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Metabolic and reproductive response to energy supplementation in dairy cows in tropical highlands

Respuesta a la suplementación energética sobre indicadores metabólicos y reproductivos en vacas lecheras en trópico alto

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ABSTRACT

Dairy farms of the department of Nariño present nutritional concerns, especially in the transition period and early lactation, because of the energy imbalance of the diets, which causes deficiencies in the productive and reproductive activity of the cows. According to the above, the main idea was to study the metabolic behavior in the transition period and early lactation of dairy cows supplemented energetically with bypass fat in the tropical highlands of Nariño (Colombia), 21 cows (Holstein x Simmental cross) of second and third lactation period were selected. They were distributed in three treatments with seven animals each; the control treatment (T1) received a base diet (forage + concentrate) without fat supplementation; the second treatment (T2) had a base diet plus 250 g/day of bypass fat and the third (T3) had a base diet plus 250 g/day bypass fat enriched with omega 3. The experimental period ranged between days 15 of prepartum to day 105 of lactation, with an interval of 15 days among sampling. To evaluate the matabolyc performance, we evaluated in serum: non-esterified fatty acids (NEFA), betahydroxybutyrate (BHB), cholesterol, triglycerides, glucose, total proteins, albumin, BUN, and globulins. In addition, body condition and reproductive behavior were determined. The results indicated that there was no efect of treatments on metabolic performance, body condition, and reproductive activity. The energy indicators showed no changes, indicating an adequate supply energy supply in the diet; however, in the metabolites associated to nitrogen, differences in protein were observed. The values found in the variables, constitute a reference of the behavior and the nutritional conditions of the dairy farms in the region.

Keywords: Negative energy, fat, nutritional supplement.

RESUMEN

Las ganaderías de leche del departamento de Nariño presentan problemas nutricionales, especialmente en el periodo de transición y la lactancia temprana, debido al desbalance energético presente en las dietas; provocando deficiencias en la actividad productiva y reproductiva de las vacas. Debido a lo anterior, el principal objetivo fue estudiar el comportamiento metabólico en el período de transición y lactancia temprana de vacas de leche, suplementadas con grasa sobrepasante en el trópico alto de Nariño. Se seleccionaron 21 vacas de la raza Holstein x Simmental, de segundo y tercer parto. Estas se distribuyeron en tres tratamientos de siete animales cada uno. El tratamiento control (T1) recibió una dieta base (forraje + concentrado) sin suplemento graso, el tratamiento dos (T2) la dieta base más 250 g/día de grasa sobrepasante y el tratamiento tres (T3) la dieta base más 250 g/día de grasa sobrepasante enriquecida con omega tres. El periodo experimental estuvo comprendido entre el día 15 preparto y el día 105 de lactancia, con un intervalo de 15 días entre muestreo. Para evaluar el comportamiento metabólico, se determinaron en suero: los ácidos grasos no esterificados (NEFA), el betahidroxibutirato (BHB), el colesterol, los triglicéridos, la glucosa, las proteínas totales, la albumina, el Nitrógeno Uréico sanguíneo (BUN) y las globulinas. Adicionalmente, se determinó la condición corporal y el comportamiento reproductivo. Los resultados indicaron no haber incidencia de los tratamientos establecidos sobre la evaluación metabólica, condición corporal y la actividad reproductiva. Al evaluar el comportamiento metabólico, durante el periodo experimental, se observó que, los indicadores metabólicos energéticos no presentaron cambios, indicando un adecuado suministro de energía en la dieta; sin embargo, al evaluar los metabolitos asociados al nitrógeno, se observaron diferencias en proteína. Por otra parte, los valores encontrados en todas las variables constituyen referencia del comportamiento y las condiciones nutricionales de las ganaderías de lechería en la región.

Palabras clave: Balance energético, grasa, suplemento nutricional.

INTRODUCTION

Over the past 30 years, Colombian dairy has been known for its dynamic, reflected in the high production increase; Colombia produces about 6717 million liters of milk per year (FEDEGAN, 2017). In Nariño, this activity has great social and economic importance, since many families depend directly and indirectly on it. In addition, this area produces more than 800 thousand liters per day. The largest amount in small farms that do not exceed 10 hectares, located at heights above 3000 masl; this implies a differential management, since, at this altitude, the land should be dedicated exclusively to the preservation of ecosystems, rather than agricultural and livestock activities (Solarte and Zambrano, 2012).

The implementation of inadequate technologies and practices for the area, have generated detriment to the soil, triggering deficiencies in the energetic-protein contributions of forages; coupled with this, the indiscriminate use of nitrogen (N), increases the ingestion of

N from the pastures, causes a greater energy expenditure by the cow to eliminate the excess of this mineral; additionally, the energetic wear caused for maintenance and production. This energy imbalance is reflected in the productivity and reproductive activity, increasing open days, services by conception, interval between births and low volumes and poor milk quality. Additionally, the specialized dairy farms of the Nariño highlands present nutritional problems especially during the transition period and early lactation, due to the energy imbalance caused by the decrease in diet consumption, due to the physiological state in which cows are found this phase and the low quality of the food consumed; which leads to a partition of the reserved energy, in order to solve the energy needs required for dairy production mainly, even to the detriment of reproductive activity, all due to the low contribution of the forage diet base and the low or inadequate supplementation that is done in the herds.

In regards Sossa and Barahona (2015) mention that the purpose of livestock activity, is based on implementing food strategies to offer and optimize the nutrient intake, being necessary to use strategic supplementation adjusted to the requirements of the animals, therefore, as an alternative of sustainability and productivity, it is necessary to study technological offers, that will meet the energy needs of cows in the transition stage and the early lactation, to improve the quality and production; for this, the bypass fats, become a food strategy, because they are inactive ruminal and do not compromise the cellulolytic activity of the bacteria, generating a significant energy input to meet the requirements of the animal.

Under the above considerations, the aim was to study the metabolic behavior during the transition period and early lactation under an energy supplementation in high producing dairy cows in the high tropic of Nariño; likewise, reference blood levels of NEFA, BHB, cholesterol, triglycerides, glucose, total protein, albumin, globulins and BUN in dairy cows for the Nariño region.

MATERIALS AND METHODS

Location. The research was conducted in the dairy watershed in Guachucal municipality, Nariño department. The herd was at 3125 masl, the climatic conditions were: temperature of 10°C, relative humidity between 82 and 86% and 1017mm rainfall per year. According to Holdridge, the life zone is lower montane dry forest (bs-MB).

Nutritional management of animals. The cows had a base diet consisting of fodder mixed with ryegrass (*Lolium perenne*) and/or kikuyo (*Cenchrus clandestinus* Hochst. Ex Chiov). The grazing of the animals consisted of a rotational system throughout the day, where the animals consumed the food ad libitum and a commercial supplement which was supplied during the morning and evening milking; for prepartum cows (21 days before parturition), three kilograms of prelactation concentrated were provided, and for dairy cows, one kilogram of concentrate per five kilograms of milk produced. The nutritional composition of both the forage mix and the balanced feed are described in Tables 1 and 2. Dry matter intake was estimated, considering the formula: CMS = 120 * PV/FDN * 100% (Ríos and Solarte, 2015). Additionally, the consumed of the balanced feed was calculated, based on an approximation of 14.95kg.MS/vaca/day, that is, 2.67% of the live weight (PV) per animal.

Table 1. Nutritional composition of the forage (L. *perenne* and *C. clandestinus* Hochst. ex Chiov) mixture.

Variable	%
Dry material	22.9
Ashes	8.87
Protein	17.66
ether extract	2,42
FDN	60.61
FDA	33.86
LDA	9.4
Hemicellulose	26.75
Cellulose	24.46
ELN	10.44
EB	430

Table 2. Antepartum commercial supplement nutritional composition and lactation.

Variable	%Prepartum	Lactation%
Dry material	89.38	88.89
Protein	15	17.9
Ether extract	5.89	5.55
FDN	60.61	33.73
FDA	33.86	11.85

Selection of experimental units and treatments. 21 animals crossing Holstein x Simmental were selected with an average weight of 560 kg, second or third delivery, producing 22±0.57liters/day. These were randomized into three experimental groups of 7 cows each. The diets were: in the control (T1) basal diet without addition of fat supplement, the treatment 2 (T2) the base diet was provided and 250g/day of commercial bypass fat unenriched, and the treatment 3 (T3) had basal diet plus 250g/day bypass fat enriched with omega 3 (equivalent to two capsules corresponding to 2400mg of omega 3). The nutritional composition and fatty acid profile of the fat used is presented in Table 3; the amount of bypass fat is established according to previous studies (Duque *et al.*, 2011; Duarte *et al.*, 2016) and the purpose of incorporating omega 3 for the third treatment was to enrich polyunsaturated fatty acids diet (without ruminal protection), in a total percentage of 0.1% in the diet. The two groups of animals supplemented with fat excess, began the diet from day 21 antepartum until day 105 of lactation. The total experimental period was 120 days with an interval between collection of samples of 15 days. The samplings were distributed in eight periods: one in the antepartum (15 days before the birth) and seven after the birth, until reaching the 105th postpartum day.

Table 3. Nutritional composition and fatty acid profile of bypass fat.

Nourishing	% Minimum	% Maximum
Fat (as fatty acid)	73	
Calcium	7	
Humidity		7
Ingredients		
Fatty acids from palm and soybean		
Calcium hydroxide		
Antioxidants		
Fatty acid profile		
Fatty acid	**units**	
Palmitic	44.533%	
Stearic	4.201%	
Oleic	31.042%	
Linoleic	18.286%	
Linolenic	0.672%	
Myristic	1.266%	

Source: Technical file of commercial product Pecutryn Energy, 2016.

Collection and analysis of samples. Cows blood samples were obtained by venipuncture coccygeal, in vacuum tubes without anticoagulant, they were centrifuged at 2500rpm for 15 minutes, the serum was removed and stored at -20ºC until the metabolites serum analysis NEFA, BHB, total proteins, BUN, cholesterol, glucose, albumin and triglycerides. The tests were carried out through colorimetric enzymatic method, using the RAYTO® optical reading semiautomatic chemistry analyzer equipment (Shenzhen, China). In addition, a professional zootecnista, was responsible for monitoring the body condition of each cow.

The reason for studying specific hematobioquímicos components, is to evaluate, diagnose and prevent metabolic disorders; besides offering offer valuable information regarding the nutritional status of the animals. In the case of the evaluation of energy metabolism, metabolites, glucose, NEFA, BHB, cholesterol and triglycerides and its association with the behavior of body condition they were considered. Additionally, the protein balance of the diet was evaluated through metabolites: total protein, albumin, globulins, and BUN. Finally, in response to the diet offered, reproductive activity was analyzed.

Reproductive characterization. The reproductive variables: services per conception (SC), open days (DA) and the interval between births (IEPE), were obtained from historical and individual records of each cow. To estimate SC, the inseminations until the effective conception were counted, the ADs taking the days from birth until the next pregnancy and finally, IEPE taking into account the pre-trial birth and estimating the date of the next one; starting from the effective insemination and confirmation of pregnancy carried out by the veterinarian.

Statistic analysis. The variables were analyzed through a mixed model measures repeated over time using PROC MIXED. The model considered the fixed effects of treatments, periods and their interaction periods. The effect of animal within each treatment constituted the random effect and the milk production recorded in the previous lactation was considered as a covariate. In each variable five covariance structures (composite symmetry, unstructured symmetry, autoregressive with random effect and Toeplitz), commonly used in repeated measures, equally spaced in time were analyzed (Littell *et al*., 2006). The lowest value of the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used as methods to determine the best covariance structure. The comparisons between means with statistical significant differences ($P < 0.05$) were made through the Dunnett test. Finally, a descriptive and exploratory analysis was performed including means and standard deviation. All the data were processed with the Statistical Analysis System (SAS), version 9.1.

RESULTS AND DISCUSSION

Serum cholesterol levels differed between treatments and periods. For Glucose, NEFA and triglycerides only significant difference were observed between the sampling periods without any variation between treatments. In BHB there were no differences for treatments nor sampling times, as shown in Tables 4 and 5.

Table 4. Statistical effect between treatments and periods in the energy metabolites evaluated in dairy cows on hightlands of Nariño.

Variable	Treatment	Period	Treatment *Period	Covariance
Glucose	0.1834	0.0109	0.0002	* NS
NEFA	0.8859	<0.0001	0.7228	* NS
BHB	0.8918	0.3224	0.0125	0.0293
Cholesterol	0.0215	<0.0001	0.0045	0.0095
Triglycerides	0.0906	<0.0001	0.5629	0.0216
DC	0.5989	0.3571	0.6776	0.0101

* NS indicates that, in the first statistical trial, the covariance was not significant and therefore was removed from the model for that metabolite.

Table 5. Serum energy metabolites from cows (Holstein x Simmental), in prepartum and early lactation.

Variable	I Prepartum	Breastfeeding (days)							p-value
	-15	15	30	45	60	75	90	105	
Glucose (mmol/L)	3.92	3.83	3.69	3.61	3.81	3.69	3.64	3.74	0.0109
NEFA (mmol/L)	0.27[c]	0.40[ab]	0.49[a]	0.45[ab]	0.41[ab]	0.38[b]	0.39b	0.46[ab]	<0.0001
BHB (mmol/L)	0.53	0.48	0.46	0.50	0.47	0.51	0.55	0.53	0.3224
Cholesterol (mmol/L)	4.40[b]	3.82[c]	4.33[b]	5.12[a]	5.30[a]	5.24[a]	5.28[a]	5.34[a]	<0.0001
Triglycerides (mmol/L)	0.32[a]	0.19[def]	0.18[ef]	0.16[f]	0.21[de]	0.23[cd]	0.26[bc]	0.28[b]	<0.0001

Different letters indicate statistical differences between groups.

Glucose values per treatment were 3.6±0.06, 3.7±0.06 and 3.8±0.06mmol/L for T1, T2 and in T3, respectively. According to Kaneko *et al*. (2008) normal serum glucose levels in bovines are from 2.5 to 4.16mmol/L; while Ceballos *et al*. (2002) report average glucose concentrations of 3.8±1.0 and 3.7±1.0mmol/L for pre and postpartum periods, respectively, indicating that the results of this study were within the ranges reported by these authors. In ruminants, the amount of glucose absorbed depends on the number of volatile fatty acids absorbed from the rumen which constitues the basis of gluconeogenic homeostatic processes (Aschenbach *et al*., 2010). No fluctuations with or without supplementation of this metabolite were observed. But nevertheless, its behavior over time of sampling, as shown in Tables 4 and 5 showed significant differences; being the higher values on day 15 before delivery, followed by day 15 postpartum. This shows that cows maintained their homeostasis and positively faced the transition period and early lactation, highlighting the importance of the narrow hormonal control of the glucose (Hanno, 2012). Meanwhile, the lowest value was recorded on day 45 of lactation due to the simultaniety with the beginning of the production peak.

NEFA serum ranged from 0.39±0.03; 0.41±0.03 and 0.42±0.03mmol/L for treatments one, two and three, respectively. Contreras and Sordillo (2011) reveal that at the end of lactation and early dry period, can be found values lower than 0.2mmol/L, while in the two weeks before delivery, NEFA levels in blood begin to increase to reach a peak between days 0 and 10 postpartum, identifying concentrations close to 0.75mmol/L depending on the degree of lipid mobilization. Similarly, Djokovic *et al*. (2013) mentioned ranges 0.27±0.14mmol/L in prepartum and 0.54± 0.26mmol/L for the time of delivery, while García *et al*. (2016) report values 0.4mmol/L at the time of birth and 15 days after. Since the concentration of NEFA in plasma is used as a diagnostic tool to assess the degree of BEN in dairy cows in early postpartum and is also considered as an indicator of lipid mobilization reserves in the body; the results indicate a minimal lipid mobilization. On the other hand, to evaluate the behavior of the metabolite at the different sampling periods differences were detected, being the highest value on day 30 of lactation, followed by day 105. The lower value was observed in the prepartum (Tables 4 and 5). These results agree with Ceballos *et al*. (2002), who set a low concentration during the antepartum, which was increasing, reaching its peak in the second week of postpartum, ie, in the last antepartum days, due to a depression of dry matter consumption, the lipolysis and fat mobilization are accelerated at the beginning of lactation, lead to an increase in non-esterified fatty acids.

The serum levels of B-hydroxybutyrate (BHB), were 0.50±0.019, 0.51±0.019 and 0.50±0.02mmol/L, for T1, T2 and T3 respectrively, therefore, the cows studied did not mobilize excessive amounts of fat that would produce a significant increase in BHB values, which agrees that no evidence of subclinical ketosis will emerge. In this regard, Suthar *et al*. (2013) indicate that animals with BHB values greater than 1.00mmol/L and up to 1,40mmol/L may have subclinical ketosis. In the present study, the results were within the normal range when compared to those reported by Campos *et al*. (2007) who established a range of 0.35 - 0.65mmol/L in tropic lowlands, in animals with an average production of 19L/day, for different racial groups and in different physiological stages. Kaneko *et al*. (2008) report 0.38 - 0.44mmol/L and Djokovic *et al*. (2013) indicate values of 0.36 to 1.36mmol/L, meanwhile, Remppis *et al*. (2011) mention that BHB values should be below 1.00mmol/L in early lactation and below 0.6mmol/L in late gestation. No significant differences were found sampling period. T2 showed marked fluctuations in periods five seven, with a decrease and an increase, respectively in the concentration of BHB, which causes a homeostatic loss of the animals in this treatment (Tables 4 and 5). For its part, the T3 tends to gradually raise its levels progressively, therefore, there is an increased lipid mobilization to meet energy needs. These fluctuations can be attributed to the energy needs required by the two groups of animals, to maintain production levels, given that they had a higher output than the control treatment (production per treatment average 21.8±0.57 (T1); 23.7±0.58L (T2) and 22.4± 0.60 (T3) L/cow/day).

The cholesterol values were 4.5±0.13mmol/L for T1, 4.8±0.13mmol/L for T2 and 5.17±0.14mmol/L for T3, showing significant differences between them (Table 4), demonstrating higher levels in T3; Likewise, differences were found during the experimental weeks. At first, 15 days before parturition (prepartum) values were within average, then they felled significantly in the second week postpartum (corresponding to day 15 of lactation) and finally, they risen gradually up to meet the highest value on day 105 of lactation (Table 5).

Campos *et al*. (2007) found values of 2.1±1.2mmol/L for Holstein cows and 1.4±0.9mmol/L in Simmental cows; likewise, Ceballos *et al*. (2002), indicate values of 2.8±0.6mmol/L in prepartum and 3.5±1.1mmol/L in postpartum. The results of this evaluation are higher than those mentioned due to geographical, environmental and management conditions of the production system; in this case the height conditions over 3125 masl, average temperature of 10°C and fluctuations during the experimental period, where it came to -2°C, where the importance of calorigenic paper and oxygen consumption evidenced by of animals exposed to high altitudes, effects associated with endocrine regulation of the thyroid gland (Campos and Rhodes, 1999).

Triglycerides showed values of 0.20±0.011 (T1), 0.24±0.011 (T2) and 0.23±0.012 (T3) mmol/L; without differences between treatments (Table 4). Kaneko *et al*. (2008), indicate values of serum concentrations between 0 and 0.77mmol/L, while Moyano and Rodriguez (2014) report values from 0.19 to 0.25 mmol/L, which shows that the animals of this assay were in referenced ranges without being affected by supplementation, additionally, the results show that there is no evidence of risk factors to the presentation of fatty liver.

The assessment carried out within different sampling periods revealed significant differences. The highest value was registered on day -15 (prepartum), followed by a decline until day 45 of lactation, where it begins to gradually rise to obtain a value of 0.27±0.015mmol/L on of lactation day 105 (Table 5). This behavior is consistent with that described by Moyano and Rodriguez (2014), who report higher triglyceride values in dry period and an increasing trend as lactation days increase. Such variations may be attributable in the prepartum period to a high level of body reserves and absence of milk synthesis, conditions that allow to the animal to face the delivery and initiation of production; however, lowering serum triglyceride level arising subsequently, reflects the energy deficit which is exposed the cow and as a normal process, it takes the necessary energy from the oxidation of triglycerides that has reserved in adipose tissue, which has in its structural conformation, glycerol, and triglycerides (Duque *et al*., 2011). From day 45 postpartum, it was observed that levels rose, as the cow stabilizes its energy status and the triglycerides increase to be transported in chylomicrons and in very low-density lipoprotein, to mammary gland, to synthesize preformed fatty acids (palmitic and stearic) (García *et al*., 2014).

Body condition. For body condition we found means of 3.5 points for T1 and 3.25 points for T2 and T3; indicating a reduced mobilization of pre and postpartum body reserves. Additionally, there were no differences between treatments or sampling periods, because the base diet provided the necessary energy so that the animals were not forced to mobilize their body reserves during the first phase of lactation. In addition, in the crossing of the racial group Holstein x Simmental, the Simmental breed for its rusticity generates greater capacity for lipid reserves after the moment of birth, as observed during the experimental time (Asociación Simmental Simbrah Mexicana, 2017). The results of this evaluation agree with the work carried out by Aguilar *et al*. (2009) and Tyagi *et al*. (2010), who evaluated the supply of fat excess, without finding differences in body condition between animals with and without fat supplement.

Table 6. Statistical effect between treatments and periods in the nitrogenous metabolites evaluated in dairy cows in tropical highlands of Nariño.

Variable	Treatment	Period	* Treatment Period	covariance
Total proteins	0.9406	0.4674	0.2684	* NS
BUN	0.0175	<0.0001	0.1202	* NS
Albumin	0.2471	0.003	0.0408	* NS
Globulins	0.5993	0.0153	0.3037	* NS

* NS indicates that in the first statistical covariance trial was not significant and therefore was removed from the model for that metabolite.

On the other hand, when evaluating the group of metabolites associated to nitrogen, present in Tables 6 and 7, the statistical analysis indicates that the BUN presented differences, between treatments and periods, while for albumin and globulins differences were observed only between the periods. In total proteins, there were no differences in treatments or sampling periods.

The values of total proteins obtained show a mean of 7.66 ± 0.07mg/L; 7.65 ± 0.07mg/L and 7.68 ± 0.07mg/L, for the control treatments, T2 and T3, respectively (see Tables 6 and 7); without significant differences between them. Expected blood levels for cows' range between 6.74 and 7.46mg/L. These values are close to or similar to those obtained by (Kaneko *et al*., 2008; Bradford, 2010 and Tóthová *et al*., 2008).

The averages obtained in this study, are within the ranges reported by Brandford (2010), in this sense, the cows had an adequate performance of protein metabolism. During the different sampling periods, as well as between treatments, no significant differences were observed (Tables 6 and 7). However, starting on day 15 postpartum, serum levels of protein began to increase gradually; this coincides with the period in which the animals regulate the consumption of dry matter, agreeing with that mentioned by Meikle *et al*. (2013), who state that the increase in plasma protein after childbirth is positively correlated with MS consumption. In addition, altitude conditions increase the levels of certain blood proteins, such as hemoglobin, in response to the lower concentration of oxygen (Campos and Rodas, 1999).

The mean albumin value of T1, T2 and T3 were 3.46 ± 0.03; 3.54 ± 0.03 and 3.47 ± 0.03mg/L, respectively, without significant differences between them; however, in the sampling periods, differences were found, a marked decline was observed between periods one and two (Tables 6 and 7); due to an inadequate supply of amino acids to the liver for the synthesis of this protein, as well as to a lower hepatic ability in some animals for the uptake of these amino acids (Campos *et al*., 2012). On the other hand, on days 30 and 75 postpartum, high values were found, produced by chronic antigenic stimulation and production of increasing immunoglobulins when freeing from the effect of high levels of cortisol in the peripartum (Bradford, 2010), as a mechanism of own defense of each cow, to respond in its stage of high production and entry to a new reproductive cycle. The average values of albumin are in the range of 3.03 - 3.55mg/L, as described by Kaneko *et al*. (2008); 3.0 - 4.3mg/L by Bradford (2010), while Campos *et al*. (2007), mention values of 2.58 ± 0.44mg/L and 2.27 ± 0.44mg/L in low tropics for Holstein and Simmental cows, respectively. The values found in this study are within the averages described in the first citations.

Table 7. Serum values of the metabolites associated to nitrogen form Holstein x Simmental cows in prepartum and early lactation.

Variable	I Prepartum	Breastfeeding (days)							p-value
	-15	15	30	45	60	75	90	105	
Total proteins (mg/L)	7.68	7.68	7.58	7.63	7.74	7.55	7.70	7.77	0.4674
Albumin (mg/L)	3.53^{ab}	3.40^{bc}	3.51^{b}	3.49^{bc}	3.44^{bc}	3.65^{a}	3.49^{bc}	3.44^{bc}	0.003
Globulins (mg/L)	4.14	4.28	4.07	4.14	4.29	3.90	4.22	4.32	0.0153
BUN (mmol/L)	5.84^{ab}	5.11^{d}	5.11^{d}	5.24^{cd}	5.11^{d}	5.48b^{cd}	6.2^{a}	5.6^{bc}	<0.0001

Different letters indicate statistical differences between groups.

Serum globulin levels were 4.19 ± 0.06, 4.10 ± 0.06 and 4.21 ± 0.06mg/L for T1, T2 and T3, respectively, without significant differences between them, as indicated in Tables 6 and 7. When comparing the results of this investigation with those found by Kaneko *et al*. (2008), indicating values between 3.0 and 3.4 mg/L, and Bradford (2010) between 3.0 - 3.5mg/L, the values are higher. However, Campos *et al*. (2007) obtained values of 4.03 ± 0.41mg/L for Holstein cows and 4.4 ± 0.35mg/L for Simmental cows at the beginning of lactation, in conditions of the Colombian tropics, due to the greater immunological response to internal and external parasitism.

Serum BUN levels reported values of 5.46 ± 0.23, 5.72 ± 0.23 and 5.22 ± 0.23 mmol/L for T1, T2 and T3, respectively; There were significant differences between treatments and sampling periods (Table 6 and 7). The different periods showed lower values on the 15th prepartum and 15th days of lactation, just when there is a low consumption of dry matter, required by the physiological state of the animal. In addition, Bargo *et al*. (2003) indicate that high production cows should consume, on average, 3.5% of their live weight in DM and the cows of this study reached an estimated consumption of 2.67% of their live weight in DM, which reveals a decrease in crude protein that affects low BUN values. Subsequently, from day 60 postpartum, the values were increased, until reaching the maximum value on day 90, as shown in Table 7. This increase is attributed to the regulation of consumption by the animal, a higher intake of degradable protein supplied through balanced feed, especially in the change from pre to post-lactation, as well as, to the scarce energy at the rumen level, which agrees with what Zarate *et al.* (2016), who maintain that an increase in urea can occur because the amount of degradable protein in the rumen is too high, or because the soluble protein/non-fibrous carbohydrate ratio, degradable in the rumen, is also high.

On the other hand, normal BUN values are between 3.33 and 5.0mmol/L (Kaneko *et al*., 2008); 1.77 - 5.5mmol/L in prepartum and 1.16 - 5.7mmol/L at the start of lactation, in high tropic (Ceballos *et al.*, 2002), 5.69mmol/L in cows with more than two births (Pedraza *et al*., 2006 and López *et al*., 2011) and 6.33mmol/L in cows supplemented energetically (Alvarez *et al*., 2006). These ranges indicate that the results of this study are acceptable.

Reproductive activity. There were no significant differences between the proposed treatments ($p > 0.05$) for the estimated interval between births (IEPE), open days (DA) and design (SC) services.

IEPE average was 363, 357 and 375 days and DA was 85, 79 and 90 days forT1, T2 and T3, respectively; while for SC mean values of 1.6 were found for T1 and T3 and 1.5 for T2. For its part, the Breeding Program (Rosero-Galindo *et al*., 2009) in Nariño found, for crossing Holstein × Simmental, an IEP of 410.96±83.31 days, DA 134.76±82.77 days and SC of 2.14±1.34. The results of this assessment show a group of animals with an efficient performance, since they reprise their reproductive activity in fewer days and express greater cyclicity, fertility, and pregnancy, compared to the Breeding Program (Rosero-Galindo *et al*., 2009). Additionally, it is important to emphasize the capacity of the experimental group to comply with the demanding production and the return to a new gestation, which agrees with the results of Tyagi *et al*. (2010) in high production mestizo cows supplemented with fat excess and with a reduction in the time for the restart of the estrous cycle and delivery interval at first service; likewise, Duarte *et al*. (2016) cited a decrease in the interval days between deliveries in supplemented cows. Additionally, the values of this study are better than those reported for the area, therefore, the contribution to the general management of the operation is relevant in aspects such as nutrition, herd health, detection of jealousy and effectiveness in artificial insemination.

CONCLUSIONS

The metabolic behavior is indicative of physiological changes to which an animal is exposed depending on the production period in which it is found, being able to identify biochemical processes that allow us to make the necessary corrections based on the diet offered.

The cows did not show excessive lipid mobilization. The metabolic energy indicators did not suffer drastic changes. The diet offered in the cattle meets the energy requirements for the cows. Serum (BUN) levels did not suggest excesses of crude protein.

The diets offered did not have effect on reproductive activity of the cows.

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