Effect of dietary variation on indicators of metabolic energy, protein, and liver function in dairy cows during the transition period

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Efeito da variação dietética sobre os indicadores de energia metabólica, proteína e função hepática em vacas leiteiras durante o período de transição

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Abstract

A metabolic profile is a useful tool for nutritional diagnosis and assessing health status, especially during the critical transition period in dairy cows. The objective of this study was to characterize blood metabolic indicators during the transition period, evaluating a diet based on pastures, corn silage, and soy in comparison to a conventional pasture and concentrate-based diet. The study was conducted in the Bijagual area, district of David, Province of Chiriquí, Republic of Panama, using 30 dairy cows. The weight, body condition score, and the temperature-humidity index were assessed throughout the transition period (one month before and one month after calving). Additionally, blood samples were collected in the field on days -28, -21, -14, -7, 0, 3, 7, 14, 21, and 28 relative to calving to analyze energy indicators (glucose, triglycerides, beta-hydroxybutyrate, non-esterified fatty acids), protein indicators (total protein, blood urea nitrogen), and liver function indicators (globulin, albumin, albumin/globulin ratio, and bilirubin). Data were statistically analyzed using a repeated measures mixed model via the SAS statistical package. The results showed that diet based on pastures, corn silage, and soy yielded better performance in energy, protein, and liver function indicators, with the exception of cholesterol, which was higher in the conventional pasture and concentrate-based treatment. It was concluded that the metabolic biomarkers related to energy, protein, and liver function remained within normal ranges for dairy cows, indicating adequate nutritional management and resilience of the cows during the transition period in both treatments.

Keywords: Metabolic biomarkers. Temperature-humidity index. Lipolysis. Metabolism. Liver function.

Resumo

O perfil metabólico é uma ferramenta útil para o diagnóstico nutricional e avaliação do estado de saúde, especialmente durante o período crítico de transição em vacas leiteiras. O objetivo deste estudo foi caracterizar os indicadores metabólicos sanguíneos durante o período de transição, avaliando uma dieta à base de pastagens, silagem de milho e soja em comparação com uma dieta convencional baseada em pastagem e concentrado. O estudo foi conduzido na área de Bijagual, distrito de David, província de Chiriquí, República do Panamá, utilizando 30 vacas leiteiras. O peso, a condição corporal e o índice temperatura-umidade foram avaliados ao longo do período de transição (um mês antes e um mês após o parto). Além disso, amostras de sangue foram coletadas em campo nos dias -28, -21, -14, -7, 0, 3, 7, 14, 21 e 28 em relação ao parto para analisar indicadores de energia (glicose, triglicerídeos, beta-hidroxibutirato, ácidos graxos não esterificados), indicadores de proteína (proteína total, nitrogênio ureico no sangue) e indicadores de função hepática (globulina, albumina, relação albumina/globulina e bilirrubina). Os dados foram analisados estatisticamente utilizando um modelo misto de medidas repetidas por meio do pacote estatístico SAS. Os resultados mostraram que a dieta à base de pastagens, silagem de milho e soja apresentou melhor desempenho nos indicadores de energia, proteína e função hepática, com exceção do colesterol, que foi maior no tratamento convencional à base de pastagem e concentrado. Concluiu-se que os biomarcadores metabólicos relacionados à energia, proteína e função hepática permaneceram dentro dos intervalos normais para vacas leiteiras, indicando manejo nutricional adequado e resiliência das vacas durante o período de transição em ambos os tratamentos.

Palavras-chave: *Biomarcadores metabólicos. Índice temperatura-umidade. Lipólise. Metabolismo. Função hepática.*

Introduction

The term metabolic profile was proposed by Payne et al. (1970) and emerged as an auxiliary method for diagnosing so-called production diseases. According to Contreras et al. (2010), the analysis of metabolic profiles can support the study of nutritional balance and the physiological status of production animals (Gao et al., 2022).

In intensive dairy production systems, cows are subjected to high productive demands. The main limitation is low dry matter intake (DMI) relative to the nutrient requirements of postpartum cows, in addition to forage quality and the environmental stress typical of tropical regions (Muzzo et al., 2025). Under these conditions, metabolic and nutritional status monitoring is of great importance, along with the correlation between milk components and the metabolic profile and biology during lactation (Toscano et al., 2023).

During the transition period, a readjustment occurs in the conditions of the animal's gastrointestinal tract especially in the rumen and in the quantity and type of volatile fatty acids produced through fermentation, as explained by Church (1988). This is due to the need for a balance between cellulolytic microorganisms and the fermentation of soluble carbohydrates (Li et al., 2021). Likewise, among the most significant hormonal changes are the serum concentrations of estrogens and corticosteroids around calving, which contribute to the reduction in DMI and coordinate metabolic changes that promote the mobilization of body fat reserves (Friggens, 2003; Friggens et al., 2004).

Moreover, any animal experiencing a metabolic disorder caused by poor management or feeding strategies during the transition period will suffer consequences in both milk production and reproduction (Drackley, 1999).

The endocrine and metabolic changes during the transition period in dairy cows are coordinated by growth hormone (GH), which stimulates hepatic gluconeogenesis to increase the supply of glucose to the mammary gland (Martin et al., 2021). Simultaneously, GH also induces insulin resistance, which reduces glucose utilization by the liver, muscle, and adipose tissue, and stimulates lipolysis, increasing the concentration of non-esterified fatty acids (NEFA) in the bloodstream (Marinković et al., 2019). These are used either for milk synthesis or as an energy source during the cow's postpartum period (Wankhade et al., 2017).

It has been indicated that negative energy balance (NEB) is an inevitable event during the dairy cow's transition period (Triwutanon and Rukkwamsuk, 2021). This is an important physiological characteristic, particularly in high-producing animals, due to the combined result of reduced intake and higher energy demand for maintenance and production. NEFA and

Beta-Hydroxybutyrate (BHB) are important energy metabolites traditionally used as indicators of NEB during the transition period (Gobikrushanth et al., 2019; Krattenmacher et al., 2019).

Effective management of cows during the transition period consists of promoting a moderate rate of lipolysis that declines rapidly as lactation progresses, with minimal impact on the animal's body reserves (Martins et al., 2020). While the release of NEFA into the bloodstream provides energy to tissues, the bovine liver has limited capacity to metabolize NEFA into triglycerides (TG) (Contreras et al., 2018). When TG levels in the liver increase and acetyl-CoA produced by fatty acid oxidation is not utilized in the tricarboxylic acid cycle, it is converted into ketone bodies such as acetone, acetoacetate, and BHB, which can be detected in blood, milk, and urine analyses (Goff and Horst, 1997).

The objective of this study was to characterize the energy, protein, and liver function metabolic profiles in dairy cows during the transition period under tropical production conditions in Panama, in order to evaluate their nutritional and physiological status and their adaptive response to the metabolic challenges typical of this critical stage.

Material and methos

The study was conducted in the Bijagual township, district of David, Chiriquí Province, Republic of Panama. This area is located under lowland agroecological conditions at an altitude of 133 meters above sea level, with temperatures ranging between 26 and 34 °C. The average relative humidity is 80% during the rainy season (June-December) and 40% during the dry season (January – May).

Study animals and experimental groups

Thirty multiparous dairy cows (Holstein × Brown Swiss) were selected in their third lactation, to ensure the stability of metabolic profile, and randomly and evenly assigned into two experimental groups of 15 cows each, with similar gestation periods to avoid heterogeneity in calving dates. Two experimental groups were established: control (CON), in grazing with management conditions and supplementation with concentrate; and mixed (MIX), in grazing with supplementation of corn silage and soybean forage.

The nutritional plan for the CON treatment cows during the pre-partum period included: *Brachiaria brizantha* grass, within a continuous rotational system, with a total of eight blocks of 5000 m^2 each, where they remained throughout the day, except during milking periods, when they were supplemented with concentrate or silage in the feeders. Tables 1 and 2 describe the nutritional composition of the ingredients in the CON and MIX diets, respectively, used for the pre and postpartum treatments of the study groups and the amount of daily diet offered per animal. The transition to prepartum diets was implemented gradually starting from day -28 of the expected parturition date, considering the actual parturition date as 0, and the point of inclusion of the postpartum diet until day +28.

Table 1 - Nutritional composition of the control diet during prepartum and postpartum periods and the amount of daily diet offered per animal

Description	DM (%)	CP (%)	Energy*	NDF (%)	ADF (%)	Offered**
Prepartum						
Prepartum concentrate	84.5	18.8	1.8	35.7	19.0	1.8
Green forage	29.5	8.7	0.9	72.7	35.9	25.0
Soybean meal	89.0	44.0	1.8	14.9	38.0	0.4
Postpartum						
Postpartum concentrate	90.1	19.4	1.8	37.9	19.4	4.5
Green forage	21.5	13.8	1.2	66.7	34.8	35.0

Note: DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.*Mcal/day. **Kg/animal.

Table 2 - Nutritional composition of the mixed diet during prepartum and postpartum periods and the amount of daily diet offered per animal

Description	DM (%)	CP (%)	Energy*	NDF (%)	ADF (%)	Offered**
Prepartum						
Forage soybean	26.2	16.2	1.4	49	28	2.3
Green forage	29.5	8.7	0.9	72.7	35.9	33.0
Soybean meal	89.0	44.0	1.8	14.9	38.0	0.4
Postpartum						
Postpartum concentrate	90.1	19.4	1.8	37.9	19.4	2.7
Corn silage	26.2	9.0	1.0	68.3	39.8	2.3
Forage soybean	26.2	16.2	1.4	49.0	28.0	2.3
Green forage	21.5	13.8	1.2	66.7	34.8	30.0
Soybean meal	89.0	44.0	1.8	14.9	38.0	0.4

Note: DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.*Mcal/day. **Kg/animal.

Feed consumption (Kg/day) in the feeder was measured daily for each cow observed, by the difference in weight between the offer and the rejection, taking samples weekly during the silage and concentrate supplementation research period.

Data collection method and instruments

The Temperature-Humidity Index (THI), weight, and body condition score (BCS) were measured in transition cows to account for environmental impact. The weight was recorded using a digital scale model LP 7510, and body condition was evaluated using the Embrapa Vetscore system (Barbosa et al., 2022) on a scale from 1 to 5. Measurements were taken on days -28, -21, -14, -7, 0, 7, 14, 21, and 28 of the transition period.

For THI calculation, dry-bulb temperature (°C), wet-bulb temperature (°C), and relative humidity (%) were obtained from the *Empresa de Transmisión Eléctrica* (ETESA) Meteorological Station, in Gualaca, located 5 km from the research site. The THI was calculated during the first morning milking (4 am) and the second afternoon milking (2 pm), using the equation proposed by Jeelani et al. (2019): THI = 1.8aT- (1 - RH) (aT - 14.3) + 32. Where: aT is the average temperature (°C), and RH is the relative humidity.

Cows in each group were identified by their corresponding farm number. Blood samples were collected weekly during the early morning milking via jugular vein puncture using a 16-gauge needle.

Samples were drawn into vacuum tubes for serum, immediately centrifuged, thus minimizing residual glycolysis by the erythrocytes, at 1096 g force for 15 minutes, and the separated serum was stored at -20 °C until analysis. The methodology proposed by Manston and Allen (1981) and Reid et al. (1983) was followed, with samples collected on days -28, -21, -14, -7, 0, 3, 7, 14, 21 and 28 of the transition period. To avoid biases arising from acute stress and alterations in lipid mobilization, dystocic births were established as an exclusion factor for the animal in the study. Only animals with eutocic births and without immediate metabolic complications, such as milk fever or severe retained placenta, were considered.

Biomarkers evaluated

NEFA and BHB were evaluated using specific commercial kits purchased from Solarbio (Beijing, China). Glucose, TG, total protein, very low-density lipoprotein (VLDL), urea, globulin, albumin, albumin/globulin (Alb/Glob), cholesterol and total bilirubin were evaluated using commercial diagnostic kits from Wiener lab. Group, Rosario, Argentina.

Energy indicators

• Non-esterified fatty acids (mmol/l), (Solarbio Cat No: BC0590). Wavelength: 550 nm (spectrophotometry). Sample volume required: 100 µL. Incubation time: 60 minutes.

• Beta-hydroxybutyrate (mmol/l), (Solarbio Cat No: BC5080). Wavelength: 340 nm. Sample volume required: 100 µL. Incubation time: 60 minutes.

• Glucose (mg/dl), (WIENER LAB Manual Cod 14 00101). Wavelength: 505–530 nm. Sample volume required: 20 µL. Incubation time: 10 minutes at room temperature or 37 °C.

• TG (mg/dl), (WIENER LAB Manual Cod 1780107). Wavelength: 510 nm. Sample volume required: 20 µL. Incubation time: 10 minutes at 37 °C.

• VLDL (mg/dl), (WIENER LAB Manual Cod 1220 104). This procedure is an estimation of VLDL cholesterol concentration in serum or plasma, calculated not directly measured enzymatically in most cases. VLDL is typically estimated using the Friedewald equation: VLDL (mg/dL) = TG/5.

Protein indicators

• Total protein (g/dl), (WIENER LAB Manual Cod 1999736). Wavelength: 546 nm. Sample volume: 20 µL. Incubation time: 10 minutes at 37 °C.

• Urea (mg/dl), (WIENER LAB Manual Cod 100 9807). Wavelength: 340 nm. Sample volume: 20 µL. Incubation time: 10 minutes.

• Globulin (g/dl), (WIENER LAB Manual Cod 169 0001). Estimation of globulin levels in serum is not directly measured. Globulin concentration is obtained by subtracting albumin from total protein: Globulin (g/dL) = Total protein (g/dL) - Albumin (g/dL).

• Alb/Glob ratio, (WIENER LAB Manual Cod 169 0001). Evaluation of the balance between albumin and globulin in serum; useful in liver function tests, nutritional assessments, and immune status, not directly measured. The Alb/Glob ratio is calculated using: Alb/Glob ratio= (Albumin (g/dL)/(Total protein (g/dL) - Albumin (g/dL).

Liver function indicators

• Albumin (g/dL), (WIENER LAB Manual Cod 100 8135). Wavelength: 630 nm. Sample volume: 20 µL. Incubation time: 10 minutes.

• Cholesterol (mg/dL), (WIENER LAB Manual Cod 1221221). Wavelength: 510 nm. Sample volume: 20 µL. Incubation time: 10 minutes at 37 °C.

• Total bilirubin (mg/dL), (WIENER LAB Manual Cod 1120008). Wavelength: 546 nm. Sample volume: 100 µL. Incubation time: 10 minutes.

All parameters were analyzed using a multiparametric Mindray PRO-240 analyzer.

Statistical analysis

Metabolic parameters were analyzed using a repeated measures design as proposed by Gill (1988) and using the MIXED procedures of SAS v.9.

The statistical model contained treatment as a fixed effect, animal nested within treatment as a random effect (whole-plot error term), period as a repeated measure, and the interaction of treatment and period. The following mathematical model was applied: $Y = \mu + T_i AT_{ij} + P_k + TP_{ik} + E_{ijk}$.

Where: Y = metabolic parameters and milk production. µ = overall mean. Ti = treatment effect (fixed effect). $A(T)_{ij}$ = error A; animals nested within treatments (random effect). P_k = effect of period (-28, -21, -14, -7, 0, +7, +14, +21 and +28 days). TP_{ik} = treatment × period interaction. E_{ijk} = residual error.

The experimental unit for treatment comparisons was the animal. Repeated measures across periods within each animal resulted in correlated observations; hence, a homogeneous variance-covariance structure. The compound symmetry variance-covariance structure was specified to model equal correlation and constant variance for repeated observations within animal. For significant main effects and interactions, Tukey's multiple comparison test was used for pairwise comparisons. Statistical significance was evaluated at $p \leq 0.05$.

Ethics statement

The experimental procedures were conducted in Panama under an institutional cooperation agreement with the National Secretariat of Science, Technology and Innovation (SENACYT), which authorized and supported the project's execution within the country. Ethical oversight was provided by the Bioethics Committee of La Salle University, Bogotá, Colombia, within the framework of an inter-institutional arrangement that extended ethical coverage to the country of execution. The research protocols complied with Panamanian regulations applicable to animal research and production, as well as with international standards for the care and use of animals in research. Written authorization and informed consent were obtained from the participating farm before the

start of experimental activities. The procedures followed internationally recognized guidelines on animal welfare, ensuring the minimization of stress and the protection of animal health throughout the study.

Results

All animals remained clinically healthy and without visible signs of infectious diseases or clinical metabolic disorders during the study period. This stable health allowed us to establish that the variations observed in the biomarkers were strictly due to the cow's metabolic adaptation and the effect of the dietary treatments evaluated.

Figure 1 shows the evolution of the THI from 30 days before to 30 days after calving (day 0), a critical stage in the physiology of dairy cows known as the transition period.

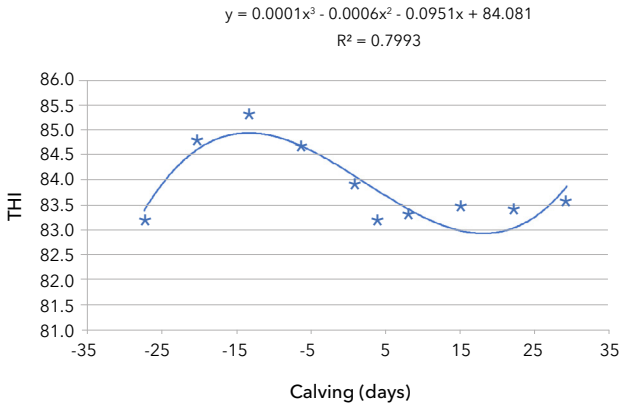

Figure 1 - Trend of the Temperature-Humidity Index (THI) during the transition period.

An increase in THI is observed during the prepartum phase, reaching its peak between days -15 and -10 with values exceeding 85, followed by a gradual decline during the postpartum period, reaching a minimum close to 83 around day +15, and a slight recovery toward the end of the monitoring period.

From a physiological perspective, this variation has important implications. THI values above 72–74 are considered thresholds for mild heat stress in high-producing cows, while values above 80 indicate severe heat stress. In this context, the values observed during the prepartum phase suggest that animals were exposed to environmental conditions that could potentially compromise thermal homeostasis.

Prepartum heat stress has been linked to detrimental effects on various productive and physiological parameters. For example: reduced (DMI) and alterations in energy metabolism, which can predispose animals to metabolic disorders such as ketosis and fatty liver; disruption of the somatotropic axis, including reduced insulin-like growth factor 1 (IGF-1) and GH levels, which impairs body reserve mobilization and readiness for lactation; immunological alterations, including suppression of neutrophil and lymphocyte function, increasing the risk of postpartum uterine infections and mastitis; decreased milk yield, attributed to reduced energy reserves, oxidative stress, and lower metabolic adaptability.

Conversely, the decline in THI observed in the postpartum phase may create more favorable conditions for physiological recovery and the successful establishment of lactation. However, if heat stress was severe during the prepartum period, its physiological consequences may persist for several weeks postpartum.

These findings underscore the importance of implementing heat stress mitigation strategies, such as forced ventilation, misting systems, or sprinkler cooling especially in warm climates or critical seasons. Environmental management during the transition period should be considered a key tool for safeguarding the health, welfare, and productivity of dairy cows.

Weight

Weight is one of the best indicators to describe changes in weight gain and/or loss in animals. In this study, during the prepartum period, cows had an average weight of 447.1 kg, while postpartum weight averaged 390.4 kg. Statistical analysis showed no significant differences ($p > 0.05$) between treatments or treatment × period interaction (Table 3); however, differences were significant for the period factor ($p < 0.001$).

However, Figure 2 visually illustrates the evolution of weight, using polynomial trend lines, in the CON and MIX groups throughout the transition period. In both treatments, weight showed a similar pattern, characterized by a slight increase at the end of

gestation, followed by a progressive decrease after delivery. This decrease is consistent with the typical physiological response associated with the onset of lactation and the negative energy balance that occurs during the early postpartum period.

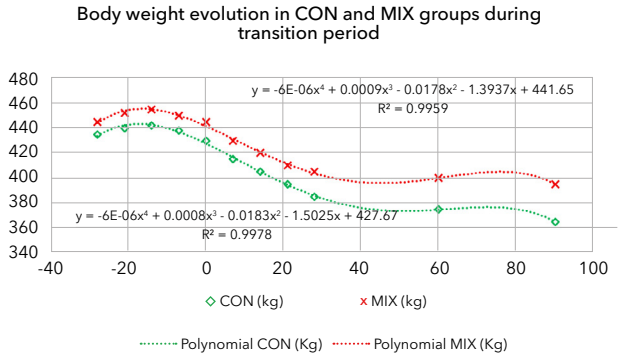

Figure 2 - Comparison of the evolution of weight (kg) over time between a control group (CON) and a test group (MIX), during the transition period.

The CON group exhibited a more pronounced decrease in weight. In contrast, the MIX group demonstrated a comparatively attenuated weight loss (Figure 2).

These findings suggest that the intervention applied to the MIX group may have mitigated weight loss during the transition period relative to the control group. Differences in the slope and shape of the fitted curves indicate a potential influence of the MIX treatment on metabolic regulation or physiological adaptation during this critical phase.

Body condition score

The average BCS was 3.05 for prepartum and 2.91 for postpartum. The average for the CON group was 2.91, and 2.89 for the MIX group. There were no significant differences ($p > 0.05$) between treatments or between treatment and period interaction (Table 3); however, the period effect was statistically significant ($p < 0.002$).

Table 3 - Evolution of weight and body condition in dairy cows during the transition period under two feeding treatments

Variables	Mean	D-28	D-21	D-14	D-7	D0	D7	D14	D21	D28	SEM	T	P	T*P
WGT														
CON	379.4	439.7	437.5	447.9	445.5	401.2	395.3	383.0	375.3	374.2	13.4	0.55	<0.001	0.79
MIX	390.5	445.5	449.7	454.2	451.0	408.3	403.3	393.5	382.2	385.8	13.4			
BCS														
CON	2.91	3.14	3.12	3.11	3.03	2.90	3.00	2.92	2.91	2.88	0.04	0.74	<0.002	0.37
MIX	2.89	2.97	3.03	3.04	2.94	2.91	2.92	2.90	2.85	2.89	0.04			

Note: ANOVA results for weight and body condition score; general means for control (CON) and MIX treatments during the transition period in dairy cows. D = day; SEM = standard error of the mean; T = Tteatment; P = period; T*P = treatment × period interaction; WGT = weight (Kg); BCS = body condition score.

Blood biomarkers

Table 4 summarizes the effects of dietary treatment, physiological period (days relative to calving), and their interaction (T×P) on various metabolic parameters in dairy cows evaluated from -28 to +28 days relative to parturition. An analysis of variance was performed to identify significant differences.

Prepartum and postpartum VLDL concentrations were 10.16 and 7.51 mg/dL, respectively. The differential between CON and MIX treatments was +0.5 mg/dL; notably, the lowest VLDL levels were observed in the MIX treatment during early lactation.

VLDL concentrations did not differ significantly between treatments ($p > 0.05$), although a tendency toward a treatment × period interaction was observed. However, as observed in Table 4, the period effect was significant ($p < 0.001$) for both treatments. A slight decrease in VLDL was noted prior to parturition, extending through day 7 postpartum, after which VLDL concentrations tended to increase in both groups.

Mean BHB concentrations were 0.134 mmol/L in the CON group and 0.126 mmol/L in the MIX group. The BHB profile indicated higher ketone body production in CON compared to MIX.

Nonetheless, from day 21 postpartum onward, BHB levels declined in both treatments, consistent with the weight trajectory showing weight loss until day 21 postpartum, followed by weight gain.

Table 4 - Analysis of variance of all metabolic variables and overall means for control (CON) and mixed (MIX) treatments during the transition period

Variables	Mean	D-28	D-21	D-14	D-7	D0	D3	D7	D14	D21	D28	SEM	T	P	T*P
GLU															
CON	51.69	49.10	50.95	51.97	49.56	69.50	48.18	48.96	45.81	51.81	51.06	1.58	0.326	<0.0001	0.628
MIX	49.44	44.60	46.43	44.97	47.64	73.68	49.31	43.87	44.00	49.75	50.12	1.59			
TRG															
CON	44.17	53.90	59.39	49.50	50.50	38.50	35.75	36.04	37.93	40.68	39.50	1.41	0.228	<0.0001	0.074
MIX	41.71	48.20	52.09	47.50	47.56	39.87	35.06	35.31	36.87	37.87	36.75	1.41			
VLDL															
CON	8.80	10.60	11.86	9.87	10.12	7.75	7.12	7.18	7.56	8.06	7.87	0.28	0.236	<0.0001	0.096
MIX	8.32	9.58	10.33	9.40	9.50	7.87	7.12	7.00	7.37	7.56	7.00	0.28			
TP															
CON	6.47	6.68	6.91	6.49	6.29	6.20	5.87	6.11	6.59	6.75	7.43	0.16	0.243	<0.0001	0.947
MIX	6.19	6.43	6.54	6.02	6.14	6.04	5.75	5.87	6.12	6.48	6.51	0.16			
BUN															
CON	12.52	10.8	12.94	13.10	15.87	18.89	12.41	9.91	9.62	10.29	11.29	0.69	0.379	<0.0001	0.063
MIX	11.64	11.6	11.53	10.42	12.62	16.18	12.10	12.94	8.66	10.59	9.66	0.70			
GLO															
CON	3.33	3.49	3.6	3.34	3.17	2.98	2.82	3.06	3.44	3.65	3.74	0.11	0.485	<0.0001	0.986
MIX	3.21	3.39	3.41	3.07	3.10	2.93	2.81	2.90	3.28	3.56	3.62	0.11			
ALB															
CON	3.14	3.19	3.3	3.15	3.12	3.21	3.04	3.04	3.14	3.10	3.09	0.08	0.182	0.0030	0.817
MIX	2.98	3.03	3.12	2.95	3.04	3.10	2.94	2.97	2.84	2.91	2.88	0.08			
ALB/GLO															
CON	0.97	0.94	0.94	0.97	1.02	1.11	1.11	1.01	0.92	0.87	0.84	0.03	0.635	<0.0001	0.937
MIX	0.95	0.90	0.93	0.97	1.01	1.07	1.05	1.05	0.87	0.83	0.81	0.03			
CHOL															
CON	100.29	94.90	100.70	92.75	97.12	82.94	84.62	92.08	110.81	118.94	127.00	4.76	0.035	<0.0001	0.871
MIX	85.29	85.70	82.24	73.8	76.83	69.18	68.76	77.37	99.03	109.31	110.62	4.77			
BILI															
CON	0.20	0.18	0.18	0.19	0.17	0.24	0.18	0.21	0.20	0.19	0.18	0.01	0.391	0.0600	0.917
MIX	0.17	0.17	0.15	0.17	0.16	0.20	0.18	0.17	0.17	0.17	0.18	0.01			
BHB															
CON	0.14	-	-	0.13	0.13	-	-	0.13	0.14	0.14	-	0.003	0.173	<0.0500	0.724
MIX	0.13	-	-	0.12	0.12	-	-	0.12	0.13	0.13	-	0.003			
NEFA															
CON	0.45	-	-	0.45	0.47	-	-	0.48	0.42	0.42	-	0.020	0.535	0.5670	0.980
MIX	0.43	-	-	0.44	0.45	-	-	0.46	0.42	0.37	-	0.020			

Note: D = day; SEM = standard error of the mean; T = treatment; P = period; T*P = treatment by period interaction; GLU = glucose (mg/dl); TRG = triglycerides (mg/dl); VLDL = very low-density lipoprotein (mg/dl); TP = total proteins (mg/dl); BUN = blood urea nitrogen (mg/dl); GLO = globulin (g/dl); ALB = albumin (g/dl); ALB/GLO = albumin/globulin ratio (g/dl); CHOL = cholesterol (mg/dl); BILI = bilirubin (mg/dl); BHB = beta-hydroxybutyrate (mmol/L); NEFA = non-esterified fatty acids (mmol/L).

NEFA concentrations did not differ significantly between treatments or periods ($p > 0.05$), though a tendency for treatment × period interaction was present. Peak NEFA concentrations occurred during the first week postpartum, with CON exhibiting higher NEFA levels than MIX, suggesting greater mobilization of body reserves and, consequently, increased weight loss.

Total protein concentrations averaged 6.41 and 6.26 g/dL prepartum and postpartum, respectively, across treatments. Prepartum total protein ranged from 3.58 to 7.83 g/dL, whereas postpartum values ranged from 3.28 to 8.24 g/dL. Treatment means were 6.47 g/dL (CON) and 6.19 g/dL (MIX). No significant differences in total protein were detected between treatments or treatment × period interactions ($p > 0.05$), but the period effect was highly significant ($p < 0.001$).

Blood urea nitrogen (BUN) levels averaged 12.54 and 12.29 mg/dL prepartum and postpartum, respectively, across treatments. The most frequent values were 12 mg/dL prepartum and 9 mg/dL postpartum. The differential between treatments was +0.88 mg/dL, with means of 12.82 mg/dL (CON) and 11.94 mg/dL (MIX). No significant differences were found for treatment or treatment × period interaction ($p > 0.05$), while period effects were highly significant ($p < 0.0001$). A progressive increase in BUN was observed prepartum, peaking at parturition for both treatments.

Globulin concentrations were 3.29 and 3.23 g/dL prepartum and postpartum, respectively, with no significant differences detected between treatments or treatment x period interaction ($p > 0.05$). However, the period effect was significant ($p < 0.0001$). Globulin levels remained relatively stable during transition, with a slight increase between days 21 and 28 postpartum; treatment means differed by only 0.12 g/dL.

The Alb/Glob ratio was 0.97 and 0.96 prepartum and postpartum, respectively, with treatment means of 0.97 (CON) and 0.95 (MIX). Neither treatment nor treatment × period interaction effects were significant ($p > 0.05$), whereas period effects were highly significant ($p < 0.0001$), with the greatest variation occurring from parturition to 3 days postpartum.

Prepartum albumin averaged 3.11 g/dL and postpartum 3.02 g/dL, with the mode at 3.27 g/dL. CON animals exhibited higher albumin concentrations than MIX (Table 3), with a treatment difference of 0.16%. Albumin showed no significant treatment or treatment × period interaction effects ($p > 0.05$), but the period effect was significant ($p < 0.001$).

Cholesterol concentrations averaged 100.54 mg/dL prepartum and 86.77 mg/dL postpartum. Prepartum mean and mode were 89.08 and 91 mg/dL, respectively; postpartum values were 96.78 and 88 mg/dL. Cholesterol was significantly different between treatments (100.29 mg/dL CON vs. 85.29 mg/dL MIX), treatment × period interaction ($p < 0.05$), and period effects ($p < 0.001$).

Mean bilirubin concentrations were 0.17 mg/dL prepartum and 0.19 mg/dL postpartum, with treatment means of 0.19 (CON) and 0.17 (MIX). No significant differences were observed for treatment, period, or interaction effects ($p > 0.05$). The greatest variation occurred at parturition; minimal changes were noted pre- and postpartum.

The correlation analysis of the metabolic biomarkers evaluated during the transition period (pre- and postpartum) allowed for the identification of relevant physiological interactions among the protein, energy, and hepatic function systems (Figure 3). These findings are key to understanding the metabolic adaptations dairy cows undergo during this critical phase.

Protein indicators

A highly significant positive correlation was observed between total protein and globulin ($r = 0.89$; $p < 0.001$), confirming that globulins constitute a structurally important fraction of total plasma protein. Likewise, total protein showed a strong correlation with albumin ($r = 0.71$; $p < 0.001$) and a moderately negative correlation with the Alb/Glob ($r = -0.38$; $p < 0.001$), suggesting coordinated regulation among protein fractions during the periparturient physiological shift.

In turn, the Alb/Glob ratio exhibited a very strong negative correlation with globulin ($r = -0.73$; $p < 0.001$) and a moderate positive correlation with albumin ($r = 0.35$; $p < 0.001$), which is consistent with the composite nature of this indicator. These associations reflect alterations in plasma protein synthesis and distribution as a response to the metabolic challenges of calving and early lactation.

	Protein indicators					Energy indicators				Liver functionality		
	BUN	**TP**	**GLOB**	**A/G**	**GLU**	**TRG**	**VLDL**	**BHB**	**NEFA**	**ALB**	**CHOL**	**BILI**
BUN	1	-0.040 p = 0.405	-0.160 p = 0.004	0.260 p < 0.001	0.250 p < 0.001	0.030 p = 0.595	0.030 p < 0.001	-0.020 p = 0.768	0.040 p = 0.571	0.150 p = 0.005	-0.070 p = 0.168	0.030 p = 0.534
	TP	1	0.890 p < 0.001	-0.380 p < 0.001	0.180 p = 0.001	0.310 p = 0.004	0.310 p < 0.001	-0.010 p = 0.886	0.210 p = 0.007	0.710 p < 0.001	0.560 p < 0.001	0.040 p = 0.484
		GLOB	1	-0.730 p < 0.001	0.120 p = 0.320	0.160 p = 0.100	0.160 p = 0.003	-0.020 p = 0.717	-0.200 p = 0.012	0.320 p < 0.001	0.470 p < 0.001	-0.060 p = 0.268
			ALB/ GLOB	1	0.003 p = 0.957	0.090 p = 0.100	0.100 p < 0.001	0.030 p = 0.639	0.080 p = 0.318	0.350 p < 0.001	-0.180 p = 0.001	0.190 p = 0.006
				GLU	1	0.100 p = 0.872	0.001 p = 0.979	-0.270 p < 0.001	-0.020 p = 0.737	0.190 p = 0.007	-0.060 p = 0.279	0.003 p = 0.948
					TRG	1	0.990 p < 0.001	0.030 p = 0.691	0.004 p = 0.553	0.400 p < 0.001	0.160 p = 0.004	0.200 p = 0.595
						VLDL	1	0.010 p = 0.847	0.050 p = 0.518	0.410 p < 0.001	0.170 p = 0.004	0.200 p = 0.002
							BHB	1	0.000 p = 0.972	0.020 p = 0.726	0.190 p = 0.014	0.090 p = 0.248
								NEFA	1	-0.120 p = 0.128	-0.150 p = 0.060	0.100 p = 0.211
									ALB	1	0.410 p < 0.001	0.180 p = 0.001
										CHOL	1	0.030 p = 0.553
											BILI	1

CC < 0 (negative correlation) – statistically significant.

CC > 0.2 (positive correlation) – statistically significant.

Figure 3 - Correlations of protein, energy and liver function indicators.

Note: BUN = blood urea nitrogen; TP = total protein; GLOB = globulin; A/G = albumin - globulin ratio; GLU = glucose; TRG = triglycerides; VLDL = very low-density lipoprotein; BHB = beta-hydroxybutyrate; NEFA = non-esterified fatty acids; CHOL = cholesterol; BILI = bilirubin; CC = Correlation coefficient.

Energy indicators

Among the energy parameters, an almost perfect correlation was found between TG and VLDL ($r = 0.99$; $p < 0.001$), which is physiologically expected given the role of VLDL as the primary carriers of TG from the liver to peripheral tissues. This strong association indicates an active and sustained lipid mobilization during the postpartum period, coinciding with states of NEB.

A negative correlation was also identified between glucose and BHB ($r = -0.27$; $p < 0.001$), supporting the use of BHB as a biomarker for subclinical ketosis. The decrease in glucose associated with the increase in BHB reflects a compensatory use of ketone bodies as an alternative energy source in the context of limited glucose availability.

Although weaker, the correlations between total protein and glucose ($r = 0.18$; $p = 0.001$), and between total protein and VLDL/TG ($r = 0.31$; $p = 0.004$ and $p < 0.001$, respectively), suggest a connection between the energy status and the protein synthesis capacity in transition cows.

Hepatic function

Regarding hepatic function indicators, albumin showed significant correlations with cholesterol ($r = 0.41$; $p < 0.001$) and globulin ($r = 0.32$; $p < 0.001$), reflecting a link between hepatic functionality and protein homeostasis. Cholesterol also correlated with TG ($r = 0.16$; $p = 0.004$) and VLDL ($r = 0.17$; $p = 0.004$), suggesting a joint role of these lipids in the postpartum metabolic response.

In contrast, bilirubin showed low or no correlation with most of the evaluated biomarkers ($p > 0.05$), except for a slight positive correlation with Alb/Glob ($r = 0.19$; $p = 0.006$), suggesting that bilirubin levels remain relatively stable during this physiological period, or that their variability is not strongly influenced by the other metabolic systems assessed.

Correlation analyses (Figure 3) revealed a strong positive relationship between total protein and albumin ($r = 0.70$, $p < 0.001$), and a moderate positive correlation with globulins ($r = 0.32$, $p < 0.001$), indicating that increases in total protein correspond with rises in albumin, globulins, cholesterol, and VLDL.

Among energetic parameters, VLDL and TG were highly correlated (r = 0.99), and VLDL correlated positively with bilirubin (r = 0.20). BHB showed a negative correlation with glucose (r = -0.27) and a positive correlation with cholesterol (r = 0.19). No correlation was detected between BHB and NEFA. NEFA correlated negatively with cholesterol (r = -0.15), globulin (r = -0.20), albumin (r = -0.12), and total protein (r = -0.21), and positively with bilirubin (r = 0.10). No significant correlations were found between NEFA and TG, glucose, or BUN.

Discussion

Two nutritional strategies were evaluated during the transition period: grazing supplemented with commercial concentrate (CON) and grazing supplemented with corn silage and forage soybean (MIX). Although both diets were formulated to meet energy and protein requirements, differences in nutrient sources may influence ruminal fermentation patterns, energy availability, and the mobilization of body reserves during early lactation. Corn silage provides fermentable carbohydrates that increase propionate production in the rumen, a key precursor for hepatic gluconeogenesis, while forage soybean contributes metabolizable protein that supports hepatic metabolism and milk synthesis. In tropical grazing systems, where forage energy density is often limited, these nutritional strategies may modulate the degree of negative energy balance and metabolic adaptation during the transition period (Drackley, 1999; Bionaz et al., 2020).

The average THI indicated that both treatments were subjected to heat stress. This elevated THI compromises the productive capacity of the mammary gland by increasing energy expenditure for maintenance (Stefanska et al., 2024), which in turn affects energy metabolism, leading to weight and condition loss. Combined with peripartum homeostasis alterations (Sammad et al., 2022; Sun et al., 2025), this scenario can be detrimental; however, both variables remained within normal physiological limits as reported by Komisarek et al. (2025), demonstrating the resilience of dairy cows to tropical environments.

Early lactation imposes a high nutrient demand on the organism, and the typically low DMI during the prepartum period (Gross and Bruckmaier, 2019; Gross, 2022) promotes the onset of metabolic imbalances and postpartum weight loss. In this study, gradual weight loss was already evident in the prepartum period, which suggests that nutrient partitioning prioritized the mammary gland's requirements, mobilizing greater body reserves to counteract the NEB (Martens, 2020).

The slightly low glucose concentration found can be attributed to a physiological hormonal imbalance of the hypothalamic-pituitary-adrenal axis during early postpartum, due to adrenal cortex insufficiency. Additionally, sodium fluoride tubes were not used to allow for the simultaneous measurement of total protein, albumin, and liver enzymes in a single serum sample, thus avoiding interference from anticoagulants on these analytes. It is acknowledged that the use of non-anticoagulant collection tubes (red-top) may have allowed continued glycolysis by erythrocytes during serum separation, thereby reducing measurable glucose levels. This represents a potential methodological limitation, despite immediate conditioning, and that reported glucose levels may exhibit a slight deviation due to this field processing of samples. Nevertheless, glucose values remained within the lower normal limit according to Drackley (2023). The highest glucose concentrations in both treatments were observed at parturition, likely driven by the glucocorticoid-induced elevation of blood glucose.TG represent the primary energy reserve in adipose tissue. When energy is required, these reserves are mobilized to the liver as NEFA (Piantoni and VandeHaar, 2023), where they are typically oxidized for ATP production (Campos-Gaona et al., 2018). When this metabolic pathway is overwhelmed, NEFA are re-esterified to TG in the liver. This suggests that the CON treatment mobilized a larger amount of body reserves, leading to a gradual reduction in weight.

Lower TG concentrations observed postpartum may be explained by their uptake by the mammary gland (Ghaffari et al., 2024). TG accumulating in the liver are transported into the bloodstream as VLDL, which require adequate availability of apolipoproteins, phospholipids, and cholesterol for synthesis. The balanced formulation of both treatments likely ensured adequate nutrient supply for normal VLDL synthesis and secretion, as reflected in the mean VLDL values observed. Nevertheless, Bionaz et al. (2020) associated high VLDL levels with a positive energy balance, stimulating lipogenesis in adipose tissue.

NEFA reflect the degree of mobilization of body reserves into the bloodstream, representing an adaptive response to NEB (Tessari et al., 2020). The CON group mobilized more body reserves in response to mammary gland nutrient demand (Orquera-Arguero et al., 2024), as evidenced by significant weight loss and without a concomitant rise in BHB levels. Conversely, the MIX group showed lower NEFA mobilization and less body condition loss. According to Barletta et al. (2017).

The liver is responsible for albumin synthesis, while globulin production is primarily associated with immune system activity (Cattaneo et al., 2021). However, the normal functioning of both systems is closely linked to dietary protein intake. In this study, both diets were balanced for protein and energy, resulting in normal levels of total protein, albumin, and globulin.

Globulin concentrations may increase in response to infection and inflammation (Warken et al., 2018). Elevated blood globulin levels are therefore associated with greater immune activity due to inflammatory states. However, the globulin values obtained in this study remained within the lower normal limits proposed by Bertoni and Trevisi (2013).

The Alb/Glob ratio values found were close to 1, which is considered normal (Cattaneo et al., 2021). Low Alb/Glob suggests high blood globulin concentrations, commonly associated with inflammation (Thongrueang et al., 2023), whereas a high ratio indicates greater albumin availability, which is essential for the transport of fatty acids in the blood (Hutapea et al., 2023).

Albumin is considered a negative acute-phase protein in dairy cows and is therefore inversely associated with inflammation. Low albumin concentrations indicate higher inflammatory states. However, this study found optimal albumin values in both groups, as described by Roa-Vega et al. (2017), suggesting minimal inflammatory response in the animals evaluated.

Cholesterol is a key hepatic metabolite, serving as a precursor for bile salt synthesis and for TG mobilization. In this study, cholesterol levels decreased around parturition in both treatments, in agreement with previous reports attributing this decline to cholesterol redistribution toward the mammary gland. Notably, cholesterol concentrations increased progressively postpartum as feed intake recovered (Wagner et al., 2023).

Conclusion

Under tropical grazing conditions, both nutritional strategies evaluated during the transition period were able to maintain productive performance and metabolic stability in dairy cows. However, the diet supplemented with corn silage and forage soybean (MIX) showed a tendency to promote a more stable metabolic adaptation during early lactation, evidenced by lower mobilization of body reserves and a more moderate loss of weight and body condition.

These results suggest that the incorporation of locally available conserved forages can represent a viable nutritional strategy to support metabolic adaptation during the transition period in tropical dairy systems while reducing dependence on commercial concentrates.

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Authors´ contributions

The first draft of the manuscript was written by AF. Conceptualization: AFC, CB, JN. Methodology: AFC, CB. Formal analysis and investigation: AFC, CB, JN, SM, PMP. Visualization: AFC, PMP. Writing, review and editing: AFC, JN, PMP. AFC. Supervision: CB. All authors commented on previous versions of the manuscript, read and approved the final version.

Data availability statement

The data of this study will be shared upon reasonable request to the corresponding author.

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