Relationship between the resumption of postpartum ovarian activity and the metabolic profile of Holstein cows at high altitude in Ecuador

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Abstract

The resumption of postpartum ovarian activity (RPOA) is influenced by the cow's metabolic profile, though the relationship between RPOA and the metabolic profile of dairy cows at high altitude in the Andean Region of Ecuador is still unclear. The goal of this study was to determine the relationship between RPOA and the metabolic profile of Holstein cows at high altitude in the Andean Region of Ecuador. Thirty cows were selected and their RPOA and metabolic profile at 30 days postpartum were evaluated. According to the RPOA, the metabolic indicators were compared using the t-Student test for independent samples. A simple linear correlation was run between the main indicators of the RPOA and the metabolic parameters. An observational analytical study was performed to establish the association between RPOA and metabolic profile. Holstein caws showing an early RPOA had lower β-Hydroxybutyrate and blood urea nitrogen ($P < 0.05$), and higher phosphate, copper, and zinc concentrations ($P < 0.05$) concentrations in blood serum as compared to Holstein cows with late RPOA. Increased blood β-hydroxybutyrate and low phosphate, copper and zinc were risk factors ($P < 0.05$) for late postpartum RPOA. In addition, the follow parameters were correlated ($P < 0.05$): appearance of the dominant follicle and zinc levels, follicle diameter and copper, occurrence of ovulation and blood β-hydroxybutyrate level, the occurrence of ovulation and phosphate , luteal activity and cholesterol, corpus luteum volume and copper, blood serum progesterone and cholesterol, and blood serum progesterone and copper levels. There was a strong correlation ($P < 0.01$) between the occurrence of ovulation and copper levels, luteal activity and blood β-hydroxybutyrate levels, and copper and zinc levels. These findings show that RPOA was related to the metabolic profile at 30 days postpartum, especially with protein excesses and energy, and phosphate, copper and zinc deficiencies.

Key words: *Ovarian reactivation; Dominant follicle; Ovulation; Luteal activity; β-hydroxybutyrate*

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Introduction

The resumption of postpartum ovarian activity (RPOA) implies the establishment of the functionality of the hypothalamus-pituitary-ovary-uterus axis and should occur in early postpartum (as quickly as possible). Its duration is influenced by the cow's metabolic state (Guáqueta et al., 2014; Folnožić et al., 2015), uterine health (Boudelal and Adnane, 2022) and parity (Folnožić et al., 2016).

Pituitary and ovarian functions are affected by dietary protein status (deficit or excess) and energy deficiency. A dietary energy deficit is the main limiting factor of cow reproduction, especially in the gestation-lactation transition period, which occurs three weeks before and three weeks after calving (Fiore et al., 2018; Butler, 2019; Turk et al., 2020).

A negative energy balance (NEB) in cows, causes disturbances in the function of the hypothalamus-pituitary-ovary axis, affecting the secretion, amplitude and frequency of GnRH and LH pulses, which can cause reproductive problems (Turk et al., 2020; Ulloa et al., 2020). The body condition score reflects the energy balance of animals and at calving time showed an effect on the duration of RPOA in dairy cows from the El Carchi province, Ecuador (Balarezo-Urresta et al., 2020).

Mineral deficiencies are associated with reproductive problems (Folnožić et al., 2019; García-Díaz et al., 2020), especially in cattle herds in tropical regions (García-Díaz et al., 2010b, 2020). However, under these conditions, parenteral copper (Cu) supplementation (García-Díaz et al., 2012), parenteral Cu, zinc (Zn) and manganese (Mn) supplementation (Noval-Artiles et al., 2016), or energetic-mineral supplementation (Balarezo-Urresta et al., 2021) during the transition period (gestation-lactation) showed favourable effects on the mineral profile and energy metabolism in cows. Reproductive activity was improved, reducing the intervals of calving-open days (COI) and the calving-calving interval (CCI), and increasing the birth rate.

In addition, the metabolic profile of bovines allows for an estimation of their nutritional status, and thus knowing the presence of subclinical alterations that affect the reproduction and production of these animals allows for the application of corrective measures (Fiore et al., 2018).

Dairy cattle herds in the province of Carchi, Andean region of Ecuador, showed a lower reproductive efficiency, caused by nutritional and metabolic alterations (Balarezo-Urresta et al., 2016). Under these conditions, the influence of body condition score at calving on RPOA was studied (Balarezo-Urresta et al., 2020). However, that study lacked information about the relationship between RPOA and the metabolic profile of dairy cows.

The goal of this study was to determine the relationship between RPOA and metabolic profile of Holstein cows at high altitude in the Andean region of Ecuador.

Materials and methods

The study was carried out from August 2015 to August 2016, in the El Carchi province, Ecuador, at an altitude of 2,990 to 3,450 meters (coordinates 1º12′3″N and 78º33′12″ W).

In the study area, the average air temperature fluctuates between 6 and 11°C (minimum 2ºC and maximum 15ºC), with average rainfall in the rainy season of 1,000 to 1,250 mm (RyS; October to April), and 700 to 850 mm in the dry season (DyS; May to September) (Balarezo-Urresta *et al.*, 2020). The soil is of the Andisol order, with an effective depth of 70 cm. The relief is undulating with a slope percentage between 10 and 20% (Balarezo-Urresta et al., 2020).

The farm uses pasture irrigation from rivers and urea fertilization (200 to 300 kg of N_2 ha^{-1}year^{-1}). In the herds, the rationed grazing system was used by means of an electric fence, with a global load of 2.5 UGM ha^{-1}. Grazing was carried out for 18 hours a day on *Lolium perenne* L., *Dactylus glomeratta* L., *Trifolium repens* L., *Pennisetum clandestinum* L. and *Holcus lannatus* L. No mineral supplements, energy or protein concentrates were administered.

Milking was manual twice a day, the first between 4 and 7 a.m. and the second between 3 and 6 p.m. Artificial calf rearing was used from the third day of birth. The herds had productive levels of 15 to 18 L/cow^{-1} day^{-1}.

Animals

All procedures followed the recommendations of the ARRIVE Guidelines (Kilkenny et al., 2010) and were approved by the Ethical Committee of the Scientific Council of the Faculty of Agricultural Sciences, Universidad Central "Marta Abreu" de Las Villas (agreement 12/2015).

In total, 30 Holstein cows from the Andean region of Ecuador were used, between the second and fourth lactation, aged between 4 to 8 years, between the second and fourth calving, the last one eutocic, with body condition score at calving between 3.0 and 4.0 points (5-point scale). The cows had no pathologies in their genital tract, no clinical signs of disease and were not receiving any medical treatment. The animals underwent palpation analysis of organs and systems, deworming and vaccinations according to the schedule of the area. RPOA and its relationship with metabolic profile 30 days after delivery were assessed.

Blood sampling

Blood was drawn by coccygeal venipuncture with vacutainer tubes, after cleaning and disinfecting the area with 70% alcohol. For haematology analysis, 5 mL blood was drawn into in vacutainer tubes impregnated with sodium EDTA (1 mg/mL blood) and stored at 4°C until analysis. For biochemical indicators, 10 mL blood was drawn into similar tubes but without an anticoagulant to enable spontaneous retraction of the clot. After 24 hours, the tubes were centrifuged at 2,500 *g* for 10 min to obtain the blood serum, which was frozen at -10°C until analysis.

Determination of haematochemical parameters

Haematochemical indicators were determined in a Star Fax 3300 equipment (Aznar Diagnóstica, USA), using commercial kits, according to the manufacturer's procedures (Table 1). All analyses were performed in the Diagnostic Laboratory of the Veterinary Clinic "Carlos Martínez Hoyos", University of Nariño, Colombia.

RPOA evaluation

Ultrasonography was performed twice a week using a CTS-800 ultrasound machine (SIUI, China), with a 5 MHz linear transducer, which offers a good relationship between image quality and depth up to 11 cm (Quintela et al., 2012). The resumption of ovarian activity was determined when the dominant follicle (DF) appeared, with a diameter ≥ 5 mm; this was monitored by ultrasound until atresia or ovulation, or progesterone (P_4) values were > 1 ng/mL (Salas-Razo et al., 2011).

Cows were classified according to the time of postpartum RPOA, using days to first ovulation (27.76 ± 7.73 days), which was used as the cut-off point to group the animals into two categories: cows with early ovarian reactivation, i.e., those that

Table 1. Haematochemical indicators assessed

Profile	Parameter	Method
Mineral	Calcium, Magnesium, Copper, Zinc	Atomic Absorption Spectrophotometry (AAS)
	Phosphorus	Phosphomolybdate. Ultra Violet (UV)
Haematology	Haemoglobin	Automated haematology module
	Haematocrit	
	Total Erythrocyte Count	
Haemochemical	Total proteins	Biuret. Colorimetric
	Albumin	Bromocresol green. colorimetric
	Globulins	Total Protein-Albumin Difference
	Cholesterol	CHOD-POD. Enzymatic Colorimetric
	Blood urea N2 (BUN)	Urease-GLDH. UV Kinetic
	Triglycerides	GPO-POD. Enzymatic Colorimetric
	β-hydroxybutyrate (β-OH)	RANBUT D-3. enzyme kinetic
Hepatic	Alanine aminotransferase (ALAT)	UV Kinetic, according to the International Federation of Clinical Chemistry (IFCC)
	Alkaline phosphatase	
	Aspartate aminotransferase	

ovulated within the first 35 days, and those with late ovarian reactivation, i.e., later than 35 days (Guáqueta et al., 2014).

The time of the first ovulation was defined as the disappearance of the preovulatory follicle from the last wave of follicular development and the subsequent appearance of the corpus luteum (CL) (Walsh et al., 2007), as well as at values of $P_4 > 1$ ng/mL (Salas-Razo et al., 2011).

The onset of luteal activity was determined when two consecutive samples were obtained, 7 days apart, with blood serum P_4 concentrations > 1 ng/mL (Hannan et al., 2010).

DF diameter and corpus luteum volume were calculated by the ultrasound scanner, which provides these measurements automatically, measuring from a single frozen image when the maximum apparent area was viewed.

Progesterone concentrations in blood serum were measured from 7 to 120 days postpartum (PPD). For these analyses, blood was drawn according to the procedures described above and determined using the Electrochemiluminescence technique, on a Cobas e 411 analyzer (Roche, Germany), according to the manufacturer's instructions. The methodology has high analytical sensitivity (CV < 10%) and a wide detection range (0 to 6 ng/mL), using 10 to 50 μL blood serum.

Statistical procedure

Metabolic profile parameters at early and late RPOA were compared using a t-Student test for independent samples. A multivariate analysis of correlations between the main RPOA parameters and those of the postpartum metabolic profile was performed. In these processes, the statistical package Statgraphics Centurion (Ver. XV.II) was used.

A cross-sectional observational analytical study was carried out to establish the association between RPOA and metabolic activity at 30 DPP. Measurements of frequency, impact and statistical significance were obtained using the EPIDAT 3.1 statistical package. The prevalence ratio (PR) indicates how many times more likely the animals exposed to each of the factors are to present late RPOA, the prevalence in exposed denotes the percentage of cows with delayed RPOA within each of the metabolic and nutritional disorders due to their exposure to them, and the prevalence in unexposed indicates the percentage of

Table 2. Comparison of metabolic parameters ($\bar{x}$ ± SE), according to the time of postpartum resumption of ovarian activity

Parameters	postpartum resumption of ovarian activity		*P* Value
	Early (*n*=18)	Late (*n*=12)	
Haemoglobin (g/L)	143.63 ± 3.06 [a]	133.38 ± 3.75[b]	0.0437
Total Erythrocyte Count (10^{12}/L)	7.58 ±0.24[a]	7.41 ± 0.30[a]	0.6611
Haematocrit (L/L)	0.43 ± 0.08[a]	0.42 ± 0.01[a]	0.4397
Total proteins (g/L)	96.23 ± 1.55[a]	93.66 ± 1.84[a]	0.6611
Albumin (g/L)	26.75 ± 0.74[a]	26.76 ± 0.90[a]	0.9925
Globulins (g/L)	69.46 ± 1.50[a]	66.90 ± 1.84[a]	0.2899
Cholesterol (mmol/L)	3.69 ± 0.11[a]	3.25 ± 0.13[b]	0.0215
Triglycerides (mmol/L)	1.57 ± 0.10[a]	1.24 ± 0.13 [b]	0.0494
β-hydroxybutyrate (mmol/L)	0.58 ± 0.06[a]	0.97 ± 0.08[b]	0.0011
BUN (mmol/L)	4.58 ± 0.32 [a]	5.82 ± 0.39[b]	0.0219
Creatinine (mmol/L)	117.40 ± 7.54[a]	123.53 ± 9.24[a]	0.6114
Alanine aminotransferase (UI/L)	30.12 ± 1.71[a]	31.51 ± 2.09 [a]	0.6105
Aspartate aminotransferase (UI/L)	85.66 ± 2.35[a]	84.70 ± 2.89[a]	0.7986
Alkaline phosphatase (UI/L)	105.87 ± 12.37[a]	112.38 ± 15.10[a]	0.7412
Calcium (mmol/L)	2.02 ± 0.08[a]	1.95 ± 0.09 [a]	0.5860
Phosphorus (mmol/L)	1.80 ± 0.04[a]	1.64 ± 0.06[b]	0.0396
Magnesium (mmol/L)	1.25 ± 0.05[a]	1.12 ± 0.07[a]	0.1823
Copper (μmol/L)	12.05 ± 0.30[a]	10.78 ± 0.37[b]	0.0139
Zinc (μmol/L)	12.47 ± 0.32[a]	11.50 ± 0.39[b]	0.0476

[ab] different letters in superscripts within the same row indicate statistical differences according to the Student's t-test for independent samples (P<0.05).

cows with prolonged RPOA, without exposure to metabolic or nutritional disorders.

Results

Haemoglobin, cholesterol, triglycerides, P, Cu and Zn had higher concentrations in blood serum (P<0.05) in Holstein cows with early RPOA, than in Holstein cows with late RPOA (Table 2). Serum concentrations of β-OH > 1 mmol/L or Cu < 11.77 µmol/L or P < 1.61 µmol/L or Zn < 12.62 µmol/L (Table 3 and Figure 1) constituted risk factors (P < 0.05) for the cows with late RPOA.

In addition, the prevalence in exposed and prevalence in unexposed (Figure 1) represent the magnitude by which late RPOA could be reduced if these exposure factors are eliminated, and can serve as a basis for implementing zootechnical

Table 3. Risk factors related to metabolic and nutritional alterations at 30 days postpartum for the late resumption of ovarian activity

Factor	PR	CI-95 %	χ^2	*P*- Value	PE	PNE
High BUN (> 4 mmol/L)	2.00	0.67-5.90	1.87	0.1709	0.50	0.25
High β-OH (> 1 mmol/L)	3.26	1.40-7.57	7.64	0.0057	0.77	0.23
Low Cu (< 11.77 µmol/L)	2.18	1.17-5.40	3.45	0.0531	0.62	0.28
Low P (< 1.61 mmol/L)	3.45	1.34-8.86	7.75	0.0054	0.72	0.21
Low Zn (< 12.62 µmol/L)	2.00	1.06-5.24	2.22	0.0046	0.66	0.38

PR: Prevalence ratio; χ^2: Chi square; **CI**: confidence interval; **PE**: Prevalence in exposed; **PNE**: Prevalence in unexposed; **BUN**: blood urea N_2; **β-OH**: β-hydroxybutyrate

PE: Prevalence in exposed; **PNE**: Prevalence in unexposed; **PR**: Prevalence ratio; **BUN**: blood urea N_2; **β-OH**: β-hydroxybutyrate

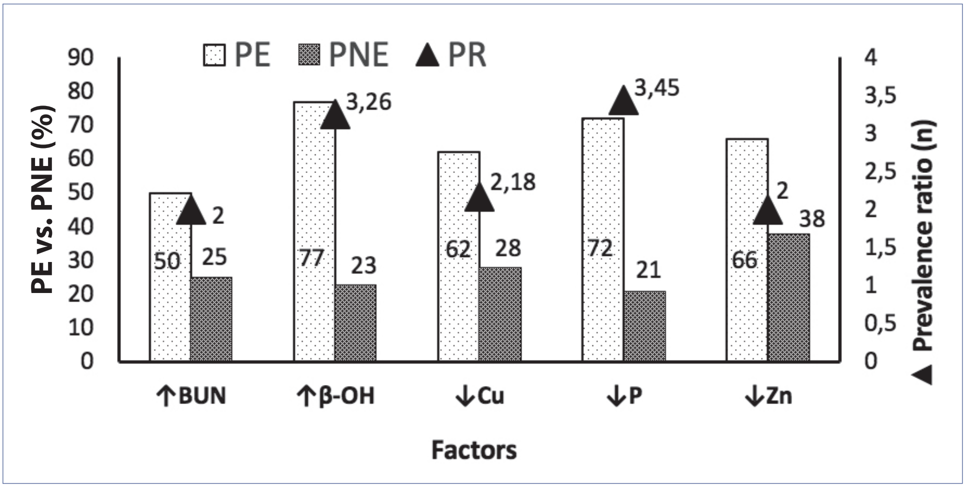

Figure 1. Risk factors related to metabolic and nutritional alterations at 30 days postpartum for late restart of ovarian activity

Table 4. Correlation coefficients between the RPOA parameters and metabolic parameters at 30 days postpartum

RPOA parameters	Metabolic parameters					
	β-OH	Cholesterol	BUN	P	Cu	Zn
Dominant follicle appearance (d)	0.282	-0.288	0.053	-0.112	-0.353*	-0.408*
Dominant follicle diameter (mm)	-0.077	-0.021	0.151	-0.043	0.325*	0.081
Occurrence of ovulation (d)	0.348*	-0.279	0.070	-0.3565*	-0.453**	-0.315
Luteal activity (d)	0.457**	-0.379*	0.184	-0.079	-0.427**	-0.433**
Corpus luteum volume (cm^3)	-0.057	-0.025	0.079	-0.022	0.354*	0.200
Serum progesterone (ng/mL)	-0.189	0.420*	0.080	-0.065	0.366*	-0.178

β-OH: β-hydroxybutyrate; **BUN**: blood urea N_2; * $P< 0.05$; ** $P< 0.01$

measures to prevent delayed RPOA and postpartum anoestrus.

The time of appearance of DF was correlated ($P<0.05$) with zinc and copper levels; while DF diameter was correlated ($P<0.05$) with copper levels. However, the time of occurrence of ovulation was correlated positively with β-OH ($P<0.05$) and negatively with P ($P<0.05$) and Cu ($P<0.01$), while luteal activity was correlated positively with β-OH ($P<0.05$) and negatively with cholesterol ($P<0.05$), Cu ($P<0.01$) and Zn ($P<0.01$). The corpus luteum volume only showed a correlation with Cu ($P < 0.05$), and blood serum P_4 concentration was correlated ($P<0.05$) with cholesterol and Cu (Table 4).

According to the correlation coefficients (Table 4), cows with an earlier RPOA showed earlier postpartum luteal activity, lower concentrations of β-OH and BUN, and higher concentrations of cholesterol, Cu and Zn in blood serum (Table 2) in comparison cows with a later RPOA.

Discussion

The higher haemoglobin levels in cows with earlier RPOA (Table 2) can be attributed to the higher copper levels in the blood (Table 2), since Cu improves ceruplasmin activity, which facilitates the absorption and transport of iron (Fe) as the central atom of haemoglobin (Feng-Li et al., 2011).

On the other hand, the increase in β-OH ($P < 0.05$) in the blood serum of cows with late RPOA (Table 2) may be due to the poorer body condition score at calving in these cows, which caused a higher negative energy balace (NEB) during the gestation-lactation transition period, which triggered lipid mobilisation and oxidation, accelerated loss of the body condition score and fertility problems (Fiore et al., 2018; Chacha et al., 2022).

However, the main cause of BUN increasing may be a consequence of the high levels of crude protein and energy deficiencies in the animal diet (Balarezo-Urresta et al., 2016), which is also by the NEB of the animals (Butler, 2019). The main effect of increased BUN on fertility is to reduce the uterus pH, affecting both semen and embryo survival (Butler, 2013; Folnožić et al., 2019).

In addition, the higher BUN in the blood serum of cows with late RPOA could be due to energy deficit and excess crude protein in the diet of herds. In these circumstances, BUN could increase because the ability of ruminal microorganisms to use NH_3 is saturated, which reaches the liver via blood and this organ converts it into urea, so an NH_3 excess leads to a high BUN concentration (Butler, 2013; Turk et al., 2016).

Consequently, hypocholesterolaemia may be due to the mobilisation and use of fat by the cow at the end of pregnancy and early lactation to compensate for the energy deficit (Butler, 2019), which is higher in cows with late RPOA and a deeper NEB and, therefore, greater mobilisation and lipid oxidation with energetic purposes, as suggested the β-OH concentrations (Table 2).

Despite the differing serum concentrations of BUN (*P*<0.05) between animals according to RPOA (Table 2), concentrations > 4 mmol/L were not a significant risk factor for the presentation of the late postpartum RPOA. In contrast, the difference in Zn (*P*<0.05) between animals according to RPOA (Table 2), i.e., Zn < 12.62 μmol/L at 30 DPP, were associated (*P*<0.05) with the presentation of late RPOA (Table 3 and Figure 1). These results confirm the usefulness of observational analytical studies to determine the impact of an exposure factor (Ajakaiye et al., 2011).

On the other hand, cows with higher blood serum cholesterol had higher blood serum P_4, which can be explained by the importance of cholesterol in the synthesis of this hormone, by a mechanism that culminates in the enzymatic conversion of the lipid metabolite into P_4 (Butler, 2013; Đuričić et al., 2020). Besides, cholesterol is necessary for the synthesis and transport of FSH, LH, E_2 and P_4, thus its deficit causes lower blood and tissue concentrations of these hormones and then low reproductive efficiency (Small et al., 1997; Quintela et al., 2011). These results corroborate that the NEB affects the development of ovarian structures and the synthesis of hormones (Ulloa et al., 2020). However, CL development and P_4 synthesis requires, in addition to cholesterol, energy, protein (Butler, 2013) and minerals (especially Cu and Zn) (Yatoo et al., 2013; Đuričić et al., 2020).

In the current study, the blood concentrations of Cu and P were higher in cows with early RPOA (Table 2). In addition, in cows with higher Cu levels, the dominant follicle had a larger diameter, ovulation occurred in less time postpartum, and the CL had a higher volume. The greater development of the latter explains why higher concentrations of P_4 were diagnosed in these cows. These results confirmed the importance of these minerals in animal reproduction (García-Díaz et al., 2012, 2020).

Moreover, the serum concentrations of cholesterol, P, Cu and Zn were affected in cows within the gestation-lactation transition period, showing that this stage is critical for the dairy cow and its deficiencies affect cow reproductive performance. These mineral deficiencies were diagnosed previously in cows under grazing conditions apparently fed an adequate mineral content but displaying reproductive issues (García-Díaz et al., 2012; Noval-Artiles et al., 2016; Balarezo-Urresta et al., 2020).

As is known, Cu modulates and modifies the stability of GnRH granules and the release of neurohormones; therefore, supplemented Cu complexes in combination with GnRH cause an efficient release of LH and FSH. Copper deficiency causes inhibition in the synthesis of these hormones, non-ovulation, poor CL development and insufficient production of P_4 (Michaluk and Kochman, 2007).

In addition, Cu participates in energy generation because it is an integral part of cytochrome-c-oxidase (CCO), the terminal enzyme of the respiratory chain, and catalyses the transfer of four electrons to O_2 to form two molecules of water and ATP (Gebhard et al., 2001). Also, through Lysyl oxidase (LOX), it coordinates the growth and development of the follicles (Harlow et al., 2003). Therefore, copper deficiency impairs preovulatory follicle growth and ovulation (Kendall et al., 2003). This is particularly the case at Cu <11.77 μmol/L, as the risk of presenting anoestrus and repetition of service in dairy cows is increased, with a negative correlation between Cu levels (9.8 ± 1.0 to 14.0 ± 0.9 μmol/L) and reproductive indexes (García-Díaz et al., 2010a).

Zinc is another important microelement for reproduction since it is involved in the animal's energy metabolism (Evans and Lawrenson, 2017), luteal function (Hackbart et al., 2010) and in the production of cholesterol, oestrogens (E_2) and P_4 (Small et al., 1997). The results obtained in the current study are in line with those obtained by García-Díaz et al. (2020). They showed that zinc deficiency is a significant relative risk factor for the presentation of anoestrus at levels less than 15 μmol/L (RR 1.74, P=0.007), with an even greater impact at Zn < 14 μmol/L (RR 2.79, P<0.001).

In addition, Zn plays an important role in the metabolism of carbohydrates, proteins, lipids, DNA, vitamin A and especially arachidonic acid, and the synthesis of $PgF_{2\alpha}$. Its deficiency causes an energy deficit and disorders of the functions of the hypothalamic-pituitary-ovarian axis, inhibiting the secretion and the amplitude and frequency of LH pulses. Together with the alteration of the prostaglandin $PgF_{2\alpha}$ production directly affects the life of the CL and the production of P_4 by the luteal cells (Hackbart et al., 2010).

Zn is also involved in the reorganisation of ovarian follicles through the enzyme metalloproteinase-2 (MMP-2), for which Zn is a co-factor (Kendall et al., 2006), and it plays an important role in the production of P_4 by cells of CL through the activity of the SOD enzyme, for which Cu and Zn are co-factors (Salas-Razo et al., 2011). In addition, Zn is essential for the secretion and function of thyroid hormones and its deficiency decreases fertility, causes anoestrus and abortion (Yatoo et al., 2013).

In herds where the current research was developed, zinc deficient animals (< 12.62 μmol/L) had a significant lengthening of the first service calving interval (FSCI) (Balarezo-Urresta et al., 2016), which indicates that this deficiency causes postpartum anoestrus. Accordingly, Zn deficiency is a significant risk factor for reproductive disorder (García-Díaz et al., 2020).

On the other hand, Cu, Zn and cholesterol intervene in the mechanism for the production of P_4 and when faced with a lack of these microelements, the activity of the enzyme superoxide dismutase (SOD) – as the first line of antioxidant defence - decreases and the superoxide anion (O_2^-) produced in CL does not split. This is transformed in the luteal cells into hydrogen peroxide (H_2O_2), which is not a free radical, but rather a luteolytic and inhibitor of cholesterol transport to the mitochondria to become pregnenolone, which decreases the production of P_4 (Márquez et al., 2011).

Hypophosphataemia and hypocupraemia might be caused by excesses of crude protein, Mg and Fe, because at the ruminal level, P and Cu form sulfates by the action of microorganisms. These sulfates bind Mg and form SO_4Mg, which decreases P absorption in the small intestine. Sulfates also bind Fe and give rise to iron sulfide, which decreases Cu absorption at the ruminal level (Djoković et al., 2019).

Conclusions

The resumption of postpartum ovarian activity is related to the metabolic state at 30 days postpartum, mainly with the indicators of energy and mineral metabolism, especially with protein excesses and energy, P, Cu and Zn deficiencies.

The cows with an earlier resumption of postpartum ovarian activity had lower concentrations of β-OH and higher concentrations of cholesterol, Cu and Zn in blood serum, with earlier postpartum luteal activity.

Serum concentration of β-OH > 1 mmol/L or Cu < 11.77 μmol/L or P < 1.61 μmol/L and Zn < 12.62 μmol/ constituted risk factors (P<0.05) for that cows showed a late postpartum resumption of postpartum ovarian activity.

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Odnos između povrata aktivnosti jajnika nakon poroda i metaboličkog profila Holstein krava na velikim nadmorskim visinama u Ekvadoru

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Na povrat aktivnosti jajnika nakon porođaja (RPOA) utječe metabolički profil krava, ali odnos između RPOA i metaboličkog profila mliječnih krava na velikim visinama u andskoj regiji Ekvadora nije jasan. Stoga je cilj ove studije bio utvrditi odnos RPOA i metaboličkog profila Holstein krava na velikim nadmorskim visinama u andskoj regiji Ekvadora. U ovom istraživanju odabrano je trideset krava i procijenjeni su njihovi RPOA i metabolički profili na 30. dan nakon teljenja. Prema RPOA, uspoređeni su metabolički indikatori uporabom studentovog t-testa za nezavisne uzorke. Provedena je jednostavna linearna korelacija između glavnih indikatora RPOA i metaboličkih parametara. Provedena je opservacijska analitička studija za utvrđivanje veze između RPOA i metaboličkog profila. Holstein krave koje su pokazivale rani RPOA imale su nižu razinu β-hidroksibutirata i urea nitrata u krvi (*P*<0,05), a veće koncentracije P, Cu i Zn (*P*<0,05) u krvnom serumu u usporedbi s Holstein kravama koje su pokazivale kasni RPOA. Povećane razine β-hidroksibutirata u krvi te hipofosfatemija, hipokupremija i hipocinkemija su predstavljale faktore rizika (*P*<0,05) za kasni RPOA nakon teljenja. Uz to, sljedeći su parametri bili povezani (*P*<0,05): pojava dominantnog folikula i cinkemija, promjer folikula i kupremija, ovulacija i razina β-hidroksibutirata u krvi, ovulacija i fosfatemija, lutealna aktivnost i kolesterolemija, obujam žutog tijela i kupremija, koncentracije progesterona u krvnom serumu i kolesterolemija i koncentracije progesterona u krvnom serumu i kupremija. S tim da je postojala velika korelacija (*P*<0,01) između ovulacije i kupremije, lutealne aktivnosti i razine β-hidroksibutirata u krvi i kupremije i cinkemije. Naši nalazi pokazuju da je RPOA povezan s metaboličkim profilom na 30. dan nakon teljenja, posebice s viškom bjelančevina i energije te nedostatkom P, Cu i Zn.

Ključne riječi: *reaktivacija jajnika, dominantni folikul, ovulacija, lutealna aktivnost, β-hidroksibutirat*