Minerals and Serum Metabolites Profile in Cows Reared on Natural Pastures in a Semi-Arid Area

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Abstract: This study aimed to profile minerals and metabolite levels in cows solely reliant on natural pastures. Mineral plays a significant role in cattle production and reproduction. Measures of serum metabolites are good indicators in disease diagnosis and nutritional status of animals. A total of 179 blood samples were collected from cases of cows of different ages and breeds experiencing dystocia (n=50), downer cow syndrome (n=34), vaginal prolapse (n=16), retained placenta (n=13), and abortions (n=69) following reports submitted to the North-West (NWU) Animal Hospital in Mafikeng Campus. Analysis of minerals and serum metabolite were done using ICP-MSand IDEXX Catalyst Chemistry Analyser. The data were coded and analysed using the Statistical Software for Social Scientists (SPSS) version 25. Descriptive statistics were used to show the distribution of the minerals and serum metabolites levels across the reproductive conditions. The results were significantly different at 5% ($P < 0.05$). Low zinc levels were seen in cows with abortions and dystocia. In downer cow syndrome, the levels of iron, magnesium and phosphorus were high. Low Iodine levels were seen in cows with vaginal prolapse, retained placenta, abortion and dystocia. Low phosphorus levels were noted in cows with vaginal prolapse. Significant differences were seen in the concentrations of Urea/BUN, Total bilirubin, Aspartate amino-transferase (AST), ammonia and lipase. The levels of minerals and serum metabolites are significant indicators in cows presenting with different reproductive conditions and could affect production.

Keywords: Minerals, serum metabolites, cow reproduction, animal health.

INTRODUCTION

In animal health, mineral imbalances have been implicated in the increase of an animal's susceptibility to infections and the risk of reproductive conditions [1]. Mineral irregularities such as deficiencies or toxicities can adversely influence the animal's health [2]. Minerals are known for their contribution to several physiological processes such as cell formation and participate in activities of vitamins, enzymes and hormones. Deficiencies and toxicities of certain mineral elements may cause reproductive disorders as minerals play an important role in the health and reproduction of livestock [3]. Animal reproduction, health and growth can be affected by immunological or physiological disorders which arise as a result of mineral deficiency [4].

Serum metabolic profiles have been widely used in animal health as a predictive tool for abnormalities in the reproductive system and in risk assessment of cattle metabolic disorders [5]. Disturbed metabolic processes are related to a rise in reproductive conditions [6]. Pathophysiological reaction and health status of the animal can be indicated by alterations in serum metabolites [7]. Alteration in metabolic status may subsequently lead to negative energy balance (NEB) due to reduced feed intake experienced in the peri-perturm period and ultimately affect the general

reproductive health [8]. Any deficiency or excess in minerals affects reproductive health and imbalances in serum biochemical parameters are indications of poor nutritional status.

Poor reproductive health in cattle directly relates to negatively affected economy and livelihoods of farmers who rely mainly on production yields. Sustainable food safety and security can be affected by the occurrence of metabolic disorders which are known to cause impaired fertility and complications in subsequent pregnancy which ultimately cause poor reproductive performance. Therefore, this study aimed to profile serum minerals and metabolites levels as biomarkers in cows (experiencing retained placenta, vaginal prolapse, dystocia, downer cow syndrome and abortion) solely reliant on natural pastures.

MATERIALS AND METHODS

Study Site

This study was conducted in the Mafikeng area of the North-West Province in South Africa. The area is geographically located at 25°38'E and 25°51'S coordinates. The area has a temperature range of 22 to 35°C which is typically experienced during summer which is encountered between August and March with an annual rainfall averages from 200 to 500 mm [9]. In this study location, the winter climate with chilly nights and sunny days are seen from May to July with temperatures ranging from 2 to 20°C [10]. The Mafikeng area, in total size, is approximated to be about 3 703 square kilometres large [9].

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Sample Collection and Preparation

A convenient sampling method was employed, and samples were collected during ambulatory farm visits. In this study cows of different ages (2-7 years), breeds (Brahman, Nguni, Bonsmara, Angus, Afrikaner, Simmental, Drakensberger and Mixed breeds), parity (1-8) and body condition score (1-5) were used. Blood samples from cows experiencing reproductive conditions were collected as the cases were reported to the North-West University Animal Hospital. A total of 179 samples were collected from cows with cases of vaginal prolapse (n=16), downer cow syndrome (n=34), retained placenta (n= 10), abortion (n= 69) and dystocia (n= 50). Blood samples were collected from the jugular vein into red stoppered tube, packed into a cooler box and transported on ice to the North-West University Laboratories for analysis. Serum was harvested through blood centrifugation at a speed of 2 500 rpm for 10 minutes at room temperature, within 2 hours after collection to allow clotting. The serum was then frozen at -20°C until analysis.

Minerals Analysis

The blood serum samples were further analysed for zinc (Zn), copper (Cu), iron (Fe), iodine (I) and selenium (Se) using NexION 300X ICP-MS (Inductively Coupled Plasma Mass Spectrometer) machine (Perkin-Elmer, Waltham, USA). Samples were first prepared as follows: 0.7 ml of defrosted serum was transferred into sterile tubes, followed by preparing Trichloro-acetic acid (TCA) (50g TCA into 500 ml of demineralised water), then adding 6.650 ml of TCA to 0.7 ml of serum, after which the tubes were covered and shaken individually on an electric stirrer, left for 10 minutes on the bench, then centrifuged at 2 500 rpm (revolutions per minute) for 10 minutes. From each tube 5 ml of the supernatant was transferred to clean test tubes, 5 ml of TCA was added to 5 ml of the supernatant fluid to make 10 ml, the preparation was left overnight. Samples were filtered using Whatman qualitative filter papers. About 10 ml of each sample was placed in 15 ml centrifuge tube, then the samples were analysed using the ICPMS 300X machine [11].

Instrument Conditions for the ICP-MS (Inductively Coupled Plasma Mass Spectrometry)

All chemicals that were used were analytical grade quality. Ultrapure water was obtained from a Millipore water system (Millipore), and the ultrapure Nitric acid (HNO_3, Merck) was used to digest the samples. Stock standard solutions of Arsenic and Mercury containing $10\mu g/mL$ in 2% HNO_3 were prepared as per the procedure described by [11]. Certified reference materials (CRM) (National Institute of Standard Technology NIST-8436) were used for the standardization and validation of the method.

Serum Metabolites Analysis

Samples were analysed using the IDEXX Catalyst Chemistry analyser according to the manufacturer's instructions. Briefly, for serum samples 300 μL is the recommended sample volume when running a Chem 17 CLIP and electrolytes. Load the sample (300 μL serum into the sample cup), then place slides and pipette tips in the sample drawer. Then close the sample drawer and press the Start button on the analyzer.

Statistical Considerations

The data were analysed using SPSS version 25. The effect of reproductive conditions on the mineral and serum metabolite composition of affected animals was tested using the Multivariate Analysis of Variance (MANOVA). Individual ANOVA tests were then used to test the effect of each reproductive condition on the mineral composition of affected animals. For the significant ANOVA tests, a *post-hoc* test (Tamhane T2) was used to determine the statistical significance and the magnitude of the differences in the mean levels of each mineral or serum metabolites between pairs of reproductive conditions (i.e. the mean of calcium in Downer cow syndrome minus (-) the mean of calcium in Vaginal prolapse = the mean difference). Tamhane T^2 is a *post-hoc* test for determining the significance and the magnitude of the difference in means based on treatments. Since the animals under each type of treatment (reproductive conditions) are unequal, equality of variances is not assumed in this study and Tamhane T^2 is known to give accurate results when the equality of variances is not assumed [12].

RESULTS

Minerals and serum metabolites were analyzed from blood samples of cows with reproductive condition. The study hypothesized that the types of reproductive conditions (retained placenta, dystocia, vaginal prolapse, downer cow syndrome and abortion) do influence the mean levels of minerals (Magnesium, phosphorus, copper, zinc, selenium, iodine and iron) and serum metabolites (Urea/BUN, phosphates,

calcium, total protein, aspartate amino-transferase, gamma-glutamyl transferase, total bilirubin, cholesterol, ammonia, triglycerides, lipase and creatinine kinase.

Table **1** shows that all the multivariate tests (Pillai's Trace, Wilk's Lambda and Hotteling's Trace) were significant at 1% (P <0.01), therefore indicating that the type of reproductive condition had an effect on at least one of the mean levels of the minerals, serum metabolites and hormones of the affected animals. Table **1** identifies the exact serum metabolites whose mean levels are affected by the types of reproductive condition.

Table **2** shows that mean magnesium (Mg) levels from animals affected by downer cow syndrome and retained placenta were significantly (P < 0.01) greater than the upper limit of normal level of magnesium (Mg) 12.019-36.603 mg/L (Table **2**). Selenium (Se) levels were significantly higher than the upper limit of normal range (0.003-0.339 mg/L) across all the conditions (Table **2**). Significantly lower zinc (Zn) concentrations were observed in abortion and dystocia cases since the normal range is 1.2115-2.25 mg/L (Table **2**).

Magnesium and phosphorus mean concentrations were the highest in downer cow syndrome and the less in dystocia across the conditions (Figure **1**). Retained

Table 1: Multivariate Tests for Minerals and Serum Metabolites in Reproductive Conditions

	Minerals Sig. (P-Value)	Serum metabolites Sig. (P-Value)
Pillai's Trace	0.000	0.000
Wilks' Lambda	0.000	0.000
Hotelling's Trace	0.000	0.000

Table 2: One-Sample Test for Mean Mineral Concentration in Affected Cow

Reproductive Conditions	Mineral mean concentration (Mg/L)						
	Magnesium (Mg)	Phosphorus (P)	Copper (Cu)	Zinc (Zn)	Selenium (Se)	Iodine (I)	Iron (Fe)
Downer cow syndrome	46.945*	42.751*	0.988	1.165	22.865*	2.789*	12.688*
Retained placenta	40.606*	37.365*	1.441*	1.818	26.614*	6.792	5.383*
Vaginal prolapse	26.194	27.076*	1.024	1.443	25.638*	0.335*	6.674*
Abortion	29.051	27.248*	1.720	0.642*	18.717*	1.489*	5.119*
Dystocia	25.007	18.782*	0.449*	0.440*	11.423*	2.245*	6.701*

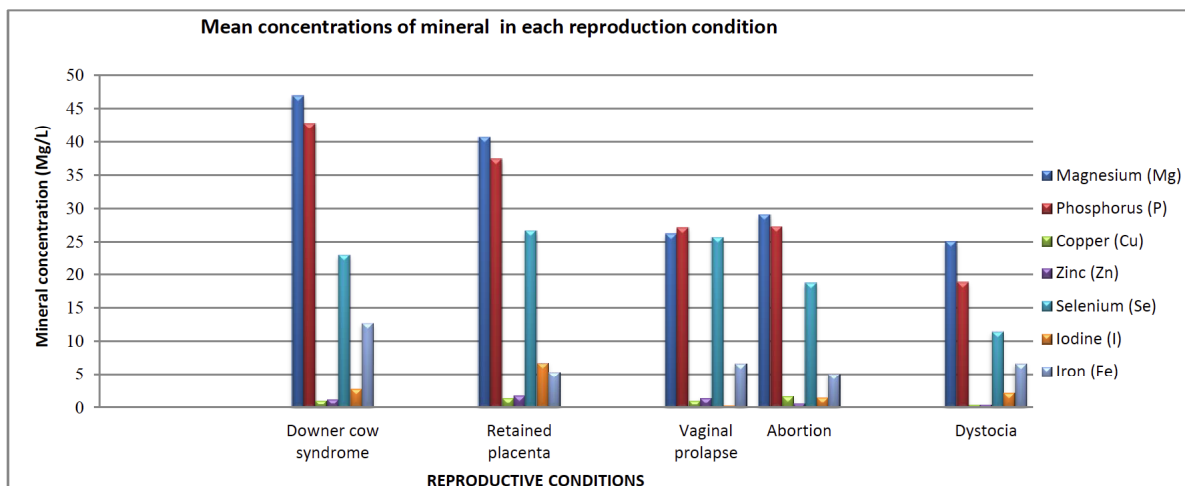**= Significant at 1% level of significance (P< 0.01).

Figure 1: Mineral mean concentrations across different reproductive condition.

placenta cases exhibited highest selenium mean concentrations while the least amounts were seen in cases of dystocia (Figure **1**). Iron mean concentrations were the highest in downer cow syndrome cases with its lowest level seen in abortion cases (Figure **1**).

The highest level of cholesterol was seen in vaginal prolapse, with the least of its measure seen in cows with retained placenta (Figure **2**). Figure **2** shows the Urea concentrations being higher in animals presented with dystocia, while the minimum was observed in animals presented with abortion cases. It is also

Table 3:   Serum Metabolites Concentrations across the Type of Reproductive Condition in Cows

Reproductive Conditions	Serum metabolite mean concentration											
	BUN (mmol/L)	PHOS (mmol/L)	CA (mmol/L)	TP (g/L)	AST (U/L)	GG (U/L)	TBIL (µmol/L)	CHOL (mmol/L)	NH3 (µmol/L)	TRIG (mmol/L)	LIPA (U/L)	CK (U/L)
Retained placenta	4.919	7.328	1.994	80.143	114.643	15.857	7.357	2.768	898.571	0.172	93.143	74.00
Downer cow syndrome	6.891	2.128	2.284	74.941	167.029	23.441	7.074	2.829	822.912	0.561	74.00	320.294
Abortion	4.12	1.932	18.179	73.754	113.493	18.942	6.472	16.234	686.51	0.327	74.71	395.058
Dystocia	7.731	5.285	2.33	71.757	201.1	36.56	9.92	2.946	629.38	0.438	132.92	325.40
Vaginal prolapse	3.565	1.754	1.836**	76.824	102.647**	24.941	7.706	17.835	613.059**	0.889	82.00	321.471**

**= Significant at 1% level of significance (P< 0.01); BUN=Urea; CA= Calcium; AST =Aspartate amino-transferase; GGT= Gamma-glutamyltransferase; Total bilirubin (TBIL); Total protein (TP); CHOL = Cholesterol; NH3 = Ammonia; TRIG = Triglycerides, LIPA= Lipase; CK = Creatinine kinase.
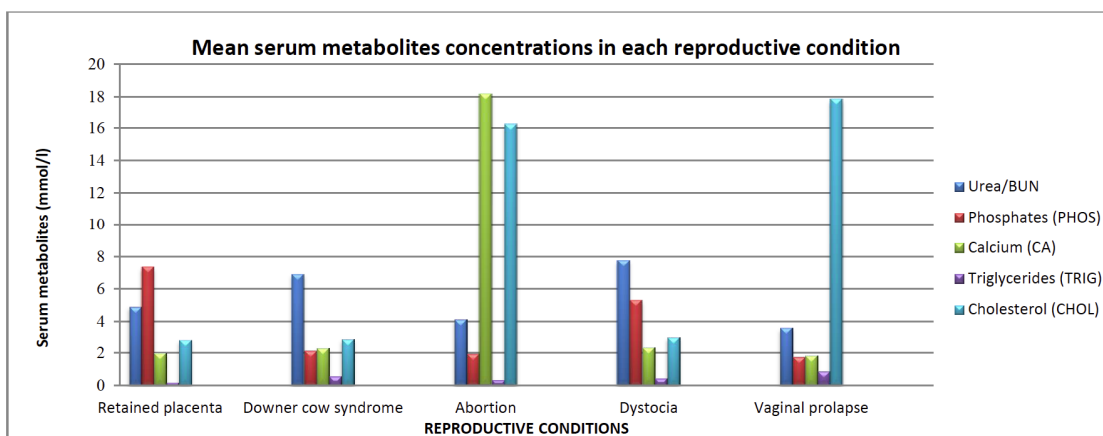

Figure 2: Mean concentrations of urea, phosphates, calcium, triglycerides and cholesterol in cows.
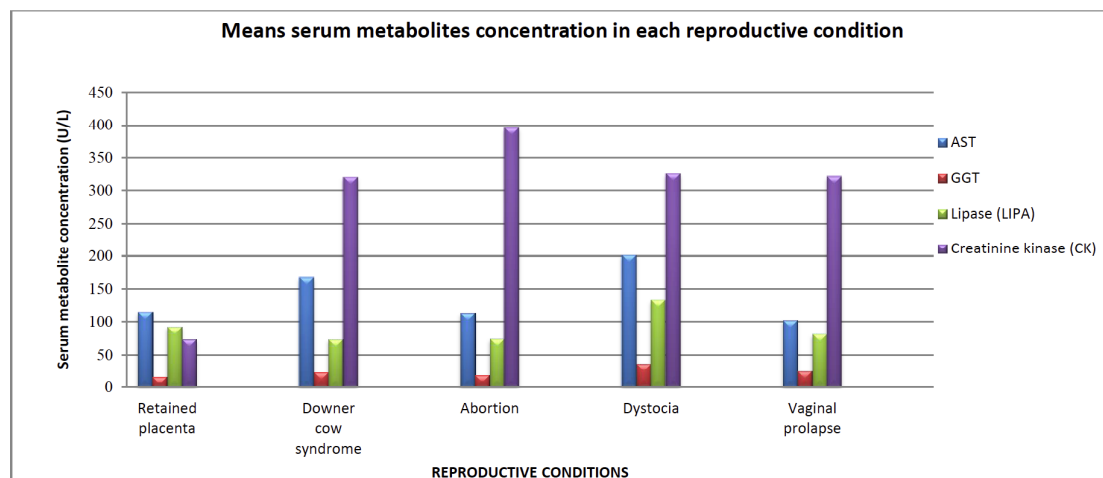

Figure 3: Mean concentration of serum AST, GGT, LIPA and CK in different condition.

Table 4: Summary of Predictive Characteristics which could Increase the Risk of Cows Experiencing Reproductive Conditions

Conditions	Serum metabolites	Reference values	MINERALS	Reference values
Dystocia	High AST -High urea/ BUN	Ammonia (0–90 µmol/L)** Aspartate amino transferase (0–91 U/L)**	-Low phosphorus, copper, zinc selenium and iodine -High Iron	Copper (7.801-23.621 µmol/L)***
Retained placenta	High AST-Low calcium	Calcium (2.2–2.62 mmol/L)* Cholesterol (1.96–5.86 mmol/L)** Creatinine kinase (0–110 U/L)**	-High magnesium, selenium, iron -Low phosphorus	Iodine (4.960-19.850 µg/L)*** Iron (12.500-44.811 µmol/l)***
Vaginal Prolapse	High AST -Low calcium	Gamma-glutamyl Transferase (0–80 U/L)** Lipase (30–400 U/L)** Phosphates (1.38–2.55 mmol/L) **	-Low phosphorus and iodine -High selenium and iron were high	Magnesium (0.4945-1.506 mmol/L)*** Phosphorus (0.720-6.400 mmol/L)***
Abortion	High AST -Low calcium	Total bilirubin (2.8-9.3 µmol/L)* Total Protein (8–80 g/L)** Triglycerides (0.08–0.20 mmol/L)*	-Low phosphorus and iodine -High selenium	Selenium (0.024-3.000 µg/dL)*** Zinc (10.710-19.890 µmol/L)***
Downer cow syndrome	High AST-High urea/ BUN	Urea/BUN (2.50–6.7 mmol/L)*	-High magnesium, selenium, copper and Iron -Low phosphorus and iodine are lower	

Sources for reference values: *[13]; **[14], ***[15].

observed that phosphates levels was found to be the highest in retained placenta cases while low in vaginal prolapses (Figure **2**).

Serum AST was seen to be the highest among dystocia cows and was the least in vaginal prolapse compared to all other conditions (Figure **3**). Dystocia cows had elevated concentration of GGT than other conditions with its lowest seen in retained placenta cases (Figure **3**). Serum lipase concentration amounting to nearly 150 U/L in dystocia cases was the highest among all the reproductive conditions and was low in downer cow syndrome (Figure **3**). Creatinine kinase was the highest (close to 400 U/L) among aborting cows and amount to above 300 U/L among downer cow syndrome, dystocia and vaginal prolapse, with its lowest measure seen in retained placenta cases (Figure **3**). Creatinine kinase was the highest (close to 400 U/L) among aborting cows and amount to above 300 U/L among downer cow syndrome, dystocia and vaginal prolapse, with its lowest measure seen in retained placenta cases (Figure **3**).

Table **4** shows summary the results of the current study, indicating the abnormal concentrations (compared to the normal values) of minerals and serum met acolytes across the different reproductive conditions.

DISCUSSION

The current study aimed to profile minerals and serum metabolites concentrations in cows presenting with dystocia, retained placenta, dystocia, vaginal prolapse and abortion. The results indicated significant differences in several minerals and serum metabolites concentrations in cows presenting with various reproductive conditions (Table **1**). Particularly, magnesium concentration was found to be high in downer cow syndrome cases (Table **2**). This could be as a result of usually calcium decrease seen in downer cow syndrome, however, this was not the case in the present study, as the calcium concentrations were normal in downer cow (Table **2**). Nonetheless, research has shown that magnesium increase in downer cows is typically due to lowered cellular uptake, bone metabolization and lowered urinary loss of magnesium [16]. The magnesium increase in this study suggests a linkage between downer cow syndromes with nutrient deficiency.

Phosphorus (P) mean concentrations were the highest in downer cow syndrome than in other conditions (Figure **1**). It is typically expected that phosphorus level will be low, because reduced phosphorus levels have been associated with metabolic disorders including downer cow syndrome [17]. Also due to the release of para-thyroid hormone during hypocalcaemia, there is an increased salivary and urinary phosphorus loss, hence hypocalcaemic cows are inclined to be hypophosphataemic [18]. However, other studies have shown that transitioning cows consuming feed mixtures high in phosphorus levels are at 6-9 times risk of developing of

hypocalcemia [19, 20]. Additionally, low blood calcium concentration is known to be the primary cause of downer cow syndrome in post-parturient cows [21]. Another research also reported that cows are predisposed to hypocalcaemia which arises from intake of excess dietary phosphorus which inhibits the renal enzyme (1a-hydroxylase) necessary for catalysing vitamin D conversion into its active form (1,25(OH)2D3); hence hypocalcemia in cows [22], which could be the reason for the high levels of phosphorus in downer cow syndrome presented in this study.

Significantly low phosphorus concentrations were seen in cows with vaginal prolapse and abortion (Table **2**). Similar finding have been presented indicating that phosphorus and calcium deficiencies are among various etiological factors linked to the incidences of vaginal prolapse, together with hormonal imbalance [23, 24]. In the dystocic cows the mean concentrations of phosphorus were low (Figure **1**). On the contrary, a previous study reported high phosphorus in cows with dystocia as compared with those that calved without difficulty [25]. Nonetheless, an earlier study of [26] indicated that the incidence of dystocia can be influenced by nutrient insufficiencies, which could explained the low P concentration in dystocia cows being due to low phosphorus feed intake.

The mean iron (Fe) concentration in retained placenta, vaginal prolapse, abortions and dystocia were all significantly low (Table **2**). Likewise, low iron levels has been associated with the incidence of retained placenta [27, 28]. Other studies have also related iron deficiency with abortion due to its role in foetal growth [29, 30]. Additionally, mineral disproportion in animals have been associated with impaired immune functions leading to deteriorated health, less appetite and poor body condition [31, 32], this could explain the out of balance iron concentrations in vaginal prolapse, abortions and dystocia in the present study.

Copper (Cu) deficiencies have been linked to increased incidence of embryonic death and foetal placental membrane retention [4]. However, such associations were not made in the present study, as copper concentrations were high in retained placenta and abortions cases (Table **2**). Similar observations were reported in a study of [33] which indicated high concentrations of copper in aborted cows and retained placenta cases, which are normally seen after dystocia. Serum copper concentrations has also been said to

increase due to excessive intake (over supplementation) and liver damage [34].

Low zinc (Zn) mean concentrations are typically evident in aborting cows than in non-aborting cows due to dietary deficiencies [35]. Zinc concentrations are also low in cows with retained placenta which increases the risk of abortion in subsequent pregnancies [36]. The lowered zinc concentrations may be as a result of high calcium and phosphorus which decrease intestinal zinc absorption [37]. The current study has as well shown significantly low mean concentration of Zn in retained placenta and aborting cows (Table **2**).

Cows with selenium deficiency are likely to abortions and have retained placenta [38, 39]. However, the present study found significantly high selenium concentration in abortions, vaginal prolapse and downer cow syndrome (Table **2**). This is in line with reports by [2] indicating that high selenium may cause pregnant cows to abort due to toxicity. Selenium has a small range differences relative to toxicity and deficiency, for that reason its addition to feeds should be regulated, as toxicity may occur due to intake of high selenium in forages or excess supplementation [38, 41]. Those could be reasons for increased levels of selenium in retained placenta cases in the current study (Figure **1**).

Significantly low mean concentrations of iodine were seen in cows with abortions, dystocia, downer cow syndrome with the lowest quantity seen in vaginal prolapse (Table **2**). A decrease in iodine concentrations may lead to abortions [40]. Vaginal prolapses may occur as a result of poor body condition with a reduction of the fat surrounding the vagina as a result of nutritional insufficiencies [42]. The incidence of downer cow syndrome may as well be seen in times of nutrient insufficiencies [43]. Iodine deficiency has functional characteristics of influencing reproductive sufficiency in cows [2].

Measures of serum metabolites have been used as predictive tools to precisely identity animal's nutritional status by indicating nutrient metabolism in the body [44]. Another study indicated that serum metabolites are also indicators for energy deficit [45]. A study by [46] linked the high urea concentrations leading to pH reduction in the uterus which can be harmful to the embryo implantation and development, hence the association between abortion and elevated blood urea nitrogen. However, the present study did not find such

association as the Urea mean concentration in abortion cases was ($\bar{x}$ =4.12 mmol/L) within normal ranges (2.50–6.7 mmol/L) seen in Table **3**. Other studies have associated urea concentrations with the incidence of dystocia in cows [47, 48]. Similarly, the present study found serum urea concentration to be higher in dystocia cases (Figure **2**). This association could be due to high urea levels known to lower calcium which is responsible for maintaining adequate contraction during parturition [49].

Changes in metabolism can elevate AST levels in blood as a result of excess fat accumulation in the liver as the cow transition during the peri-partum period [50]. AST enzymes are also significant predictors for soft tissue damage in various organs, primarily liver and cardiac muscle [14]. Furthermore, authors have associated high AST concentration with dystocia, retained placenta, metritis, endometritis and poor reproductive performance in cows [45]. This is in agreement with the findings of the current study which indicates that serum AST concentrations were significantly high in dystocia and low in vaginal prolapse (Table **3**). Proper muscular function is important to allow smooth parturition and avoid difficulty during birth [50]. This could explain AST imbalances seen in dystocia cases.

High total bilirubin concentrations is associated with dystocia in cows [45]. This is in line with current results showing significantly high serum total bilirubin concentrations in cows with calving difficulties (Table **3**). Figure **2** shows the highest measure of TBIL cows with dystocia. The multiple comparison of TBIL has indicated that across the reproductive conditions, significant associations were only seen in the abortion and dystocia interaction with the mean difference showing higher TBIL in dystocia (Table **3**). Total bilirubin is a liver function indicative metabolite, and its increase is significantly association with fatty liver condition known to negatively influence fertility, production, immune function and increased energy deficits [51].

Intensive energy deficit is related to the high circulatory ammonia concentration, due to increased energy requirements during amino acid transamination necessary for excess NH_3 excretion [52]. Significantly high values of ammonia were evident in the present study in the cases of abortion (Table **3**). This is in agreement with previous reports showing that high systemic NH_3 compromise initial embryo growth in the

oviduct which could lead to embryo mortality [53]. Elevated ammonia concentration has also been linked to impaired fertility and poor reproductive health [54]. Serum ammonia concentrations were the highest in cows with dystocia and the least in those with retained placenta (Figure **2**). This could be linked to the excessive energy deficiency which has been recognised as a cause of dystocia in cows [55]. Excess ammonia in the blood is toxic; this may be seen not as converting ammonia to less toxic form for excretion as a result of liver disorders [53].

CONCLUSION

Magnesium levels are usually high in downer cow syndrome and retained placenta than in vaginal prolapses. Iron, copper, zinc and selenium concentrations are much higher in downer cow syndrome as compared to those in dystocia cases. The phosphorus levels in dystocia are lower than in retained placenta, vaginal prolapse and abortion. In domestic animals, adequate amounts of minerals in their biologically useful forms are essentially required for optimum production and reproduction. Urea/BUN concentrations in downer cow syndrome and dystocia were higher than normal. In case of abortions, retained placenta, and vaginal prolapse calcium concentrations were low. The concentrations of AST in dystocia, retained placenta, vaginal prolapse, abortion and downer cow syndrome were higher than normal. This study concludes that the incidences of reproductive conditions do affect serum metabolites in cows. Therefore, serum metabolites are useful predictors of peri/postpartum disorders, productivity and reproductive performance in cows.

ETHICS APPROVAL, AND CONSENT TO PARTICIPATE, HUMAN AND ANIMAL RIGHTS

The Animal Health Research Ethics Committee approved and granted permission for the study to be conducted after receiving approval from the North-West University Research Ethics Regulatory Committee (NWU-RERC); Ehics number: NWU-00409-18-A5. All animal owners gave consent to participate and allowed us to work with their animals. All animal subjects were handled humanely, blood samples were collected by qualified animal health technicians.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

ABBREVIATIONS

TBIL	=	Total Bilirubin
NH3	=	Ammonia
CHOL	=	Cholesterol
UREA/BUN	=	Blood Urea Nitrogen
PHOS	=	Phosphates
TRIG	=	Triglycerides
CA	=	Calcium
TP	=	Total Protein
AST	=	Aspartate Amino-Transferase
Zn	=	Zinc
Cu	=	Copper
Fe	=	Iron
I	=	Iodine
Se	=	Selenium
TCA	=	Trichloroacetic Acid
SPSS	=	Statistical Package for the Social Sciences
ANOVA	=	Analysis of Variance
MANOVA	=	Multivariate Analysis of Variance
ICP-MS	=	ICP-MS
CRM	=	Certified Reference Materials
NRF	=	National Research Foundation
HWSETA	=	Health and Welfare Sector Education and Training Authority

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