed restriction; Supplementary Fig. S1 [see the online version of the article at <http://journalofanimalscience.org>]). In short, the experiment was composed of 8 blocks within 80 DOP (6 complete blocks and 2 incomplete blocks), 7 blocks within 110 DOP (6 complete blocks and 1 incomplete block), and 7 blocks within 140 DOP (6 complete blocks and 1 incomplete blocks; Supplementary Fig. S1 [see the online version of the article at <http://journalofanimalscience.org>]).

Pregnant does started being subjected to feed restriction at, on average, 36.8 ± 0.998 DOP (i.e., after ultrasonographies) and continued until the target slaughter DOP was reached. Animals without feed restriction (0%) were fed ad libitum, with the daily feed amount offered adjusted to allow 15%orts. The amount of feed offered daily to animals subjected to 20 and 40% feed restriction was based on the amount of feed consumed on the previous day by goats fed ad libitum within the block. Orts from goats fed ad libitum were collected daily, and composite samples were taken every 30 d. The diet (Table 1) was formulated to meet the nutritional requirements of pregnant goats as defined by the NRC (2007). Animals were fed twice daily, at 0730 and 1700 h. Maintenance requirements were estimated using the comparative slaughter

Table 1. Chemical composition of the ingredients in the experimental diet, expressed on a DM basis

Ingredient	Ingredient, % of diet, DM basis	DM, %	GE, MJ/kg of DM	CP, % of DM	SolP, ¹ % of CP ³	SolNP, ² % of CP ³	SDP, ³ % of CP ⁴	ADIP, ⁴ % of CP ⁵	EE, ⁵ % of DM	NDF, ⁶ % of DM	Ash, % of DM
Cracked corn	32.9	82	16.3	10.2	24	17.5	73	3.2	2.9	16.9	1.97
Soybean meal	12.3	83.1	17.4	51.8	30.4	16	67.2	23.7	1.86	22.2	6.31
Dehydrated corn plant	44	85.2	16.1	10.22	9.5	9.03	50.6	39.9	1.71	58	4.24
Tifton 65 hay	10	87.2	16.1	7.76	29.3	4.5	50.2	16	0.94	78.4	6.6
Mineral premix ⁶	0.38	99	–	–	–	–	–	–	–	–	99.9
NaCl	0.07	98	–	–	–	–	–	–	–	–	99.1
Limestone	0.33	95	–	–	–	–	–	–	–	–	99.1
Diet composition		84.2	16.2	15.0	18.7	12.1	59.6	23.1	2.03	41.7	4.73

¹SolP = soluble protein; SolP is nonprotein N assumed to be instantly degraded in the rumen and the true protein that rapidly escapes from in situ bags.

²SolNP = nonprotein N.

³SDP = insoluble protein (that is potentially degradable); this is the B fraction.

⁴ADIP = acid detergent insoluble protein.

⁵EE = ether extract.

⁶Premix contained (per kg) 190 g Ca, 90 g Cl, 73 g P, 62 g Na, 44 g Mg, 30 g S, 940 mg Mn, a maximum of 730 mg F, 340 mg Cu, 16 mg I, 10 mg Se, 3 mg Co, 1.35 mg Zn, and 1.06 mg Fe.

technique according to the method proposed by Lofgreen and Garrett (1968).

Body weight was measured during the experiment (every 15 d) and immediately before slaughter without fasting. The slaughter procedure involved stunning the animal with a pneumatic pistol followed by exsanguination by cutting the jugular veins and carotid arteries, and the animal's blood was collected, weighed, packaged, and frozen at -20°C to be reincorporated into the MB at grinding. Upon cessation of vital signs, the gravid uterus (uterus, placenta, placentomas, fetuses, and fetal fluid) and mammary gland were removed from the goat's body. The gastrointestinal tract (GIT) was weighed before and after emptying and flushing with water to determine empty body weight (EBW), which was calculated as the body weight at slaughter minus the weight of the contents of the GIT, bladder, and gallbladder. The MB weight was obtained by subtracting the gravid uterus and mammary gland weights from EBW. The gravid uterus, mammary gland, and MB (including empty GIT, blood, heart, lungs, spleen, and other viscera) were weighed, packaged, and frozen at -20°C . The gravid uterus, mammary gland, and MB were subsequently ground, homogenized, and sampled for further chemical analysis.

Digestibility Trial

To determine to the ME intake (MEI) and N balance (NBAL), the set of goats to be slaughtered at 140 DOP ($n = 18$) was subjected to 3 digestibility assays during pregnancy at around 80, 110, and 140 DOP. While they were still on trial, the goats were adapted to the metabolic cages for 2 d followed by a 5-d collection period. The goats were individually housed in metabolic cages in

a randomized block design according to the feed intake treatments previously mentioned. The goats were placed in metabolism cages at 73, 103, and 133 DOP. Feed, orts, feces, and urine were collected daily over the 5-d trial. A total of 20% of the excreted feces was collected, producing a 5-d composite. Urine was collected in buckets containing 50 mL of 7.2 N H_2SO_4 , and a 10% aliquot was removed daily and frozen for further analysis.

Chemical Analyses

Samples of feed, orts, and feces were dried in a forced-air oven at 55°C for 72 h. The gravid uterus, mammary gland, and MB samples were freeze-dried for 72 h. The DM of feed, orts, and feces was determined following Association of Official Analytical Chemists (1990) procedures (method number 930.15), and the fat content of the feed ingredients was also determined following Association of Official Analytical Chemists (1990) procedures (number 920.39). Feed ingredients were analyzed for ash (complete combustion at 600°C for 6 h; AOAC, 1990; method number 924.05) and NDF with amylase and without sulfite (Robertson and Van Soest, 1981). The N content of the feed, orts, urine, feces, gravid uterus, mammary gland, and MB were determined using Dumas combustion using a LECO FP-528 LC (LECO Corp., St. Joseph, MI; Etheridge et al., 1998). Gross energy of feed, orts, urine, feces, gravid uterus, mammary gland, and MB was determined using a calorimetric bomb (Parr Instrument Co., Moline, IL).

Calculations

The MEI was estimated as the GE intake minus energy losses in feces, urine, and gases. The losses in feces

Table 2. Equations obtained from baseline goats slaughtered at 50 d of pregnancy used to estimate energy and protein retention during pregnancy

Dependent variable	Intercept	SEM	Slope	SEM	Independent variable ¹	P-value	RMSE ²
Maternal BW (MBW), kg	1.96	5.77	0.736	0.112	BW ₅₀ ¹	<0.01	1.82
Energy, kJ	-43.0	111	13.0	2.74	MBW ₅₀ ²	<0.01	37.5
Protein, g	-128	931	163	22.9	MBW ₅₀	<0.01	313

¹BW₅₀ = BW at 50 d of pregnancy; MBW₅₀ = MBW at 50 d of pregnancy.

²RMSE = root mean square error.

and urine were estimated using the digestibility data at 80, 110, and 140 DOP. The energy lost through gas production was estimated using the equation proposed by Blaxter and Clapperton (1965). The MEI as a proportion of GE intake was considered to be the metabolizability (**qm**).

Energy and N daily retention in the MB were estimated using the difference between the energy and protein content of the MB at slaughter (80, 110, or 140 DOP) and the energy and protein content of the MB at 50 DOP as estimated using the equations obtained from the baseline goats. The equations used to predict the MB energy and N content at 50 DOP are presented in Table 2.

The energy requirement for maintenance during pregnancy was estimated as the nonlinear relationship between daily heat production (**HP**) and daily MEI according to Eq. [1]. The HP was estimated as the difference between MEI and retained energy (retained energy in the MB + retained energy in the pregnancy products) as follows:

$$HP_{ijk} = W_{0j} \times \exp(b_j \times MEI_{ijk}) + bl_{k(j)} + error_{ijk} \quad [1]$$

in which HP_{ijk} is the daily HP (kJ/kg EBW^{0.75}) for the *i*th animal in the *k*th block on the *j*th DOP; W_{0j} is the intercept to be estimated, which was considered equal to the NE_m of the *j* = 1, 2, or 3 DOP; b_j is the slope of the exponential equation to be estimated for each *j* = 1, 2, or 3 DOP; MEI_{ijk} is the daily MEI (kJ/kg EBW^{0.75}) for the *i*th animal in the *k*th block on the *j*th DOP; $bl_{k(j)}$ is the effect of block *k* nested in DOP *j*; and $error_{ijk}$ is the residual error $\sim N(0, \sigma_e^2)$, in which σ_e^2 is the residual variance.

The NE_m (kJ/kg EBW^{0.75}), which is equal to the fasting HP, was estimated as the intercept of the exponential equation. The requirements of ME for maintenance (ME_m ; kJ/kg EBW^{0.75}) was computed by interactively solving the exponential equation until HP was equal to MEI. The efficiency of ME utilization for maintenance (k_{mE}) was computed as NE_m/ME_m .

The MP requirement for maintenance (MP_m) was estimated using regression of the retained protein on MB and pregnancy products (g/kg EBW^{0.75}) against MP intake (**MPI**; g/kg EBW^{0.75}). The MPI was estimated using

the chemical composition of the diet according to Luo et al. (2004a). The MP_m was obtained by assuming the retained protein on MB and pregnancy products as equal to 0.

$$RP_{ijk} = a_j + b_j \times MPI_{ijk} + bl_{k(j)} + error_{ijk} \quad [2]$$

in which RP_{ijk} is the daily retained protein in MB and pregnancy products estimated using the comparative slaughter technique (g/kg EBW^{0.75}) for the *i*th animal in the *k*th block on the *j*th DOP; a_j is the intercept representing the endogenous and metabolic losses of the *j* = 1, 2, or 3 DOP; b_j is the slope of regression of the *j* = 1, 2, or 3 DOP; MPI_{ijk} is the daily MPI (g/kg EBW^{0.75}) for the *i*th animal in the *k*th block on the *j*th DOP; $bl_{k(j)}$ is the effect of block *k* nested in DOP *j*; and $error_{ijk}$ is the residual error $\sim N(0, \sigma_e^2)$.

The daily net protein requirement for maintenance (NP_m) during pregnancy was estimated using a linear regression of retained N against N intake (g/kg EBW^{0.75}; Eq. [2]). The retained N was estimated using the comparative slaughter (N retained on MB and pregnancy products) and NBAL (from the digestibility trial) techniques. The intercept of the regression was assumed to be the endogenous and metabolic losses of N, which are assumed to represent the net N requirement for maintenance. This value multiplied by a factor of 6.25 provided the estimate of NP_m .

$$RN_{ijk} = a_j + b_j \times NI_{ijk} + bl_{k(j)} + error_{ijk} \quad [3]$$

in which RN_{ijk} is the daily retained N estimated using the comparative slaughter or NBAL (g/kg EBW^{0.75}) technique for the *i*th animal in the *k*th block on the *j*th DOP; a_j is the intercept representing the endogenous and metabolic losses of the *j* = 1, 2, or 3 DOP; b_j is the slope of regression of the *j* = 1, 2, or 3 DOP; NI_{ijk} is the daily N intake (g/kg EBW^{0.75}) for the *i*th animal in the *k*th block on the *j*th DOP; $bl_{k(j)}$ is the effect of block *k* nested in DOP *j*; and $error_{ijk}$ is the residual error $\sim N(0, \sigma_e^2)$.

The efficiency of MP utilization for maintenance (k_{mP}) was computed as NP_m/MP_m .

The efficiency of ME utilization for pregnancy (k_{pE}) and the efficiency of MP utilization for pregnancy (k_{pP}) were estimated using regressions of energy and protein daily retention in the MB plus pregnancy products against daily MEI and MPI above maintenance. For this calculation, we used the ME_m and MP_m determined using Eq. [1] and [2]. These regressions were set with an intercept equal to 0, and the k_{pE} and k_{pP} were obtained using the inverse of slope for pregnancy product retention (Eq. [4]). In this study, we did not consider the slope for the MB as an efficiency because the energy and protein retention are a result of the deposition and mobilization processes and we were not able to quantify them separately. Therefore, there was no interpretation of the slope as related to retention in the MB.

$$IAM_{ijk} = a_j \times RMB_{ijk} + b_j \times RPP_{ijk} + bl_{k(j)} + error_{ijk} \quad [4]$$

in which IAM_{ijk} is the intake of ME above maintenance or MP above maintenance for the i th animal in the k th block on the j th DOP; a_j is the slope related to the retention on the MB of the $j = 1, 2, \text{ or } 3$ DOP; RMB_{ijk} is the retained energy or protein on the MB for the i th animal in the k th block in the j th DOP; b_j is the slope related to the retention on pregnancy products of the $j = 1, 2, \text{ or } 3$ DOP; RPP_{ijk} is the retained energy or protein on pregnancy products for the i th animal in the k th block on the j th DOP; $bl_{k(j)}$ is the effect of block k nested in DOP j ; and $error_{ijk}$ is the residual error $\sim N(0, \sigma_e^2)$.

Statistical Analyses

The regression equations obtained from the baseline goats to predict the MB energy and protein content at 50 DOP were obtained using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Statistical significance was declared at $P < 0.05$. The slopes and intercepts of each equation were estimated using the ESTIMATE statement of the MIXED procedure of SAS.

The results obtained from the digestibility trial were evaluated in a completely randomized block design with repeated measures over time. The data were analyzed as mixed models with fixed effects of level of feed restriction (2 df), days of gestation (2 df), and the interaction of these factors and the random effects of block and residual error using the MIXED procedure of SAS (version 9.4). The covariance matrix that best fit the data according to the corrected Akaike information criterion (AICc) was selected. Orthogonal contrasts

were performed to verify linear or quadratic trends. Statistical significance was declared at $P < 0.05$.

The results of MB composition were evaluated in a randomized block design as mixed models with the fixed effects of level of feed restriction (2 df), days of gestation (2 df), and the interaction of these factors and the random effects of block and residual error using the MIXED procedure of SAS (version 9.4). Orthogonal contrasts were performed to verify linear or quadratic trends. Statistical significance was declared at $P < 0.05$.

Maintenance requirements and efficiencies were obtained by fitting nonlinear equations (i.e., Eq. [1] to Eq. [4]) to the data using the %NLINMIX macro of SAS (version 9.4), considering DOP as a fixed effect. The between-blocks variability was modeled by introducing a parameter μ into equation parameters (W_0 or a). The block effect was included in the random statement (subject = block [days]) using Variance Components as the variance-covariance matrix structure (Littell et al., 2006). The residual variance was modeled using the power-of-the-mean variance function to obtain a homogeneous variance of the residuals (Littell et al., 2006). Restricted maximum likelihood or maximum likelihood was used as the method of estimation, and the choice between them was based on the AICc value. The equations were fitted using a linearization around the empirical best linear unbiased prediction, which was based on the parameter estimation procedure of Lindstrom and Bates (1990). Parameters of the models for each DOP were compared using the CONTRAST statement. When there was no effect of DOP on the equations parameters, the fixed effect of DOP was removed from the model. Differences between parameters were declared significant at $P < 0.05$.

Monte Carlo simulations were performed to obtain the lower 90% confidence interval (LCI) and upper 90% confidence interval (UCI) of the ME_m , NE_m , MP_m , and NP_m estimates. We calculated 10,000 simulated values for each of these energy and protein requirements with a multivariate normal distribution for the parameters and error estimates using the algorithm of Fan et al. (2002).

RESULTS AND DISCUSSION

Digestibility and Maternal Body composition

The DMI linearly decreased as pregnancy progressed ($P < 0.01$) as well as with increasing feed restriction ($P < 0.01$; Table 3). Goats used in this study were carrying twins, which likely led to greater rumen compression as the pregnancy progressed than at the beginning of pregnancy, resulting in a considerable decrease in DMI (Forbes, 2007). In addition, with the progress of pregnancy, feed intake is affected by an increase in the levels of plasma estrogen released from the placenta, increasing the estrogen-to-progesterone ratio (Grum-

mer, 1995). Although the decrease in feed intake led us to expect an increase in nutrient digestibility, this was not observed for GE and CP digestibility (69.6 ± 6.28 and 73.2 ± 4.78 , respectively), which did not change with DOP or feed restriction. However, our results are in accordance with studies performed on pregnant ewes limit-fed chopped alfalfa hay and beef cows fed ad libitum, which also reported no change in apparent total tract DM digestibility with advancing pregnancy (Gunter et al., 1990; Hanks et al., 1993).

The qm was greatest at 0% of feed restriction and decreased at a decreasing rate ($P = 0.03$) with feed restriction; qm was 63.3 ± 2.16 , 55.7 ± 2.35 , and $58.2 \pm 2.30\%$ at 0, 20, and 40% of feed restriction, respectively. A significant interaction between DOP and feed restriction was observed for energy balance; at 80 and 110 DOP, goats fed ad libitum showed the greatest energy balance; however, at 140 DOP, the energy balance was similar among goats irrespective of feed restriction ($P = 0.025$; Table 3). The NBAL linearly decreased as feed restriction increased ($P < 0.05$; Table 3) and was 6.39 ± 2.13 , 1.36 ± 2.40 , and -0.468 ± 2.32 g/d at 0, 20, and 40% of feed restriction, respectively. In addition, losses of energy and N in the urine (% of energy and N intake, respectively) linearly increased ($P = 0.02$) in these females as feed restriction increased. Irrespective of DOP, pregnant goats subjected to 0, 20, and 40% feed restriction showed, on average, energy losses in urine of 2.53, 2.70, and 3.72% energy intake, respectively, and N losses in urine of 62.5, 67.6, and 92.6% N intake, respectively. This may explain the decrease in qm and NBAL with feed restriction, which could also indicate a mobilization of MB reserves. Goats at 140 DOP showed lighter MB weight than goats at 80 and 110 DOP, especially those fed at 40% feed restriction ($P = 0.011$; Table 4). On the other hand, protein, fat, and energy MB composition were not affected by DOP and feed restriction ($P > 0.05$; Table 4). Therefore, it is clear that goats decreased their body tissues composition proportionally to MB weight. In addition, a linear decrease was observed in the protein and energy retention on the MB with the increase of feed restriction ($P < 0.05$; Table 4). Therefore, these changes in the energy and protein retentions confirm the mobilization of MB reserves.

Through these results, we can infer that due to the high protein and energy demand of the pregnancy products, the female mobilizes body reserves when the diet supply does not meet those requirements (Bauman and Currie, 1980; McNeill et al., 1997). Therefore, as digestibility of CP and GE did not increase with feed restriction, mobilization of maternal reserves to meet pregnancy demands was needed, leading to the decrease in qm, energy balance, and NBAL. In addition,

Table 3. Results of digestibility trial performed with pregnant goats subjected to feed restriction from 80 to 140 d of pregnancy

Variable	Treatments												P-value												
	80 d						140 d						Main effect ¹			Interaction ²			Contrast ³						
	0%	20%	40%	0%	20%	40%	0%	20%	40%	0%	20%	40%	SEM	DOP	R	DOP × R	DOP	R	DOP	R	Lin;	Quad;			
DMI, g/d	1,449	825	606	1,185	781	549	601	587	477	76.7	63.4	70.0	2.15	0.01	<0.01	0.17	0.45	0.54	<0.01	<0.01	Lin;	<0.01	Lin;	<0.01	
Digestibility coefficient, %																									
GE	73.8	68.2	74.1	75.6	57.8	67.1	63.4	76.7	70.0	67.1	63.4	70.0	6.28	0.45	0.54	0.07	0.45	0.54	0.07	0.07	0.07	0.07	0.07	0.07	0.07
CP	77.1	72.4	75.5	77.5	65.0	70.0	69.9	80.2	71.3	70.0	69.9	71.3	4.78	0.43	0.27	0.01	0.43	0.27	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Metabolizability, %	64.8	57.3	64.1	65.3	46.7	54.2	59.5	63.1	56.2	46.7	54.2	56.2	4.88	0.11	0.01	0.07	0.11	0.01	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Energy balance, MJ/d	16.7	9.01	8.24	11.1	6.63	5.84	5.96	7.15	5.40	6.63	5.84	5.40	1.91	<0.01	<0.01	0.02	<0.01	<0.01	0.02	<0.01	Lin;	<0.01	Lin;	<0.01	Lin;
N balance, g	8.23	1.95	1.45	13.0	1.96	-0.98	-2.10	0.156	-1.87	1.96	-0.98	-1.87	4.54	0.06	0.04	0.14	0.06	0.04	0.14	0.14	0.14	0.14	0.14	0.14	0.14

¹DOP = days of pregnancy; R = feed restriction.

²DOP × R = interaction between days of pregnancy and feed restriction.

³Orthogonal contrasts were tested only when the main effect was significant ($P < 0.05$). Lin = linear effect; Quad = quadratic effect.

we also suggest that more studies are needed to better elucidate energy and protein partition in pregnant does.

In addition, protein retention linearly decreased with the progress of pregnancy ($P < 0.01$). Irrespective of feed restriction, a negative retention at 140 DOP was observed, indicating that not only feed restriction but also the progress of pregnancy affects protein mother reserves. This can be due to the decrease of DMI that may reduce the microbial protein synthesis, decreasing the AA supply for the MB. However, this decrease of protein supply with the progress of pregnancy affected NBAL less than feed restriction.

Energy Requirements for Maintenance

There was no effect of DOP on the intercepts and slopes of the equations for estimation of energy requirements for maintenance during pregnancy ($P > 0.05$), and therefore, a unique equation (Eq. [5]) was applied from 80 to 140 DOP (Fig. 1). The NE_m was 197 (kJ/kg $EBW^{0.75}$) with an LCI and UCI of 120 and 210 kJ/kg $EBW^{0.75}$, respectively. The ME_m , when MEI was set equal to HP, was 315 kJ/kg $EBW^{0.75}$; the LCI and UCI for ME_m were 169 and 330 kJ/kg $EBW^{0.75}$, respectively.

$$HP = 197 \pm 22.6 \times \exp(0.00149 \pm 0.00018 \times MEI), [5]$$

in which HP is the daily HP (kJ/kg $EBW^{0.75}$) and MEI is the daily MEI (kJ/kg $EBW^{0.75}$; root mean square error [RMSE] = 16.5, AICc = 554, $\sigma_e^2 = 272$).

The NE_m for mature goats reported by the Agricultural and Food Research Council (1998) of 315 kJ/kg $BW^{0.75}$ is greater than that observed in our study. In addition, the observed NE_m (197 kJ/kg $EBW^{0.75}$) was also lower than those reported for sheep of 269 (Ratnayake et al., 1973) and 259 kJ/kg $BW^{0.75}$ (Blaxter, 1967). The ME_m found in this study were considerably lower than those previously reported for goats: 400 kJ/kg $BW^{0.75}$ for pregnant and lactating goats up to 8 mo of age (Voicu et al., 1993), 438 kJ/kg $BW^{0.75}$ for mature goats (AFRC, 1998), 462 kJ/kg $BW^{0.75}$ for mature goats over 18 mo of age (Luo et al., 2004b), 501 kJ/kg $BW^{0.75}$ for mature dairy goats (NRC, 2007), and 542 kJ/kg $BW^{0.75}$ for mature and growing goats (Salah et al., 2014).

To our knowledge, this is the first study measuring requirements for maintenance during pregnancy in goats, and no standard values of NE_m or ME_m have been established for goats of similar condition to allow comparison. Before the estimation of NE_m (i.e., using Eq. [5]), we tested the effect of feed restriction on the parameters of Eq. [5], and they were not significantly affected by feed restriction ($P > 0.05$), which means

Table 4. Maternal body (MB) composition of pregnant goats at from 80 to 140 d of pregnancy

Variable	Treatments												P-value						
	80 d						110 d						140 d			Interaction ²	DOP × R	DOP	R
	0%	20%	40%	40%	0%	20%	20%	40%	40%	0%	20%	40%	SEM	DOP	R				
BW, kg	62.9	55.2	50.3	62.0	64.4	63.4	61.6	61.7	56.3	3.77	0.27	0.03	0.06	—	—	Lin; <0.01			
$EBW^{0.75}$, kg	55.4	46.3	41.3	55.0	57.4	55.2	55.9	55.9	51.7	3.53	0.11	<0.01	0.03	—	—	Lin; <0.01			
MB, kg	51.0	42.7	37.8	45.8	48.1	47.2	40.8	42.5	38.2	3.29	0.25	0.03	0.01	—	—	Lin; <0.01			
Protein, g/kg MB	151	152	150	150	145	156	150	138	151	6.55	0.72	0.28	0.64	—	—	—			
Fat, g/kg MB	284	229	237	273	291	259	264	276	253	25.0	0.59	0.38	0.43	—	—	—			
Energy, MJ/kg MB	14.7	12.6	12.8	14.3	14.9	13.9	13.9	14.1	13.5	0.895	0.52	0.36	0.41	—	—	—			
Protein retention, g/kg MB	23.5	7.33	-19.2	2.72	-8.47	-1.74	-23.4	-22.3	-36.4	9.17	<0.01	<0.01	0.05	Lin; <0.01	Lin; <0.01	Lin; <0.01			
Fat retention, g/kg MB	104	40.0	19.8	83.1	93.6	54.4	39.2	68.5	12.8	35.4	0.50	0.09	0.37	—	—	—			
Energy retention, MJ/kg MB	4.55	1.72	0.298	3.32	3.46	2.08	0.983	2.15	-0.359	1.41	0.28	0.03	0.26	—	—	Lin; 0.01			

¹DOP = days of pregnancy; R = feed restriction.

²DOP × R = interaction between days of pregnancy and feed restriction.

³Orthogonal contrasts were tested only when the main effect was significant ($P < 0.05$). L = linear effect.

⁴EBW = empty body weight.

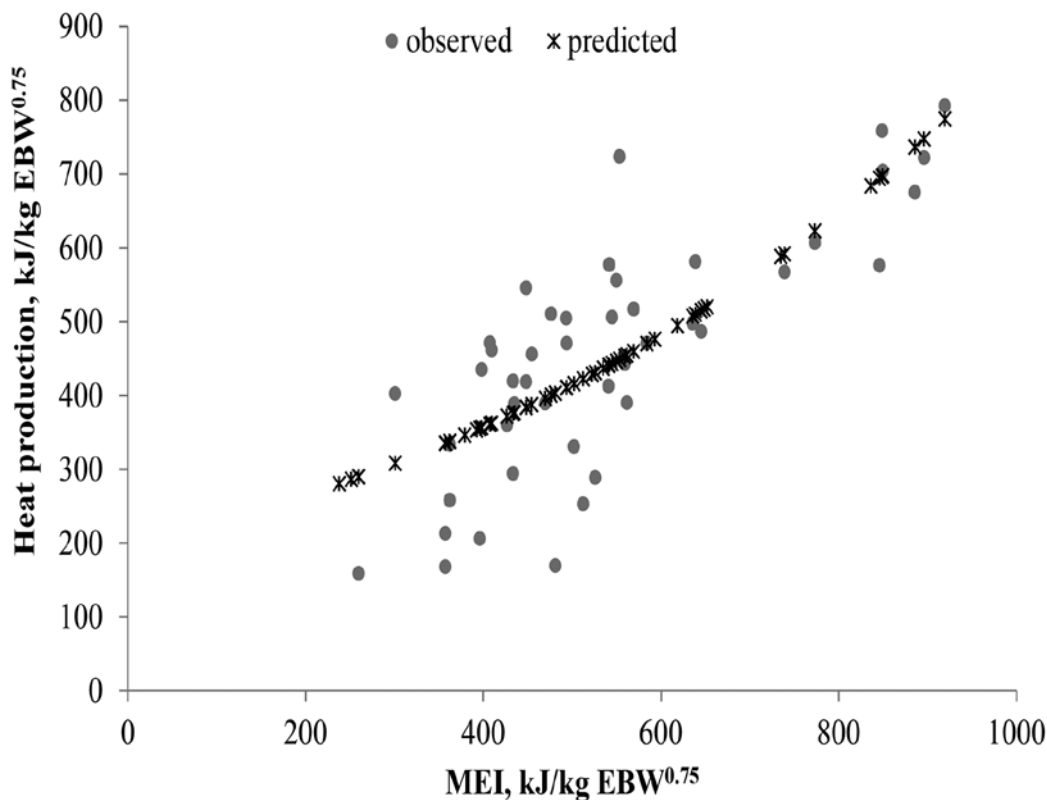


Figure 1. Relationship between daily ME intake (MEI; kJ/kg empty body weight [EBW]^{0.75}) and daily heat production (HP; kJ/kg EBW^{0.75}) of goats during pregnancy (Eq. [5]; $HP = 197 \pm 22.6 \times \exp(0.00149 \pm 0.00018 \times MEI)$).

that feed restriction did not affect the fast HP of goats in this study. Although some studies showed an effect of feeding level on FHP in lambs and calves (Ferrell et al., 1986; Labussière et al., 2011), this was not observed in our study using adult female goats. Our experiment was not designed to evaluate the feeding level effect on FHP (i.e., comparative slaughter technique), making further discussions about it difficult, and therefore, we suggest that more studies on this subject are still needed with adult ruminants, especially pregnant goats.

The low energy requirements observed in this study may be related to the decrease in basal metabolic rate (BMR) observed with aging. Graham et al. (1974) showed that the BMR of adult sheep declines 8% per annum with aging. According to these authors' findings, the NE_m of a 4-yr-old sheep is 205 kJ/kg $BW^{0.75}$, which is close to our findings and also in accordance with the age of goats used in our experiment, which were at approximately their third or fourth parturition. Aging has also been reported to decrease BMR in humans, and BMR can be more sharply decreased when associated with a tropical climate. With regard to the climate, our experiment was performed in a region with climate classification "Aw" (Rolim et al., 2007) and is characterized as tropical with a wet summer season and dry winter season, with an average annual minimum

temperature of 15.7°C and maximum temperature of 27.7°C. In accordance with our findings, preliminary results of a study conducted at the same facility with mature goats using respirometry reported fasting HP of 183 kJ/kg $BW^{0.75}$ (Fernandes et al., 2016), which is close to our result for NE_m . Therefore, based on this discussion, we can affirm that age also influences energy requirements for maintenance in goats; however, more studies related to the influence of climate on the energy requirements of goats are still needed for better understanding of this aspect.

Protein Requirements for Maintenance

The NP_m estimated using N retention in the MB and pregnancy products, obtained using the comparative slaughter technique and regressed against N intake, provided an estimate of total endogenous losses of 1.38 ± 0.512 g/kg $EBW^{0.75}$ (Eq. [6]; $P = 0.003$), which was similar among DOP ($P > 0.05$). The LCI and UCI for NP_m were 0.488 and 2.44 g/kg $EBW^{0.75}$, respectively. Furthermore, the NP_m estimated using the NBAL regressed against N intake during the digestibility trials was 2.49 ± 0.594 g/kg $EBW^{0.75}$ (Eq. [7]; $P < 0.01$); this was similar among DOP ($P > 0.05$) and was greater than the NP_m estimated using the comparative slaughter technique.

For the comparative slaughter technique,

$$RN_{CS} = -0.222 \pm 0.082 + 0.212 \pm 0.059 NI, \quad [6]$$

in which RN_{CS} is the daily retained N on the MB and pregnancy products (g/kg $EBW^{0.75}$) obtained using the comparative slaughter technique (g/kg $EBW^{0.75}$) and NI is the daily NI (g/kg $EBW^{0.75}$; $n = 58$, $RMSE = 0.159$, $AICc = -32.8$, $\sigma_e^2 = 0.0254$).

For the NBAL technique,

$$RN_{NBAL} = -0.399 \pm 0.095 + 0.605 \pm 0.093 NI, \quad [7]$$

in which RN_{NBAL} is the daily retained N using the NBAL technique (g/kg $EBW^{0.75}$) and NI is the daily NI (g/kg $EBW^{0.75}$; $n = 18$, $RMSE = 0.229$, $AICc = 8.2$, $\sigma_e^2 = 0.0528$).

The MP_m was not affected by DOP, and when retained protein was set equal to zero, a daily requirement of 3.22 g $MP/kg EBW^{0.75}$ was found (Eq. [8]; $P < 0.01$). The LCI and UCI for MP_m were 1.36 and 4.39 g/kg $EBW^{0.75}$, respectively.

$$RPMB = -0.436 \pm 0.184 + 0.135 \pm 0.0355 \times MPI, \quad [8]$$

in which RPMB is the daily retained protein on MB and pregnancy products (g/kg $EBW^{0.75}$) and MPI is the daily MPI (g/kg $EBW^{0.75}$; $RMSE = 1.92$, $AICc = 125$, $\sigma_e^2 = 3.72$).

The NP_m appears to depend on the method of assessment used. It has been reported that for many reasons, the NBAL method overestimates NP_m for goats (Almeida et al., 2015a). In accordance, our result of NP_m as estimated using NBAL was practically double that determined using the comparative slaughter technique. In addition, it is noteworthy that the requirements found using the NBAL method resulted in greater variation for the parameters than those found using the comparative slaughter method. However, the NP_m estimated herein using NBAL is similar to the NP_m reported by the Agricultural and Food Research Council (1998; 2.19 g/kg $BW^{0.75}$), which is a feeding system that bases its recommendations on studies of endogenous urinary N plus part of the metabolic fecal N and dermal losses.

On the other hand, the NP_m determined using the comparative slaughter technique in the present study was slightly lower than those reported for growing Saanen goats from 20 to 45 kg BW (1.63 and 1.46 g/kg $BW^{0.75}$; Almeida et al., 2015a; Ferreira et al., 2015), which were also obtained using this technique. Assuming an average relationship of $BW^{0.75}:EBW^{0.75}$ of 1.09 as observed in our study, the MP_m was 2.95 g/kg $BW^{0.75}$, which is close to the 3.07 g/kg $BW^{0.75}$ reported for growing goats (Luo et al., 2004a) and 2.85 g/kg $BW^{0.75}$ for mature does of

50 kg BW (NRC, 2007). Comparing our results for MP_m with the available literature on growing goats, contrary to what happens with energy, it is clear that aging has little to no influence on phenomena related to protein requirements for maintenance. There is no evidence in the literature that NP_m decreases with aging, but our findings suggest that this subject deserves further attention. Conversely, it is known that pregnancy products' growth cannot be achieved solely through the feed ingested and that pregnant goats experience a progressive decrease in protein retention in their bodies during pregnancy whereas urea recycling increases (Castagnino et al., 2015). Although the MB can suffer such adjustments to protein metabolism to ensure pregnancy success, the results of this study show that these physiological changes during pregnancy did not affect NP_m or MP_m . Furthermore, due to the greater number of observations and low error, the results of NP_m obtained using the comparative slaughter technique seem to give a more reliable reference value for adoption in mature goats.

Efficiencies of Energy and Protein Use for Maintenance and Pregnancy

The k_{mE} found in this study was 0.63. The k_{mE} is related to diet quality (ARC, 1980), and in studies using a similar diet, similar values of 0.63 (Almeida et al., 2015b) and 0.65 (Figueiredo et al., 2016) were observed. However, using the equation proposed by the Agricultural and Food Research Council (1998), which is based on qm , our simulation resulted in a k_{mE} of 0.72 (an average qm of 0.63 for animals fed ad libitum; Table 3), which was approximately 20% greater than that obtained in our study. This finding suggests that the environment may influence diet energy use, because irrespective of physiological status, we observed similar k_{mE} with similar diets, which were lower than the value predicted by the Agricultural and Food Research Council (1998) approach.

Using the results obtained using the comparative slaughter technique, we found a k_{mP} of 0.43. There appear to be no reports on k_{mP} in goats. The Agricultural and Food Research Council (1998) adopts an efficiency of absorbed AA of 1.0, and the NRC (2001) adopts an efficiency of converting MP to net protein for maintenance of 0.67 for dairy cows, which is close to the 0.70 adopted by the Commonwealth Scientific and Industrial Research Organisation (1990). However, there is huge variability among these efficiency values, which also are much greater than the value observed in this study. Some of these differences may be related to the experimental techniques used to estimate the k_{mP} as well as to differences related to the animals' species or physiological stage. Therefore, we suggest

Table 5. Equations to estimate efficiency of energy and protein use for pregnancy (efficiency is estimated using the inverse of slope for pregnancy products retention) at 80, 110, and 140 d of pregnancy (DOP)

DOP	Equations ¹	
	Energy (RMSE = 57.5, AICc = 669, $\sigma_e^2 = 3,308$)	Protein (RMSE = 1.92, AICc = 227, $\sigma_e^2 = 3.70$)
80 d	$MEI_{am} = 0.250 \pm 0.157 \text{ REMB} + 17.3 \pm 3.70 \times \text{REPP}$	$MPI_{am} = 0.588 \pm 0.225 \text{ RPMB} + 8.20 \pm 1.26 \times \text{RPPP}$
110 d	$MEI_{am} = 0.250 \pm 0.157 \text{ REMB} + 10.2 \pm 2.36 \times \text{REPP}$	$MPI_{am} = 0.588 \pm 0.225 \text{ RPMB} + 4.79 \pm 0.860 \times \text{RPPP}$
140 d	$MEI_{am} = 0.250 \pm 0.157 \text{ REMB} + 5.38 \pm 1.38 \times \text{REPP}$	$MPI_{am} = 0.588 \pm 0.225 \text{ RPMB} + 2.35 \pm 0.622 \times \text{RPPP}$
Contrasts for the equation parameters ²	<i>P</i> -value	
	Equations for energy	Equations for protein
MB at 80 d vs. SMB at 110 d	0.98	0.71
MB at 80 d vs. SMB at 140 d	0.91	0.15
MB at 110 d vs. SMB at 140 d	0.94	0.35
PP at 80 d vs. SPP at 110 d	0.07	0.03
PP at 80 d vs. SPP at 140 d	<0.01	<0.01
PP at 110 d vs. SPP at 140 d	0.08	0.03

¹Based on Eq. [4] of Material and Methods. MEI_{am} = ME intake above maintenance; REMB = retained energy on maternal body; REPP = retained energy on pregnancy products; MPI_{am} = MP intake above maintenance; RPMB = retained protein on maternal body; RPPP = retained protein on pregnancy products; RMSE = root mean square error; AICc = corrected Akaike information criterion; σ_e^2 = residual variance.

²SMB = slope for maternal body; SPP = slope for pregnancy products. The intercept of both equations was similar among DOP ($P > 0.05$).

that more studies related to efficiency of protein use in maintenance are still needed, especially for goats.

The k_{pE} and k_{pP} increased with the progress of pregnancy and were 0.058 and 0.12, respectively, at 80 DOP; 0.10 and 0.21, respectively, at 110 DOP; and 0.19 and 0.43, respectively, at 140 DOP (Table 5). These results show that efficiency is lower in earlier pregnancy than at term. It is known that the association of hormonal actions and rumen compression by the pregnant uterus leads to a decrease in pregnant females' capacity for DMI during the second half of pregnancy (from, on average, 76 d on for goats; Forbes, 2007) at the same time that pregnancy products increase their protein and energy growth rate (Härter et al., 2016). Therefore, as we observed an increase at an increased rate of k_{pE} and k_{pP} , it is plausible to assume that the MB metabolism became more efficient at nutrient distribution to the pregnancy products during the second half of pregnancy, which was the period evaluated in this study.

Additionally, our results include energy and protein deposition in the mammary gland, whereas the Agricultural and Food Research Council (1998) adopted a k_{pE} of 0.13 for 63 d after conception until the end of pregnancy for goats, and this value was obtained based only on the energy required for conceptus growth and ignores that required for mammary gland growth, which could result in a lower efficiency value. Rattray et al. (1974) reported a k_{pE} (which includes the conceptus and mammary gland energy demand) of 0.16 for sheep at 125 and 140 DOP, and these reports are very close to our k_{pE} result observed at 140 DOP.

Our results for k_{pP} at the end of pregnancy are also close to those reported for conceptus growth in preg-

nant sheep (0.40; Jarrige, 1989) and in cows (0.33; NRC, 2001), which do not include mammary gland protein accretion. However, there is no information about the period that these k_{pP} reported by the INRA and the NRC are based on. To our knowledge, this is the first study to identify these differences in protein and energy efficiencies of utilization for pregnancy during the second half of pregnancy, and there is no such available information in the literature to allow further comparison.

The k_{pE} and k_{pP} results suggest that the retention process is less efficient than the energy and protein efficiencies for maintenance. However, as shown in Fig. 2, the accretion of energy and protein in the pregnancy products is not directly related to intake level over maintenance but results mainly from maternal physiological adjustments. This is in accordance with the report by Bauman and Currie (1980), which draws attention to the fact that this apparent inefficiency is derived from ignoring the sizeable cost of maintenance of products of conception and that fetal growth and development are energetically comparable to other productive processes (i.e., growth and milk production). In addition, pregnancy imposes significant maternal adaptations, and homeorhetic controls are involved in meeting energy and nutrient requirements ahead of the increase in nutrient demands by the pregnancy products at the same time that the fetus's nutrient uptake gains a certain degree of autonomy (Bauman and Currie, 1980). Therefore, such low values for k_{pE} and k_{pP} are reported because pregnancy products' growth is irrespective of energy and protein intake.

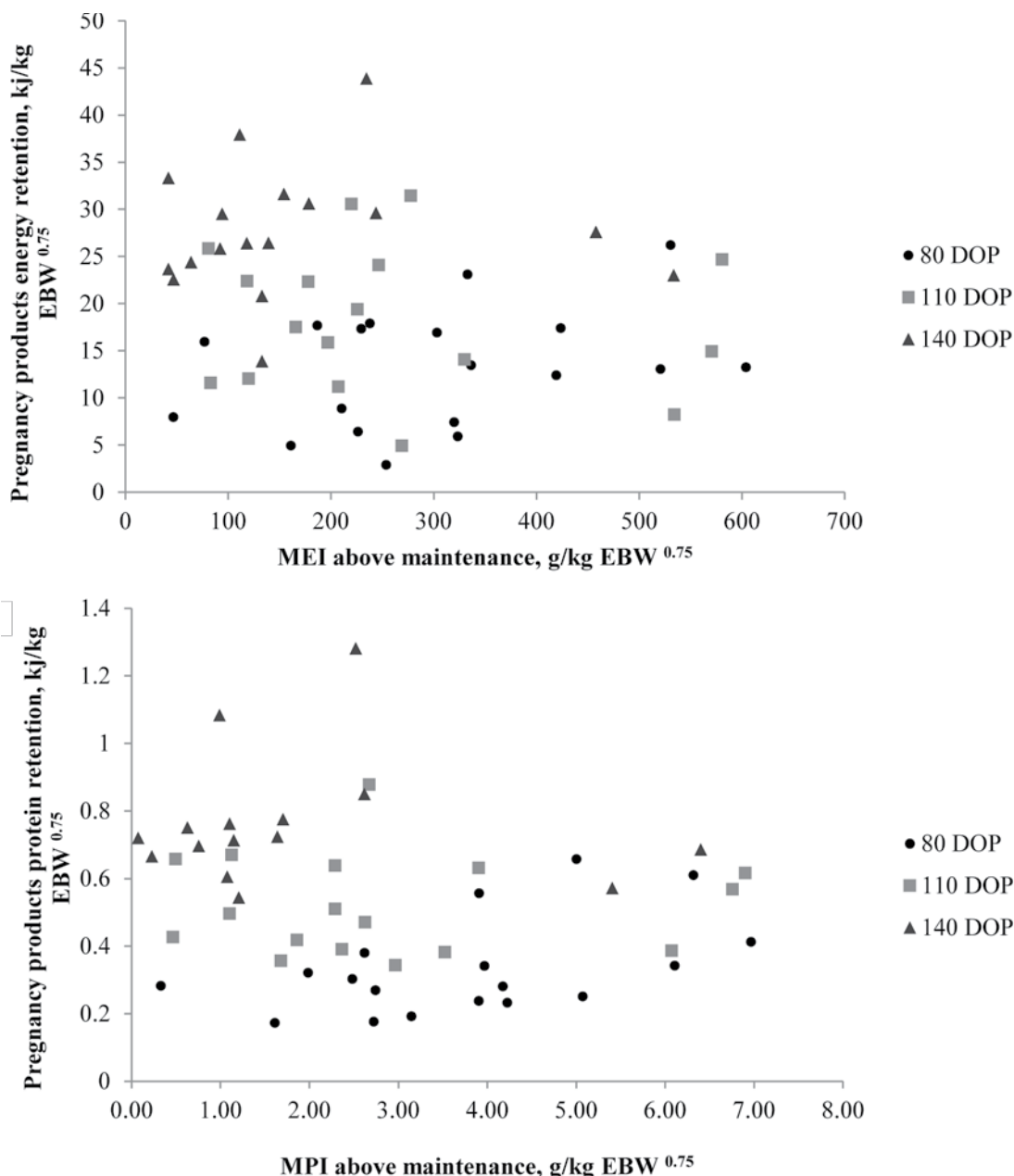


Figure 2. Metabolizable energy intake (MEI) and MP intake (MPI) above maintenance related to the energy and protein accretion on pregnancy products, respectively, from 80 to 140 d of pregnancy (DOP). EBW = empty body weight.

Conclusion

We partially reject our hypotheses because there was no evidence that pregnancy or DOP affects NE_m , ME_m , NP_m , and MP_m or k_{mE} and k_{mP} . The NE_m and ME_m were 197 and 315 kJ/kg EBW^{0.75}, respectively, and k_{mE} was 0.63. The NP_m and MP_m , both estimated using the comparative slaughter technique, were 1.28 ± 0.314 g/kg EBW^{0.75} and 3.22 g MP/kg EBW^{0.75}, respectively, and the k_{mP} was 0.40. However, k_{pE} and k_{pP} increased with pregnancy progress and were 0.058 and 0.12, respectively, at 80 DOP; 0.10 and 0.21, respectively, at 110 DOP; and 0.19 and 0.43, respectively, at 140 DOP. Finally, these findings show potential

for reducing the production cost of goats through the formulation of more precise formulation of diets.

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