

Biochemical Indicators In Dairy Cows Under Grazing And Grazing With Supplementation In Tropical Climate

Yadira Marcillo Alcívar¹, Ernesto Antonio Hurtado², Tommy Cueva Navia³

¹Admission and Leveling Unit, Escuela Superior Politécnica Agropecuaria de Manabí (Manuel Félix López), yjmarcillo@espm.edu.ec, <https://orcid.org/0009-0001-8497-5782>

²Veterinary Medicine School, Escuela Superior Politécnica Agropecuaria de Manabí (Manuel Félix López), ernesto.hurtado@espm.edu.ec, <https://orcid.org/0000-0003-2574-1289>

³Chemistry Laboratory of the Veterinary Medicine, Escuela Superior Politécnica Agropecuaria de Manabí (Manuel Félix López), tommy.cueva@espm.edu.ec, <https://orcid.org/0000-0001-5995-1730>

Abstract

Background: The main goal of this study was to examine the biochemical profiles of dairy cows that were fed two different diets: one was a diet of grass only, and the other was a diet of grass and corn stingray supplementation.

Methods: This study was conducted in tropical conditions to understand how these diets affected cholesterol, urea, glucose, creatinine, albumin, and plasma protein levels. The research was done on two farms in Manabí province. The study included 44 lactating cows (22 per farm). The research was descriptive and not experimental. Venipuncture blood samples were collected and analyzed by UV-visible spectrophotometry, processing the data with descriptive statistics and tests such as ANOVA, Student's t, and Kruskal-Wallis.

Results: The results showed that cholesterol levels stayed within a healthy range (80–150 mg/dl), with a slight increase in supplemented cows during the second third of lactation (166.42 mg/dl). Urea levels were much lower in animals that ate maize stingrays (1.20–1.35 mg/dl) than in animals that grazed exclusively (6.71–7.07 mg/dl). Glucose levels in the system reflected low blood sugar (hypoglycemia) with supplementation (17.65–43.99 mg/dl) and adequate levels in grazing (69.75–92.30 mg/dl). Creatinine levels stayed high in both systems (1.20–1.93 mg/dL), while albumin and plasma proteins remained within the normal range.

Conclusion: To sum it up, the type of diet had a direct impact on metabolism. Grazing exclusively was better for energy and protein balance than supplementation with corn stingray.

Keywords: Metabolic balance, blood biomarkers, nutritional management, protein-energy metabolism, tropical dairy systems.

INTRODUCTION

Pastures-based milk production systems encounter challenges in meeting the nutritional requirements of cows, particularly during periods of drought. Consequently, this predicament leads to the necessity of exploring alternative supplementation strategies [1]. Meeting these nutritional needs is the primary technological factor that can cause significant changes in the metabolic profile of animals [2].

Dairy cows are particularly susceptible to metabolic disorders, which have been observed to affect more than 50% of herds [3]. These alterations have the potential to compromise both productivity and reproductive efficiency, resulting in significant economic losses within production systems. The most prevalent associated diseases include hypocalcemia, gestosis, osteodystrophy, ketosis, obstetric paralysis, and alterations associated with microelement deficiencies [4,5]. Conversely, elevated milk production has been identified as a risk factor for subclinical ketosis, with a prevalence ranging from 57% to 61% during the 60 to 90 days postpartum [6].

Metabolic imbalances are more prevalent during the peripartum and early lactation periods, due to negative energy balance [7]. In this context, the evaluation of blood biochemical profiles enables the assessment of the animal's metabolic and nutritional status, thereby facilitating the identification of deficiencies that affect reproduction and productivity [8]. This tool is essential for monitoring homeostasis and correcting alterations that compromise the efficiency of the production system [9].

The analysis of the metabolic profile is a pivotal tool for the identification of the protein and energy status of animals from blood samples [10]. In dairy cows, parameters such as glucose and cholesterol

allow for the determination of the energy balance, which is essential for optimal productive performance [11]. Conversely, urea serves as an indicator of the relationship between protein and energy levels in the diet. Its deviations from the normal range can serve as a signal of nutritional imbalances that compromise feed efficiency [12]. Concentrations of plasma proteins and creatinine have been identified as significant indicators for detecting protein deficiencies, metabolic alterations, and potential liver or kidney pathologies [13].

These parameters are valuable for detecting subclinical metabolic disorders that can negatively affect milk production and fertility in dairy cows [14]. Laboratory analyses are imperative to support nutritional supplementation, whether applied tactically or strategically, and also assess animal response in pastoral systems, where measurement of consumption is difficult due to the possibility of animal selection and variation in pasture quality [15]. In light of this scenario, the following inquiry emerges: A study of the biochemical indicators of dairy cows in two distinct environments is imperative to ascertain the differences present. The first environment is that of dairy cows on pasture, and the second is that of dairy cows with supplementation in tropical climates.

The present study aims to evaluate the biochemical profile of dairy cows under two feeding systems: exclusive grazing and grazing with corn stingray supplementation in tropical conditions, to determine the influence on cholesterol, urea, glucose, creatinine, and plasma protein levels. This analysis will facilitate the establishment of useful biochemical reference parameters for monitoring the state of health and optimizing productive performance in tropical livestock systems. Consequently, the results obtained from the biochemical profile in dairy cows will be estimated according to lactation period, breed crosses, number of calvings, origin, body condition, and type of feed.

MATERIALS AND METHODS

Study Area

The development of this research was carried out in two cattle ranches in the province of Manabí, which correspond to the canton of Chone cattle ranch La Tostada located at coordinates 0°37'20.9"S 80°08'21.1"W and in the canton Flavio Alfaro cattle farm "VERZAM" with coordinates 0°12'20.0"S 79°51'33.6"W (Google Maps, 2024).

Experimental unit

The experimental unit consisted of 44 lactating cows, distributed equally among two farms (22 animals per establishment), selected under the criterion of being actively in production.

Type of research, approach and methods

It is a non-experimental and descriptive research, developed under a mixed approach that combines quantitative and qualitative analysis, thus allowing a broader and deeper understanding of the object of study.

Variables evaluated

The biochemical variables evaluated include cholesterol, urea, glucose, creatinine, albumin, and plasma proteins. Likewise, categorical variables such as breastfeeding period, number of births, origin, body condition, and type of diet will be considered.

Sample collection and processing

The selected stud farms were identified as Livestock 1 and Livestock 2. The first corresponded to the La Tostado farm, located in the city of Chone, where the diet is based on grazing complemented with tender corn Tartary. The second, called VERZAM, was in the Flavio Alfaro canton and its feeding system is limited exclusively to grazing.

Once the establishments were identified, the cows in the lactation stage were selected and individual data such as code, age, number of calvings, lactation period and milk production were recorded. Subsequently, blood was extracted by venipuncture in the coccygeal vein (Vacutainer®), after disinfection of the area, and the samples were stored in tubes with anticoagulant (EDTA), duly labeled. Finally, the tubes were placed in a rack and transferred in a MOR® cooler to the chemistry laboratory of the Veterinary Medicine career of the Higher Polytechnic Agricultural School of Manabí.

When the samples were received in the laboratory, the UV-visible spectrophotometry technique was applied, based on the absorption of ultraviolet radiation. As described by [14], tubes containing

1 mL of blood were centrifuged (Unico®, Spain) for 5 minutes at 3000–5000 rpm. Once the plasma was separated, 50 µl and 10 µl were extracted with micropipettes, to which 3.5 ml of the reagents for cholesterol, creatinine, urea, glucose, albumin and plasma proteins were added (Wiener lab® kit). For the blank control, 50 µl of sterile water was used. Finally, the samples were left to stand for 20 minutes at room temperature before reading.

The spectrophotometer (HANNA Instruments®, USA) was turned on for 15 minutes for calibration, before selecting absorbance at 540 nanometers (nm), according to cholesterol, creatinine, urea, glucose, and plasma protein reagents. Quartz cuvettes were used for measurements, and the equipment was set to zero using a sterile water target (50 µL). Subsequently, the absorbance of the standard was measured, and the plasma samples were analyzed with the corresponding reagents. The absorbed values obtained were recorded in the Wiener lab® table, which allowed the concentration of cholesterol, creatinine, urea, and glucose to be calculated. Albumin and plasma proteins according to the volume of standard solution used.

Statistical analysis

The results of the biochemical profiles were organized using descriptive statistics, with measures of central tendency and dispersion processed in Excel (2019 version). To verify the distribution of the data, the Shapiro-Wilk normality test was applied. Subsequently, in the inferential analysis, Student's t-test was used to compare the two farms and an analysis of variance (ANOVA) to evaluate the effect of the lactation period. In the case of variables such as the number of births and body condition, ANOVA was applied whenever the assumptions of normality and homogeneity were met; if it was not met, the non-parametric Kruskal-Wallis test was used, using the Infostat software (2020 student version).

RESULTS

Table 1 presents the analysis of biochemical indicators in dairy cows supplemented with corn stingray and grazing, where notable variations are evident throughout the different lactation periods. However, when considering statistical significance, it is observed that all p-values were greater than 0.05, which indicates that these variations did not reach significant differences. Consequently, the changes recorded could be attributed to chance and not necessarily to an actual effect of the breastfeeding period.

Regarding the metabolites evaluated, cholesterol remained within the physiological range in the first and third thirds but increased in the second (166.42 mg/dl). Urea and glucose remained well below baseline values in all periods, being more critical in the second third (urea: 1.20 mg/dl; glucose: 17.65 mg/dl). In contrast, albumin remained stable within normal values, and total plasma protein showed a slight increase in the third third (9.43 mg/dl). However, creatinine showed values above the reference physiological range in the three periods of lactation, with concentrations that progressively decreased from 1.93 mg/dl in the first third to 1.20 mg/dl in the third. This behavior suggests a higher-than-expected renal load, although with a tendency to stabilize as lactation progresses.

Table 1. Biochemical indicators in dairy cows under supplementation with corn stingray and grazing, in relation to the different lactation periods (Livestock 1).

VARIABLES	BREASTFEEDING PERIOD			REFERENCE VALUES	P- Value
	FIRST THIRD	SECOND THIRD	THIRD THIRD		
Cholesterol (mg/dl)	120.95 ±42.68	166.42 ± 32.97	130.65± 55.64	80-150 mg dl	0.1188
Urea (mg/dl)	1.35 ±0.15	1.20 ± 0.08	1.22 ± 0.15	24.5-50.3 mg dl	0.0608
Glucose (mg/dl)	43.99 ±46.50	17.65 ±3.03	24.26 ± 15.63	57-79 mg dl	0.2069
Creatinine (mg/dl)	1.93±0.76	1.60±0.53	1.20± 0.28	0.4-0.9 mg dl	0,0933
Albumin	3.21 ±0.52	3.32 ±0.38	3.36 ± 0.47	3.3-4.3 mg dl	0.7920

(mg/dl)					
Plasma Protein (mg/dl)	8.82 ± 0.31	8.55 ± 0.31	9.43, ±1.26	6.7-8.8 mg dl	0.1212

Table 2 shows the behavior of different biochemical indicators in grazing dairy cows, considering the three lactation periods. In general terms, there was no significant difference in any of the variables analyzed, since all p values were higher than 0.05. This indicates that, although certain numerical variations were observed between the lactation thirds, these were not statistically relevant, so the changes respond more to physiological fluctuations than to a marked effect of the period. In the case of cholesterol, the values remained within the physiological range (80–150 mg/dl) in all thirds of lactation, reflecting stable lipid metabolism. Urea, on the other hand, showed concentrations well below the reference interval (24.5–50.3 mg/dl), suggesting limited availability of degradable protein in the rumen and, consequently, restricted nitrogen metabolism.

As for glucose, levels increased progressively from the first to the third third, even exceeding the upper limit of the physiological range (57–79 mg/dl), a phenomenon that could be linked to metabolic adjustments typical of the productive stage and food management. Albumin remained within normal values (3.3–4.3 mg/dl) and showed a slight increase throughout lactation, reflecting adequate protein and liver status. Finally, total plasma protein presented values above the reference range (6.7–8.8 mg/dl), with averages between 9.1 and 9.5 mg/dl, which may be associated with physiological variations typical of the herd. For creatinine, the values recorded in three-thirds of lactation (1.41, 1.39, and 1.23 mg/dL) remained above the expected reference range (0.4–0.9 mg/dL). This finding reflects a possible overload in the protein and renal metabolism of cows, which could be associated with both diet and physiological effort in milk production.

Table 2. Biochemical indicators in grazing dairy cows and their relationship with the different lactation periods (Livestock 2).

VARIABLES	BREASTFEEDING PERIOD			REFERENCE VALUES	P-Value
	FIRST THIRD	SECOND THIRD	THIRD THIRD		
Cholesterol (mg/dl)	131.52 ± 38.53	135.24 ± 54.45	115.76 ± 52.73	80-150 mg dl	0.7132
Urea (mg/dl)	6.71 ± 0.29	6.83 ± 0.26	7.07 ± 0.26	24.5-50.3 mg dl	0.0619
Glucose (mg/dl)	69.75 ± 7.25	82.58 ± 17.11	92.30 ± 29.70	57-79 mg dl	0.2617
Creatinine (mg/dl)	1.41 ± 0.54	1.39 ± 0.66	1.23 ± 0.68	0.4-0.9 mg dl	0.0933
Albumin (mg/dl)	3.11 ± 0.31	3.38 ± 0.35	3.53 ± 0.37	3.3-4.3 mg dl	0.1633
Plasma Protein (mg/dl)	9.16 ± 0.74	9.21 ± 1.00	9.56 ± 0.54	6.7-8.8 mg dl	0.5806

In both farms, cholesterol levels remained within the physiological reference values (80–150 mg/dl), with the exception of an increase in the second third of lactation in the supplemented animals (166.42 mg/dl). This behavior could be associated with metabolic adjustments typical of the productive transition, coinciding with what was described by Takahashi et al. [16], who reported variations in lipoprotein fractions during early lactation. Despite this peak, in general the observed

values reflect an adequate lipid homeostasis, similar to that reported in dairy cows raised in tropical systems, where concentrations tend to remain stable and close to the physiological range [13,17]. Urea was one of the metabolites that showed contrasting results between the two farms evaluated. On the farm with maize supplementation, its levels remained very low (1.20–1.35 mg/dl), far from the reference range, which shows a protein deficiency and a low availability of degradable nitrogen in the rumen. This finding coincides with what was described by Quinteros et al. [8] and Ríos et al. [18], who associated reduced urea concentrations with diets with protein limitations. On the other hand, cows in exclusive grazing presented higher values (6.71–7.07 mg/dl), close to normal, in line with what was reported by Franco-Schafer et al. [19] in cattle from Panama. However, even in this livestock farm, the levels were below the reference range, which suggests similar restrictions in nitrogen intake. Overall, the results confirm that the availability of degradable protein in the diet is a key factor for protein metabolism and that both farms have limitations in this aspect.

The results show a contrasting behavior in glucose levels. In livestock farming with supplementation, the values were always below the physiological range (17.65–43.99 mg/dl), reflecting a marked energy deficit. On the other hand, in the exclusive grazing system, glucose rose progressively, reaching 92.30 mg/dl in the third third, exceeding the reference interval. This divergence could be explained by the quality and availability of forage, as well as by the absence of supplementation, as noted by Jordán et al. [11]. Barson et al. [20] warn that imbalances in glucose and urea affect reproductive efficiency, reinforcing the need for adequate nutritional control. In particular, the second third of breastfeeding showed the most severe hypoglycemia (17.65 mg/dl), a situation that according to Jiménez and Restrepo [21] increases the risk of postpartum ketosis. However, high values in grazing could be associated with better carbohydrate utilization or metabolic adaptations, as described by Andjelić et al. [22].

In both farms, creatinine values were above the physiological range (0.4–0.9 mg/dl). In supplemented cows they ranged from 1.20 to 1.93 mg/dl, while in grazing cows they ranged from 1.23 to 1.41 mg/dl. These elevated levels could be associated with renal overload or more intense protein metabolism, as noted by Franco-Schafer et al. [19] in dairy cattle. Nozad et al. [23] also link creatinine with protein and milk quality, highlighting its value as a metabolic indicator. The hypercreatinine observed may be related to greater muscle catabolism or renal alterations. Similarly, Coutinho et al. [24] reported increases linked to digestive disorders and dehydration. Although there were no significant differences in this study ($p > 0.09$), the slight decrease towards the third third suggests a possible metabolic adjustment or recovery in the animals.

In this study, both albumin and plasma proteins remained within physiological ranges, with no significant variations between production systems or between different lactation periods. This stability reflects adequate liver function and good protein status, in accordance with what was described by Filipejová and Kováčik [25], who highlight albumin as a sensitive indicator of nutritional status in cattle. On the other hand, total plasma protein presented values above the reference range in both farms (8.55–9.56 mg/dl), suggesting an active and constant protein metabolism. This finding coincides with what was pointed out by Cozzi et al. [26], who observed variations in plasma proteins linked to the lactation stage and productive conditions. Overall, the results show a favorable nutritional balance in the animals evaluated.

In summary, the two farms have biochemical profiles that, although within physiological parameters in some cases, reveal clear deficiencies in protein availability and alterations in energy metabolism. These conditions reflect the close relationship between feed management and the metabolic balance of dairy cows, as indicated by previous studies in different production systems [14,8,22].

Table 3 presents the results obtained in both farms with respect to biochemical indicators and their relationship with categorical variables. Together, the data provide a clear view of the energy and protein metabolism of dairy cows under different feeding schemes. A relevant finding was that neither age, body condition, nor number of births showed an association with biochemical indicators, since all comparisons yielded $p > 0.05$ values.

Table 3. Association of biochemical parameters with categorical variables (age, body condition and number of calvings) in the two farms evaluated

	Livestock 1	Livestock 2
--	-------------	-------------

VARIABLES	P- Value	P- Value
Cholesterol * Age	0,9935	0,6589
Cholesterol * Body Condition	0,9997	0,5517
Cholesterol* Number of births	0,9837	0,4179
Urea* Age	0,9314	0,9172
Urea* Body Condition	0,9496	0,7448
Urea* Number of births	0,9161	0,4400
Glucose* Age	0,9935	0,4913
Glucose* Body Condition	0,9997	0,1775
Glucose* Number of births	0,9837	0,5557
Creatinine* Age	0,3447	0,5456
Creatinine* Body Condition	0,1006	0,7015
Creatinine* Number of births	0,4978	0,2393
Albumin* Age	0,9785	0,0141
Albumin* Body Condition	0,9813	0,5442
Albumin* Number of calvings	0,9664	0,1899
Plasma Protein* Age	0,9368	0,7457
Plasma Protein* Body Condition	0,9352	0,6215
Plasma Protein* Number of farrowings	0,9087	0,5882

The results obtained are not completely aligned with what has been reported in the literature. For example, Ceballos et al. [14] describe that cholesterol dynamics are closely related to the body condition and energy balance of dairy cows; however, no such correspondence was observed in our research, suggesting that other factors specific to management or the feeding system could be modulating this metabolite. Similarly, when evaluating Brown Swiss cows in Puno, no significant variations in total protein or albumin was found with respect to the number of calvings, findings that differ from those obtained in this work. These differences with previous studies highlight the influence of the production environment and the type of system evaluated, which highlights the need to continue exploring how nutritional and management factors affect the metabolic profile of cattle.

The findings demonstrated that cholesterol levels remained unaffected by factors such as age, body condition, and the number of births. This conclusion was substantiated by the analysis of p values, which ranged from 0.6589 to 0.9935, thus indicating that the data were not significantly influenced by these variables. This finding aligns with the observations reported by González et al. [27], who have previously argued that variations in this metabolite are predominantly influenced by dietary and energy balance factors, rather than by individual characteristics such as age or reproductive history. Conversely, urea exhibited no substantial associations in any of the evaluated scenarios ($p > 0.44$), which aligns with the findings reported by Campos et al. [13]. These researchers posited that the concentrations of urea are predominantly influenced by the availability of degradable

protein in the rumen and the synchronization with fermentable energy, rather than by the physiological characteristics of the animal.

A salient finding was noted in Livestock 2, wherein albumin exhibited a substantial correlation with age ($p = 0.0141$). This finding indicates that variations in hepatic protein synthesis capacity may be present in both young and older animals, which is consistent with the observations reported by Arias-Islas et al. [28]. They identified albumin as a sensitive marker of nutritional status and liver function. Conversely, total plasma protein exhibited no substantial associations ($p > 0.58$), thereby reinforcing the notion that this marker predominantly reflects the overall equilibrium between protein intake and metabolism, independent of individual physiological attributes.

The results of the study indicate that most of the biochemical parameters evaluated remained stable and were not influenced by factors such as age, body condition, or number of calvings of the cows. The investigation revealed a correlation between albumin and age exclusively in Livestock 2, underscoring the significance of acknowledging albumin as a sensitive biomarker in metabolic studies. These findings align with research emphasizing the predominant influence of diet and productive management on biochemical profiles, superseding the individual factors of the animal [13,27,29].

Table 3 presents a comparison of biochemical indicators in cattle from two management systems, contrasting their values with the reference ranges. In the case of cholesterol, both groups exhibited normal values and did not demonstrate significant differences, suggesting that the type of management did not markedly affect this lipid parameter. In addition, albumin and plasma protein levels remained within normal physiological ranges, with no evidence of significant variations between the systems. This suggests that there are adequate protein and nutritional status in both cases.

The most significant results were observed in the urea and glucose levels. In the context of livestock farming with supplementation, urea values were found to be significantly lower than the reference values. Conversely, in the grazing system, urea values were found to be within the normal range, exhibiting a highly significant difference between the two groups ($p < 0.0001$). This finding suggests the potential for supplementation to modify protein metabolism, thereby reducing the concentration of nitrogenous products in the blood. With respect to glucose, supplemented cattle exhibited levels significantly below the reference range, while grazing animals demonstrated levels within the expected range, exhibiting a substantial difference ($p < 0.0001$). It is conceivable that this circumstance is associated with the nature and accessibility of energy from the diet in each system.

Table 3. Comparison test of biochemical indicators in cattle according to management system

Biochemical indicators	Livestock 1 (Grazing + supplementation)	Livestock 2 (Grazing)	Reference values	P-Value
Cholesterol	140.13±46.21	126.59 ±49.88	80-150 mg dl	>0.3557
Urea	1.26±014	6.91±0.29	24.5-50.3 mg dl	<0.0001
Glucose	29.03±30.38	84.22±22.89	57-79 mg dl	<0.0001
Creatinine	1.61 ±0.63	1.33±0.64	0.4-0.9 mg dl	>0.1387
Albumin	3.29±0.44	3.39±0.37	3.3-4.3 mg dl	>0.4009
Plasma Protein	8.89±0.81	9.34±0.78	6.7-8.8 mg dl	>0.0649

The results show that the type of feeding (maize supplementation versus grazing exclusively) was a determining factor in the metabolic profile of the cows. Urea and glucose were revealed as the most sensitive metabolites in the food system, presenting highly significant differences ($p < 0.0001$). While maize supplementation failed to adequately stabilize nitrogen and energy metabolism, grazing alone favored a more efficient metabolic balance. These findings coincide with what was pointed out by Ceballos et al. [14], who highlight that diet and forage quality are determinants in glucose and cholesterol variations, and with Burgos-Castro et al. [30], who observed that supplementation with nutritional nuclei did not produce significant effects on blood metabolites under grazing conditions. attributing this response to the characteristics of the system and energy availability.

In both management systems, cholesterol values were within the physiological reference range (80–150 mg/dl), with no statistically significant differences ($p > 0.35$). These findings are consistent with those described by Andjelić et al. [22], who point out that cholesterol variations are usually more associated with energy status and lipid metabolism during breastfeeding than with the type of management. Thus, the observed stability suggests that both grazing and supplementation ensured sufficient energy intake to maintain adequate lipid metabolism.

The most evident contrast was observed in urea: Livestock 1 presented extremely low values (1.26 mg/dl), while in Livestock 2 they reached 6.91 mg/dl, both well below the expected physiological range (24.5–50.3 mg/dl), although with a significant difference ($p < 0.0001$). This deficit reflects a low availability of degradable protein in the rumen or an inadequate protein-energy balance, as pointed out by Roa-Vega et al. [31], who report that supplementation with legumes and yeasts tends to raise blood urea levels by improving rumen fermentation. Likewise, Barrios et al. [32] propose that serum urea is a good indicator of protein status, so low levels confirm nutritional deficiencies in the herds studied.

Glucose showed highly significant differences ($p < 0.0001$). Grazing animals (Livestock 2) reached 84.22 mg/dl, slightly above the reference range (57–79 mg/dl), while in Livestock 1 the values were much lower (29.03 mg/dl). These contrasts reflect effects of the feeding system: according to Coroian et al. [2], glucose tends to decrease under conditions of negative energy balance, particularly in early stages of lactation or under deficient diets. The marked hypoglycemia in the supplemented group could be due to an imbalance between energy provided and productive demand, which suggests inadequate nutritional management despite the use of supplements.

Creatinine values ranged from 1.33 to 1.61 mg/dL, exceeding the physiological reference range (0.4–0.9 mg/dL), but without significant differences ($p > 0.13$). Hypercreatinine is often associated with increased muscle catabolism or variations in kidney function. Studies in Holstein cows have shown that creatinine tends to increase in animals with high production or subjected to metabolic stress [33]. Although no differences were found between managements, both systems could be inducing some protein overload or physiological stress.

Albumin remained within the reference values in both systems, with no significant differences ($p > 0.40$), indicating adequate protein and liver status. Regarding total plasma protein, slightly higher than the reference range (8.89–9.34 g/dl vs. 6.7–8.8 g/dl) was observed, especially in Livestock 2, although without reaching statistical significance ($p = 0.0649$). These results are consistent with what was pointed out by Stefańska et al. [34], who relate the increase in plasma proteins with the mobilization of reserves and metabolic adaptation during lactation.

Similarly, these findings are aligned with what was described by Marini and Di Masso [35], who highlight that pastoral systems, although limited, can sustain a more balanced metabolism if the quality of the forage is adequate. Overall, the indicators most sensitive to management variations were urea, glucose, and creatinine, which confirms their usefulness as biomarkers to monitor the metabolic and nutritional status of the herd, as proposed by Quinteros et al. [8] and Ceballos et al. [14].

CONCLUSIONS

The study demonstrated that the metabolic profile of dairy cows is influenced by feeding conditions, with urea and glucose exhibiting the greatest sensitivity to the nature of management. However, supplementation with baby corn stingray did not adequately balance protein and energy metabolism. In contrast, grazing alone resulted in a more stable and efficient balance. The findings of this study serve to reinforce the notion that grazing, when managed in a manner that ensures the availability of high-quality forage, has the capacity to promote a state of enhanced metabolic function and productivity in herds.

Author contributions statement: Y.M., E.A., T.C. designed the study. Y.M., E.A., T.C. collected data. Y.M., E.A., T.C. curated and analyzed the dataset. Y.M., E.A., T.C. wrote the first version of the manuscript. T.C. supervised the project. Y.M., E.A., arranged funding. All authors read, reviewed and approved the final version of the manuscript.

Acknowledgement: The authors would like to express their gratitude to all the participants and the authorities of the Escuela Superior Politécnica Agropecuaria de Manabí, who allowed this investigation to take place.

Conflicts of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding: The research was conducted independently by the researchers, and no funding was received.

REFERENCES

1. Pons M, Adrien M, Mattiauda D, et al. Bienestar de vacas lecheras en sistemas de alimentación mixta bajo dos condiciones diferentes de confinamiento: Indicadores comportamentales, bioquímicos y fisiológicos. *Cienc Apl Comport Anim.* 2023;265:105995. doi:10.1016/j.applanim.2023.105995
2. Coroian CO, Mireşan V, Coroian A, et al. Biochemical and haematological blood parameters at different stages of lactation in cows. *Bull UASVM Anim Sci Biotechnol.* 2017;74(1):32-36. doi:10.15835/buasvmcn-asb:12283
3. Batista C, Castro S, Correa H, et al. Relation between liver lipid content and plasma biochemical indicators in dairy cows. *Acta Sci Vet.* 2020;48:1720. doi:10.22456/1679-9216.100806
4. Ferreira MF, Rennó LN, Detmann E, et al. Rendimiento, respuestas metabólicas y hormonales de vacas Nellore en pastoreo a una suplementación de energía y proteína durante la fase preparto. *BMC Vet Res.* 2020;16:108. doi:10.1186/s12917-020-02317-7
5. Ventsova I, Safonov V. Biochemical criteria for the development mechanisms of various reproduction disorders in dairy cows. *Biodiversitas.* 2021;22(1):135. doi:10.13057/biodiv/d221135
6. Redrovan Passato DC, García Flores JM, Durán Aguillón LE. Identificación de factores de riesgo para la presentación de cetosis subclínica en ganado bovino lechero. *Ecuador Calidad: Rev Cient Ecuat.* 2020;7(1):43-52. doi:10.36331/revista.v7i1.101
7. Chavarria Rivera JR, García Guerrero F, Treminio Alcántara B, Reyes-Sánchez N. Cetosis subclínica como trastorno metabólico en vacas lecheras durante las etapas productivas de periparto, lactancia temprana y lactancia media. *La Calera.* 2023;23(41):154-160. doi:10.5377/calera.v23i41.17267
8. Quinteros Pozo OR, Vargas Burgos JC, Barbosa I, Marini PR. Indicadores metabólicos sanguíneos de genotipos lecheros en pastoreo en la provincia de Napo, Ecuador. *La Granja. Rev Cienc Vida.* 2017;26(2):101-115. doi:10.17163/lgr.n26.2017.10
9. López-García G, Góngora-Orjuela A, Díaz-González F, Rojas-Rodríguez A. Evaluación del estado metabólico, el peso vivo y la condición corporal durante la gestación en vacas de carne en la Altillanura colombiana. *Orinoquia.* 2019;23(2):13-24. doi:10.22579/20112629.565
10. Nascimento JC, Silva TGP, Rizzo H, et al. Indicadores bioquímicos e corporais para avaliação do perfil metabólico e nutricional em ruminantes. *Ciênc Vet Tróp.* 2016;19(3):75-84.
11. Jordán D, Villa NA, Gutiérrez M, et al. Indicadores bioquímicos sanguíneos en ganado de lidia mantenido en pastoreo en la cordillera central colombiana. *Rev Colomb Cienc Pecu.* 2016;19(1):18-26. doi:10.17533/udea.rccp.324027
12. Pedraza C, Mansilla A, Merucci F, Pinedo P, Contreras H. Niveles de urea láctea en vacas de la Región del Bío-Bío, Chile. *Agric Téc.* 2006;66(3):264-270. doi:10.4067/S0365-28072006000300005
13. Campos R, Cubillos C, Rodas ÁG. Indicadores metabólicos en razas lecheras especializadas en condiciones tropicales en Colombia. *Acta Agronómica.* 2007;56(2):77-84.
14. Ceballos A, Gómez PM, Vélez ML, Villa NA, López LF. Variación de los indicadores bioquímicos del balance de energía según el estado productivo en bovinos lecheros de Manizales, Colombia. *Rev Colomb Cienc Pecu.* 2002;15(1):13-25.
15. Torres JC, Lara JE, Viana A, et al. Evaluación de indicadores metabólicos en novillas Braford recruzadas durante el invierno en dos sistemas de producción: Pastoral y silvopastoril. *Arch Latinoam Prod Anim.* 2024;32(4):179-189. doi:10.53588/alpha.320403
16. Takahashi T, Mori A, Oda H, et al. Comparison of cholesterol levels among lipoprotein fractions separated by anion-exchange high-performance liquid chromatography in periparturient Holstein-Friesian dairy cows. *J Vet Med Sci.* 2021;83(2):260-266. doi:10.1292/jvms.20-0361
17. Mokhber Dezfouli M, Eftekhari Z, Sadeghian S, Bahounar A, Jeloudari M. Evaluation of hematological and biochemical profiles in dairy cows with left displacement of the abomasum. *Comp Clin Pathol.* 2013;22(2):175-179. doi:10.1007/s00580-011-1382-5
18. Ríos C, Marín MP, Catafau M, Wittwer F. Concentraciones sanguíneas de β -hidroxibutirato, NEFA, colesterol y urea en cabras lecheras de tres rebaños con sistemas intensivos de producción y su relación con el balance nutricional. *Arch Med Vet.* 2006;38(1):19-23. doi:10.4067/S0301-732X2006000100003
19. Franco-Schafer S, Rangel-Tapia G, Jaén-Torrijos M. Parámetros hematológicos y bioquímicos en bovinos de leche y cría en Panamá. *Cienc Agropecuaria.* 2022;35(13-29).
20. Barson RK, Padder S, Sayam ASM, et al. Serum glucose, urea nitrogen, cholesterol, and total proteins in crossbred repeat breeder and normally cyclic cows. *J Adv Vet Anim Res.* 2019;6(1):82-85. doi:10.5455/javar.2019.f316
21. Jiménez AF, Restrepo GJ. Indicadores metabólicos del estatus nutricional en vacas lecheras. Presented at: Seminario "Fundamentos bioquímicos de los trastornos metabólicos"; 2017; Medellín, Colombia.
22. Andjelić B, Djoković R, Cincović M, et al. Relationship between biochemical parameters of milk and blood and metabolic status in dairy cows during lactation. *Metabolites.* 2022;12(8):733. doi:10.3390/metabo12080733

23. Nozad S, Ramin AG, Moghadam G, et al. Relationship between blood urea, protein, creatinine, triglycerides and macro-mineral concentrations with the quality and quantity of milk in dairy Holstein cows. *Vet Res Forum*. 2012;3(1):55-59.
24. Coutinho LT, Mendonça CL, Soares GSL, et al. Biochemical indicators of dairy cows affected by fermentative digestive disorders. *Res Soc Dev*. 2022;11(5):e5071158459. doi:10.33448/rsd-v11i5.28459
25. Filipejová T, Kováčik J. Evaluation of selected biochemical parameters in blood plasma, urine and milk of dairy cows during the lactation period. *Slovak J Anim Sci*. 2009;42(Suppl. 1):8-12.
26. Cozzi G, Ravarotto L, Gottardo F, et al. Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. *J Dairy Sci*. 2011;94(8):3895-3901.
27. González FD, Muiño R, Pereira V, Campos R, Benedito JL. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. *J Vet Sci*. 2017;18(4):429-437. doi:10.4142/jvs.2017.18.4.429
28. Arias-Islas E, Morales-Barrera J, Prado-Rebolledo O, García-Casillas A. Metabolismo en rumiantes y su asociación con analitos bioquímicos sanguíneos. *Abanico Veterinario*. 2020;10:1-15. doi:10.21929/abavet2020.15
29. Contreras GA, Strieder-Barboza C, De Koster J. Symposium review: Modulating adipose tissue lipolysis and remodeling to improve immune function during the transition period and early lactation of dairy cows. *J Dairy Sci*. 2019;102(3):2783-2795. doi:10.3168/jds.2018-15340
30. Burgos-Castro D, Rojas-Bourrillón A, Campos-Granados CM. Suplementación con un núcleo nutricional y su efecto sobre variables productivas y metabólicas en vacas Jersey. *Nutr Anim Trop*. 2023;17(2):24-61. doi:10.15517/nat.v17i2.56511
31. Roa-Vega ML, Ladino-Romero EA, Hernández-Martínez MC. Indicadores de bioquímica sanguínea en bovinos suplementados con *Cratylia argentea* y *Saccharomyces cerevisiae*. *Pastos Forrajes*. 2017;40(2):121-134.
32. Barrios M, Sandoval E, Sanchez D, et al. Valores de referencia de diferentes parámetros bioquímicos en vacunos mestizos de doble propósito del Valle de Aroa, estado Yaracuy. *Mundo Pecuario*. 2013;9(1):25-30.
33. Teneva A, Hristov K, Stoimenov G. Blood biochemical profiles of dairy cows and their calves from Bulgarian Black and White cattle breed. *Bulg J Agric Sci*. 2020;26(4):890-893.
34. Stefańska B, Nowak W, Pruszyńska-Oszmałek E, et al. The effect of body condition score on the biochemical blood indices and reproductive performance of dairy cows. *Ann Anim Sci*. 2016;16(1):129-143. doi:10.1515/aoas-2015-0064
35. Marini PR, Di Masso RJ. Evaluación histórica de indicadores productivos en vacas lecheras en sistemas a pastoreo. *La Granja. Rev Cienc Vida*. 2018;28(2):102-114. doi:10.17163/lgr.n28.2018.08