

# Metabolic indicators and their relationship with reproductive efficiency in dairy cows in the high tropics

## Indicadores metabólicos y su relación con la eficiencia reproductiva en vacas lecheras en trópico alto

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### ABSTRACT

The specialized herds of the high tropics of Nariño are characterized by semi-intensive breeding models, that is, feeding based on grazing and supplementation. The objective was to analyze the milk production of grazing Holstein cows under conditions of the high tropics, taking into account the energetic and protein metabolites, and their relationship with the dry matter intake (CMS/Kg/d), the body condition (BC), and the reproductive variables. Three periods of lactation were evaluated, thus, between 4-10 days (P1), 45-55 days (P2), and between 185-200 days (P3). The investigation was carried out in four dairy production farms. The forage supply consisted of a mixture of *Lolium hybridum*, *Cenchrus clandestinus* Hoschst ex Chiov, *Holcus lanatus* and *Trifolium repens*, plus commercial concentrate. A total of 24 Holstein animals (six per productive system) from three births onwards were used, from which blood samples were obtained to determine: non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), triglycerides, cholesterol, glucose, total protein (PT), and blood urea nitrogen (BUN). The results showed that milk production during P1 is influenced by CC and body weight (Kg) by +0.04 liters of milk per unit of change in this variable. In P2, the positive incidence variables were PT, BUN, glucose, NEFA, BHB, triglycerides, and cholesterol, and in P3, the increase in CMS made it possible to show that as lactation progresses in more than 180 days, the animals substantially improve their body condition; likewise the NEFA and BHB indicators are related to a better reproductive response.

**Key words:** Non-esterified fatty acids; BUN;  $\beta$ -Hydroxybutyrate; calving interval; services per conception.

### RESUMEN

Los hatos especializados del Trópico Alto de Nariño se caracterizan por modelos de crianza semi-intensiva, es decir la alimentación basada en pastoreo y suplementación. El objetivo fue analizar la

producción de leche de vacas Holstein en pastoreo, bajo condiciones de trópico alto, teniendo en cuenta los metabolitos energéticos y proteicos, y su relación con el consumo de materia seca (CMS/Kg/d), la Condición Corporal (CC), y las variables reproductivas. Se evaluaron tres periodos de lactancia, entre 4-10 días (P1), 45-55 días (P2) y entre 185-200 días (P3). La Investigación se realizó en cuatro fincas lecheras. La oferta forrajera consistió en mezcla de *Lolium hybridum*, *Cenchrus clandestinus* Hoschst ex Chiov, *Holcus lanatus* y *Trifolium repens*, más concentrado comercial. En total fueron empleados 24 animales Holstein (seis por sistema productivo) de tres partos en adelante, de los cuales se obtuvieron muestras de sangre para determinar: ácidos grasos no esterificados (AGNE), beta-hidroxibutirato (BHB), triglicéridos, colesterol, glucosa, proteínas totales (PT) y nitrógeno ureico sanguíneo (BUN). Los resultados evidenciaron que la producción de leche durante el P1 está influenciada por la CC y el peso corporal (Kg) en +0.04 litros de leche por unidad de cambio en esta variable. En P2, las variables de incidencia positiva fueron PT, BUN, glucosa, AGNE, BHB, triglicéridos, y colesterol, y en el P3 el aumento del CMS, permitió evidenciar que a medida que avanza la lactancia en más de 180 días, los animales mejoran sustancialmente la condición corporal, así mismo los indicadores AGNE, BHB, se relacionan a una mejor respuesta reproductiva.

**Palabras clave:** Ácidos grasos no esterificados; BUN;  $\beta$ -Hidroxibutirato; intervalo entre partos; servicios por concepción.

## INTRODUCTION

Dairy farming in Nariño is one of the important economic activities, on which approximately 40,000 families depend, and which, according to DANE (2017), represents 14.1% of the regional agricultural GDP. Currently, according to the Society of Farmers and Ranchers of Nariño (SAGAN, 2022), in the department there are 133,526 cows, of which 80% are Holstein breed, and since 2012 to date, reproductive problems have been evidenced related to the wide calving intervals, which vary between  $444.5 \pm 96.2$  days, and open days of  $166.3 \pm 94.5$  (Solarte & Zambrano, 2012). The diet is based on monoculture meadows of kikuyu (*Cenchrus clandestinus* Hoschst ex Chiov), ryegrass (*Lolium spp*), white clover (*Trifolium repens*), false pomace (*Holcus lanatus*) and New Zealand-type improved pastures such as: *Lolium hybridum* (sterlingohau) and *Lolium perenne* (*nui-samson*) or in mixtures of these, plus food supplementation consisting of commercial concentrates or silages (Apráez *et al.*, 2016).

The high nutritional requirements of the breed, associated with the productive challenges with lactations of more than 5400 liters, and the non-compensation of nutritional requirements during the beginning of lactation due to the decrease in dry matter intake, induce the mobilization of body reserves, to satisfy the energy demands derived from the mammary gland, with a high risk of not being able to compensate energy homeostasis, which leads the animal to a negative energy balance (NEB), and as a consequence, the pulsatile secretion of luteinizing hormone is affected (LH), reducing ovarian responsiveness to LH, resulting in low pregnancy rates (Mann *et al.*, 2015; Wang *et al.*, 2017; Yeshambel, 2023).

On the other hand, protein mobilization is much more limited than fat, and once its reserves are depleted, milk production and immunoglobulin synthesis are compromised.

Another important indicator of the nutritional situation is the evaluation of the body condition, which, during the early postpartum period and the lactation peak, allows indirectly assess the reproductive efficiency, since if an-animal loses more than one unit between the parturition and the 100 days in milk, it is likely to present lower reproductive performance, especially in grazing animals (Drackley & Cardoso, 2014; Akbar *et al.*, 2015). For this reason, the objective of this study was to analyze milk production, taking into account energy and protein metabolites such as Non-Esterified Fatty Acids (NEFA), Beta-hydroxybutyrate (BHB), triglycerides, cholesterol, glucose, total protein (PT ), Blood Ureic Nitrogen (BUN); and their relationship between dry matter intake (CMS/Kg/d), Body Condition (BC), and their incidence on the reproductive variables of calving interval (CI) and services per conception (SC) in three periods of lactation, in grazing Holstein cows, under conditions of the Alto de Nariño tropics

## MATERIAL AND METHODS

**Location.** This investigation was carried out in four dairy herds, which were identified as F1, F2, F3, and F4, located in the municipalities of Guachucal, Túquerres and Pasto, considered the ones with the largest bovine inventory in the region, where 80% of the department's total milk volume is concentrated, in accordance with the provisions of the Nariño Farmers and Ranchers Society (SAGAN, 2022). These farms were selected, according to the livestock inventory, to cover the experimental requirement of animals in a similar gestation phase. From the records, adult cows with three or more calvings were chosen; these were grouped according to the days remaining until calving (between 4-8 days); additionally, it was considered that they had productive, reproductive and health information. The geographic coordinates and main climatic conditions of the farms are described below (Table 1).

**Table 1.** Geographic coordinates and climatic conditions of the four selected herds under conditions of the high tropics of Nariño.

Municipality	Farms	Geographical coordinates	Temperature °C	Hight Masl	Precipitation mm/year
Pasto (F1,F2)	2	1°12'41''N 77°16'52''O	11.5	3013	771.6
Guachucal (F3)	1	0° 57' 50"N 77° 43' 50"O	10.8	3158	959
Túquerres (F4)	1	1°250'77''N 77°45'04''O	11.0	3150	1117

**Selection of animals and type of feeding.** Six Holstein cows were selected from each farm, and the measurements were made during lactation (4-10 days) called the beginning and identified as P1, at the peak of lactation or P2 (between 45-55 days) and third lactation or P3 (185-200 days). Table 2 indicates the basis of forage feeding in each herd (F1, F2, F3 and F4) by lactation period.

**Table 2.** Type of forage supply supplied in the four dairy herds, by lactation period in conditions of the high tropics of Nariño.

Dairy herds	Type of mixed pastures by lactation periods		
	P1	P2	P3
F1	<i>Lolium hybridum</i> y <i>Lolium perenne</i>	<i>Lolium spp</i> <i>Trifolium repens</i> <i>Holcus lanatus</i>	<i>Lolium hybridum</i> <i>Cenchrus clandestinus</i> Hoschst ex Chiov
F2	<i>Cenchrus clandestinus</i> Hoschst ex Chiov <i>Holcus lanatus</i> <i>Trifolium repens</i>	<i>Cenchrusclandestinus</i> Hoschst ex Chiov <i>Trifolium repens</i> <i>Lolium spp</i> <i>Holcus lanatus</i>	<i>Trifolium repens</i> <i>Cenchrus clandestinus</i> Hoschst ex Chiov
F3	<i>Trifolium repens</i> <i>Holcus lanatus</i> <i>Lolium spp</i>	<i>Lolium spp</i> <i>Holcus lanatus</i> <i>Trifolium repens</i>	<i>Holcus lanatus</i> <i>Lolium spp</i>
F4	<i>Lolium spp</i>	<i>Lolium spp</i> <i>Holcus lanatus</i>	<i>Holcus lanatus</i> <i>Lolium spp</i> <i>Cenchrus clandestinus</i> Hoschst ex Chiov

Management conditions were similar in all systems, with mechanical milking, supplemented rotational grazing, and 'ad libitum' access to water. The addition of the amount of commercial concentrate was carried out according to the productive level. Additionally, and during the morning milking, 5% mineralized salt was supplied in an approximate amount of 150g/animal per day.

Regarding the supply of commercial concentrate, on all the farms evaluated, the cows in production were given one kilogram of concentrate for every five kilograms of milk during the morning and afternoon milking, that is, a 5:1 ratio, without exceeding the supply of 8 kg of supplement per animal per day. Table 3 shows the chemical-nutritional composition of the commercial supplement by production system. In all the lactation periods evaluated, the same concentration was provided. The bromatological analyzes, both of the pastures and the commercial type supplements, were carried out according to the procedures described in the food chemical analysis manual of the Specialized Laboratory of the University of Nariño, following the methodology of the Association of Official Agricultural Chemists (AOAC, 1995).

**Table 3.** Chemical - nutritional composition of the commercial supplement supplied in each dairy herd, under conditions of the high tropics of Nariño.

<sup>1</sup> Component (%)	Nutritional component			
	F1	F2	F3	F4
CP	20.4	19.3	16.8	12.2
EE	4.6	6.2	5.8	3.3
NDF	23.3	33.7	40.3	31.3
AFD	7.8	11.8	17.4	10.2

<sup>1</sup>CP = Crude Protein; EE = Ethereal Extract; NDF = Neutral Detergent Fiber; AFD = Acid Detergent Fiber.

Table 4 shows the main nutritional components of the forage mixture, for each dairy herd and lactation period.

**Table 4.** Nutritional composition of the forage mixture, by dairy herd and lactation period under conditions of the high tropics of Nariño.

<sup>1</sup> Component (%)	Lactation periods											
	P1				P2				P3			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
CP	22.3	23.9	21.8	29.8	29.6	30.9	18	21.4	13.5	19.4	16.3	19.3
EE	3.6	2.3	2.1	4.8	4.1	4.5	2.7	3.14	2.3	3.0	3.0	3.1
NDF	50.3	55.7	54.0	43.6	52.5	48.7	50.2	54.8	61.9	51.1	57.3	46.4
AFD	24.4	27.3	26.3	20.8	24.2	22.2	25.9	26.5	31.1	24.4	27.4	30.5

<sup>1</sup>CP = Crude Protein; EE = Ethereal Extract; NDF = Neutral Detergent Fiber; AFD = Acid Detergent Fiber.

The quantification of the total milk production was carried out taking into account the volume of milk obtained in the morning (am) and afternoon (pm) milkings. The protein content was determined using a Julie C2® milk analyzer (Scope Electric Ltd; Razgrad, Bulgaria), and the estimation of the Dry Matter Intake (CMS/Kg/d) under grazing conditions, was carried out by supplying 15g of Chromium Oxide (Cr<sub>2</sub>O<sub>3</sub>) as an external marker, taking into account the methodology described by Correa *et al.* (2009). In addition, on each farm, by lactation period, the body weight (Kg) of the animals was recorded using a bovine metric tape for dairy breeds, and an adjustment of 2% was made to estimate the final value.

For the determination of metabolic indicators, after milking in the morning and in each lactation period (P1, P2, and P3), blood samples were taken from each cow by coccygeal venipuncture, using vacutainer tubes without anticoagulant. The samples were centrifuged at 2500rpm for 15 minutes. This procedure was done with the purpose of obtaining blood serum, which was stored at - 20°C, in the Laboratory of the University of Nariño. Afterward, they were transferred under refrigeration to the Reproduction Laboratory of the National University of Colombia - Palmira Campus, to carry out the metabolic analyses.

Five indicators of energy metabolism (NEFA, BHB, cholesterol, glucose, and triglycerides) and two of protein metabolism (PT, BUN) were determined in the serum samples. Specific commercial reagents from Randox® (Crumlin, United Kingdom) were used for each test, using the RAYTO® brand optical reading semi-automatic chemistry analyzer (Shenzhen, China) and using bovine control 2 as intra-assay control of the same type. Comercial House. The body weight (Kg) and Body Condition (BC) scores of the animals (Ural, 2016) and the average milk production were evaluated on the day of each sampling. The characterization of the reproductive parameters, such as Calving Interval (CI) and Services per Conception (SC), were obtained from the records of each farm.

**Statistical analysis of the information.** The metabolic indicator data was analyzed by multiple regression using random coefficients for each lactation period (P1, P2, P3) and by farm, from the option of General and Mixed Linear Models. The quantitative

regressor traits were dry matter intake (CMS/Kg/d), body weight (Kg), calving interval (CI/days), NEFA, glucose, cholesterol, triglycerides, BHB, total protein, and BUN, and as categorical traits body condition (BC) and services per conception (SC). Subsequently, using the same model, the backward variable elimination method was applied, establishing a variable retention p-value of 5% (Carrasco, 2016).

The transformation of categorical to quantitative variables was done through the dummy process. For body condition, there were 2.5 and 3.0 points. In this case, the BC 2.5 points was taken as the base reference, and when the value was 2.5, a zero (0) was placed; when it was 3.0, the value would be one (1), and thus the variable “CC3” was created. For the SC variable, the value of a service was used as a base. This is because this variable contained four possible values. Therefore, it was necessary to create four dummy variables, named SC1, SC2, SC3, and SC4. The replicates considered in the model were the animals selected by the farm, as described at the beginning of the methodology.

## RESULTS AND DISCUSION

Table 5 shows the results obtained from the estimation of Dry Matter Consumption (CMS), and total milk volume, by farm and lactation period. The CMS values (Kg/d) show that the highest consumption occurred on second farm (F2), and the lowest consumption was on first farm (F1), these differences can be explained by the type of management carried out on each farm, related to the type of pastures offered to the animals, where in F1 there was a greater presence of improved pastures, such as: *Lolium hibridum*, *Lolium perenne*, *Lolium spp*, *Lolium hybridum*; farm F2 the majority of dominance in the pastures was made up of *Cenchrus clandestinus Hoschst ex Chiov*, *Holcus lanatus*, *Trifolium repens*.

**Table 5.** Consumption of dry matter and quantification of the total volume of milk, percentage of protein and milk fat by dairy farm and lactation period in conditions of the high tropics of Nariño.

Productive System	Variables		
	Milk (kg/d)	Milk protein (%)	DMI <sup>1</sup> (kg/d)
F1	18.8	3.23	11.80
F2	24.8	3.12	17.20
F3	26.1	3.19	16.60
F4	25.2	3.19	14.50
Lactation Period			
P1	26.34	3.19	16.10
P2	26.86	3.17	15.50
P3	19.18	3.18	14.90

<sup>1</sup>DMI Dry Matter Intake.



When the values are analyzed by lactation period, no differences are observed, which indicates that the animals behave in a similar way, during the lactation periods evaluated, effect that is related to the type of Holstein animal which has a similarity in the level of production (Kg/d/milk) per lactation period, and therefore the consumption of dry matter, associated with the requirements of energy and metabolizable protein are the same.

Table 6 shows the mean and standard deviation of the reproductive indicators CI, DA and SC for each production system. The reproductive efficiency of the herds evaluated does not bear similarity in similar contexts of cattle farms in the high altitude tropics of Colombia, being, in the case analyzed, manifestly lower. The mean of the CI and DA does not coincide with the results reported by Zambrano *et al.* (2019) and Zambrano *et al.* (2014) for conditions in Antioquia. The open days obtained in all the herds were above the range considered optimal for Holstein cows, which causes a significant increase in economic losses and an increase in the discard rate since this is an important indicator of the reproductive efficiency of the herd.

**Table 6.** Mean and standard deviation of the reproductive indicators per dairy herd in conditions of the high tropics of Nariño.

Productive system	Traits		
	CI	DA	SC
F1	428±59	143±59	1.75±0.45
F2	461±70	175±70	1.17±0.38
F3	492±97	207±97	2.83±1.38
F4	425±49	139±49	1.60±0.50

These results coincide with those indicated by Solarte & Zambrano (2012) for the Holstein breed in Nariño. On the other hand, Zambrano *et al.* (2014) found DA values below those obtained in this research for Holstein cows in Antioquia. In relation to services per conception, the results of farms F1, F2 and F4 are similar to those reported by Zambrano *et al.* (2014). However, for F3, it coincides with the values found by Solarte & Zambrano (2012). These results may be mainly related to the nutritional quality of the forage supply in the evaluated herds, especially the energy and protein contributions during the beginning and peak of lactation. To know the relationship between metabolism and productive and reproductive behavior, multivariate analysis was used through General and Mixed Linear Models, whose results are presented in Table 7. The equations obtained after the multiple regression analysis with random coefficients for milk production, which includes the selected variables, according to the fixed effects, for each lactation period (P1, P2, and P3) were

Equation 1, corresponding to period 1, beginning of lactation (4-10 days).

$$\text{Milk production P1} = (5.73 + (0.04) (\text{body weight Kg}) + (- 7.30) (\text{CC3}))$$

Equation 2, corresponding to period 2, lactation peak (45-55 days).

$$\text{Milk production P2} = (195.24) + (0.07) (\text{body weight Kg}) + (8.55) (\text{CC3}) + (4.83) (\text{PT}) + (1.26) (\text{BUN}) + (4.74) (\text{glucose}) + (8.19) (\text{NEFA}) + (4.22) (\text{BHB}) + (19.80) (\text{triglycerides}) + (1.44) (\text{cholesterol}) + (-0.93) (\text{CI}) + (9.0) (\text{SC2}) + (14.25) (\text{SC3}) + (-2.60) (\text{SC4}) + (9.7 \times 10^{-4}) (\text{IBC2})$$

**Table 7.** Variables associated with metabolic indicators, body condition, dry matter intake and reproductive efficiency indicators that affect milk production, by lactation period, under conditions of the high tropics of Nariño.

Period	R <sup>2</sup>		Valor	SE	GL	T-value	P-value
P1	0.69	(Intercept)	5.73	5.30	18	1.08	0.2936
		Body weight (Kg)	0.04	0.01	18	4.92	0.0001
		CC3	-7.30	1.73	18	-4.22	0.0005
			Value	SE	GL	T-value	P-value
P2	0.64	(Intercept)	195.24	8.98	3	21.73	0.0002
		Body weight (Kg)	0.07	3.8E-03	3	17.27	0.0004
		CC3	8.55	0.42	3	20.50	0.0003
		PT (g/dL)	4.83	0.41	3	-11.70	0.0013
		BUN (mmol/L)	1.26	0.26	3	4.88	0.0164
		Glucose (mmol/L)	4.74	0.40	3	11.90	0.0013
		NEFA (mmol/L)	8.19	1.07	3	7.66	0.0046
		BHB (mmol/L)	4.22	0.59	3	7.14	0.0056
		Triglycerides (mmol/L)	19.80	2.30	3	8.62	0.0033
		Cholesterol	1.44	0.12	3	12.43	0.0011
		CI (days)	-0.93	0.03	3	-30.17	0.0001
		SC 2	9.00	0.57	3	15.66	0.0006
		SC3	14.25	1.13	3	12.58	0.0011
		SC4	-2.60	0.86	3	-3.03	0.0563
CI2 (days)	9.7E-04	3.1E-05	3	30.70	0.0001		
			Value	SE	GL	T-value	P-value
P3	0.90	(Intercept)	-49.99	10.53	12	-4.75	0.0005
		DMC (Kg/d)	2.75	0.31	12	8.92	<0.0005
		CC3	8.41	2.54	12	3.31	0.0062
		NEFA (mmol/L)	45.67	10.48	12	4.36	0.0009
		BHB (mmol/L)	19.25	5.44	12	3.54	0.0041
		Triglycerides (mmol/L)	-37.31	9.29	12	-4.02	0.0017
		IBC (days)	0.02	0.01	12	2.14	0.0535
		SC 2	5.73	1.28	12	4.47	0.0008
SC3	12.48	3.41	12	3.66	0.0033		

Equation 3, corresponding to period 3, lactation peak (185-200 days).

$$\text{Milk production P3} = (-49.99) + (2.75) (\text{CMS Kg / d}) + (8.41) (\text{CC3}) + (45.67) (\text{NEFA}) + (19.25) (\text{BHB}) + (-37.31) (\text{triglycerides}) + (0.02) (\text{CI}) + (5.73) (\text{SC2}) + (12.48) (\text{SC3})$$

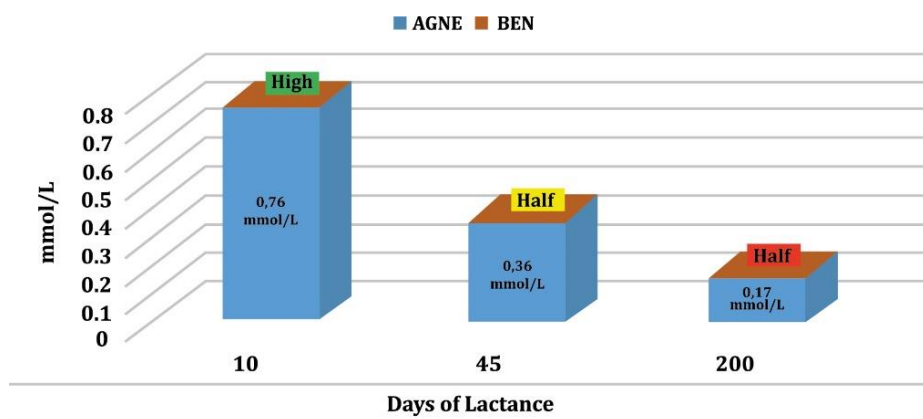
For the variables of equation 1 (P1), it is observed that the regression coefficient for body weight (Kg) indicates that the milk volume increased by 0.04 liters of milk per unit of change in this variable. In the case of body condition, the negative value -7.30 shows that, for each unit of condition lost, milk production will decrease during the onset of lactation. These results are the normal physiological response of the animal during the postpartum period, mainly due to the decrease in dry matter intake (DMS) and the high



milk production during this period, where the DMC influences the productive response of the lactating cow (Giraldo *et al.*, 2013; De Mol *et al.*, 2016).

Regarding the variables that make up prediction equation 2 (P2), it can be concluded that the metabolic indicators PT, BUN, glucose, NEFA, BHB, triglycerides, and cholesterol positively affect milk production, with values of 4.83, 1.26, 4.74, 8.19, 4.22, 19.80, and 1.44, respectively. In this case, the NEFA metabolite and triglycerides play an important role in milk production since as long as the values are within the normal ranges (<0.60 mmol/L) and 0.77mmol/L, they favor the productive response of animals in high tropical conditions (Seifi *et al.*, 2007; Kaneko *et al.*, 2008). In addition, NEFA are considered as markers of energy metabolism, allowing the evaluation of the negative energy balance (NEB) during the transition period (McCarthy *et al.*, 2015). On the other hand, non-energy compensation would lead the animal to NEB, and, as a consequence, the animal presents drastic hormonal changes, which reduce reproductive response capacity, would cause a greater number of services per conception, increase the interval between births, and therefore low efficiency in the dairy herd (Mann *et al.*, 2015; Wang *et al.*, 2017; Campos *et al.*, 2018).

The NEB determined, from the NEFA metabolite, made it possible to show that the animals, during P2, corresponding to the peak of lactation, present a medium NEB as a mechanism for mobilizing body reserves so that it compensates the NEB caused during the postpartum period and the beginning of lactation (Figure 1).



**Figure 1.** Negative energy balance of Holstein cows grazing, under conditions of the high tropics of Nariño.

In no case did the animals show clinical signs associated with ketosis, such as loss of appetite, evident loss of body weight, low milk production, and in some cases, nervous signs such as continuous licking (Garzón & Oliver, 2018), as a sequel to the elevated levels of NEFA during the beginning of lactation, called P1 (10 days of lactation).

Subsequently, the values decreased in the lactation peak, determined as period two (P2) of this investigation. Between 185 and 200 days of lactation (P3), the concentrations of this metabolite (NEFA) are lower, as a normal mechanism during the end of the second third of milk production, which allows for reducing the impact on fat mobilization, since as lactation progresses, milk production decreases, and DMS increases.

Among the risk factors associated with a greater mobilization of NEFA are the number of lactations, where animals between two and three births present a higher risk; the presence or absence of ketone bodies, especially BHB; the body condition at calving; and the dry season of calving, since the availability of food is drastically reduced during dry seasons, which has a direct effect on the dry matter intake, as well as the greater number of lactations related to dairy production (Vanholder *et al.*, 2015; Garzón & Oliver, 2018).

In the case of estimating milk production for period three (P3), the variables involved, especially DMS, show that as lactation progresses for more than 180 days, the animals substantially improve body condition, as well as some energy metabolic indicators (NEFA, BHB), which gives as a response better reproductive efficiency observed in the intervals between births and services per conception. This efficacy in the NEB is consistent with the reports presented by García *et al.* (2011), López *et al.* (2017), and David *et al.* (2018). In addition, it is important to highlight that the dietary scheme of dairy cows described in Table 1 favored that they do not present major changes in metabolic indicators during the periods evaluated.

## CONCLUSIONS

The metabolic variables selected in equation two; and those described in equation three, are the main reflection of the negative energy balance (NEB), which directly influences milk production, and that could also negatively affect the reproductive efficiency of grazing Holstein cows under conditions of the high tropics of Nariño. Dry matter intake under grazing conditions became the main variable that influences milk production during lactation of Holstein cows under high-altitude tropical conditions, and that this parameter is a key tool to assess the productivity of cows dairy farms in the Nariño region.

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**Conflict of interest:** The authors declare that there is no conflict of interest.

## BIBLIOGRAPHIC REFERENCES

- Akbar, H.; Grala, T.M.; Riboni, M.V.; Cardoso, F.C.; Verkerk, G.; McGowan, J.; ... Matthews, L. (2015). Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. *Journal of dairy science*. 98(2): 1019-1032. 10.3168/jds.2014-8584
- AOAC. (1995). Official methods of analysis: International suite. *AOAC INTERNATIONAL*. 78(1-1): 9A-26A. 10.1093/jaoac/78.1.9A
- Apráez, G.J.E.; Delgado, J.D.; Solarte, P.C. (2016). Z en variedades de pastos neozelandeses del altiplano de Nariño. *Veterinaria y Zootecnia*. 10(2): 90-105. 10.17151/vetzo.2016.10.2.8
- Carrasco, M.C. (2016). Técnicas de regularización en regresión: Implementación y aplicaciones. <https://idus.us.es/xmlui/bitstream/handle/11441/43746/Carrasco%20Carrasco%2C%20Mar%C3%ADa%20TFG.pdf?sequence=1&isAllowed=y>
- Campos, R.; Orozco, A.C.; Burbano, G.L.; Córdoba, P.A.O. (2018). Alteraciones bioquímicas y metabólicas en el periodo de transición en vacas lecheras. *Revista RIAA*. 9(2): 11. <https://doi.org/10.22490/21456453.2123>
- Correa, H.J.; Pabón, M.L.; Carulla, J.E. (2009). Estimación del consumo de materia seca en vacas Holstein bajo pastoreo en el trópico alto de Antioquia. *Livestock Research for Rural Development*. 21(4): 1-20. <http://www.lrrd.org/lrrd21/4/corr21059.htm>
- David, D.M.; Gaona, R.C.; Apráez, J.E.; Terranova, M.V.; Zambrano, G.L. (2018). Metabolic and reproductive response to energy supplementation in dairy cows in tropical highlands. *Revista de Ciencias Agrícolas*. 35(2): 51-63. 10.22267/rcia.183502.91
- Drackley, J.K.; Cardoso, F.C. (2014). Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. *Animal*. 8(s1): 5-14. 10.1017/S1751731114000731
- De Mol, R.M.; Goselink, R.M.A.; van Riel, J.; Knijn, H.M.; Van Kneegsel, A.T. (2016). The relation between eating time and feed intake of dairy cows. In: Kamphuis, C.; Steeneveld, W. *Precision dairy farming 2016*. pp: 387-394. Netherlands: Wageningen Academic Publishers. <https://doi.org/10.3920/978-90-8686-829-2>
- García, A.; Cardoso, F.C.; Campos, R.; Thedy, D.; González, F. (2011). Metabolic evaluation of dairy cows submitted to three different strategies to decrease the effects of negative energy balance in early postpartum. *Pesquisa Veterinária Brasileira*. 31: 11-17.
- Garzón, A.A.; Oliver, E.O. (2018). Epidemiología de la cetosis en bovinos: una revisión. *CES Medicina Veterinaria y Zootecnia*. 13(1): 42-61. 10.21615/cesmvz.13.1.4
- Giraldo, L.A.; Carulla, J.E.; Calle, M.A.; Giraldo, L.; Carulla, J.; Calle, M. (2013). Metabolismo digestivo de razas bovinas (bon, Holstein, cebú) en pasturas tropicales de calidad contrastante. En: Campos, G.R.; Durán, C. *Consideraciones sobre el mejoramiento genético y factores asociados en bovinos criollos colombianos y grupos multirraciales*. pp. 103-127. Palmira, Colombia: Universidad Nacional de Colombia.
- Kaneko, J.; Harvey, J.; Bruss, M. (2008). *Clinical biochemistry of domestic animals*. 6th ed. San Diego-California: Academic Press. Inc. 589p.
- López, O.R.; Tinajero, P.T.; López, O.R.; Mendoza M.G.D.; Montiel, R.; de Dios, J.; ... Ruíz, F.A. (2017). Relaciones entre calcio, ácidos grasos no esterificados, e insulina sanguínea en preparto y leche bovina perdida en el inicio de la lactancia. *Nova scientia*. 9(19): 306-328.
- DANE-Departamento Administrativo Nacional de Estadística. (2017). Informe de Coyuntura Económica Regional Departamento de Nariño. p.16, 17, 54. [www.dane.gov.co](http://www.dane.gov.co)
- Mann, S.; Yepes, F.L.; Overton, T.R.; Wakshlag, J.J.; Lock, A.L.; Ryan, C.M.; Nydam, D.V. (2015).

- Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. *Journal of Dairy Science*. 98(5): 3366-3382. <https://doi.org/10.3168/jds.2014-9024>
- McCarthy, M.; Mann, S.; Nydam, D.V.; Overton, T.R.; McArt, J.A. (2015). Concentrations of nonesterified fatty acids and  $\beta$ -hydroxybutyrate in dairy cows are not well correlated during the transition period. *Journal of Dairy Science*. 98(9): 6284-6290. <http://dx.doi.org/10.3168/jds.2015-9446>
- Seifi, H.A.; Gorji-Dooz, M.; Mohri, M.; Dalir-Naghadeh, B.; Farzaneh, N. (2007). Variations of energy-related biochemical metabolites during transition period in dairy cows. *Comparative Clinical Pathology*. 16: 253-258. <https://doi.org/10.1007/s00580-007-0682-2>
- SAGAN-Sociedad de Agricultores y Ganaderos de Nariño. (2022). Censo Bovino Histórico. Censo: predios y población bovina/bufalina por sexo y categorías de edad-año 2022. <https://sagan.com.co/informacion-estadistica/>
- Solarte, C.; Zambrano, G. (2012). Characterization and genetic evaluation of Holstein cattle in Nariño, Colombia. *Revista Colombiana de Ciencias Pecuarias*. 25(4): 539-547.
- Ural, D.A. (2016). The use of new practices for assessment of body condition score. *Revista MVZ Córdoba*. 21(1): 5154-5162.
- Vanholder, T.; Papen, J.; Bemers, R.; Vertenten, G.; Berge, A.C.B. (2015). Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *Journal of dairy science*. 98(2): 880-888. 10.3168/jds.2014-8362
- Wang, Y.; Hou, Q.; Cai, G.; Hu, Z.; Shi, K.; Yan, Z.; Wang, Z. (2017). Effects of Dietary Energy Density in the Dry Period on the Production Performance and Metabolism of Dairy Cows. *Advances in Bioscience and Biotechnology*. 8(03): 104. 10.4236/abb.2017.83008
- Yeshambel, M. (2023). Negative energy balance and its implication on productive and reproductive performance of early lactating dairy cows: review paper. *Journal of Applied Animal Research*. 51(1): 220-229. <https://doi.org/10.1080/09712119.2023.2176859>
- Zambrano, J.C.; Echeverri, J.; Herrera, A.L. (2019). Genomic Evaluation of Colombian Holstein Cattle Using Imputed Genotypes at Medium Density. *Revista MVZ Córdoba*. 24(2): 7248-7255.
- Zambrano, J.C.; Rincón, J.C.; Echeverri, J. (2014). Parámetros genéticos para caracteres productivos y reproductivos en Holstein y Jersey colombiano. *Archivos de zootecnia*. 63(243): 495-506