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Effects of mineral supplementation on reproductive performance of pregnant cross-breed Bonsmara cows: An experimental study

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Abstract

Minerals in animal feed occur in variable structures, most of which determine the uptake and usage in biological processes in the body. Effective chemical breakdown of minerals may ensure efficient utilization in metabolism. The aim this study was to evaluate the effects of mineral supplementation on reproduction in cows. A farm was selected for the experiment due to the fact that it previously experienced different reproductive conditions in the farm. The farm comprises cross-breed cows with Bonsmara dominating in the farm. Twelve pregnant primiparous and multiparous cows of different ages, parity and weight, that had previously experienced reproductive conditions, were randomly selected for this study. The cows were then randomly sub-divided into two groups (experimental and control group) of six. The experimental group was injected with MULTIMIN™ + Se + Cu at a dosage of 1 ml/45 kg BW and Calci 50 p.i. at a dosage of 100–150 ml/500 kg BW at an interval of 6 weeks (from June to October 2017). Blood samples were collected before every injection date. The *t* test was used to relate the mean weight gain and serum metabolite between the experimental and control groups. The body weight gain was significantly higher in the experimental group compared to the non-supplemented group. Supplemented cows had significantly ($p < .05$) high levels of triglycerides and creatinine kinase. A case of retained placenta and dystocia among non-supplemented cows were noted. Thus, mineral supplementation can be used to improve productivity and reproductive well-being.

KEYWORDS

communal rearing, cows, minerals, reproductive condition, supplementation

1 | INTRODUCTION

Globally, contribution of livestock production has a significant influence on agricultural growth (Randolph et al., 2007). Nutrient requirements increase as the pregnancy progresses and failure to account for nutritional demands during this period can affect reproductive performance and foetal growth (Caldow & Riddell, 2015). Minerals, in particular, are greatly essential, as any alteration in supply during gestation can predispose cows to reproductive failure (Andrieu, 2008; Griffiths, Loeffler, Socha, Tomlinson, & Johnson, 2007). In

mammals, reproductive needs for minerals are commonly consistent with the foetal, conception product (i.e. foetal fluid, uterus and placenta) and mineral content (Suttle, 2010).

Deficiencies in calcium, magnesium, phosphorus, copper, selenium, zinc and manganese have been associated with occurrences of hypocalcaemia, retained placenta, abortion, dystocia, vaginal prolapse, downer cow syndrome and overall depressed reproductive performance in cows (Amen & Muhammad, 2016; Mokolopi, 2019; Sepúlveda-Varas, Weary, Noro, & Keyserlingk, 2015; Velladurai, Selvaraju, & Napolean, 2016; Yatoo et al., 2018).

Whenever natural grasslands are the main or only source of nutrition supply, it is essential to determine the nutritional content of pastures, to quantify and measure different elements in order to account for deficiencies and improve the feed in order to enhance production and reproduction (Al-Ghareebawi, Almansor, & Muhammad, 2017). Increased attempts to reduce mineral deficiencies have as well increased the risk of toxicity. It is a common practice to offer cow mineral lick in an effort to balance requirements; however, this is not achievable unless the concentrations of minerals in the supplement are exactly the amount required. Thus, proper supplementation is necessary in order to reduce incidences of reproductive problems and animal losses due to nutritional imbalances. Hence, the aim of this study was to assess the role of mineral supplementation on pregnant cows in the prevention of peri-partum reproductive conditions.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was conducted in Mafikeng, North-West Province, South Africa. The area is situated on the following coordinates: 25°51′S and 25°38′E. Mafikeng is a semi-arid area comprising both rural and commercial farms, most of which are rural farms. Temperatures range from 22 to 35°C in summer (between August and March) and rainfall fluctuates between 200 and 500 mm/year.

2.2 | Animals

A farm in Mogosane Village (Mafikeng) with cross-breed Bonsmara cows was selected for the study due to its previous history of exposure to reproductive cases such as dystocia, retained placenta, vaginal prolapses, downer cow syndrome and abortions. The breeding history (as observed by farmers) for all cows selected for the experiment was recorded.

2.3 | Experimental design

Twelve pregnant cows, between the ages of 3–4 years and 3–4.5 months, were selected for the study. The cows were tagged and randomly allocated to one of the two treatment groups with six cows in each group: experimental (supplemented) or control (non-supplemented) group. A selected sample of primiparous and multiparous cows (3–5 years old, 347–540 kg initial body weight and parity of 1–2) were assigned to the experimental and control groups and the experiment performed for 18 weeks (from June to October 2017).

2.4 | Treatment

The experimental group was given three injections of MULTIMIN™ + Se + Cu at a dosage of 1 ml/45 kg BW and Calci 50 p.i. at a dosage of 100–150 ml/500 kg BW at an interval of 6 weeks during mid-late gestation. MULTIMIN™ + Se + Cu contains 15 mg Cu/ml (as Cu disodium EDTA), 40 mg Zn/ml (as Zn disodium EDTA), 10 mg Mn/ml (as Mn disodium EDTA), 5 mg Se/ml (as sodium selenite) and Calci 50 p.i. containing calcium 45.6 mg, magnesium 7.8 mg and phosphorus 1.32 mg. All cows were allowed to graze in the veld.

2.5 | Chemical analysis

Blood samples were collected from both groups once before the beginning of the experiment and on three occasions, prior every injection date. Samples were later analysed for serum biochemical parameters such as magnesium (Mg), total protein (TP), creatinine kinase (CK), lipase (LIPA), triglycerides (TRIG), blood urea nitrogen (Urea/BUN), uric acid (URIC), aspartate amino-transferase (AST), cholesterol (CHOL), total bilirubin (TBIL), gamma-glutamyltransferase (GGT) and ammonia (NH_3) using IDEXX Catalyst chemistry analyser accordance with the instructions in the manufacturer's manual.

2.6 | Analysis of pasture and preparation of grass samples

Pasture samples around the area (Mogosane Village), where the cows were grazing, were also harvested according to species and analysed for their mineral content. Different species of the grass were randomly collected across the grazing area using a pair of scissors in June 2017. The different species were as follows: *Eragostis rigidor* (Curly grass); *Bothriochloa radicans* (stinking grass); *Eragrostis lehmanniana* (Lehmann's Love Grass); *Panicum maximum* (White buffalo grass); *Aristida Congesta* Subsp. *barbicollis* (Spreading Three-awn); *Eragostis rotifer* (Pearly Love Grass); *Urochloa oligotricha* (Perennial Signal Grass); *Themeda triandra* (Red grass); *Heteropogon contortus* (Spear grass); *Erogostic superba* (Saw-tooth Love grass); and *Brachiaria nigropedata* (Black footed grass). These species were identified according to Van Oudtshoorn and Van Wyk (2012). The pastures were picked at the stage of maturity and about 10 cm from the ground (van Niekerk, Hassen, & Bechaz, 2010). The different types of grass were then taken to the Animal Health laboratories of the North-West University for analysis. During analysis, a portion of each grass sample was mixed in one plastic bag and a representative

sample (1 kg) used for analysis. The samples were later placed on benches to air dry. Samples were ground using a POLYMIX PX-MFC 90D (Thermo Fisher Scientific) grinder. Powdered samples were subsequently placed in sample containers and stored until analysis.

Preparation of samples was done following the procedure described by Ndou and Dlamini (2012). Laboratory tools (crucibles) required for the digestion and preparation of grass samples were soaked overnight in 36% hydrochloric acid (HCl). They were then rinsed three times with distilled water and placed for 16 hr (at 60°C) in a hot air oven to dry. After drying, the crucibles were placed in a desiccator for 6 hr to allow cooling, and later weighed to obtain crucible weight before adding the samples. Grass samples were sun-dried and ground. An analytical scale calibrated to four decimal places was used to weigh the grass samples. The difference between the mass of the crucible and fresh grass samples and the weight of the empty crucible were used to calculate the fresh weight {fresh weight = (crucible + weight of fresh sample) − (weight of empty crucible)}. Exactly 1 g of the powdered grass sample was weighed into the clean crucible. Crucibles containing ground grass samples were placed in the oven to dry at 106°C for 16 hr to remove excess moisture. After removing the crucible from the oven, samples were then placed in a desiccator to cool and weighed after 6 hr. The differences between the weight of the crucible, the dry sample and weight of the empty crucible were recorded as the dry weight of the sample {Dry weight = (crucible + weight of dry samples) − (weight of empty crucible)}. After weighing, the samples were ashed in a muffle furnace at 800°C for 16 hr.

The ash was removed from the furnace and cooled, then 1 ml Nitric acid and 9 ml hydrochloric acid were added to the crucible, and the mixture transferred to the rotors of the microwave digester and properly placed in the microwave for digestion. The samples were digested using a microwave digestion system MD2100 (CEM, Mathews, NC). After digestion, samples were transferred to 100 ml volumetric flasks and topped up with distilled water to reach the 100 ml mark using a clean glass funnel. The solution was left overnight on the bench. The following day, the sample was filtered using Whatman filter papers into sterile centrifuge tubes. Then, analysis of digested samples was performed using the Inductively Coupled Plasma Mass Spectrometry-NexION 300Q ICP-MS (PerkinElmer®).

2.7 | Conditions of instrument used for ICP-MS (inductively coupled plasma mass spectrometry)

All chemicals used were of analytical grade quality. Ultrapure water was obtained from a Millipore water system (Millipore) and ultrapure Nitric acid (HNO_3, Merck) used to digest the samples. Stock standard solutions of Arsenic and Mercury containing 10 μg/ml in 2% HNO_3 were procured from Sigma Aldrich, USA, and prepared in accordance with the procedure described by Uluozlu et al. (2017). Certified reference materials (CRM) were purchased from the National Institute of Standard Technology (NIST-8436) and used for standardization and validation of the method.

2.8 | Statistical analysis

The data were captured in excel and analysed using the Statistical Package for the Social Sciences (SPSS) Version 25. The *t* test was used to compare the mean differences (body weight and serum metabolites) between the control (non-supplemented) and the experimental (supplemented) groups. The level of significance was set at $p < .05$.

3 | RESULTS

The results of this study are summarized in Table 1, showing the experimental mean weight gain in pregnant cows given mineral supplements (Multimin) compared to those not supplemented. The mean values indicate both positive and negative weight gain (i.e. for June–July, the experimental group gained on an average weight of about 61.5 kg while in the control group, there was a weight loss of 19.5 kg). Table 2 shows variations in mean weight gain between the experimental and control groups. The results of the *t* test show that the mean weight gain (June–July) differed significantly ($p < .05$) between the experimental and control groups with the former exceeding the latter by 81.

Table 3 shows the results of the *t* test. The results show the means of weight gain (June–July) were not significantly different

TABLE 1 Experimental mean weight gain in pregnant cows given mineral supplements (Multimin) compared to those not supplemented

Group statistics				
Months	**Group ID**	**Mean weight gain**	**Std. deviation**	**Std. error mean**
June–July	Experimental	61.500	58.037	23.694
	Control	−19.500	46.561	19.008
July–August	Experimental	−13.500	75.931	30.999
	Control	17.500	22.314	9.110
August–September	Experimental	−20.000	30.835	12.588
	Control	−12.667	18.811	7.680
September–October	Experimental	19.000	53.051	21.658
	Control	−51.500	33.441	13.652

TABLE 2 Mean live weight gain variations between supplemented and non-supplemented cows

	t	*df*	Sig. (2-tailed)	Mean difference	Std. error difference
Weight gain (June–July)	2.667*	10	0.024	81.000	30.376
Weight gain (July–August)	−0.959	10	0.360	−31.000	32.309
Weight gain (August–September)	−0.497	10	0.630	−7.333	14.746
Weight gain (September–October)	2.754*	10	0.020	70.500	25.602

*Significantly different ($p > .05$) across the ages and parities of the animals.

TABLE 3 Mean differences of the experimental (supplemented) and control (non-supplemented) groups within a particular age and parities

	Age in years	Parity	Mean ± Std. deviation	Std. error mean	Sig. (*p*-value)
Weight gain (June–July)	3.00	1st	42.750 ± 89.574	44.787	.538
	4.00	2nd	10.125 ± 54.057	19.112	
Weight gain (July–August)	3.00	1st	−33.500 ± 81.847	40.924	.287
	4.00	2nd	19.750 ± 30.570	10.808	
Weight gain (August–September)	3.00	1st	−12.750 ± 18.839	9.420	.704
	4.00	2nd	−18.125 ± 28.140	9.949	
Weight gain (September–October)	3.00	1st	−6.500 ± 76.857	38.429	.745
	4.00	2nd	−21.125 ± 48.230	17.052	

TABLE 4 Comparison of serum metabolite between the experimental (supplemented) and the control (non-supplemented) groups before the first injection (June- 2017)

Serum metabolites	*t*	*df*	Sig. (*p*-value)	Mean difference
UREA/BUN	−0.955	10	0.362	−0.433 mM
Phosphates (PHOS)	−0.114	10	0.911	−0.018 mM
URIC Acid	2.983*	10	0.014	17.667 μM
Total protein (TP)	1.136	10	0.282	3.667 g/L
ALT	0.656	10	0.527	3.833 U/L
AST	−0.064	10	0.950	−1.167 U/L
GGT	0.649	10	0.531	2.333 U/L
Total bilirubin (TBIL)	0.327	10	0.751	1.167 μM
Cholesterol (CHOL)	−0.029	10	0.978	−0.012 mM
Ammonia (NH3)	1.270	10	0.233	86.167 μM
Triglycerides (TRIG)	−0.791	10	0.448	−0.003 mM
LIPA	0.285	10	0.781	3.667 U/L

*Sig. = significant differences ($p < .05$).

($p > .05$) across the ages and parities of the animals. Results of comparison of serum metabolite between the experimental and control groups are shown in Table 4. The results also show that serum uric acid concentrations differed significantly between the experimental and control groups (Table 4). Table 5 shows comparison of serum metabolite between the experimental and the control groups after the second supplementation. Serum triglycerides (TRIG) and creatinine kinase (CK) were significantly different between the experimental and control groups.

Table 6 shows significantly altered serum metabolites levels at the first sampling in the experiment, the concentrations of uric acid, triglycerides and creatinine were significantly variable between the groups. The results in Table 7 show that the concentration of P, Zn, Cu and I in the grass was lower than the normal range.

4 | DISCUSSION

The aim of this study was to evaluate the effects of mineral supplementation on the reproductive performance of pregnant crossbreed Bonsmara cows reared in semi-arid areas of South Africa.

TABLE 5 Comparison of serum metabolite between the experimental (supplemented) and control (non-supplemented) groups after the second supplementation, before the last injection of minerals (6-October-2017)

Serum metabolites	*t*	*df*	Sig. (2-tailed)	Mean difference
UREA/BUN	1.916	10	0.084	0.917 mM
Phosphates (PHOS)	−0.025	10	0.981	−0.008 mM
URIC Acid	2.072	10	0.065	12.500 μM
Total protein (TP)	−0.499	10	0.628	−6.667 g/L
ALT	−0.517	10	0.616	−7.500 U/L
AST	−0.837	10	0.422	−20.833 U/L
GGT	−1.027	10	0.328	−2.000 U/L
Total bilirubin (TBIL)	0.850	10	0.415	0.833 μM
Cholesterol (CHOL)	−0.451	10	0.662	−0.195 mM
Ammonia (NH3)	−1.083	10	0.304	−99.167 μM
Triglycerides (TRIG)	−4.661*	10	0.001	−1.800 mM
LIPA	−2.018	10	0.071	−45.833 U/L
Creatinine kinase (CK)	−4.817*	10	0.001	−110.333 U/L

*Sig. = significant differences ($p \leq .001$).

TABLE 6 Serum metabolites mean ± standard errors of cows given mineral supplements from 3–4.5 months of pregnancy

	Serum metabolites means ± standard error		
	Uric acid	Triglyceride	Creatinine kinase
Experimental groups			
Treatment	52.1667 ± 3.986	0.2167 ± 0.034	69.5 ± 6.312
Control	34.5 ± 4.379	2.0167 ± 0.384	179.833 ± 22.015
Normal ranges	2.81–3.93 mg/dl	0.08–0.20 mM	0–110 U/L

Note: Normal ranges for uric acid, tryclycerides and creatinine kinase were sourced from Mamun et al. (2013) and Cozzi et al. (2011).

TABLE 7 Composition of grass nutrient in the grazing area of the experimental farm chosen for mineral supplementation experiment, mean standard deviation and reference ranges

Nutrients	Mean ± std (μg/ml)	Reference ranges	Level of concentration
Phosphorus (P)	9.412 ± 1.622	81.4464–723.968 mg/L*	L
Magnesium (Mg)	17.946 ± 2.279	12.0188–36.6033 mg/L*	N
Manganese (Mn)	4.97 ± 4.135	0.18–0.19 mg/L***	H
Iron (Fe)	8.827 ± 1.6118	1–2 μg/ml***	H
Zinc (Zn)	0.209 ± 0.125	0.8–1.2 μg/ml***	L
Copper (Cu)	0.389 ± 0.125	0.57–0.96 μg/ml***	L
Selenium (Se)	10.66 ± 9.832	0.7–1.3 μg/ml***	H
Iodine (I)	2.858 ± 1.943	4.96–19.85 mg/L**	L

Note: Sources of reference ranges: *Djoković et al. (2014); **Cozzi et al. (2011); ***Yatoo et al. (2018).

Keys: L-below the normal range; N-within the normal range; H-Higher than normal range.

NB: μg/ml = mg/L.

The results that pasture concentrations of phosphorus (mg/L) 9.412 ± 1.622, zinc (μg/ml) 0.209 ± 0.125, copper (μg/ml) 0.389 ± 0.125 and iodine (mg/L) 2.858 ± 1.943 were lower than the normal ranges of 81.446–723.968 mg/L, 0.8–1.2 μg/ml, 0.57–0.96 μg/ml and 4.96–19.85 mg/L, respectively, as shown in Table 7 (Cozzi et al., 2011; Djoković et al., 2014; Yatoo et al., 2018).

Rainfall patterns in semi-arid areas affect the quality and availability of pastures during the dry season, and improvements in quality and availability of grasses are seen as more rainfall is experienced (Bezabih, Pellikaan, Tolera, Khan, & Hendriks, 2014). Consequently, animals reared under such conditions are prone to nutritional imbalance due to fluctuating nutrient consumption, leading to poor reproductive performance (Velladurai et al., 2016). Phosphorus is frequently stated as a 'fertility' mineral (Fageria, He, & Baligar, 2017). An earlier study on mineral content revealed that phosphorus levels in natural pastures are not adequate to improve productivity (Ateba

& Beighle, 2011). Hadžimusić, Krnić, and Hodžić (2013) found very high phosphorus requirements in cows and that what is provided by plants may be less due to low levels in the soil. Natural pastures are generally low in phosphorus, which predisposes cows to impaired muscle function, retained placenta and downer cow syndrome (Ate, Rekwot, Nok, & Tekdek, 2009). Thus, cow productivity and improved reproductive performance are directly connected to phosphorus intake (Wang et al., 2017).

The study revealed low zinc, copper and iodine in grass consumed by cows (Table 7). These deficiencies have been linked with disease susceptibility and, reproductive disorders, such as abortions and retained placenta in cows (Balamurugan et al., 2017; Kumar, 2014; Suttle, 2010; Velladurai et al., 2016). This is an indication that cows consuming mineral-deficient grass are predisposed to reproductive failures, making mineral supplementation important to avoid and reduce reproductive complications.

The study also revealed that concentrations of iron (8.827 ± 1.6118 μg/ml), manganese (4.97 ± 4.135 mg/L) and selenium (10.66 ± 9.832 μg/ml) in the different types of grass were higher than the normal ranges of 1–2 μg/ml, 12.0188–36.6033 mg/L and 0.7–1.3 μg/ml, respectively, as indicated in Table 7 (Djoković et al., 2014; Yatoo et al., 2018).

Increase in dietary iron causes copper deficiency, leading to impaired reproduction, and high selenium may lead to stillbirths and abortions due to toxicity (Abramowicz, Kurek, Dębiak, Madany, & Lutnicki, 2019; Omeje, 2016). The results show that increases in the grass minerals could influence the reproductive health of cows. The occurrence of mineral imbalances in communal pastures could explain incidences of reproductive conditions in cows (Bindari, Shrestha, Shrestha, & Gaire, 2013; López-Alonso, 2012; Taylor, 2007). Deficiencies observed and excess of mineral in the grass suggest that supplementation could influence reproduction.

The role of supplementary trace mineral during pregnancy has revealed controversial (positive, negative and sometimes neutral) results on reproductive performance (Joksimović-Todorović, Davidović, & Bojanić-Rašović, 2016). Nonetheless, the current study showed that the non-supplemented group experienced retained placenta and dystocia, while the supplemented group did not experience any reproductive disorders. Another similar study associated mineral deficiencies with increased incidences of retained placenta and dystocia (Tucho & Ahmed, 2017). These results suggest that minerals influence reproductive aptitude and health of cows reared on natural pastures.

In the current study, significant differences ($p < .05$) in body mass and serum metabolites were seen between pregnant cows injected with Multimin mineral supplements and those that were not. It is difficult to measure the state of nutrition in cows traditionally reared on natural pasture; hence, live-body weight measures become a very useful management tool for monitoring nutritional status during and after pregnancy (Dhakal et al., 2013).

Changes in body mass and metabolic profile measures in cows are known to simplify interpretations of reproduction and nutrition interactions (Caldow & Riddell, 2015). The current results show that mean weight gain (June–July) differed significantly ($p < .05$) between the experimental and the control groups, with the former exceeding the latter by 81 (Table 2). During winter (June–July), the experimental group (61.5 ± 58.037 kg) gained more weight than the control group, showing an average weight loss of 19.5 ± 46.56 kg (Table 1).

The results also revealed that that mean weight gain in spring (September–October) differed significantly ($p < .05$) between the experimental and the control groups, with the former exceeding the latter by 70.5 (Table 2). Variations in body mass have traditionally been used to monitor energy balance, which is an important nutritional indicator related to both animal health and reproduction (Salehi, Colazo, Oba, & Ambrose, 2016). Lack of mineral supplementation (as observed in the study) can cause production losses mainly in animals relying only on natural pasture (Dhakal et al., 2013). Losses in body mass have been associated with poor productivity and incidences of reproductive conditions (Rossato, Gonzalez, Dias, Riccó, Valle, Rosa, & Wald, 2001). The results imply that supplementation improves animal production during the dry season and maintains a healthier body condition compared to non-supplementation.

Serum metabolites are used as physiological indicators, which better reflect metabolic disorders, animal health, nutritional status and reproductive performance (Wu et al., 2018). Significantly altered serum metabolite levels were seen at the beginning of the experiment, before and after supplementation. Concentrations of uric acid, triglycerides and creatinine significantly varied between supplemented (treatment) and non-supplemented (control) cows (Table 6). Serum mean concentrations of uric acid were significantly high in both the experimental and control groups before the commencement of the supplementation (Table 4). Additionally, the mean difference for uric acid (17.667 mg/dl) showed that the experimental mean was greater than the control mean concentration (Table 4). Uric acid is an inorganic compound (2,6,8 trioxypurine-C5H4N4O3) and an endogenous product of purine metabolite in animals (De Oliveira & Burini, 2012). Cardiovascular diseases, chronic kidney diseases, increase body mass, insulin resistance and leptin production, and decreased excretion of renal uric acid are some of the factors known to increase concentrations of uric acid (Dórea, Danés, Zanton, & Armentano, 2017; Laughon, Catov, Powers, Roberts, & Gandley, 2011; Zhu, Peng, & Ling, 2017). Additionally, reduced body weight has been associated with high level of uric acid, due to oxidative stress, resulting from excess production of uric acid (De Oliveira & Burini, 2012). This could explain the increase in uric acid in the current study before the commencement of the experiment (Table 4).

Serum triglycerides (TRIG) and creatinine kinase (CK) were significantly different between the experimental (supplemented) and control (non-supplemented) groups (Table 5). The level of CK (179.833 ± 22.015 U/L) was significantly higher in the control group, in which dystocia and retained placenta were observed (Table 6). It has been documented that high CK levels may be due to the impairment or exertion of skeletal muscles resulting from difficult parturition (Murray et al., 2015). This could be the reason for the high

creatinine kinase levels seen in the control group of cows, which experienced difficult birth in the present study.

The present study showed the mean TRIG (2.0167 ± 0.384 mM) level was above normal in the control group (Table 6). Other researchers have associated negative energy feedback and high nutritional requirements with low levels of triglycerides (Alves et al., 2014; Petkova, Kitanov, & Girginov, 2008). In situations of positive energy balance, triglycerides are likely to be high (Sevinç, Başoğlu, Güzelbektaş, & Boydak, 2003). These associations could explain the lower body mass in the control group with the commencement of supplementation. Fatty liver syndrome is also related to decrease in TRIG concentrations, which are directly proportional to the level of cholesterol (Qureshi et al., 2016). Other serum metabolites (urea, phosphates, total protein, AST, GGT, TBIL, CHOL, NH_3 and LIPA) did not show significant differences between supplemented and non-supplemented groups ($p > .001$) throughout the experiment (Table 4).

5 | CONCLUSION

The levels of phosphorus, zinc, copper and iodine in natural pastures were low. Cows supplemented with Multimin gained more weight compared to the non-supplemented group. High levels of triglycerides and creatinine kinase were seen in pregnant cows given Multimin. Cases of retained placenta and dystocia occurred in the non-supplemented group. The results obtained in this study show that the concentration of minerals in forage gets depleted during the dry periods and cows encounter nutrient insufficiencies, thus impairing reproduction. Mineral supplementation during pregnancy could improve productivity. Further studies on mineral and reproduction interactions should be conducted on a larger scale to increase available data.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

AUTHOR CONTRIBUTIONS

MK collected the samples, analysed the data and wrote the manuscript. MM conceived the idea, supervised the study and also participated in the writing of the manuscript.

ETHICAL APPROVAL

Permission to conduct the study was granted by the Animal Health Research Ethics Committee after receiving approval from the North-West University Research Ethics Regulatory Committee (NWU-RERC), Ethics number: NWU-00409-18-A5.

DATA AVAILABILITY

Data for this study can be obtained from the corresponding author upon reasonable request.

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REFERENCES

Abramowicz, B., Kurek, Ł., Dębiak, P., Madany, J., & Lutnicki, K. (2019). Hematological parameters in dairy cows with copper deficiency. *Journal of Elementology*, *24*, 501–510.

Al-Ghareebawi, A. M. A., Almansor, K. J. A., & Muhammad, M. J. (2017). Effect of some minerals in soil and forage on same minerals in cattle plasma at three sites in the province of Thi-Qar. *Kufa Journal for Veterinary Medical Sciences*, *7*(1B), 55–60.

Alves, B. G., Alves, K. A., Martins, M. C., Braga, L. S., Silva, T. H., Alves, B. G., … Gambarini, M. L. (2014). Metabolic profile of serum and follicular fluid from postpartum dairy cows during summer and winter. *Reproduction, Fertility and Development*, *26*, 866–874. https://doi.org/10.1071/RD13102

Amen, M. H. M., & Muhammad, S. S. (2016). Effect of zinc supplementation on some physiological and growth traits in local male rabbit. *Veterinary Journal*, *6*, 151–155.

Andrieu, S. (2008). Is there a role for organic trace element supplements in transition cow health? *The Veterinary Journal*, *176*, 77–83. https://doi.org/10.1016/j.tvjl.2007.12.022

Ate, I. U., Rekwot, P. I., Nok, A. J., & Tekdek, L. B. (2009). Serum electrolyte values of cows during third trimester of pregnancy and early lactation in settled cattle herds in Zaria, Northern Nigeria. *African Journal of Biomedical Research*, *12*, 125–130.

Ateba, T., & Beighle, D. (2011). Effect of layer chicken litter and yellow maize meal on mineral status of bovine. *Life Science Journal*, *8*, 8–11.

Balamurugan, B., Ramamoorthy, M., Mandal, R. S. K., Keerthana, J., Gopalakrishnan, G., Kavya, K. M., …Katiyar, R. (2017). Mineral: An important nutrient for efficient reproductive health in dairy cattle. *International Journal of Science, Environment and Technology*, *6*, 694–701.

Bezabih, M., Pellikaan, W. F., Tolera, A., Khan, N. A., & Hendriks, W. H. (2014). Nutritional status of cattle grazing natural pasture in the Mid Rift Valley grasslands of Ethiopia measured using plant cuticular hydrocarbons and their isotope enrichment. *Livestock Science*, *161*, 41–52. https://doi.org/10.1016/j.livsci.2013.12.016

Bindari, Y. R., Shrestha, S., Shrestha, N., & Gaire, T. N. (2013). Effects of nutrition on reproduction-A review. *Advances in Applied Science Research*, *4*, 421–429.

Caldow, G., & Riddell, I. (2015). *Auditing Beef Cow Herd Reproduction. Bovine Medicine* (pp. 501–505). Chichester, UK: John Wiley & Sons, Ltd.

Cozzi, G., Ravarotto, L., Gottardo, F., Stefani, A. L., Contiero, B., Moro, L., … Dalvit, P. (2011). Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. *Journal of Dairy Science*, *94*, 3895–3901. https://doi.org/10.3168/jds.2010-3687

De Oliveira, E. P., & Burini, R. C. (2012). High plasma uric acid concentration: Causes and consequences. *Diabetology & Metabolic Syndrome*, *4*, 12. https://doi.org/10.1186/1758-5996-4-12

Dhakal, K., Maltecca, C., Cassady, J. P., Baloche, G., Williams, C. M., & Washburn, S. P. (2013). Calf birth weight, gestation length, calving ease, and neonatal calf mortality in Holstein, Jersey, and crossbred cows in a pasture system. *Journal of Dairy Science*, *96*, 690–698. https://doi.org/10.3168/jds.2012-5817

Djoković, R., Cincović, M., Kurćubić, V., Petrović, M., Lalović, M., Jašović, B., & Stanimirovic, Z. (2014). Endocrine and metabolic status of dairy cows during transition period. *The Thai Journal of Veterinary Medicine*, *44*, 59–66.

Dórea, J. R. R., Danés, M. A. C., Zanton, G. I., & Armentano, L. E. (2017). Urinary purine derivatives as a tool to estimate dry matter intake in cattle: A meta-analysis. *Journal of Dairy Science*, *100*, 8977–8994. https://doi.org/10.3168/jds.2017-12908

Fageria, N. K., He, Z., & Baligar, V. C. (2017). *Phosphorus Management in Crop Production: CRC Press*. Boca Raton, FL: Taylor and Francis Group. ISBN 13-978-1-4987-05868.

Griffiths, L. M., Loeffler, S. H., Socha, M. T., Tomlinson, D. J., & Johnson, A. B. (2007). Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. *Animal Feed Science and Technology*, *137*, 69–83. https://doi.org/10.1016/j.anifeedsci.2006.10.006

Hadžimusić, N., Krnić, J., & Hodžić, A. (2013). Concentrations of albumin and total protein in cattle blood plasma depending upon locality and seasonal changes. *Veterinaria*, *61*, 129–138.

Joksimović-Todorović, M., Davidović, V., & Bojanić-Rašović, M. (2016). The effects of some microelements supplementation-selenium, zinc and copper into dairy cow feeds on their health and reproductive performances. *Biotechnology in Animal Husbandry*, *32*, 101–110.

Kumar, A. S. (2014). Blood biochemical profile in repeat breeding crossbred dairy cows. *International Journal of Veterinary Science*, *3*, 172–173.

Laughon, S. K., Catov, J., Powers, R. W., Roberts, J. M., & Gandley, R. E. (2011). First trimester uric acid and adverse pregnancy outcomes. *American Journal of Hypertension*, *24*, 489–495. https://doi.org/10.1038/ajh.2010.262

López-Alonso, M. (2012). Trace minerals and livestock: Not too much not too little. *ISRN Veterinary Science*, *704825*, https://doi.org/10.5402/2012/704825

Mamun, M., Hassan, M., Shaikat, A., Islam, S., Hoque, M., Uddin, M., Hossain, M. B. (2013). Biochemical analysis of blood of native cattle in the hilly area of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *11*, 51–56.

Mokolopi, B. G. (2019). Phosphorus, calcium, and magnesium contents of pasture and their effect on body condition scores and body mass of communal cattle depending on natural pasture of Mogosane Village, of the North-West Province, South Africa. *Journal of Tropical Animal Health and Production*, *51*, 1295–1787.

Murray, C. F., Veira, D. M., Nadalin, A. L., Haines, D. M., Jackson, M. L., Pearl, D. L., Leslie, K. E. (2015). The effect of dystocia on physiological and behavioural characteristics related to vitality and passive transfer of immunoglobulins in newborn Holstein calves. *Canadian Journal of Veterinary Research*, *79*, 109–119.

Ndou, R., & Dlamini, M. (2012). An investigation into the cause of high abortion rates of goats in the Limpopo Province, South Africa. *Continuing Education Presenters*, *28*, 28–41.

Omeje, V. I. (2016). *Effect of dietary supplementation of organic selenium at different levels on reproductive performance of rabbit does*. (Doctoral dissertation, PhD. thesis, University of Nigeria, Nsukka).

Petkova, M., Kitanov, I., & Girginov, D. (2008). Blood lipids profile in lactating cows fed with supplement of OVOCAP®. *Biotechnology in Animal Husbandry*, *24*, 19–28. https://doi.org/10.2298/BAH0804019P

Qureshi, M. U., Qureshi, M. S., Khan, R., Rahman, A., Sohail, S. M., Ijaz, A., … Basit, A. (2016). Relationship of blood metabolites with reproductive cyclicity in dairy cows. *Indian Journal of Animal Research*, *50*, 338–348. https://doi.org/10.18805/ijar.10713

Randolph, T. F., Schelling, E., Grace, D., Nicholson, C. F., Leroy, J. L., Cole, D. C., … Ruel, M. (2007). Invited Review: Role of livestock in human nutrition and health for poverty reduction in developing countries 1,2,3. *Journal of Animal Science*, *85*, 2788–2800. https://doi.org/10.2527/jas.2007-0467

Rossato, W., Gonzalez, F. H. D., Dias, M. M., Riccó, D., Valle, S. F., Rosa, V. L. L. A., & Wald, V. (2001). Number of lactations affects metabolic profile of dairy cows. *Archives of Veterinary Science*, *6*, 83–88.

Salehi, R., Colazo, M. G., Oba, M., & Ambrose, D. J. (2016). Effects of prepartum diets supplemented with rolled oilseeds on calf birth weight, postpartum health, feed intake, milk yield, and reproductive performance of dairy cows. *Journal of Dairy Science*, *99*, 3584–3597. https://doi.org/10.3168/jds.2015-10186

Sepúlveda-Varas, P., Weary, D. M., Noro, M., & von Keyserlingk, M. A. (2015). Transition diseases in grazing dairy cows are related to serum cholesterol and other analytes. *PLoS ONE*, *10*, e0122317. https://doi.org/10.1371/journal.pone.0122317

Sevinç, M., Başoğlu, A., Güzelbektaş, H., & Boydak, M. (2003). Lipid and lipoprotein levels in dairy cows with fatty liver. *Turkish Journal of Veterinary and Animal Sciences*, *27*, 295–299.

Suttle, N. F. (2010). *Mineral nutrition of livestock* (4th ed.). London UK: Cabi.

Taylor, G. J. (2007). *Factors affecting the production and reproduction performance of tropically adapted beef cattle in Southern Africa* (Doctoral dissertation, University of Pretoria).

Tucho, T. T., & Ahmed, W. M. (2017). Economic and reproductive impacts of retained placenta in dairy cows. *Journal of Reproduction and Infertility*, *8*, 18–27.

Uluozlu, O. D., Tuzen, M., Mendil, D., & Soylak, M. (2007). Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. *Food chemistry*, *104*, 835–840.

van Niekerk, W. A., Hassen, A., & Bechaz, F. M. (2010). Influence of growth stage at harvest on fermentative characteristics of Panicum maximum silage. *South African Journal of Animal Science*, *40*, 334–341.

Van Oudtshoorn, F., & Van Wyk, E. (2012). *Guide to grasses of Southern Africa* (3rd revised ed.). Pretoria: South African National Biodiversity Institute.

Velladurai, C., Selvaraju, M., & Napolean, R. E. (2016). Effects of macro and micro minerals on reproduction in dairy cattle a review. *International Journal of Scientific Research in Science and Technology*, *1*, 68–74.

Wang, K., Cui, K., Liu, G., Luo, X., Huang, J., Nie, L., … Peng, S. (2017). Low straw phosphorus concentration is beneficial for high phosphorus use efficiency for grain production in rice recombinant inbred lines. *Field Crops Research*, *203*, 65–73.

Wu, X., Sun, H., Xue, M., Wang, D., Guan, L. L., & Liu, J. (2018). Serum metabolome profiling revealed potential biomarkers for milk protein yield in dairy cows. *Journal of Proteomics*, *184*, 54–61. https://doi.org/10.1016/j.jprot.2018.06.005

Yatoo, M., Parray, O., Ashraf, T., Ashraf, S., Nisar, M., & Taifah, S. (2018). Longitudinal study on evaluation of serum micromineral and hormone interrelation in cattle. *Journal of Pharmacognosy and Phytochemistry*, *7*, 1201–1203.

Zhu, Y., Peng, X., & Ling, G. (2017). An update on the animal models in hyperuricaemia research. *Clinical and Experimental Rheumatology*, *35*, 860–864.

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