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Association between metabolic and immunological changes during the transition period of dual-purpose cows in the Veracruz tropic, Mexico

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Abstract

The objective of this study was to identify changes and associations in body condition score (BCS), serum glucose and β-hydroxybutyrate (BHB) concentrations, and white blood cell populations in dual-purpose cows during the transition period in the Veracruz tropic. BCS was evaluated and blood samples were taken weekly to determine white blood cell populations, serum glucose and BHB concentrations of 30 multiparous dual-cows (*Bos taurus* × *Bos indicus*) from 3 weeks before the expected date of calving to 3 weeks postpartum. During the prepartum period, BCS (3.56 vs 3.11 points), leukocyte (8.964 vs 7.032×10^3 cells μL^{-1}), neutrophils (3.353 vs 2.201×10^3 cells μL^{-1}), lymphocytes (4.750 vs 4.051×10^3 cells μL^{-1}), and monocytes populations (222 vs 126×10^3 cells μL^{-1}) were higher compared to the postpartum period. Contrarily, BHB concentration was higher in the postpartum period (1.34 vs 0.84 mmol L^{-1}) than in the prepartum period. No differences in basophils and eosinophils populations and glucose concentration were identified. Associations between BCS, BHB, glucose, and populations of neutrophils, monocytes, and basophils were detected. The higher BCS, the higher the monocyte population (r = 0.22). The lower the glucose concentration, the higher the BHB concentration (r = −0.51). The higher the concentration of BHB, the lower the number of neutrophils (r = −0.22), monocytes (r = −0.32) and basophils (r = −0.23). In conclusion, low-producing dual-purpose cows experienced fluctuations in BCS, BHB, and immune cell populations during the transition period, suggesting similar metabolic and immune changes as in high-producing dairy cows.

Keywords: energy, immunosuppression, metabolism, peripartum, white blood cells

1 Introduction

The peripartum period in dairy cows is defined as the transition from the non-lactating pregnant to the lactating non-pregnant state (Goff & Horst, 1997). During this period, dairy cows undergo several metabolic, hormonal, inflammatory, immunological, nutritional, and even behavioural adaptations. These changes occur in a chain reaction through several physiological processes that begin three weeks before and conclude three weeks after calving (Drackley, 1999). During this period, one of the most occurring events is the mobilisation of adipose tissue to obtain energy to meet the nutritional demands of maintenance and milk production (Ingvartsen & Moyes, 2015). However, this mobilisation of the body reserves results in the increase of β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) concentrations, which have been shown to have undesirable effects on the organism of the dairy cow (LeBlanc, 2020). Similarly, another of the most important changes during the transition is the reduction in the populations and activity of immune cells involved in both the innate and adaptive immune responses (Ingvartsen & Moyes, 2015; LeBlanc, 2020). The exacerbation of one or more of these peripartum associated metabolic and immunological factors may compromise the success of lactation (Drackley, 1999).

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It is noteworthy that in countries with tropical and subtropical ecosystems, most of the bovine population is raised in grazing systems (Yamamoto *et al*., 2007). The main type of cattle is double-purpose with two main income sources: [1] sales of milk yield, and [2] sales of calves after weaning for beef production (Ramírez-Rivera *et al*., 2019). Dual-purpose cattle predominate in the tropics due to their rusticity, adaptation to climatic conditions, and the lower capital investment and technical support required compared to specialized dairy production systems (Rojo-Rubio *et al*., 2009).

Although the transition period has been widely studied in dairy cows, there is a lack of information related to this period in dual-purpose cows. Therefore, the aim of this study was [1] to identify changes in immune cell populations, body condition score (BCS), and glucose and BHB concentrations, as well as [2] to identify associations between these variables over the transition period of dual-purpose cows.

2 Materials and methods

This study gathered data from previous experiments including Lammoglia *et al*. (2019). This study was performed on a farm located in the northern area of Veracruz state, Mexico from October 2018 to September 2019. The farm is located in a tropical region with an average annual temperature of 24.9 ºC, relative humidity of 80 % and annual rainfall of 1,241 mm. The average herd milk production was 16.11 ± 3.71 kg/cow/day. The multiparous cows (n= 30; *Bos taurus* × *Bos indicus*) were managed under an intensive rotation system of native tropical grasses, Brizanta (*Brachiaria brizantha*) and African Star (*Cynodon plectostachyus*). Additionally, the cows had access to orange pulp silage (10 ± 1.5 kg/cow/day), mineral salts and fresh water. The cows had a dry period of 65 ± 10 days prior to their expected calving date. Dry cows were separated from lactating cows. Calving was assisted by farm staff when required. The cows were followed up to 21 days postpartum to monitor their health.

Two blood samples were retrieved from the plexus from the base of the tail through a double-needle system on a weekly basis, starting from 3 weeks before the expected date of calving until 3 weeks postpartum. The first sample was collected in a tube without anticoagulant while the second sample was stored in an EDTA tube (4 ml, BD Vacutainer®). Blood samples were stored in an isothermal container at 4 ºC until the samples were processed in the laboratory.

Determination of body condition, glucose and β-hydroxybutyrate

At each farm visit, BCS was assessed using the scale of 1 to 5 established by Ferguson *et al*. (1994), where a BCS of 1 corresponds to a lean cow and a BCS of 5 corresponds to an obese cow. Serum glucose and BHB concentrations were evaluated immediately after collecting the blood sample in the tube without anticoagulant. A cytometer (FreeStyle®, Optium Neo™ Abbott) was used to calculate the BHB concentration (mmol L^{-1}), while the glucose concentration (mg/dL) was measured with a glucometer (OneTouch®, UltraMini™ LifeScan Inc).

Determination of white blood cells

In the laboratory, blood was extracted from the EDTA tubes using the Thoma pipette up to the 0.5 mark, then filling with Turk's solution up to the 11 mark. Solutions were homogenized for 3 minutes. At the end, the first three drops were discarded, and the fourth drop was inserted between the Neubauer chamber and a coverslip. After one minute, the leukocytes were counted in the four quadrants of the Neubauer chamber. The number of leukocytes per microlitre (µL) is obtained using the following formula:

$$\text{leukocytes } (\mu L^{-1}) = \frac{\text{cells in 4 squares}}{4} \times 10 \times 20$$

A blood smear was stained following the indications of a commercial rapid blood staining kit (Hycel®). Subsequently, the smears were observed under a microscope using the 100× objective in immersion oil. The different types of white blood cells (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were counted with a cell counter (ICB-Counter®) until reaching 100 cells. The population of white blood cells per µL was obtained with the following formula:

$$\text{cell type } (\mu L^{-1}) = \frac{(\text{\% cell type}) \times (\text{leukocytes } \mu L^{-1})}{100}$$

Statistical analysis

Data were analysed with the STATISTICA v.10.0 software (StatSoft Inc). Continuous data (cell populations, serum glucose and BHB concentrations, and BCS) per week were analysed with the One-way ANOVA model. Differences of Least Square Means (LSM) between weeks were analysed using Fisher's LSD method.

A Pearson correlation analysis of continuous variables was performed to establish the correlation coefficient between two variables in the prepartum, postpartum, and transition periods. Variables with a significant correlation were analysed following the linear regression model. Statistical significance was characterized by $p < 0.05$.

3 Results

Cows had a higher ($p < 0.05$) BCS during the prepartum period compared to the postpartum period (Fig. 1A; Table 1). Serum glucose concentration during the first postpartum week (42 mg dL^{-1}) was higher ($p < 0.05$) than in the third postpartum week (33 mg dL^{-1}), however, these two weeks were similar to the rest of the weeks during the entire transition period (Figure 1B), with no differences between the prepartum and postpartum periods (Table 1). Serum BHB concentration during the third postpartum week (1.66 mmol L^{-1}) was like the second postpartum week (1.43 mmol L^{-1}), but higher ($p < 0.05$) at weeks −3, −2, −1 and 1 relative to calving, averaging 0.88 mmol L^{-1}. Additionally, a difference ($p < 0.05$) was detected between the second week before (0.71 mmol L^{-1}) and the second after calving (Fig. 1C). When comparing both periods, a higher ($p < 0.05$) concentration of BHB was identified during postpartum compared to the prepartum period (Table 1). A decrease ($p < 0.05$) in the leukocytes, neutrophils, monocytes and lymphocytes population was detected during the transition period (Fig. 2A-D), while the population of eosinophils (Fig. 2E) and basophils (Fig. 2F) remained constant over the experimental weeks. The leukocyte population in the second (7.090×10^3 cells μL^{-1}) and third (6.512×10^3 cells μL^{-1}) postpartum weeks were lower ($p < 0.05$) than in the third week prior to parturition (9.475×10^3 cells μL^{-1}), and the number of leukocytes in the third postpartum week was different ($p < 0.05$) compared to the number of leukocytes in all weeks of the prepartum period. No differences were observed between -2, -1, 1 and 2 weeks relative to parturition (Fig. 2A). Similarly, compared to the weeks of the prepartum period, the second (2.228×10^3 cells μL^{-1}) and third (2.020×10^3 cells μL^{-1}) postpartum weeks had lower ($p < 0.05$) numbers of neutrophil cells. The third (3.611×10^3 cells μL^{-1}) and second (3.578×10^3 cells μL^{-1})

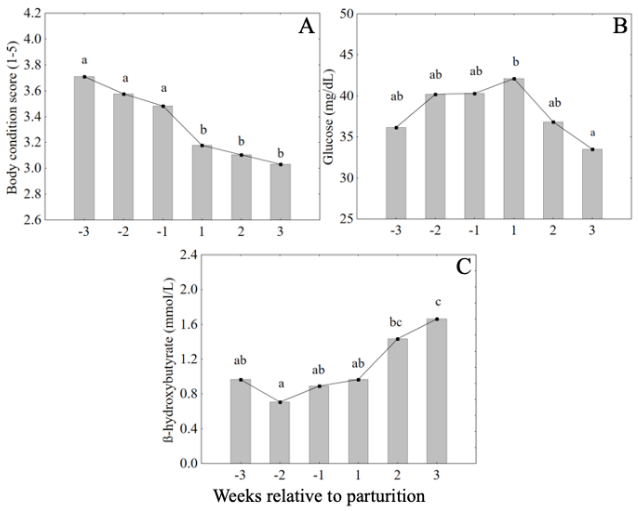

Fig. 1: *Changes in [A] body condition score and concentrations of [B] glucose and [C] β-hydroxybutyrate during the transition period in dual-purpose cows [Values with different superscripts ([a,b,c]) differ p < 0.05].*

Table 1: *Changes in body condition score (BCS), glucose, β-hydroxybutyrate (BHB) and white blood cell populations during the prepartum and postpartum periods in dual-purpose cows (LSM ± SEM).*

	Period		
Variable	*Prepartum*	*Postpartum*	*P*
BCS (1-5)	3.56 ± 0.07^a	3.11 ± 0.04^b	<0.01
Glucose (mg dL^{-1})	39.41 ± 2.14	37.66 ± 1.29	0.48
BHB (mmol L^{-1})	0.84 ± 0.17^a	1.34 ± 0.10^b	0.01
Leukocytes*	8.964 ± 479^a	7.032 ± 291^b	<0.01
Neutrophils*	3.353 ± 202^a	2.201 ± 123^b	<0.01
Lymphocytes*	4.750 ± 202^a	4.051 ± 123^b	<0.01
Monocytes*	222 ± 18^a	126 ± 11^b	<0.01
Eosinophils*	612 ± 105	564 ± 64	0.69
Basophiles*	103 ± 14	73 ± 9	0.07

* in 10^3 cells μL^{-1}. Values within a row with different superscripts ([a,b]) differ $p < 0.05$.

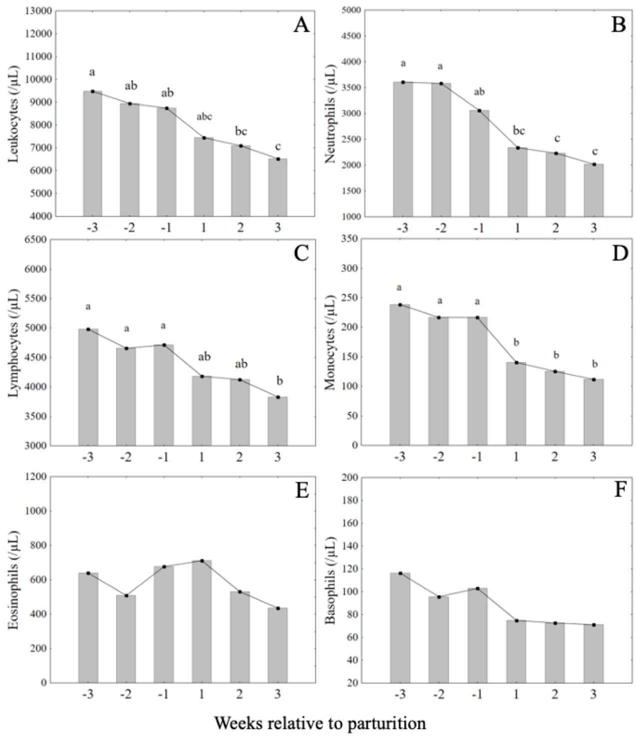

Fig. 2: *Changes in the population of [A] leukocytes, [B] neutrophils, [C] lymphocytes, [D] monocytes, [E] eosinophils, and [F] basophils during the transition period in dual-purpose cows [Values with different superscripts ([a,b,c]) differ p < 0.05].*

Table 2: *Correlation analysis between body condition score (BCS), glucose, β-hydroxybutyrate (BHB) and immune cell populations during the prepartum, postpartum, and entire transition period of dual-purpose cows.*

	Period								
	Prepartum			Postpartum			Transition		
Variable	*BCS*	*Glucose*	*BHB*	*BCS*	*Glucose*	*BHB*	*BCS*	*Glucose*	*BHB*
Glucose	−0.05			0.12			0.1		
BHB	0.23	−0.24		−0.06	−0.55 *		−0.12	−0.51 *	
Leukocytes	−0.03	−0.2	−0.27	−0.05	−0.06	−0.03	0.12	−0.05	−0.12
Lymphocytes	−0.07	−0.27	−0.14	−0.01	−0.15	0.13	0.12	−0.14	0.03
Monocytes	−0.02	0.1	−0.39 *	0.05	−0.01	−0.23 *	0.22*	0.03	−0.32 *
Eosinophils	0.02	−0.21	0.06	−0.09	0.08	−0.13	−0.04	0.04	−0.11
Basophils	0.13	−0.12	−0.23	−0.06	0.17	−0.2	0.08	0.11	−0.23 *
Neutrophils	−0.04	−0.08	−0.28	−0.09	−0.03	−0.11	0.15	−0.01	−0.22 *

Correlations with * are significant ($p < 0.05$).

week prior to parturition had a higher ($p < 0.05$) number of neutrophils compared to all weeks of the postpartum period (Fig. 2B). The largest difference in the lymphocyte population was detected when comparing all weeks of the prepartum period with the third postpartum week (3.830×10^3 cells μL^{-1}; Fig. 2C). The monocyte population at all postpartum weeks was lower ($p < 0.05$) compared to all prepartum weeks (Fig. 2D; Table 1).

The correlation between serum BHB concentration and the monocyte population was significant ($p < 0.05$) during the prepartum, postpartum, and entire transition period, while The correlation between BHB concentration and glucose was significant ($p < 0.05$) during the postpartum and transition period. During the entire transition period, BCS and serum BHB concentration were significantly ($p < 0.05$) associated with the monocyte population and the neutrophil and basophil population, respectively (Table 2). All mentioned correlations were negative, except the association between BCS and monocyte population which was positive.

4 Discussion

The transition period is very important for the productive cycle and has been widely studied in dairy cattle, while there is lack of studies for dual-purpose cattle due to several factors, including poor or no technology application, such as data management software, genetic selection and reproductive biotechnologies (Galina & Geffroy, 2023).

In our study, leukocyte, neutrophil, lymphocyte, and monocyte populations of dual-purpose cows decreased from three weeks prior to calving until three weeks after calving. By the third week postpartum, there was no indication of recovery, which could indicate a longer period of immunosuppression compared to dairy cows, which is also supported by Lammoglia *et al.* (2021) who reported a decrease in leukocyte and neutrophil populations in dual-purpose cows up to week 9 postpartum. On the contrary, various studies in the Holstein breed under intensive production systems indicated that immune cell populations reached their lowest level between the second and third postpartum week, recovering their normal levels between the third and fourth week after calving (Moyes *et al.*, 2014; Trimboli *et al.*, 2019). This peripartum immunosuppression is considered physiological due to its association with glucocorticoids released during the parturition process, among other factors related to energy metabolism such as BCS, NEFA, BHB and glucose availability (Ingvartsen and Moyes, 2013). Two of the main cells affected by this immunosuppression are neutrophils and lymphocytes. Neutrophils are considered the first line of defence against pathogenic microorganisms and inflammatory processes (Paape *et al.*, 2003), while lymphocytes are the cells responsible for producing immunoglobulins and other immunological factors (Sordillo *et al.*, 1997). Accordingly, a reduction in the number and function of neutrophils (chemotaxis, phagocytosis and oxidative burst; Schukken *et al.*, 2011) and lymphocytes cells (Ingvartsen & Moyes, 2013) was observed during the period around parturition. However, the recovery of both cells may differ. While lymphocytes can recover after one to two weeks postpartum, neutrophils take longer to recover (Martinez *et al.*, 2012).

A common phenomenon in dairy cows during the peripartum period is the loss of BCS. In our study, there was a BCS reduction from 3 weeks before calving until the end of the sampling period. This is in agreement with a study by Lammoglia *et al.* (2021) who reported that dual-purpose cows under grazing conditions suffered a reduction in BCS from one week prior to calving to the ninth week postpartum. Similar results were obtained in Holstein and Simmental cows kept in a confinement system under a silage and concentrate diet (Knob *et al.*, 2021). An opposite pattern was observed for BHB levels which increased from the second week before calving until the end of the postpartum sampling period of our study. Although the serum glucose reached its maximum level in the first week postpartum, it remained below the optimal reference level of 40 mg/dL (Ruoff *et al.*, 2017) throughout the entire transition period. Similar changes in BHB and glucose have also been identified in dairy cattle between the prepartum and postpartum periods (Martinez *et al.*, 2012; Zarrin *et al.*, 2016; Gärtner *et al.*, 2019; Knob *et al.*, 2021).

The interaction between BHB and glucose in dual-purpose cows from our study suggests a process similar to dairy cows (Zarrin *et al.*, 2016). The peripartum period is a key timelapse where the cows undergo metabolic changes associated with the mobilisation of body energy reserves since glucose has been identified as the main source of energy for immune cells and other cell types (Ingvartsen and Moyes, 2013). The transition from a non-lactating to a lactating status requires large amounts of energy. When nutritional demands are not covered, a process of adipose tissue mobilisation is initiated. This lipomobilisation elevates the serum NEFA and consequently BHB concentrations. At this moment, the dairy cow enters a negative energy balance (NEB) state characterized mainly by a metabolic and oxidative imbalance (Drackley, 1999). In this sense, low BCS, high concentrations of BHB and low glucose levels (among other factors) have been identified as indicators of NEB (Han van der Kolk *et al.*, 2017). Absalón-Medina *et al.* (2012) showed that dual-purpose cows experiment an energy deficit during their dry period, especially in the last trimester of gestation, due to the poor nutritional quality of some tropical grasses and management factors. So, taking our results into consideration, we suggest that dual-purpose cows undergo NEB and therefore experience immunosuppression even more exacerbated, probably due to specific factors related to the production system.

Ketone bodies, but mainly BHB, have been shown to have a negative impact on the proliferation and immune response of neutrophils and lymphocytes. As previously described, neutrophil chemotaxis and phagocytosis, as well as lymphocyte blastogenesis are reduced in the presence of high levels of BHB (Grinberg *et al.*, 2008). This is because immune cells depend exclusively on glucose to perform their functions (migration, chemotaxis, phagocytosis, etc.), and therefore cannot resort to ketone bodies as an alternative source of energy (Ingvartsen & Moyes, 2013). Accordingly, we observed fewer neutrophil cells at higher levels of BHB. The same interaction has been reported in cattle specialized in milk production (Ingvartsen and Moyes, 2013; LeBlanc, 2020). By contrast, there was no association between BHB and lymphocytes. Our study found associations between indicators of energy metabolism (BCS and BHB) with the monocyte population. A higher BCS indicated a higher number of monocytes. While a higher concentration of BHB was related to a lower number of monocytes in circulation. Macrophages are tissue cells that originate from monocytes. When monocytes leave the circulation to infiltrate toward different tissues, a differentiation process is initiated according to the target tissue. As previously discussed, during the transition period, a large proportion of dairy cows mobilize adipose tissue reserves due to a lack of energy. There is evidence that the inflammatory processes caused by this lipomobilisation, as well as the necrosis of the adipocytes themselves, work as chemotactic factors for macrophages, which are the main immune cells of adipose tissue (Ampem *et al.*, 2016). There is evidence that a greater number of macrophages have been found in the adipose tissue of those cows that lost a greater BCS during the postpartum period (De Koster *et al.*, 2018; Newman *et al.*, 2018). It has been found that higher levels of BHB in those cows with a greater BCS loss were associated with a 93 % increase of macrophages in adipose tissue extending up to the fourth postpartum week compared to the average during prepartum (Newman *et al.*, 2018). This may explain why cows with lower BCS and higher BHB concentration during the transition period had a lower number of circulating monocytes. Perhaps, a greater proportion of circulating monocytes migrated to adipose tissue as macrophages, attracted by the inflammatory process induced by lipomobilisation.

5 Conclusion

Dual-purpose cows of this study experienced changes in energy metabolism indicators and immune cell populations during the transition period. It was possible to identify a reduction in BCS and leukocytes, neutrophils, lymphocytes and monocytes, as well as an increase in BHB levels from the prepartum to the postpartum period. The variations and correlations between immune response and metabolic indicators suggest a peripartum immunosuppression and negative energy balance status that are similar to dairy cows but exacerbated due to factors related to the dual-purpose system.

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Conflict of interest

The authors declare no conflict of interest.

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