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Impacts of trace element supplementation on productive/reproductive postpartum performances of grazing dairy heifers from volcanic soils

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Abstract This study has been designed to evaluate the effect of trace elements (TE) on the reproductive and productive performances of postpartum Holstein Friesian heifers, fed on pasture with origin on volcanic soils, poor in TE. Twenty-six heifers pregnant were divided into two groups: experimental (EG) and control (CG) groups (n=13, each group). For animals belonging to the EG, two intra-ruminal capsules with TE were administrated 60 days before partum, while in the CG, no boluses were administered. All animals' blood was collected weekly to assess progesterone levels by the ELFA technique for 11 weeks after partum. TE were evaluated on blood by AAS when the experience started, on the day of delivery, and after 60 days. Before calving, no statistical differences were observed between groups for the trace elements, although it has been noticed that heifers had deficiencies in Selenium, Copper, and Iodine. On the calving day and 60 days after, a statistical increase ($p<0.05$) in serum Copper and Selenium was observed in the animals belonging to the EG. For the other TE, no statistical differences were observed. Concerning reproductive characteristics, the EG, at five weeks postpartum, 70% of the cows were cyclic, while in the CG in the same period, 33% of animals showed signs of ovarian activity. At the level of productive parameters, no differences were observed between groups. The results obtained by the present study allow concluding that, under our experimental conditions, the administration of trace elements, in addition to reducing postpartum anestrus, increases the quality of the corpus luteum in the postpartum period of heifers.

Keywords: copper, iodine, manganese, pasture, reproduction, selenium, trace minerals

1. Introduction

Soil-climatic conditions and fertile soils of volcanic origin allow the Azores to present excellent conditions for creating dairy and beef cattle based on pastures throughout the year. However, cattle grazing in the Azores lacks some essential TE such as cobalt, copper, iodine, selenium, and zinc (Pinto et al 2007a,b), because of the low levels of these elements in the pasture due to the volcanic genesis of Azorean soils (Linhares et al 2021), compromising the normal functioning of the body which can cause health problems for cattle, specifically in terms of the immune and reproductive systems (Suttle 2010). Particularly in heifers, it has been demonstrated that TE are crucial for the reproductive apparatus development, keeping animals healthy and well prepared for their first lactation. Moreover, as the period between the first calving and the resumption of oestrus in heifers is longer than compared of adult cows, one can disclose that, in this period, the needs for TE, namely Iodine, manganese, selenium, and copper, are also higher in heifers than in cows.

Regarding dairy cows, Nazari et al (2019) found that irregular luteal phase (short or prolonged) and delayed or no ovulation are often associated with low copper levels. Moreover, manganese can promote successful pregnancy through steroidogenesis by stimulating progesterone produced in the corpus luteum (Van Emon 2020). Iodine is critical for thyroid hormones involved in foetal metabolism and development (Lannaccone 2019). For selenium, El-Shahat and Abdel-Moem (2011) have proven several functions on the reproduction of cattle, highlighting its direct action on the oocyte to protect against cell damage on the anoestrus by acting on the follicle, promoting folliculogenesis. Studies carried out in regions such as New Zealand, Ireland, or England have already proven that marginal deficiencies of TE in ruminants affect cattle productivity (Grace 1994), even when there are no clinical manifestations (Gonzaléz and Silva 2019), being, therefore, often necessary to supplement cattle with these elements. Supplementation of TE in cattle fed on pasture is not as linear as in other dietary constituents, as their absorption depends on the concentrations existing in the food that the animals have access to, as well as other factors such as age, race, sex, environmental conditions, intestinal pH, and physiological state of the animal (Peers and Philips 2011; López-Alonso 2012), among others. Existing interactions between minerals,

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namely antagonism, can lead to a lack of TE in the body, although recommended supplementation levels have been met (McDowell 2003).

Despite all the above, farmers often underestimate the role of TE in their animals, as these minerals are present in different cellular metabolisms in low amounts, believing, thus, that the amount ingested by animals naturally in pastures and forages is sufficient to cover the needs.

Given the importance of iodine, manganese, selenium, and copper in the reproductive function of cattle, this work aimed to determine the levels of TE in heifers fed on pasture, as well as to evaluate the influence of supplementation of these minerals on the resumption of reproduction in the postpartum period.

2. Materials and Methods

2.1. Experimental design

A total of 26 Holstein Friesian heifers at 200±21 days of gestation were randomly assigned to two experimental and control groups. In the experimental group (n=13), each animal received two intraruminal slow-release capsules, designed to remain in the reticulum for 240 days, ensuring a regular and constant supply of trace elements, whose composition is presented in Table 1; in the control group (n=13) no intraruminal capsules provided to heifers.

Table 1 Trace element concentrations in the basal diet (mg/kg DM) and the slow-release intraruminal capsules.

Active Component	Basal diet (mg/Kg DM)	Daily dose released by capsules (mg/day)
Copper	3.21	138.0
Selenium	0.39	2.0
Manganese	61.20	70.0
Iodine	15.19	4.2

Animals from both groups remained separated from lactating cows up to one week before calving, feeding only on pasture. The heifers were joined to the lactating herd one week before the expected calving date to adapt to the milking routine. In addition to pasture, animals were total mixed ration (TMR) consisting of corn silage and concentrate. The diet was formulated according to the requirements of the NRC (2001), showed in Table 2.

2.2. Blood samples and data collection

Blood samples were collected from the coccygeal vein into glass tubes to obtain serum and heparinised tubes (Vacutainer, Becton Dickinson, NJ, USA) to obtain plasma for progesterone and TE levels determination. Regarding the collection dates, these were carried out when the intraruminal capsules were placed, two months before the expected day of calving (Day – 60) and on Day -30. After calving, blood was collected one day after calving and weekly for 11 weeks after delivery.

After collection, blood was transported to the laboratory, centrifuged for 10 min at 1000 x G, and plasma and serum were stored in microtubes at –20 °C until analysis, according to Nunes et al (2016). The analytical procedures for determination of the concentration of progesterone and TE were performed by the Enzyme-Linked Fluorescent Assay technique and the TE by Atomic Absorption Spectrophotometry, respectively.

The same classifier determined the body condition score at the beginning of the experiment and on days 30 and 60 after calving, using a 5-point scale (1 = emaciated, 5 = fat) according to Edmonson et al (1989).

After parturition the type of parturition (dystocia or eutocia), placental retention and peripartum-associated diseases (hypocalcaemia, mastitis, metritis) were recorded. Milk production, fat, and protein content of milk, as well as somatic cell counts, were also evaluated. The amount of milk produced was recorded and milk samples were collected by the milk contrast service of the Terceira Island Agricultural Association. The samples were analysed in the laboratory of the milk classification service of Terceira Island, by the NP EN ISO/IEC 17025:2018. The Milkoscan equipment was used to determine the crude protein content and the fat content. Somatic cell count analysis was performed with the Fossomatic equipment using a previously described method (Gonzalo et al 1993; IDF 1995).

Table 2 Chemical composition of the pasture and the total mixed ration (TMR).

	Pasture	TMR
Dry matter (DM)	21,42	45,306
Chemical Composition (%DM)		0
Crude Protein (CP)	21,43	12,61
Neutral detergent fibre (NDF)	57,91	54,47
Acid detergent fibre (ADF)	28,8	32,39
Organic matter	91,38	95,4
Ingrediente (%DM)		
Corn silage	-	80
Concentrate	-	20

2.3. Statistical analysis

All the statistical analyses were performed using the SPSS v.26 software. Before statistical differences analysis, the normality and homogeneity evaluations were obtained for the different variables. Percentage data were normalised through arcsine transformation. The Kolmogorov-Smirnov test was employed to assess the different variables' normality distribution. Levene's statistic was performed to test the equal homogeneity, and a one-way ANOVA was performed using the Tukey test to assess the statistical differences among groups. The level of significance accepted was from

5% to less. Results are presented as mean ± standard error of the mean (SEM).

3. Results and Discussion

In extensive pasture systems, ruminants are more likely to have marginal TE deficiencies, causing decreased productive performance in livestock and increased susceptibility to various diseases (McDowell 2003; Gonzaléz and Silva 2019). Despite acting in small concentrations, TE plays a key role in regulating several biological functions, including metabolic processes, reproduction, and immunity (Underwood and Suttle 1999; Spears 2003). The trace minerals rumen bolus (TMB) is the most effective supplementation method in grazing animals compared to other supplementation methods as it provides a large variety of TE for longer periods and reduces labour costs (Grace and Knowles 2012).

In the present study, it has been observed that, at the beginning of the experimental period, none of the animals had adequate levels of iodine, cobalt, and selenium, and only 41.6% of them had adequate levels of manganese, showing a lack of TE in Azorean pasture conditions (Table 3). These results agree with those previously published by Pinto (2007a,b), which revealed a general deficiency of TE in grazing cattle in most of the Azores' islands, which can be associated with the characteristics of the volcanic soils existing here. Linhares et al (2021) reported that in S. Miguel, the concentrations of Cu and Zn are insufficient to cover the minimum needs of ruminants, while Fe, Co, Cu, I, and Zn in the soils and pastures of this same island of the Azorean archipelago exist in very low concentrations.

Table 3 Mean concentration of trace elements present in blood plasma 60 days before parturition, at parturition and 60 days after parturition.

Trace Elements	-60 days		calving day		60 days	
	Ctrl	Exp	Ctrl	Exp	Ctrl	Exp
Copper (µg/dL)	30.86±4.34^{a}	33.83±6.31^{a}	73.86±6.41^{b}	84.14±6.93^{b}	71.75±3.80^{b}	82.57±8.90^{b}
Manganese (µg/L)	5.58±0.62^{a}	6.64±0.66^{a}	5.84±0.60^{a}	6.30±0.46^{a}	5.51±0.59^{a}	6.63±0.59^{a}
Selenium (µg/L)	24.43±2.22^{a}	26.33±3.63^{a}	38.75±3.01^{b}	51.57±2.89^{b}	41.77±3.13^{b}	48.61±3.56^{b}
Iodine (T_4) (µg/dL)	45.87±3.04^{a}	40.67±2.55^{a}	43.52±2.54^{a}	52.85±3.32^{a}	48.61±5.99^{a}	52.73±2.89^{a}

The values represent the means ± SEM (Standard error of the mean). Means with different superscripts within the same line are significantly different *P* < 0.05. Ctrl – control group; Exp – Experimental group.
Values with different letters in same columns indicate differ significantly (*P* < 0.05).

For animals into which TMBs were introduced, a significant increase (*P* < 0,05) in the mean concentration of Cu and Se was observed on the parturition day and 60 days after. The mean concentration of Cu increased from 33.83±6.31 µg/dl to 84.14 ± 6.93 µg/dl in the experimental group and from 30.86±4.34 to 73.86 ± 6.93 µg/dl in the control group on the day of parturition. Sixty days after partum, Cu levels in both groups were like those found at day zero, 71.75±3.80 µg/dl and 82.57±8.90 µg/dl. These results are in line with those obtained by Mehere et al (2002) and by Akhtar et al (2009), in which, in the last month of pregnancy, there is an increase in the concentration of copper in ruminants. The reasons given by Yokus and Cakir (2006) for an increase in serum copper levels in cattle at the time of calving is linked to the rise in the rate of ceruloplasmin synthesis. The increase in copper concentration at the time of parturition in the last ten days of pregnancy is related to the physiological process of parturition being necessary for high copper concentrations for the endocrine glands related to childbirth to start their activity (Pathak et al 2004).

Concerning the levels of Selenium, 60 days after the administration of trace elements, the values increased significantly (*P* < 0.05), passing all animals in the experimental group to marginal levels (30-70 µg/dl) according to the values reference indicated by Kincaid (1999). At the end of the trial period, the values found were like those for parturition. In all periods analysed, heifers did not show adequate levels of selenium in plasma. The record of significant increases in selenium and copper in the control group, between the collection carried out before and after calving, could be due to the improvement in the feeding of these animals since, before calving, heifers were grazing on poor pastures without supplementation, being placed together with the cows to produce milk, thus improving feeding. Therefore, not only did the animals begin to graze on improved pastures, but they also started to be supplemented with commercial concentrates, which in its analytical constitution refers to the existence of copper and vitamin E. Due to the nutritional interdependence between vitamin E (α-tocopherol) and selenium, which are involved in similar functions, makes it possible, in case of deficiencies, for selenium to be compensated by vitamin E and *vice versa* (Arthington and Ranches 2021; Mehdi and Dufrasne 2016).

In the case of iodine and manganese, there was a non-significant decrease between the first and second analyses. Despite the decrease observed at parturiated, sixty days later, when analysed trace elements, the concentration values were higher than those found before calving. In the case of iodine, determined through the concentration of T_4 in the blood, the results are according with obtained by Magdub and Johnson (1977), Thilstead (1985), and more recently by Lana Ferreira (2020), who reported that at the beginning of lactation serum T_4 concentration is very low. Contreras et al (1999) state that there may be a relationship between the excretion of iodine into colostrum and the decline in T_4 concentration. The same author also states that there may be a link between negative energy balance and low levels of T_4 in the blood.

The TMB supplementation did not affect milk production, composition, or somatic cell counts (Table 4). Values recorded for milk production were identical in both groups, with no evidence of the effect of TMB supplementation on this parameter. There were also no significant differences between the control and experimental groups for milk composition, such as fat and protein content. As far as SCC (Somatic cells count) is concerned, although there was a decrease in the group supplemented with trace elements under the conditions in which this study was developed, it was not enough to validate statistically significant differences. On the other hand, as all animals were in their first lactation, a low somatic cell count would be expected, as postulated by Ganda et al (2016).

Table 4 Mean values of production and composition (fat and protein) of milk and SCC, per day, in the control and experimental groups in the first 3 months after parturition. Data represent Mean ± standard deviation; SCC – Somatic cells count.

Parameters	Milk production (L)			Fat (%)			Protein (%)			SCC (10^3 cells/ml)		
Months after parturition	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Experimental group	27.90 ±5.06	27.58 ±3.12	28.16 ±5.49	3.90 ±0.41	3.28 ±0.40	3.36 ±0.33	2.87 ±0.30	2.83 ±0.17	2.85 ±0.22	52.43 ±29.09	30.00 ±7.87	25.75 ±8.05
Control Group	24.94 ±3.85	27.68 ±4.37	27.92 ±4.10	3.80 ±0.87	3.43 ±0.51	3.52 ±0.68	3.09 ±0.37	2.70 ±0.20	2.80 ±0.22	73.38 ±36.49	30.71 ±13.64	28.04 ±8.54

Eight weeks before partum, as expected, progesterone plasma concentration was identical in both groups: 11.94±3.12 ng/ml and 11.83±2.76 ng/ml, respectively, for the experimental and the control group, dropping to baseline levels after parturition, and remaining so for five weeks. It should be noted that all deliveries were considered normal, with no dystocic deliveries, no need for veterinary help, or retained placenta. From the fifth week after partum, the experimental group showed an increase in P_4 levels, with a peak at the week eighth and tenth. In the control group, P_4 levels remained at baseline until week seven, slightly increasing from the eighth week onwards (Figure 1).

According to Alvarez et al (2009), if the management of the animals is adequate, ten weeks after delivery, only 10% of the animals do not show signs of oestrus. In this study, five weeks after calving, the percentage of cows showing oestrus signs in the experimental group was 70% *vs* 33% in control, and this trend continued to the end of the study. At the end of data collection, eleven weeks after parturition, it was observed for the control group that only 50% of females were cycling *vs* 80% in the experimental group.

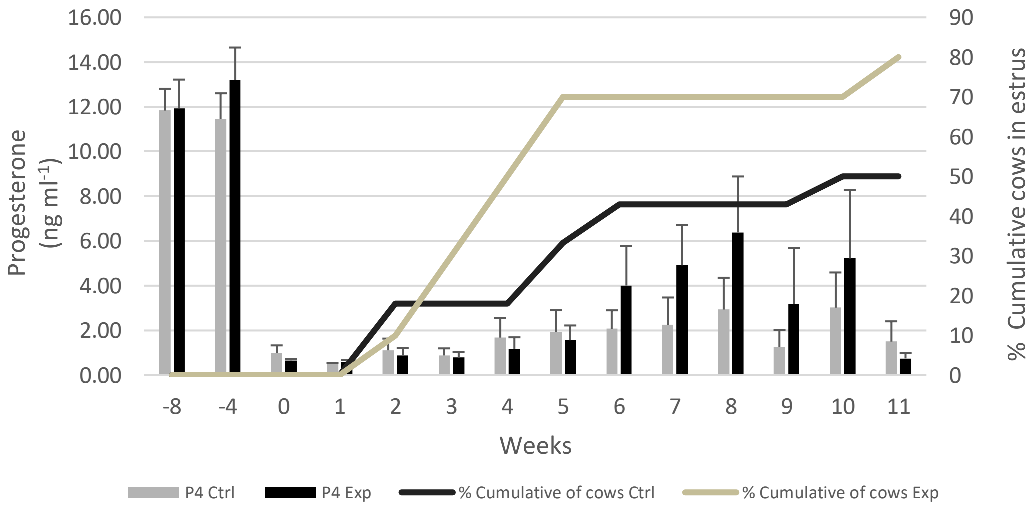

Figure 1 Serum progesterone concentration at 8 weeks before and 11 weeks after calving (week 0 represents the partum). The columns represent the progesterone values, and the bars represent SEM. Lines represent the cumulative percentage of animals that came into oestrus after parturition.

4. Conclusions

In conclusion, the results of the present study extend the results of previous studies on the beneficial roles of trace elements at the reproductive level. The supplementation of heifers with trace elements, two months before calving, in our conditions, will give rise to a greater development of the corpus luteum, and the animals start their reproductive activity in a shorter period after parturition when comparing the two groups under study, thus reducing the period of anestrus. We highlight the increase in copper and selenium levels after delivery due to the prolonged action of TMB. Supplementation of pasture-fed cattle with TMB is essential in preventing TE deficiencies, especially in grazing cattle. New studies based on differences in application throughout the year should be implemented since the availability of ET in pastures varies according to the seasons. Moreover, it would also be interesting to test groups of animals of different ages and lactations, allowing us to conclude about the real needs in the different physiological states of cattle.

Conflict of Interest

The authors declare that there is no conflict of interest.

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