

**The role of mineral supplementation on the incidence of
bovine reproductive conditions in communal areas
around Mafikeng of the North West Province**

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DECLARATION

I, Molefe Keitiretse hereby declare that THE ROLE OF MINERAL SUPPLEMENTATION ON THE INCIDENCE OF BOVINE REPRODUCTIVE CONDITIONS IN COMMUNAL AREAS AROUND MAHIKENG OF THE NORTH WEST PROVINCE is an original research done by me and it has never been published or submitted for a degree at any other institution.

.....

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ABSTRACT

The aim of this study was to determine the metabolic characteristics of bovine presenting with reproductive conditions in order to establish important predictors for their incidence in communal farms in the Mafikeng District Municipality in South Africa. To achieve that, the current study evaluated concentrations of serum minerals, biochemical parameters and reproductive hormones along with the management of the cows that presented with abortion, dystocia, vaginal prolapse, retained placenta and downer cow syndrome. This research was conducted in Mafikeng by following up cases of reproductive conditions brought to the North West University (NWU) Dale Beighle Animal Health Centre. A convenient sampling method was used in the collection of blood samples from cows presenting with reproductive conditions during ambulatory trips to different communal farms. A total of one hundred and eighty two (182) cases were attended to and blood samples from cows presenting with retained placenta (n=13), abortion (n=69), downer cow syndrome (n=34), dystocia (n=50) and vaginal prolapse (n=16) were collected. Additional to that, a structured questionnaire was administered to assess the management issues in the different affected herds, for which 135 farmers were interviewed on each reproductive condition or case attended. Furthermore, for confirmation of data, a controlled study on the effect of mineral supplementation during pregnancy was run on an identified farm which previously had presented several reproductive conditions among its animals. In this study, 12 pregnant cows were chosen and divided into two groups: a control and an experimental group with six cows in each. A randomly selected sample of primiparous and multiparous cows aged between 3-5 years, with body weight between 347 and 540 kg and parity of 1 to 2 were assigned to the control (non-supplemented) or experimental group (supplemented). The cows in the experimental group were supplemented, at 6-week intervals, from mid- to late gestation with MULTIMINTM + Se +Cu at 1ml/45 kg BW and Calci 50 p.i. at 100-150 ml/500kg BW dosage. Blood samples were collected before the experiment and after every six weeks when supplements were given. Sample analysis for minerals, serum metabolites and hormones were performed using the Inductively Coupled Plasma Mass Spectrometer (ICP-MS), IDEXX Catalyst and ELISA kits,

respectively. The data were statistically analysed using SPSS version 25 to calculate frequencies and descriptive statistics. Pie charts were used to summarise the responses for each variable in the questionnaire. The Chi-square test of association was used to determine the possible association between the occurrence of the reproductive conditions and the various attributes of the cows as well as the farm management practices. A two-stage cluster analysis was used to segment the animals based on the variables associated with the reproductive conditions as identified by the Chi-square test. The effect of the reproductive conditions on the serum biochemistry, hormonal and mineral profiles of affected animals was estimated by means of the Multivariate Analysis of Variance (MANOVA). A post hoc test (Tamhane T2) was used for the significant ANOVA tests, to determine the statistical significance and the magnitude of the differences in the means of each serum metabolite and mineral between pairs of reproductive conditions. The data generated from interviewing farmers showed that most communal farms were headed by males (79%), aged between 40-55 years; most had primary level of education (76%), were single (68%) and the majority had monthly income of <R1000.00. The cows which were affected by the reproductive conditions were mostly in their first parity (49%), they were from free-range farms (58%), from herds with only one annual veterinary checkup (71%) and had unknown Brucella status (100%). In this study, analysis of blood in cows with reproductive disorders revealed a significant ($P<0.05$) relationship between dystocia cases and high selenium (11.423 mg/L) and iron (6.701 mg/L) concentration. Cows with dystocia had significantly ($P<0.05$) low mean concentrations of phosphorus (18.782 mg/L), copper (0.449 mg/L), zinc (0.440 mg/L) and iodine (2.245 mg/L). The serum metabolic profile in cows that presented with dystocia also revealed significantly ($P<0.05$) lower mean concentrations of urea/BUN (7.731 mmol/L), AST (201.1 U/L), creatinine kinase (325.40 U/L) and ammonia (629.38 μ mol/L). The hormonal assessment in cows with dystocia showed significantly higher mean concentrations of estradiol (862.09 ± 123.44 pg/ml), oxytocin (370.50 ± 71.66 pg/ml), as well as lower progesterone (8.59 ± 0.402 ng/ml). In cases of downer cow syndrome, concentrations of magnesium, selenium and iron were significantly ($P<0.05$) higher with respective means of 46.945 mg/L, 22.865 mg/L and

12.688 mg/L. Significant differences were seen in mean levels of urea/Bun (6.891 mmol/L), AST (167.029 U/L), ammonia (822.912 μ mol/L) and creatinine kinase (320.294 U/L) which were found to be higher in downer cows. Additionally, significantly ($P<0.05$) lower mean progesterone (4.40 ± 1.222 ng/ml) and higher estradiol (781.32 ± 135.70 pg/ml) concentrations were noted in downer cows. Significantly higher levels of magnesium (40.606 mg/L), selenium (26.614 mg/L) and iron (5.383 mg/L) were seen in cows having retained placenta. The results also indicated that significantly ($P<0.05$) lower calcium, high AST and ammonia were noted in cows with retained placenta with mean concentrations of 1.994 mmol/L, 114.643 U/L and 898.571 μ mol/L, respectively. The study also found that high prostaglandins (92.06 ± 45.57 pg/ml) and lower progesterone (3.78 ± 0.151 ng/ml) concentrations were significantly ($P<0.05$) related to incidence of retained placenta. The mean concentrations of phosphorus (27.248 mg/L), zinc (0.642 mg/L) and iron (5.119 mg/L) were significantly lower than the normal range in aborting cows. In addition, mean concentration of calcium (18.179 mmol/L), AST (113.493 U/L), ammonia (686.51 μ mol/L) and creatinine kinase (395.058 U/L) were significantly ($P<0.05$) higher in cases of abortion. Highly significant variations were seen in aborting cows with higher estradiol (1122.99 ± 71.99 pg/ml), prostaglandin (300.41 ± 24.48 pg/ml), oxytocin (574.73 ± 60.65 pg/ml) concentrations as well as lower progesterone (2.45 ± 1.509 ng/ml). The incidences of vaginal prolapse was significantly ($P<0.05$) related to high selenium (25.638 mg/L) and iron (6.674 mg/L) concentrations, as well as lower concentrations of phosphorus (27.076 mg/L) and iodine (0.335 mg/L). The study also found significantly ($P<0.05$) lower progesterone (4.67 ± 0.301 ng/ml) and high prostaglandins (241.84 ± 28.35 pg/ml) concentrations in cows presenting with vaginal prolapse. In the study, all six cows in the experimental group (supplemented with MULTIMINTM + Se +Cu and Calci 50 p.i.) during pregnancy did not experience any reproductive difficulties, whereas two of the cows from the non-supplemented group (control) presented with a retained placenta and dystocia each.

The general profile of farmers in the communal areas showed older males, with low educational level and not earning more than a thousand rand per month. These characteristics play a significant role in

a production process as they can limit the farmer's adoption of innovative practices due to information inaccessibility and ultimately lower the farmer's contribution to profitable livestock agriculture. The breed, parity, knowledge about brucellosis, feeding system and access to veterinary services may influence incidence of reproductive disorders. Prioritisation of veterinary services through farmer training is necessary for knowledge transfer could help reduce the occurrence of reproductive losses resulting from poor farm management practices in communal farming. The occurrences of abortion, downer cow syndrome, vaginal prolapse, dystocia and retained placenta in communally reared cows are directly related to farm management practices. The measurements of minerals and serum metabolites provide a useful index for studying potential risk related to the occurrence of reproductive conditions and are very important supportive management tools for improving production as well as minimizing animal losses in communal farms. Hormonal disproportion during pregnancy limits and hinders reproductive performance and can be the cause of reproductive disorders and reduced production. The results of this study also suggest that proper mineral supplementation during pregnancy can improve reproductive capacity by increasing the energy and nutrient content in the feed consumed.

This study therefore recommends regular determination of mineral, serum metabolites, and hormone levels in expectant cows as important biomarkers for monitoring animal reproductive health. These parameters serve as good and useful indicators for reproductive conditions. In addition, this study provides a guiding tool useful in improving preventive strategies for reproductive conditions, which veterinary practitioners can use to reduce bovine losses and increase reproductive performance. It has also shown that management and adequate mineral supplementation during pregnancy reduces the risk of reproductive conditions in cows reared on natural pastures under semi-arid conditions.

DEDICATION

I dedicate my work to my family for believing that I have the capacity to achieve greatness and offering me all that they could for me to obtain my degree. To my brother, son, cousins, friends, all relatives and all who both directly and indirectly contributed to my success I say thank you a thousand times. All of us could not choose where we were born but we can choose where we want to go. Aim high, never stop believing, anything is possible. ‘God is no respecter of persons’, what He did for me He can also do for you.

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LIST OF ABBREVIATIONS

ALB	Albumin
ALT	Alanine amino transferase
AST	Aspartate amino-transferase
BCS	Body condition score
Ca	Calcium
CHOL	Cholesterol
CK	Creatinine kinase
Cr	Creatinine
Cu	Copper
Fe	Iron
GGT	Gamma-glutamyl Transferase
GLOB	Globulin
I	Iodine
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
MANOVA	Multivariate analysis of variance
Mg	Magnesium
OT	Oxytocin
P	Phosphorus
PGF2 α	Prostaglandins
PRG	Progesterone
Se	Selenium
TBIL	Total bilirubin
TP	Total protein
TRIG	Triglycerides
Urea	Blood Urea Nitrogen (BUN)
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The majority of communal farmers in South Africa rely mostly on livestock production. The different reasons for keeping cattle affect the level at which communal farmers contribute to the cattle production market. Except for monetary benefits (selling - mostly to the local villagers and rarely to the formal market), communal farmers have other reasons for keeping cattle such as cultural and social purposes (Jooste, 2001; Mazibuko, 2013). Some of the reasons why cattle are kept in many communities include for meat and milk supply for the families, crop fertiliser from the dung, religious rituals, funerals, wedding ceremonies and income generation. Some keep livestock as a form of savings to be used in times of economic downturns (Musemwa *et al.*, 2010; Mazibuko, 2013).

In communal areas, the state of cow reproductive ability is not precisely identified but generally presumed to be low (Ndlovu *et al.*, 2007; Thornton, 2010; Molefe, 2016). The incidence of disease and disorders increases during the transition period (late pregnancy to early lactation) consequently affecting productivity (Goshen & Shpigel, 2006; Santos *et al.*, 2009; Molefe *et al.*, 2016). The occurrence of either vaginal prolapse, downer cow syndrome, dystocia, abortion and retained placenta inflicts an extensively negative impact on the reproductive capacity in many communal herds. The relationship between reproductive ability and nutrition is a topic that has been receiving much attention from veterinary professionals, cattle producers as well as researchers in the field of nutrition (Pradhan & Nakagoshi, 2008).

The incidence of metabolic disorders may be seen as a result of the insufficiency of nutritional components such as proteins, minerals, water, disturbance in metabolic developments, extra

distribution of mineral to the foetus, increased forfeiture through milk, urine and faeces (Fikadu *et al.*, 2016). Proper adjustments and supplementation of the nutritional and hormonal balance in cows during pregnancy are essential to prevent the occurrence of reproductive conditions. In communal farming, a lack of proper record keeping, such as professionally designed and monitored disease control programmes are major constraints in cattle production and better livelihood in rural communities. Therefore, it was essential to conduct this research to address problems of cow reproductive performance in rural communities by means of identifying possible causes of reproductive conditions.

1.2. Problem statement

Farmers, particularly emerging farmers in communal set up are faced with losses due to reproductive conditions such as downer cow syndrome, dystocia, retained placenta, vaginal prolapse and abortion. Losses of cattle due to these reproductive disorders are high in communal farms and consequently affect productivity. Resultant from that, most rural farmers remain in poverty and farm production is also reduced and so retarding economic development by placing farmers in financially stagnant state. Increased knowledge about factors influencing the occurrence of reproductive conditions in communal farming is required to improve the effectiveness of treatment methods to apply, as well as in introducing appropriate and viable management plans which can lead to optimum cattle reproduction and raise general production on rural farms. After the identification of factors predisposing cows to the incidence of reproductive conditions, farmers could be trained to follow and properly implement farm management protocols which can prevent and reduce the level of reproductive losses in their herds. It is thus necessary to study factors linked to reproductive problems in communal farms, profile them and develop a tool as a reference parameter to be used as a predictor in healthy cows to prevent the occurrence of reproductive conditions.

1.3. Justification

Livestock production plays a significant role in the livelihood of communal farmers and is a source of employment for most poor rural communities in South Africa (Ainslie *et al.*, 2002). Good and proper reproductive health in cattle is directly related to production yield and contributes to the economy. Effective cattle production is necessary to help sustain food safety and security particularly in rural communities. An increase in the incidence of dystocia, retained placenta, vaginal prolapse, abortions and downer cow syndrome hinders growth and contributes to reduced productivity in cattle farming. Communal farmers in South Africa make up to half of the 14.1 million cattle ownership (Agriculture, 2008). However, the country still faces very high numbers of poor people and unemployment in most rural communities. The role and impact of reproductive wellbeing of cattle in communal farms requires much consideration and proper evaluation. Mafikeng is a semi-arid area and has numerous developing communal farms with cattle facing reproductive difficulties.

1.4. Hypothesis

Reproductive associated conditions such as abortion, vaginal prolapse, downer cow syndrome, retained placenta and dystocia in cattle are related to abnormalities in mineral, hormonal and serum biochemical balances. These conditions can be prevented by assessing serum mineral, hormonal and serum biochemical prior to parturition and by supplementing the pregnant cow with needed elements.

1.5. Research aim and objectives

1.5.1 Aim

This study aimed to establish physiological profiles of minerals, serum metabolites and hormonal levels for each specific reproductive condition (downer cow syndrome, dystocia, retained placenta, vaginal prolapse and abortion) and to study possible correlations between these profiles and each condition for all cases presented to the Animal health Hospital, North

West University between 2014 and 2019. This was done with the aim of developing a reference tool as a predictor for each condition that would assist animal care practitioners to detect or identify earlier the risk of the occurrence of these conditions and be able to prevent them or reduce that risk by supplementing with the missing elements.

1.5.2. Objectives

1. To identify the cattle production management practices that may predispose the animals to increased incidence of reproductive conditions in communal farming system in the Mafikeng local Municipality.
2. To profile each reproductive condition based on mineral, biochemical and hormonal levels.
3. To determine the correlation between each profile and the reproductive condition.
4. To evaluate the possible effects of minerals supplementation on reproduction of bovine that had previously presented any of the reproductive conditions.
5. To develop for each reproductive condition, a reference tool with indicative parameters that would assist animal care practitioners in preventing these conditions from occurring.

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

In cow reproduction, the transition period (late pregnancy and early lactation) is a significant contributor to both production efficiency and viability (Roche *et al.*, 2017). Normally as a means to support the new physiological state of lactation, there are hormonal changes to coordinate the transformation, through the process called homeorhesis (Neave *et al.*, 2017). Cows undergo several metabolic alterations as they transition from late pregnancy to early lactation (Ngangkham & Ajit, 2016). Nutritional demands for cows in the transitional period are usually very high (Dale *et al.*, 2016). The effectiveness and sustainability of nutritional and management programs during this period are directly related to prevalence of metabolic disorder and overall reproductive capacity thereafter (Ngangkham & Ajit, 2016). Simultaneously, the cow's inability to acclimatize to the environmental effects predisposes it to peri-parturient conditions.

Increased knowledge about adaptive processes and nutritional requirements during the transition period can improve cow management. Inability to account for the nutritional requirements to maintain development, production and reproduction in livestock still remains a challenge due to the imbalanced feed supply (Martinez *et al.*, 2014). The high demand for energy during the final 2-4 weeks of gestation is mostly to provide for foetal growth and colostrum production requirements, which if not met may result in negative energy balance weeks prior to delivery (Fikadu *et al.*, 2016). Cattle are capable to recompense for the insufficiencies of energy by metabolising fat in the body, yet metabolic diseases and problems in reproduction may occur because of over metabolism of fat (Bezerra *et al.*, 2014).

The incidences of dystocia, abortion, retained placenta, downer cow syndrome and vaginal prolapse in communal farms have been shown to have negative effects on cow reproduction (Molefe *et al.*, 2016). These conditions, in most cases, are seen concurrently or as a result of the other, which make them difficult to be effectively managed. According to McDougall (2001) hypocalcaemia may increase the occurrence of dystocia, while high numbers of retained placenta may arise as a result of dystocia. Another study further indicated that there is good evidence that both hypocalcaemia and dystocia predispose to post-partum diseases such as retained foetal membranes (RFM) and metritis (Benzaquen *et al.*, 2015).

In the application of treatment measures, factors such as hypocalcaemia, have to be given some consideration. Metabolic diseases have huge economic influences owing to the fact that they affect cows near the peak of their reproductive life (Fikadu *et al.*, 2016). Production loss impacts are felt more by the communal farmers due to the occurrence of reproductive conditions. Reduced dry matter intake (DMI) may lead to occurrence of other metabolic disorders (Melendez *et al.*, 2002). What is not well established is the mineral and energy status of cows affected with metabolic disorders (Benzaquen *et al.*, 2015). Therefore, highlighting the nutritional involvement on the incidence of reproductive conditions in communal farm management and disease control is necessary.

2.2. Reproductive conditions in cows

2.2.1. Downer cow syndrome in bovines

In communal farms of South Africa, downer cow syndrome is a limitation of production mainly due to poor nutrition and farm management. It is a disorder characterised by the animal's inability to rise (Ménard & Thompson, 2007). The aetiology of this condition is not entirely understood, because of its strong association with the energetics or electrolyte metabolism, as well as infectious diseases or trauma (Guyot *et al.*, 2017). The condition normally arises at the

initial stages of the postpartum period; it is also globally deemed a major production disturbance in dairy farms (Kalaitzakis *et al.*, 2010). Anxiety, low body temperature and a lack of appetite are clinical manifestations seen in downer cows prior and post calving (Houe *et al.*, 2001).

The condition is mostly seen in the peri-partum period where disturbance in the immune function, utilization of nutrients and alterations in tissue metabolism can be observed (Kimura *et al.*, 2006). At this time, the primary phases of uterine involution and provisions for the succeeding reproductive cycle occur (Abuom *et al.*, 2012). Low blood calcium and energy disproportion are the primary causes of downer cow syndrome (Radostits *et al.*, 2000). It has further been emphasised that cows experiencing downer cow syndrome often have low blood calcium (Fleischer *et al.*, 2001; FitzGerald, 2011). The occurrence of hypocalcaemia at calving predisposes cows to other additional infections as a result of reduced concentrations of calcium ion having effects on immune system and muscle functions (Kimura *et al.*, 2006; Reinhardt *et al.*, 2011).

Interruptions in cervical involution and myometrial lethargy may subsequently be seen causing cows to have uterine prolapse (Roberts, 2004). Other symptoms of hypocalcaemia include postpartum conditions which are birth canal injuries, metritis, fatty liver, nerve paralysis, ketosis, mastitis mostly seen in older cows, a decrease in smooth muscle contraction, suppression of dry matter intake and an increase in body fat mobilization in the form of non-esterified fatty acids (Kimura *et al.*, 2006). Decreased rumen function and low feed consumption have similarly been associated with subclinical hypocalcaemia (Hansen *et al.*, 2003). Feed mixtures containing high phosphorus consumed by cows in the dry period have been recognized as a risk factor for the development of hypocalcemia by approximately six to nine times (Lean *et al.*, 2006).

Cows may become hypercalcaemic due to mineral disproportions as there are no precise hormonal regulators for magnesium (Mg) and potassium (K) homeostasis in ruminants (DeGaris & Lean, 2008). Another study reported that downer cow syndrome was more frequent in cows than in heifers (Kutanaee *et al.*, 2014). This was also found in an earlier study showing that the degree of predisposition differs based on age and lactation levels in cows (Abuom *et al.*, 2012b). Soon after calving, it is essential to ensure that cows are closely monitored for early detection of downer cow syndrome as part of control measures (Kreplin & Yermcio, 2009). The beginning of lactation is linked with milk fever, probably because calcium is largely present in milk and its quantity in colostrum is nearly twice as much as that in milk in late lactation (Tsioulpas *et al.*, 2007a).

Most of the affected cows die when the condition advances with the animal not being able to rise in situations where the cows are not treated (Higgins *et al.*, 2013). If a cow is unresponsive to calcium therapy, then magnesium, phosphorus and potassium must be provided with other treatments while awaiting laboratory examination results (Adams *et al.*, 2017). Measures of blood mineral can be used as a monitoring tool for the management of downer cows in addition to other suitable approaches used in milk fever prevention (Kreplin & Yermcio, 2009). These strategies are significant in decreasing downer cow syndrome (Agger & Renney, 2004). The maintenance of a normal fertility state in cows is very important for successful livestock farming (Ghosh *et al.*, 2001).

2.2.2. Dystocia in bovines

Proper management of pregnant cows prior to calving is essential for the prevention of metabolic disorders. Dystocia can be defined as unusual difficult birth (Savc *et al.*, 2016). In cattle, causes of dystocia can be categorized as direct, phenotypic, genetic and non-genetic factors (Gaafar *et al.*, 2011). Factors directly related to dystocia are foetal mal-presentations, and uterine torsion; while, phenotypic factors involve the cow pelvic area, body weight,

prenatal death, calf birth weight, parity, gestation length and body condition at calving (Gaafar *et al.*, 2011; Mee, 2012a). Retained placenta and oversized calves are some of the non-genetic factors related to dystocia in cows (Johanson & Berger, 2003).

In addition, cow age and parity may influence dystocia (Ettema & Santos, 2004). In a study by Zaborski *et al.* (2009) they stated that nutritional inadequacies do influence the incidence of dystocia. Another study reported that hormonal irregularities, weakness and stress can lead to abnormal parturition (Gaafar *et al.*, 2011). Susceptibility to dystocia was also reported to be greater in very young heifers and very old cows, and age at first calving did not influence the occurrence of dystocia (Mee, 2012a). Persistent stage two dystocia (characterized by prolonged duration or arrested descent) and uterine inertia can occur due to fat mobilisation in over-conditioned heifers resulting from decreases in calcium utilization and magnesium availability (Gaafar *et al.*, 2011).

2.2.2.1. Nutritional impacts in bovine dystocia

Studies have associated dystocia with reduced milk yield (Berry *et al.*, 2007) and poor productivity (de Maturana *et al.*, 2007). Some studies stated that there is lack of understanding with regards to nutritional impact during the early stages of pregnancy on development of the placenta or calf birth weight (Hickson, 2006). Nonetheless, Zaborski *et al.* (2009) linked the occurrence of dystocia with nutritional imbalances. Feed concentration manipulation during gestation can be very useful as an alternative approach intended for regulating calf birth weight (Gaafar *et al.*, 2011).

Many studies on beef cattle have reported negative effect of feed imbalances and its influence on dystocia and calf birth weight (Freetly *et al.*, 2000). Some published data in dairy cattle studies on older cows and heifers presented little effect of maternal nutrition in the final stages of gestation (Sorge, 2005). Dystocia may occur due to lowered foetal and placental weight as

well as size of the pelvic area. In addition, insufficient pelvic ligaments relaxation and uterine inertia can induce dystocia which can result in stillbirth (Mee, 2012a).

2.2.2.2. Bovine body weight and dystocia

Previous research has revealed that the likelihood of dystocia occurrence rises through a birth weight escalation of approximately 13%/kg in cows (Johanson & Berger, 2003). A study by Mee (2008) indicated that oversized calves can be caused by maternal pelvic size and calf weight at birth accounts for 5 to 10% of the phenotypic change in cows with difficult parturition. Also, the study indicated that Holstein-Friesian heifers with low body weight (<260 kg) at service are more susceptible to dystocia; similarly, overweight (>360 kg at breeding) heifer have a greater risk of calving difficulty (Mee, 2008).

Overfeeding in the final trimester of gestation, to such an extent that an increase in body condition is observed, can lead to extra deposition of adipose in the birth canal in heifers and oversized foetus with resultant calving difficulties predisposing to stillbirths (Mee, 2012). It is essential to indicate that excess or poor body condition at calving increases the risk to dystocia and calving assistance in Holstein-Friesian heifers (Mee, 2008). In addition, studies also reported that variations in the frequency of dystocia were not noted in Holstein-Friesians first calving cows having body weight ranging from 520-600 kg before-calving and a 2.75-3.50 body condition score ranges (Carson *et al.*, 2004).

2.2.2.3. Bovine dystocia and Foetal mal-position

A report by Mee (2012) outlined that breech mal-presentation or cranial mal-posture, foreleg mal-posture and posterior mal-presentation during gestation are the most frequent forms of foetal position abnormality. An earlier study of Mee (1991a) found that there was less than 5% occurrence of foetal mal-presentation. Furthermore, Mee (2008) reported that in about 20-40% of incidences, mal-presented foetuses in older cows are the usual reasons for dystocia. In

addition to that, about twice and five-times mal-presented calves have a greater risk of dystocia and stillbirth incidence, respectively (Molefe, 2016).

Studies have also indicated less heritability and most importantly no repeatability in abnormal position of foetuses (Mee, 2012a). Atypical foetal presentation is commonly subjective to multiple births having about four-times greater possibility of dystocia (Molefe, 2016). Another risk factor posing a five-times greater risk of dystocia is twin ovulations (Mee, 2008). It is also indicated that parity in older cows lead to a three-times higher risk of experiencing dystocia. In addition calving difficulty may occur due to seasonal variations, type of herd, increase in intake of dry matter and high milk production (Molefe, 2016). It is as well, indicated that breed, sire and gender can influence foetal death and mal-presentation (Mee, 2012a).

2.2.2.4. Bovine housing and dystocia

Research has related housing type with difficult calving, showing that tie-stall confinements predispose to dystocia in dairy cows (Ostojić-Andrić *et al.*, 2011). Increased incidence of dystocia can arise from inadequate exercise, immobility and psychological stress (Mee, 2012). Furthermore, probable differences between health, welfare, systems of management and calving performance, are poorer in confinement systems as compared to rich pastures (Mee, 2012a; Molefe, 2016). Nonetheless, successful calving does not only rely on the management systems but also can be influenced by management of the system (Vasseur *et al.*, 2010b).

A Canadian study showed that approximately 51% of farmers used tie-stalls as their calving areas and this type of housing predisposes cows to difficult calving (Vasseur *et al.*, 2010a). Previous studies have indicated that beef heifers and cows confined in calving pens are most predisposed to dystocia than cows in a yard or pasture (Dufty, 1981; Mee *et al.*, 2014). A study by Molefe (2016) also stated that calving in confined facilities led to increased beef calf mortality.

2.2.2.5. Effects of dystocia on reproduction and production capacity

According to Mee *et al.* (2011) cows which encounter dystocia are more likely to experience dystocia at a subsequent calving. Of many reproductive conditions which cows come across during the transition period, dystocia greatly affects subsequent cow fertility (Bonneville-Hébert *et al.*, 2011). Several studies have also shown that dystocia increases the risk of placental membrane retention and metritis, which predisposes to cow culling and stillbirth (Mee, 2004; Molefe, 2016). A study by de Maturana *et al.* (2007) indicated that at a fraction of 12% dystocia may reduce success after first insemination as a consequence of impaired fertility. A significant difference was observed in association of dystocia to the tendency of a cow's delayed pregnancy (Gaafar *et al.*, 2011).

In a study by Berry *et al.* (2007), they reported that a huge drop in milk production is likely to occur in cows having dystocia compared to those without. In confined production system, the cost of dystocia affects about 41% of production, 34% of subsequent fertility, 25% in cow/calf morbidity and mortality, apart from management, veterinary and high culling costs (Molefe, 2016). In relation to animal health, dystocia worsens the possibility of breathing and gastrointestinal illnesses in calves (Eaglen *et al.*, 2011). It is equally essential to indicate that the total cost of dystocia is four-times greater than treatment costs alone, when taking into consideration the costs related to the interrelated sequelae of dystocia (Molefe, 2016).

2.2.3. Vaginal prolapse in bovines

Vaginal prolapse is a condition of the reproductive tract frequently seen in 24 months old cows in both pregnant and non-pregnant animals (do Nascimento *et al.*, 2016). The condition is commonly seen during the transition period (in the last third of gestation and postpartum) and occurs much in multiparous cows usually in the winter (Criado & Esteban, 2001). It is a reproductive condition of both beef and dairy cows which is accountable for poor reproduction

and losses in economic growth; however, it is not a very frequent condition in cattle reproduction (Ahmed *et al.*, 2005). Nonetheless, vaginal prolapse is considered as a dreadful condition which requires sufficient control as it results in contamination, mucosal trauma, excessive oedema and fatal haemorrhage leading to a serious prognosis (Miesner & Anderson, 2008; Yimer *et al.*, 2016).

Prolapse of the vaginal mucosa is significantly associated with overfeeding, intensive management system and follicular aspiration of cows (do Nascimento *et al.*, 2016). Reports by Kreplin and Yaremicio (2009) indicated that the exact causes of vaginal prolapses are not known and all variations of prolapses remain undefined. However, Abuom *et al.* (2012a) reported that increased occurrence of vaginal prolapse is seen in the dry periods and is due to low body condition and reduced nutrition, as well as low peri-vaginal fat (for its pelvic cavity support to the uterus and vagina). Other studies show that oestrogenic substances present in feeds, for instance mouldy maize, soya-bean meal, subterranean clover pasture and barley, also cause an increase in incidence of prolapse (Noakes *et al.*, 2001a; Molefe, 2016).

Elevated frequencies of vaginal prolapse are typically encountered during the dry season as pasture lose their nutritional value, causing cows to lose optimal body condition, thus a decrease in peri-vaginal fat which functions to support the uterus and the vagina in the pelvic cavity (Abuom *et al.*, 2012b). It may also be predisposed by parity, injured peri-vaginal area, poor vulvar conformations, as well as a lack of oestrogen which is responsible for connective tissue movements such as relaxation and contractions (Criado & Esteban, 2001). The majority of the cows treated by suture of the vulva and mechanical organ replacement may become sterile and have chronic cervicitis (Molefe, 2016). It is therefore important to take precautions to avoid all possible complications resulting from this reproductive condition.

2.2.4. Abortions in bovines

Abortion is the ejection of a foetus or embryo (dead or alive) from the uterus between 42 and 280 days of pregnancy before it can survive outside (Peter, 2000). Abortions normally are outcomes of an event that occurred weeks to months before the actual event, and the cause is possibly imperceptible at the time of abortion itself (Kreplin & Yaremicio, 2009). The causes of abortion are in most instances never discovered, also most abortions occur during the winter season (Molefe, 2016). Research has also shown that mycotic infections (caused by mycotoxins) commonly influence abortions in different animals, affecting approximately three to ten percent in all cases (Williams, 2012). In some cases, factors that might cause abortions are a number of bacterial, viral, fungal, protozoal and non-infectious agents (Anka *et al.*, 2014). Transmission of the infection passing to the foetus through the placenta is characterised by plaque or crust development on the skin of the foetus (Molefe, 2016). A study by Anderson (2007) showed that foetuses are invaded by fungi in about 25% of abortions and red or white worm-like lesions are usually seen. In addition, retained placenta may occur in cases where the aborted foetus is not removed from the uterus in time and many other complications may occur. Nutritional deficiencies are considered to be the cause of non-infectious abortion in both heifers and cows (Akar & Yildiz, 2005). In addition, nutritional deficiencies are as well linked to the development of foetal defects and embryo mortality early in the pregnancy.

Nutritional insufficiency during the time of mating, may lead to reproductive failure in ruminants and early embryonic mortality may occur as a consequence (Dunne *et al.*, 2000). Although the embryonic mortality is an acute symptom of nutritional imbalance on fertility and the effects of nutrition on the quality of the foetus (by oocyte capacity development and embryo development variations) are not entirely clear (Boland *et al.*, 2001). In laboratories, diagnosis can be made by culture of organism from the aborted tissues (Kreplin & Yaremicio, 2009). It

has been found that even with careful laboratory assessment, causes of abortions in approximately 70% of the cases cannot be established (Anka *et al.*, 2014; Molefe, 2016). Reports from many laboratories indicate that fetuses submitted are between the second and third trimester of pregnancy and are mostly caused by infections. Furthermore, making a diagnosis is very challenging and the causes for abortion are usually unknown in more than a half of the cases (Anderson, 2007).

A case control study by Enjalbert *et al.* (2006) found that of two thousand beef and dairy herds, the incidence of prenatal mortality and abortion were highest seen in cows with selenium (Se) deficiency. A study conducted in West Canada reported that the average selenium concentrations in aborted fetuses were lower compared to that in non-aborted ones. In addition, clinical manifestation such as cardiac failures were evident in selenium deficient aborted fetuses in comparison to aborted fetuses showing no lesions (Waldner & Van De Weyer, 2011). The inadequate supplementation of a ration with vitamins A, vitamin E, beta-carotene iodine, Selenium, Copper and Zinc may also induce abortion in the advanced stage of gestation in cows (Akar & Yildiz, 2005; Molefe, 2016).

It has been indicated that cows most likely to abort are replacement heifers, cows that are more than 10 years of age, and cows with a body condition score of less than 5 or equal to 9 at pregnancy testing. Also, cows with twin pregnancies and cows bred on community pastures that were not vaccinated were also more likely to abort than cows that were not on community pastures regardless of vaccination status (Molefe, 2016). Moreover, calving-related events such as retained placentas, severe dystocia, problems such as uterine prolapse, abortion or calf death within one hour of birth remained connected to an increased risk of abortion in subsequent calving season after accounting for all other factors (Waldner, 2014). Losses from abortions and stillbirths are the most important determinants of economic success (Waldner & García Guerra, 2013). The incidence of abortion directly influences profitability in a herd and

negatively impacts cow fertility as it consequently increases financial losses incurred through replacement in communal herds.

2.2.5. Retained placenta in bovines

In southern Africa, the occurrence of placental membrane retention in cattle is around 4% (Esslemont & Kossaibati, 2000; Yusuf *et al.*, 2012). Normally, expulsion of the foetal placental membranes during parturition of cow should happen within 8 hours after calving; if the membrane remains attached for longer than 12 hours the condition is termed retained placenta (Kumar *et al.*, 2017; Rokde *et al.*, 2017). After parturition, sufficient and systematic contraction of the uterus are essential in the physical placental removal (Akar & Yildiz, 2005). Retained placenta is a postpartum uterine disorder which is often seen in cattle and usually predisposes cows to the development of puerperal metritis (Cui *et al.*, 2017). Placental membrane retention may result from inadequate prostaglandins (PGF₂ α) secretion, oxytocin emission and calcium concentrations, all of which maintain enough uterine contractions and might delay uterine involution and increase incidence of dystocia (Akar & Yildiz, 2005). Stress may also lead to the incidence of retained placenta (Kornmatitsuk *et al.*, 2000).

Other causes of retained placenta include selenium deficiency, weight of the placenta, calf birth weight, the use of prostaglandins and/or glucocorticoids in the early stimulation of parturition, reduced concentration of plasma estrogen, twinning, vitamin E and selenium deficiency, uterine atony, stillbirth, negative energy balance (ketosis), dystocia, hypocalcaemia, delayed gestation, abortion, seasonal and hormonal conditions, early parturition and also infections (Swieffy, 2003; Akar & Yildiz, 2005; Jemal, 2016). Retained placenta incidence is higher in winter than in summer, due to nutritional and environmental variations such as concentration of dietary forage ratio and temperature; in addition, during the initial half of the gestation, seasonal nutritional changes may impact development and growth (Jemal, 2016). The incidence

of reproductive conditions can be associated with subsequent requirements for more services for conception, the pregnancy rate is lowered and first service conception is reduced, which is also seen in cases of retained placenta (Jemal, 2016; Molefe, 2016; Cui *et al.*, 2017).

A study of Vitamin E in association to retained placenta prevention found no connection between the condition and concentrations of tocopherol (Vitamin E in its natural form) in circulation (LeBlanc, 2008). Mineral deficiencies are also highly associated with retained placenta (Alaçam, 2002). Other studies have reported that low serum concentrations of potassium (K), zinc (Zn) and magnesium (Mg) before parturition might predispose cows to the incidence of placental membrane retention (Akar & Yildiz, 2005). Low plasma concentrations of selenium-dependent glutathione peroxidase (GSH- Px) have been related to retained placenta (Molefe, 2016). Animals supplemented with selenium do not usually get retained placenta whereas about 17.4% to 20% prevalence was seen in non-supplemented control animals (Abuom *et al.*, 2012b). Studies showed that cows previously affected by retained placenta are prone to encountering the condition in subsequent pregnancies than the ones that have never experienced the condition before (Fleischer *et al.*, 2001).

2.3. Minerals in cow reproduction

2.3.1. Introduction

In various body processes, animals require minerals for a successful reproduction. Diseases of minerals can be seen as both deficiencies and toxicities. Understanding of the nutritional factors related to reproductive disorders is necessary to create appropriate and viable interventional strategies to increase reproductive efficiency in communal farms. Assessing cattle mineral and nutritional status in order to improve production and reproduction is as well a significant aspect in the mineral-reproduction relationship. Increased knowledge of current improvements in mineral nutrition is necessary for efficient production. The inability of natural forage to supply sufficient nutrient requirements leaves farmers with a challenge of having to supplement.

However, it is important to not just supplement but to properly prepare, administer and monitor amounts given, as less or too much nutrient intake can also lead to health problems.

Minerals are structural components of the body (Velladurai *et al.*, 2016). They are also defined as inorganic substances found in all body tissues and fluids (Soetan *et al.*, 2010). They can be classified into macro (major) and micro (trace) elements (Kumar, 2015). Macro minerals are the compounds required in large quantities, while micro minerals are needed in small amounts (Al-Ghareebawi *et al.*, 2017). There is also a third category of minerals termed ultra-trace elements (Velladurai *et al.*, 2016). The macro-minerals class comprise of phosphorus, magnesium, calcium, sodium chloride and potassium chloride, whereas micro-elements include iron, copper, cobalt, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulphur (Sujayil & Dhanaraj, 2017).

The ultra-trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for animals (Singaravadivel & Santhanaraj, 2017). Other minerals such as cadmium, lead, tin, lithium and vanadium have not been shown to be essentially required (Soetan *et al.*, 2010). Minerals have functional properties including the maintenance of certain physiochemical movements in the body such as activities of enzymes and hormones; they also play a role as regulators of cell replication and differentiation (Velladurai *et al.*, 2016). For normal functioning of the body processes these inorganic substances (minerals) are necessary in all living organisms (Soetan *et al.*, 2010).

In animal health, minerals are very important since any deficit lowers the animal's disease resistance and may increase the incidence of reproductive condition (Yatoo *et al.*, 2013). Mineral deficiencies and imbalances have long been known to be responsible for low production among cattle and buffaloes (Velladurai *et al.*, 2016). Though they yield no energy, they significantly aid in numerous metabolic processes in the body (Eruvbetine *et al.*, 2003; Olufayo & David, 2017). Requirements of minerals for reproduction and immunity are

generally higher than maintenance requirement of animals (Velladurai *et al.*, 2016). The macro-minerals are required in amounts greater than 100 mg/dl while micro-minerals are required in amounts less than 100 mg/dl (Kumar, 2015).

Imbalances and toxicity of certain mineral elements may cause reproductive disorders as minerals play an important role in health and reproduction of the livestock (Sharma *et al.*, 2007). Animal reproduction, health, and growth can be affected by immunological or physiological disorders which arise as a result of mineral deficiency (Velladurai *et al.*, 2016). Beside energy and protein, deficiency of calcium, phosphorus, iron, zinc and copper in blood have been reported to be a predisposing factor to the occurrence of placenta retention and repeat breeding in dairy cows (Kumar, 2015).

Mineral deficiencies or imbalances in soils or forages have been implicated, in part, for low animal production and poor reproductive performance in the developing regions of the world (Gonçalves *et al.*, 2017; Jones, 2017). Primarily, the use of mineral supplements has been encouraged as a measure to prevent production losses and combat clinical manifestations of insufficiency (Spears, 2000; Enjalbert *et al.*, 2006). Nonetheless, trace mineral's influence on the immune function has been highlighted in recent research (Arzate-Vázquez *et al.*, 2017; Dunn *et al.*, 2017). A study by López-Alonso (2012) indicated that provision of mineral supplements is an affordable investment and a sound value in costs. However, that is not the case for most communal farmers as they cannot afford supplementary feeding as they mostly rely on natural pastures.

2.3.2. Calcium (Ca)

Calcium (Ca) is an essential macro nutrient required by the body (Harper, 2017). It is needed in large amounts in both animals and humans (Martinez *et al.*, 2016). Its functional properties include muscle contraction, nerve conduction, blood clotting, maintaining healthy bones, teeth

and also aid in the correct functioning of the heart (Trailokya *et al.*, 2017). Approximately 99% of Ca is present in the skeleton; it is also known to be the most available mineral in the body (Trailokya *et al.*, 2017). Nonetheless, a lesser percentage of calcium in the body outside the skeleton is found in the blood and is important for survival (Suttle, 2010).

In the course of severe decline in calcium to levels less than normal, the homeostatic maintenance mechanism of blood calcium concentration will cause the reabsorption of calcium from the bones, for that reason, consumption of adequate calcium is important to lessen undesirable effects (Trailokya *et al.*, 2017). Feeding calcium-rich diet to animals is a significant way to provide correct daily consumption (Trailokya *et al.*, 2017). Nonetheless, in animals which cannot consume sufficient calcium from feed, supplementation is a necessity in order to increase calcium supply (Trailokya *et al.*, 2017).

The calcium: phosphorus ration should be maintained at between 1.5:1 and 7:1 in order to prevent disproportion (Norman *et al.*, 2012). Calcium concentrations in serum or plasma are very useful measures which can be used in the diagnosis of subclinical hypocalcaemia; in addition, 2.0 mM and 1.88 mM are the cut-off points of total calcium concentrations (Reinhardt *et al.*, 2011). An increase in calcitriol and parathyroid hormone warrants an immediate adaptation to begin new supplies; nonetheless, ionised calcium concentrations in plasma may possibly decline to an extent that the cow develops milk fever (Penner *et al.*, 2008)

Low blood calcium predisposes cows to a number of peri-parturient disorders, for instance mastitis, ketosis and retained placenta (Sepúlveda-Varas *et al.*, 2015). Low calcium levels in the blood serum is typically concurrent with increasing plasma magnesium concentrations (Fikadu *et al.*, 2016). Studies have also indicated that high parity Jersey cows are more prone to hypocalcaemia, while heifers rarely experience milk fever (Roche & Berry, 2006). Calcium

requirements change depending on animal age and production status (Mokolopi & Beighle, 2006).

Table 2.1 Calcium requirements in cows

Production stage	% per dry matter intake (DMI)
Non lactating	0.18%
Pregnant	0.27%
Growing and finishing	0.3%

Sources: (Mokolopi & Beighle, 2006)

Oral calcium supplements in cows usually reduce calcium concentration after calving. Therefore, magnesium must concurrently be provided in supplementation with calcium as magnesium plays an important part in the homeostasis of calcium (Rude & Gruber, 2004). The association of hypocalcaemia with potassium reduction may be seen as a result of reduced dry matter intake leading to the recommendation of supplementing K in cows with low blood calcium (Benzaquen *et al.*, 2015).

2.3.3. Magnesium (Mg)

Magnesium is the second most abundant intracellular divalent cation (Blaine *et al.*, 2014). Several enzymes rely mainly on intracellular magnesium for the regulation of their metabolism (Mahon *et al.*, 2003). Nerve transmission is one of the most important functional characteristics of intra-cellular magnesium (Wang *et al.*, 2017b). In mature ruminants, magnesium absorption can occur in two mechanisms: one of which is the most important and occurs in the transference during low magnesium levels is the one dependent on potential difference above the rumen epithelium (Blaine *et al.*, 2014). The second mechanism is characterised by magnesium

transportation over the epithelium with no dependency on the potential differences (Martens & Schweigel, 2000).

It has been shown that deficient magnesium absorption hinders the cow's capacity to stimulate the stored magnesium from bones, which is required for sustaining magnesium concentrations in cells and in the fluids outside the cells (Kronqvist *et al.*, 2011). Other studies have indicated that decreased concentrations of potassium prevent the uptake of magnesium, whereas ammonia content in the rumen stimulates magnesium uptake (Blaine *et al.*, 2014). In addition, research has also shown that when the concentration of magnesium increases there is a decline in plasma calcium concentrations during calving (Goff *et al.*, 2002). Moreover, the quantity of magnesium necessary for reproduction exceeds the amount required for growth by approximately 30% (Kreplin & Yaremicio, 2009).

It has been reported that magnesium deficiency has severe effects on reproductive performance in cows (Amen & Muhammad, 2016). Clinical signs such as no heat, low rates of conception, low calf birth weight and abortions may result from magnesium deficiency in cows (Musa *et al.*, 2016). Additionally, the common observations of magnesium deficits are that calves are born weak and deformed with enlarged joints and legs twisted (Kreplin & Yaremicio, 2009). Quantifying plasma magnesium level is a useful technique in hypomagnesemia diagnosis, with a suggested cut-off point of 0.74 mM (Vusikala *et al.*, 2016). On the other hand, other studies have indicated that total plasma magnesium does not at all times decline during magnesium deficiency, therefore to accurately estimate magnesium status, measures of ionised plasma magnesium are very useful (Vera *et al.*, 2016).

Hypomagnesaemia is also recognized as grass tetany, winter tetany and grass staggers dependent on the conditions in which it may possibly arise (Kronqvist *et al.*, 2011). Another study also made indications that a deficiency of magnesium in the cerebrospinal fluid can lead to death as a result of hypomagnesaemia and tetany (McCoy *et al.*, 2001). Generally, the

reproductive status of animals is not directly affected by magnesium and that is because of its indirect proportional relations with calcium so a disorder of any sort in Ca-P-Mg concentrations can affect reproduction (Velladurai *et al.*, 2016). Additionally insufficient magnesium may be seen in animals with low reproductive efficacy due to a loss of appetite (Velladurai *et al.*, 2016)

2.3.4. Phosphorus (P)

Phosphorus (P) is a vital nutrient required in all living organisms (Yang *et al.*, 2017). An essential form of phosphorus consumed by the majority of organisms is called inorganic orthophosphate (PO_4^{3-} or Pi), nonetheless its presence in the environment limits the growth of plants and algae (Juneja *et al.*, 2013). In animals, phosphorus has the most identified roles compared to other minerals in the body, mainly for the role it plays in tissue and bone development, milk production as well as utilisation of energy (Abrams & Atkinson, 2003).

It is the second most abundantly available mineral at percentages between 80-85 in the skeleton (teeth and bones); it also plays a role in enzyme transfer and metabolic reactions in the body (Velladurai *et al.*, 2016). Phosphorus is also frequently stated as a “fertility” mineral (Fageria *et al.*, 2017). It is also the structural component of phospholipids and nucleic acids, having a significant role in the metabolism of energy, protein modification as well as signalling transduction cascades (Yang *et al.*, 2017).

Phosphorus deficiency is a serious concern in most rural farms of the semi-arid regions in South Africa, and the clinical manifestations associated with this deficiency include osteomalacia and stiffness (Bakunzi *et al.*, 2012). A South African study of mineral content revealed that phosphorus level in natural pastures is not adequate to improve productivity; furthermore, sick cattle reared on these veld and craving dead animal bones has brought about suggestions that there is a deficiency of phosphorus in most cattle herds (Ateba & Beighle, 2011).

A study by Hadžimusić *et al.* (2013) showed that cow's phosphorus requirements are very high and that what is provided by plants may be due to the low levels in the soil. The detection of P insufficiency has been reported in some classical studies early in the century (Suttle, 2010). Previous studies have indicated that reproductive performance can be affected by phosphorus producing conditions such as an increase in the required services per conception, delayed puberty (related to growth rate and poor appetite), and one of the highest challenges is monitoring phosphorus (P) intake and outputs in cattle feeds, mainly in rural farming regions (Kreplin & Yaremcio, 2009).

Additionally, satisfying requirements for phosphorus in cattle (not decreasing or increasing consumption) can serve as a technique useful in regulatory and managing phosphorus imbalances (Erickson *et al.*, 2002). Phosphorus deficiency predisposes to low conception rates, delayed sexual maturity, increased occurrence of cystic ovaries, uneven oestrous cycles, ovarian activity, reduced fertility rate, feed intake and low milk production (Velladurai *et al.*, 2016). Low blood phosphorus contributes to the incidence of metabolic disorders in dairy cows; for instance post-parturient haemoglobinuria and downer cow syndrome (Kachhwaha *et al.*, 2010).

A study reported that lowering dietary P from 0.57 to 0.37% did not negatively affect milk production, but did significantly reduce P excretion into the environment (Wang *et al.*, 2014). There is no improvement in reproductive performance even when increasing the phosphorus dietary requirements from the NRC revised recommendations of 0.3 – 0.4 % to a dietary phosphorus level of 0.38- 0.4% (NRC, 2001). Productivity and improved reproductive performance have a direct connection with phosphorus intake (Wang *et al.*, 2017a).

2.3.5. Selenium (Se)

Selenium is an important component of enzyme systems and interacts with vitamin E to prevent tissue damage (Atli *et al.*, 2012). Detrimental effects associated with selenium insufficiency in animals include low growth, poor health and fertility (Glombowsky *et al.*, 2017). As selenium has a narrow margin of safety, toxicities are mostly seen than deficiencies in farm animals and selenium deficiency in animal reproduction is associated with abortions, still birth or weak offspring and retained foetal membranes in females, cystic ovaries, metritis and mastitis (Balamurugan *et al.*, 2017). On the other hand, selenium toxicity in cows that are pregnant is related to the incidence of weak and lethargic calves, still births and abortions (Omeje, 2016). Studies have associated selenium insufficiency in cattle with infertility, occasional abortions, higher cases of retained placentas, premature or weak calves, low disease resistance and “white muscle disease” in calves (Kreplin & Yaremicio, 2009). In terms of preventing mammary gland infections dietary Vitamin E and Se supplements may be given to animals (Ata & Zaki, 2014). Amino acids such as seleno-cysteine and seleno-methionine are the main forms of selenium present in foodstuffs and selenite is the major form in supplemental selenium in feed (Whanger, 2002).

In selenium yeast, seleno-methionine retention in blood and tissues has shown to be more than the content in inorganic sources (Givens *et al.*, 2004). Selenium has small range differences relative to toxicity and deficiency, for that reason its addition to feeds is regulated (Kreplin & Yaremicio, 2009). Research has indicated that supplementing dairy cows with selenium and Vitamin E controls the risk of retained placenta, further suggesting that dietary supplements with either or both can decrease susceptibility to retained placenta and also reduce the services per conception to about 1.54 -2.05 lower (Mee, 2004; Balamurugan *et al.*, 2017).

2.3.6. Copper (Cu)

Copper (Cu) is an important micronutrient required for normal functioning of body metabolism in animals (El Basuini *et al.*, 2017). Cu is an important mineral which has been known to affect reproductive performance, production and growth (Balamurugan *et al.*, 2017). Copper also has functional roles in red blood cells, connective tissues and enzymes in the body (Suttle, 2010). It also plays important roles in the animal's immune system and it is as well involved in several reactive proteins, enzyme cofactors, copper-oestrogen interactions, copper-progesterone interactions, melanin pigmentation, iron transportation, and cellular respiration (Balamurugan *et al.*, 2017).

Copper insufficiency has the same influence on reproduction as other mineral deficiencies predisposing cows to poor fertility and delayed puberty (Suttle, 2010). Additional indicators of low copper concentration include very high numbers of retained placenta and repeat breeding (Kreplin & Yaremicio, 2009). Copper insufficiency is also related to early embryonic death, reduced immune competency, decreased conception rate, low libido, lowered semen quality, severe testicular tissue damage and bull sterility (Balamurugan *et al.*, 2017).

Copper deprivation in ruminants leads to ataxia, abnormal wool and hair pigmentation, anaemia, bone disorders, connective tissue disorders, cardiovascular disorders, scouring or diarrhoea, growth retardation and disease susceptibility (Suttle, 2010). Ratios such as Zn: Cu 4:1, Cu: Mo 6:1 and Fe: Cu 40:1 are very supportive in the maintenance of blood mineral copper (Balamurugan *et al.*, 2017). In addition, the standard requirement of copper in cattle is said to be 10 ppm. In ruminants, copper requirements for maintenance per dry matter is 8mg/kg (Suttle, 2010). In dairy cows the normal copper serum concentration should be between 0.576–0.96 mg/l and any value less indicate a deficiency (Xinhuan *et al.*, 2017).

2.3.7. Iron (Fe)

Fe is required in every animal for maintaining natural processes, for example oxygen transportation and electron transfer of enzymes (Sánchez *et al.*, 2017). Iron in animals either carries or activates a number of enzymes (Molefe, 2016). Above fifty percent of bodily iron (Fe) content is present in haemoglobin (NRC, 2000). The cellular uptake of iron occurs in many tissues through a process called transferrin cycle (da Silva & Rita, 2017). Transferrin is a plentiful plasma protein which fixes two atoms of Fe having very high affinity and it functions to keep iron soluble, consequently avoiding precipitation of aqueous plasma Fe^{3+} ions out of aqueous plasma (Molefe, 2016).

Fe distribution in foetal development is cell mediated by the extra embryonic visceral endoderm and syncytiotrophoblast in the placenta (Lieu *et al.*, 2001). In foetal development the intra-placental transfer of iron happens during gestation, therefore iron performs a highly significant role in foetal growth as its deficiency may lead to foetal mortality (Andrews *et al.*, 2005). Iron deficient animals are likely to require many inseminations per conception (they are likely to be repeat breeders) and may rarely abort (Kumar *et al.*, 2011a).

The NRC (2001) indicated that high amounts of iron predisposes to metabolic acidosis, low feed intake, weight reduction, hypothermia and diarrhoea. Additionally, a feeding requirement of about 1,000 mg Fe/kg is estimated as the tolerable level of iron (NRC, 2001). The quantity of iron available in feed is understood to be smaller than iron concentration in supplements and animals mainly receive iron from water and soil ingestion (NRC, 2000). As a result of anaemia, a lack of appetite and low body condition, impairment in reproductive performance may be seen (Kumar, 2015).

Typically, iron deficiency has characteristic clinical signs including pale mucous membranes signifying anaemia, low weight gain, reduced feed intake and pica (Pietzak, 2014). Iron

insufficiencies may as well occur most commonly due to high parasite infestation in a rearing area as a result of chronic blood loss; also in calves housed in confinement setup and those given milk become very vulnerable (NRC, 2000). Iron loss through faeces and urine is very minimal in cases where there is no blood loss (Molefe, 2016).

2.3.8. Iodine

Iodine (I) is an essential micro-nutrient in cattle reproduction. Generally, iodine is found in the diet as iodide and is required for thyroid hormone synthesis (Kumar *et al.*, 2011b). Metabolic energy rate regulation is by the use of triiodothyronine (T₃) and thyroid hormone, thyroxine (T₄)-both requiring iodine for their synthesis and this is an important function of iodine in the body (NRC, 2000). Reproductive insufficiency can result from the influence of iodine on the thyroid gland, as a decrease in conception rate and ovarian activity usually occur due to impaired thyroid gland (Balamurugan *et al.*, 2017). It is also involved in brain development of progeny as well as growth and foetal development (Velladurai *et al.*, 2016).

A deficiency of iodine can lead to failure of fertilization, early embryonic death, still birth with weak calves, abortion, delayed puberty, irregular oestrous, increased frequency of retained placenta in females, a decrease in libido and deterioration of semen quality in males (Balamurugan *et al.*, 2017). Goitre is also seen especially in young animals, increased perinatal mortality and impaired reproduction (Grace & Waghorn, 2005). Increased iodine intake has been linked to a number of health problems including increased frequency of retained placentas, suppressed oestrous, extended gestation periods, abortion and decreased resistance to infection and disease (Franke *et al.*, 2009; Balamurugan *et al.*, 2017). Lugol's iodine has been used to treat poor conception ability, silent heat and repeat breeding (Kumar, 2015).

2.4. Serum metabolites

2.4.1. Introduction

Blood biochemical analysis in veterinary medicine is an essential tool in animal health evaluations (Oetzel, 2004). In many herds blood chemistry testing has been used to diagnose reproductive disorders in cattle (Nozad *et al.*, 2011). The health status of cows during the peripartum period is fundamental to the rate of production and to the subsequent reproductive performance. Alterations in the metabolic profile may result from impaired fertility and abnormal puerperal physiology due to nutrient imbalances (Dhami *et al.*, 2017). Metabolic profiles in cattle are used as biomarkers for health status, to identify production limitations of cattle in subsistence farming and also aid in disease diagnosis, treatment as well as prognosis determination (Ndlovu *et al.*, 2007; Giri *et al.*, 2017; Kalyani *et al.*, 2018).

Serum constituents are good indicators of reproductive disorders, for example retained placenta, dystocia, and endometritis in cows (Kruip *et al.*, 2001; Taylor *et al.*, 2003). Biochemical profiles of cow's reproductive performance have shown significant differences among various breeds of cattle (Aggarwal *et al.*, 2016; Kalyani *et al.*, 2018). Another study has also indicated that normal serum metabolite concentrations are vital aspects in the proper functioning of different systems in the body (Saqib *et al.*, 2018). Serum metabolites essential to the precise measuring of reproductive health in cows include urea/BUN, creatinine, aspartate aminotransferase (AST), alanine amino transferase (ALT), calcium, total protein, albumin, globulin, cholesterol, glucose, minerals and non-esterified fatty acids (Ndlovu *et al.*, 2007; Magnus & Lali, 2009).

The physiology of the animal, age, nutritional status, breed and seasonal changes may influence alteration in serum metabolite concentrations (Ndlovu *et al.*, 2007). Optimum reproductive performance in cows requires early resumption of postpartum cyclicity in order to obtain

increased productivity (Utama *et al.*, 2018). The incidence of postpartum disorders may increase due to low concentrations of magnesium, urea nitrogen, albumin, cholesterol as well as low body condition score (BCS) (Utama *et al.*, 2018). Previous studies have reported that activities of serum enzymes could be low or absent in cows of good health and those nutrient sensitive metabolites in the blood stream influence ovarian functioning (Ndlovu *et al.*, 2007; Dampney *et al.*, 2014). The balance in metabolic indicators is crucial to the proper functioning of the reproductive system and essential to improved production status in small scale farming.

2.4.2. Cholesterol

Cholesterol (CHOL) is a structured fat or lipid circulating in the blood stream which is present in all body cells and is also necessary for life maintenance (Dampney *et al.*, 2014). CHOL gives the structural framework for the essential metabolic processes including production of steroid hormones; it influences the synthesis of bile acids necessary for fats digestion and it also promotes embryonic growth and supports cell membrane formation (Guzel & Tanriverdi, 2014). Cholesterol levels were reported to be significantly low in non-cycling cows (Jeong *et al.*, 2015a). On the contrary, other reports has shown that cholesterol concentration in blood is usually low in early cycling cows and high in the late-cycling cows (Obese *et al.*, 2015).

Cholesterol level in cows tends to increase as a result of high energy feed intake; furthermore, serum cholesterol is associated with negative energy balance and it is as well a good indicator for decreased growth performance and lack of energy (Utama *et al.*, 2018). Elevated CHOL concentrations are indicators of biliary or hepatic diseases, insufficient thyroid hormone production, Cushing disease and or high fat meal intake (Kahn *et al.*, 2010). High cholesterol concentration raises the probability of reproductive disorders, diabetes mellitus and cardiovascular diseases in obese cows (Kumar, 2014). The level of cholesterol has been reported to be high in multiparous cows as compared to primiparous cows and it was also noted

to gradually increase after calving due to body fat metabolism (Saqib *et al.*, 2018). Cholesterol is also known by its influence on ovarian steroid hormones' synthesis (Jeong *et al.*, 2015a).

A study by Matras *et al.* (2014) reported significantly high cholesterol levels in cows during lactation and further indicated that increased cholesterol concentration is useful in ruminant production. A high cholesterol index may also be seen due to the profile of fatty acids and fat supplementation (Rabiee & Lean, 2000). A study of Tombuku *et al.* (2017) showed that repeat breeder cows commonly exhibit low cholesterol concentrations. According to Maciel *et al.* (2001) low CHOL concentration may be responsible for reduced growth factors of plasma insulin and lack of ovulation in cattle. Natural pastures are said to have low cholesterol concentration as compared to silage mixed diets (Barlowska *et al.*, 2011). It is also known to improve other reproductive factors and the overall animal herd health (Gonthier *et al.*, 2005).

2.4.3. Urea nitrogen

In mammals, urea/BUN (Blood urea nitrogen) is a naturally occurring product of metabolism, which is endogenously produced by amino acid and protein catabolism in living organisms (Tshuma *et al.*, 2014). Urea formation mainly takes place through typical nitrogen excretion by the liver. Nitrogen removal occurs through Krebs-Henseleit cycle, a metabolic process also known as ornithine or the urea cycle (OECD-SIDS, 2008). The microorganism in the rumen and reticulum convert urea into protein (Kertz, 2010). Embryo growth and implantation can be negatively affected by high urea levels which reduce the pH in the uterus (Tshuma *et al.*, 2014). Blood urea nitrogen is a useful indicator in the process of metabolism of protein and in the functioning of the kidney (Dampney *et al.*, 2014).

In animal feed, Urea concentrations can replace protein fractions. Urea concentrations normally range between 3.3–6.7 mmol/L (200 to 400 mg/L) (OECD-SIDS, 2008). Reduction of protein content and increased dietary starch can be used to maintain urea consumption, as high protein

diets increase the production of urea and elevate the level of BUN in plasma (Kertz, 2010). Previous studies have shown that as a result of protein insufficiency, reproductive performance is impaired when urea/BUN concentrations get lower than 7 mg/dl (Tshuma *et al.*, 2014). A study of metabolism reported that a cow's fertility is likely to be affected by urea in the process of cleavage and blastocyst formation in the fertilised embryo (Jorritsma *et al.*, 2003).

High urea concentration is related to renal disease, urinary obstruction, congestive heart disease and gastrointestinal disorders (Kahn *et al.*, 2010). High urea concentration may lead to shock, starvation, and dehydration, whereas the opposite symptoms are seen when the urea level is reduced (OECD-SIDS, 2008). Elevated urea level decreases calcium required to reduce contraction potency (Ok *et al.*, 2005). A study by Jeong *et al.* (2015b) found that there was no correlation between the level of urea and cyclic resumption. However previous studies have shown a lowered urea concentration in non-cyclic cows for 2 to 6 weeks postpartum (Kalyani *et al.*, 2018). A decline in urea concentration may be connected to lower liver ureagenesis and/or low dietary intake of proteins (Macrae *et al.*, 2006).

The normal ranges for urea/BUN in exotic cows is 20–30 mg/dl (Kalyani *et al.*, 2018). The high concentrations of urea/BUN are normally associated with the body's ability to utilize nitrogen while its low concentration raises effectiveness of nitrogen in the body (Nonaka *et al.*, 2008). Urea measurements are good biomarkers for nutritional status changes and the metabolism of protein because of its relationship with catabolism of protein and the amount of ammonia in the rumen in the body (Carvalho & Soeparno, 2010). Urea toxicity is common in ruminants; urea's reaction with water becomes a biological hazard as it actively degrades to ammonia (Raidal & Jaensch, 2006). The liver detoxifies circulatory ammonia and removes it from the body as BUN, yet if this protection mechanism is hindered, severe ammonia poisoning can occur (OECD-SIDS, 2008).

2.4.4. Creatinine

Creatinine (Cr) is a micro-molecular metabolite produced from creatinine and phosphocreatine; additionally Cr is normally present in muscles (Tesfaye *et al.*, 2009). In clinical practice today, serum creatinine is used as a biomarker for detecting and assessing severe and chronic renal disorders (Thomas *et al.*, 2017). According to Issi *et al.* (2016) Cr is the best predictor for kidney function and may be elevated due to about 75% decrease in functional nephrons. Additionally, a normal concentration of creatinine in the animal implies that there is low kidney (nephron) damage. The degree of creatinine release typically relies on the mass of the body muscle since it is produced from the muscle creatinine phosphatase breakdown (Kalyani *et al.*, 2018).

A previous study associated the concentration of creatinine with live body weight (Whittet *et al.*, 2004). Alterations in serum creatinine in the body can occur due to malnutrition, age, gender, post-surgical states, movement or action (Tesfaye *et al.*, 2009). Myopathological differences have also been associated with lowered change in muscle tension and irregular calcium control (Wyss & Kaddurah-Daouk, 2000). A South African study found significantly lower creatinine concentrations in Nguni cattle than in cross breed cattle (Mapiye *et al.*, 2010). The values for normal range of creatinine in cows is 1-2 mg/dl in exotic cattle (Kalyani *et al.*, 2018).

Serum creatinine concentration relates directly in proportion to the body mass and the maximum concentration of creatinine is typically found in skeletal muscle. As a result, it is concluded that creatinine actively participates in tissue energy metabolism (Wyss & Kaddurah-Daouk, 2000; Tesfaye *et al.*, 2009). In agreement with this idea, several investigational outcomes have shown that there is an association between the disturbance of creatinine concentration and muscular diseases (Wyss & Kaddurah-Daouk, 2000).

2.4.5. Total protein (TP)

A study by Dampney *et al.* (2014) indicated that total protein (TP) status in cows can be determined by monitoring blood urea nitrogen, globulin and albumin. There are significant differences observed in different breed types and the concentrations of total protein (Kalyani *et al.*, 2018). Significantly higher concentrations of total protein in cyclic cows (9.19 ± 0.45 g/dL) may be seen as a result of environmental effects, nutritional imbalances and breed differences (Ahmad *et al.*, 2004). Total protein concentration is a good indicator of liver and kidney damage (García *et al.*, 2017). Endometritic cows (19.16 ± 1.00 g/dL) may also show elevated concentrations of total protein which may lead to reduced fertility (Ahmad *et al.*, 2004). Based on the direct proportion of total protein and serum globulin, TP concentrations have been associated with an increase in parity because older cows have a more developed immune system and have been exposed to pathogens (Bobbo *et al.*, 2017).

In older cows, increased protein intake can cause impaired fertility and also lead to increase of services per conceptions (Ahmad *et al.*, 2004). The total protein concentration in gravid cows tends to increase approximately 8 week before calving and rapidly decrease at calving (Žvorc *et al.*, 2000). The colostrum loss of globulin can cause the concentration of total protein to be reduced during parturition (Bobbo *et al.*, 2017).

2.4.6. Globulin

In gravid cows, plasma globulin concentration decreases as a result of the γ -globulins from blood being transferred to the colostrum (Bobbo *et al.*, 2017). The concentrations of globulin differs significantly amongst various cow breeds (Kalyani *et al.*, 2018). The concentration of globulin rises until the eighth month of gestation, followed by an increase caused by mammary gland immunoglobulin concentration and an abrupt decrease is seen basically before calving (Žvorc *et al.*, 2000). In cases of inflammation, globulin concentration tends to increase (García

et al., 2017). Variations in serum globulin concentration have been used as a biomarker for immune response indications in animals (Bobbo *et al.*, 2017).

2.4.7. Albumin

The measurements of albumin (ALB) serve as indicator of liver and kidney function as well as serving as useful tools in the assessment of the nutritional status of cows (Irfan *et al.*, 2014). Low albumin concentration is significantly different in non-cyclic cows as compared to albumin in cyclic cows (Obese *et al.*, 2015). Due to a lack of energy a decrease in albumin level may result from excessive albumin catabolism (Jeong *et al.*, 2015a). Albumin has been reported to be low in repeat breeding cows (Tombuku *et al.*, 2017). Reduced blood albumin concentration is known to influence libido, causes a lack of oestrus, early embryo mortality and uterine wall absorption of deceased embryos (Bearden *et al.*, 2004). Early cycling cows display high albumin concentrations all through the peri-partum period compared to cow with postponed reproductive cyclicity (Krause *et al.*, 2014).

A study reported a lack of significant variances in cattle breed types and the values of albumin to be within the normal ranges 3.03-3.55 g/dl in exotic breeds as compared to communal breeds (Kalyani *et al.*, 2018). In pregnant cows, a decrease in albumin concentration is usually followed by elevation in globulin concentration; also the ALB levels may decrease in mid-gestation and rise to acceptable levels until calving (Žvorc *et al.*, 2000). The concentration of albumin in cows is inclined to rise in cases of acute infectious diseases (Ahmad *et al.*, 2004). Low albumin concentration is related to hepatic failure due to inflammation (García *et al.*, 2017). During early lactation, a significantly low concentration of albumin may occur due to fatty liver disorder and an increase in ALB may be noted from about 15 up to 90 days post calving (Bobbo *et al.*, 2017).

Some association has been made between high concentration of albumin and reduced inflammation rate during infections; moreover, in order to support globulin synthesis the liver production of albumin must be low (Bertoni *et al.*, 2008). In cows with mammary gland infections there is low albumin production (Bobbo *et al.*, 2017). Downer cows may have normal calcium levels due to low albumin concentration, which could lead to a misleading diagnosis (Seifi *et al.*, 2005). Low albumin levels have been associated with a delay in postpartum cyclic resumption (Krause *et al.*, 2014). Reduced albumin concentration may also be seen as a result of increased catabolism of ALB which is observed in low energy instances (Jeong *et al.*, 2015b).

2.5. Hormones

2.5.1. Introduction

In animal health assessment of hormones has been increasingly used to monitor disturbing changes associated with cow reproduction (Zhang *et al.*, 2018). Hormones play a significant role in supporting reproductive processes (Rosselli & Dubey, 2006). Several researchers have identified the relationship between postpartum reproductive efficiency and the metabolic hormones in cattle (Samadi *et al.*, 2013). Predisposition to metabolic disorders may result from biological alterations in the neuro-hormonal system, which may lead to impaired immune function (Dudhatra *et al.*, 2012). Whether or not the animal develops postpartum disorders primarily depends on the state of the immune function (Manimaran *et al.*, 2016). Due to the endocrinal and metabolic alterations known to suppress the uterine defence mechanism, the incidence of metabolic disorders is increasing in cattle (Mateus *et al.*, 2002; Kim *et al.*, 2005).

The effectiveness of a hormone is not merely subject to hormonal levels; it is also dependent on the number of receptors in the targeted organ, since the hormonal binding to the receptors precedes effect production on the target organ (Heppelmann *et al.*, 2018). The reproductive

competency in cows can be extremely affected by the prevalence of reproductive disorders (Katiyar *et al.*, 2016). Early detection of pregnancy is a suitable approach to improve reproductive performance and is also important in the prevention of early embryonic death which often occurs between 8-17 days of pregnancy in both dairy and beef cows (Kubo *et al.*, 2018). Reproductive performance is one of the most vital aspects in maximizing production yields and increasing profitability in cattle production (Palomares *et al.*, 2010), hence proper management during peri-partum periods requires great consideration to ensure sustainability in small scale farming and increase contribution to the greater economy.

2.5.2. Oestrogen

Oestrogen is among many hormones produced by the ovaries. Oestrogen is manufactured in the extra-gonadal regions of the body such as the mesenchymal cells of adipose tissue and skin, bone chondrocytes and osteoblasts, cells of the aortic smooth muscle and vascular endothelium and also in numerous brain parts such as medial basal hypothalamus and anterior hypothalamus (Çiftci, 2013). Estradiol 17β (E2), Oestrone (E1) and Estriol (E3) are the three main forms of oestrogen naturally produced in an animal body (Çiftci, 2013). The pro-reproductive mediation of estradiol (the predominant form of oestrogen) occur in various organs and a number of biological processes (Schulster *et al.*, 2016).

The mode of action for estradiol is to bind oestrogen receptors activating production of autocrine and paracrine factors (growth factors and cytokines such as transforming growth factor β , leukemia inhibitory factor, endothelin-1, nitric oxide) facilitating reproductive processes (Rosselli & Dubey, 2006). Estradiol is also known to influence growth (stimulate the secretion of growth hormone), maturation, reproductive tract function, sexual differentiation and sexual behaviour (Çiftci, 2013).

Time dependent and cyclic-oestrogen has functional properties in reproduction; it controls oocyte development to fertilization, embryo implantation and embryo development (Rosselli & Dubey, 2006). Oestrogen is an intra-ovarian hormone functionally characterised by influencing calcium homeostasis, hypothalamus, liver, pituitary gland and the skeleton (Çiftci, 2013). The release of oestrogen in late pregnancy for the preparation of subsequent gestation, cows grazing on pasture with high oestrogen content such as *Trifolium subterraneum* and the use of growth implant containing oestrogen predispose cows to vaginal prolapse (Patel & Forestry, 2017).

2.5.3. Progesterone

Progesterone (PRG) is a significant controller of numerous reproductive processes, for instance ovulation, menstrual cycle, implantation and maintenance of pregnancy in mammals (De Amicis *et al.*, 2012). In different animal species and humans, progesterone (PRG) is a sexual hormone that participates in the reproduction of both females and males (Valadez-Cosmes *et al.*, 2016). Progesterone is a steroid hormone mainly synthesized and secreted by ovaries, placenta, adrenal glands, and testis (Blavy *et al.*, 2016). It has a known characteristic of maintaining pregnancy in cows (Stevenson & Lamb, 2016). In female, the 4-pregnene-3,20-dione (progesterone) has an important role in the reproductive cycle regulation in mammals (Yu & Maeda, 2017).

Cholesterol and circulating pregnenolone in the brain, spinal cord and peripheral nerves *de novo* synthesises progesterone (Valadez-Cosmes *et al.*, 2016). The global occurrence of preterm births is up to 5-18% and they are connected to calf mortalities and morbidity (Minguet-Romero *et al.*, 2014). In order to avoid pre-term labour, progesterone treatment is mostly used to improve management in reproduction (Romero *et al.*, 2014). At the beginning of the luteal phase, low levels of progesterone have negative impacts on embryo survival due

to its influence on the interval to first ovulation, conception rate, oocyte development, and embryo quality which are known to be directly linked to the negative energy balance in the post-partum period (Martin *et al.*, 2013).

Determination of progesterone levels in cows has been used to evaluate the status of reproduction, for pregnancy diagnosis so as to improve fertility (Blavy *et al.*, 2016). There have been developments in technologies which can be used for progesterone measurements on farms. To establish normality in assessment of progesterone levels, consideration of different reproductive cycle stages (e.g., luteal phase, follicular phase) is essential for pre-conversion in order to obtain proper measurement interpretation (Gorzecka *et al.*, 2011). Variation of progesterone concentration in blood plasma is occasional; it is normally high during egg maturation prior to ovulation and subsequently decreases at the time of ovulation (Blavy *et al.*, 2016). Progesterone can also be measured from faeces, saliva and milk (ideal in dairy industries) (Yu & Maeda, 2017). A high concentration of progesterone is sustained during gestation and it is the best biomarker for evaluation of reproductive status (Valadez-Cosmes *et al.*, 2016).

Consistent assessment of progesterone measurements can increase reliability of the knowledge of reproduction data in mammals (Blavy *et al.*, 2016; Yu & Maeda, 2017). Progesterone, by its inhibitory effect, prevents myometrial contraction and the corpus luteum is the main source of progesterone in oestrus animals (Stevenson & Lamb, 2016). The use of early progesterone detection in pregnancy diagnosis and reproductive disorders increases productivity, reproductive efficiency and welfare in cows (Nyman *et al.*, 2014).

2.5.4. Prostaglandins

Prostaglandins are a set of biological lipid complexes having diverse hormone –like effects in animals (Dudhatra *et al.*, 2012). These tissue hormones are also described as autacoids and are

known to be present in every tissue in both humans and animals (Katiyar *et al.*, 2016). According to structure and function, prostaglandin A, B, E and F are the four groups of prostaglandins of which only F (PGF2 α) and E are most essential and directly affect fertility and reproductive system (Tsai *et al.*, 2001). In cows, Prostaglandin F2 α (PGF2 α) synthesized in the uterus can be stimulated by oxytocin in the tissue of the endometrium and result in degradation of the corpus luteum and recurring oestrus (Kubo *et al.*, 2018). The use of prostaglandins (PGF2 α) in pregnant cows has been to prompt uterine contraction and elevate phagocytosis inside the uterus (Younger *et al.*, 2014).

Prostaglandins have other functional properties of stimulating contractions of the myometrium in cattle (Sauls *et al.*, 2018). In nearly all mammals, prostaglandins are recognised for their function in local luteal regulation as well as a means to advance breeding proficiency and as treatment for retained placenta, pyometra, anoestrus as well as endometritis (Dudhatra *et al.*, 2012). Natural uterine clearance of bacterial infections occur in cows, only about 20% of the infections may last for up to three weeks after calving (Younger *et al.*, 2014). Due to prostaglandin's ability to induce uterine contraction leading to foetal expulsion (ecbolic properties), it is also useful in the elimination of intraluminal uterine substances and in corpus luteal lysis (Rigby *et al.*, 2001).

Low breeding capacity, anoestrus, infertility and sub-fertility may as well successively occur as a result of deferred uterine clearance (Younger *et al.*, 2014). Termination of pregnancy can also be executed by the use of PGF2 α and its analogues as they are able to suppress the peripheral levels of progesterone by inducing luteal regression, prompting abdominal contraction and cause part of the cervix to relax (Katiyar *et al.*, 2016). The use of prostaglandins (PGF2 α) in the first five months (150 days) of pregnancy lead to abortion and has also been shown to stimulate calving as soon as 255 days of pregnancy (Dudhatra *et al.*, 2012). Furthermore, cloprostenol drug (synthetic analogue of prostaglandins F2 α) has been used in

cows as a means to improve breeding capacity (Katiyar *et al.*, 2016). Subsequent reproductive efficiency of cows relies much on proper uterine involution and contaminants expulsion (Younger *et al.*, 2014).

2.5.5. Oxytocin

Oxytocin (OT) is a nonapeptide posterior pituitary hormone secreted through the paraventricular nuclei (PVN) and supraoptic nuclei (SON) of the hypothalamus which is also deposited into the blood stream by the posterior pituitary neurons (Uvnäs-Moberg *et al.*, 2001). Increase of calcium (Ca^{2+}) in the cells results from oxytocin receptors which trigger contractions of the uterus and plays a role in both parturition and lactation processes in animals (Arrowsmith & Wray, 2014). In mammals, the secretion of oxytocin is through the corpora lutea (Stormshak, 2003). Oxytocin is known to positively influence uterine contractility such as when used in instances of forcefully causing uterine contractions after labour to discontinue postpartum haemorrhages by clamping the blood vessels of the uterus (Heppelmann *et al.*, 2018).

In the induction of labour, oxytocin can be useful and is mostly ideal when the advantages for foetal ejection prior to normal delivery exceed the danger associated with the maintenance of pregnancy (Arrowsmith & Wray, 2014). Characteristic functions of oxytocin include induction of uterine contraction, uterine involution, uterine motility, calving, expulsion of uterine contaminants and stimulation of prostaglandins (PGF_2 alpha) synthesis (Palomares *et al.*, 2010). Both neurogenic and circulatory oxytocin play a part in the coordination processes such as foetal-mother attachments and other parental connections (Uvnäs-Moberg *et al.*, 2001). Oxytocin has also been used in contractions inhibition or activation of the myometrium in health management problems and preterm labour (Arrowsmith & Wray, 2014).

In labour dysfunction treatment programs such as dystocia management, oxytocin is commonly used to correct the disorder although it is only known to succeed in about 50% of the cases (Arrowsmith & Wray, 2014). Infusion of oxytocin causes hyper activity of the uterus of which the placenta-foetal unit is unable to recompense, this subsequently leads to reduced oxygen supply and blood flow to the foetus causing distress or foetal-hypoxia, tetanic contractions and ultimately foetal-death (Liston *et al.*, 2002). Following the released hormone from the pituitary gland, the maternal circulatory increase of oxytocin concentration is usually seen at the onset of parturition in mammals (Blanks & Thornton, 2003). Additionally, the ejection of oxytocin in animals has been reported to be rhythmic for the period of foetal expulsion (Arrowsmith & Wray, 2014). The inconsistency in oxytocin role during labour indicates a greater need to further study its role during parturition.

Mineral and hormonal balances in production animals are very significant to reproductive efficiency of cows. The disregard for risks related to mineral and endocrinal imbalances can reverse both hormonal and mineral benefits in cow reproduction. Hence, mineral requirements and reproductive hormones in livestock farming should be greatly considered in terms of farm management and optimising reproductive yields.

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CHAPTER 3

FACTORS PREDISPOSING COWS TO THE INCIDENCE OF REPRODUCTIVE CONDITIONS IN THE COMMUNAL FARMING SYSTEM

Abstract

This study aimed to identify cattle production management practices that predispose the animals to incidents of some reproductive conditions in communal farming. Based on the statistical data obtained from the Molopo State Veterinary offices in the Department of Agriculture at Mahikeng local municipality, the population of registered communal farmers in the area was approximately 4370 in 2017. The Rao Soft sample size calculator was used to determine the sample size of 135 from the population of communal farmers whose cows encountered reproductive conditions (dystocia, downer cow syndrome, retained placenta, vaginal prolapse and abortion). Data were collected during farm visits following cases reported to the Dale Beighle Animal Hospital at the North West University (NWU). Pre-tested questionnaires were designed and disseminated from which the farmer's demographic information as well as data about the cows were obtained. The data included information such as the age, parity, breed, body condition scores, feeding system, feed type, whether the animals were supplemented, types of supplements, cow vaccination status, type of vaccine were used, cows' Brucellosis status and access to veterinarian services. Pie charts were used to summarize the frequencies. In this study, factors significantly related ($P < 0.05$) to the incidence of reproductive conditions were cows breed, parity, feeding system, whether the farmer has heard about Brucellosis or not and the frequency of getting animals checked by a veterinarian. The results showed that first parity cows most of which were Afrikaner breed, reared in free range conditions, where farmers have not heard about Brucellosis and having access to veterinarian

services only once a year experienced reproductive conditions. Implementation of strategies for the improvement of farming methods in communal farming is necessary to change the state of livestock agriculture and improve the economic status of communal farmers.

Key words: Cows, Management practices, Production, Reproductive condition.

3.1. Introduction

The incidence of reproductive conditions during parturition is a complex subject influenced by factors associated with management and systemic health (Ball and Peters, 2004). Planning for effective reproduction begins prior to parturition during the dry period. Research has emphasised and produced substantial evidence on the impact of proper management in the reproduction of transitioning cows (Drackley and Cardoso, 2014). Even with many developments and innovation strategies to increase production and reduce losses, the occurrence of reproductive disorders such as dystocia, retained placenta, vaginal prolapse, downer cow syndrome and abortion in cows reared in communal areas is seen during almost every calving season.

Moreover, the status of cow reproductive efficiency in communal farms is not exactly known but only presumed to be low, mainly due to a lack of data and because of the inaccessibility of animal health care for poorer farmers (Butler, 2005; Drackley and Cardoso, 2014; Lucy *et al.*, 2014). Hence, for efficient reproduction, it is important to ensure adequate nutrient supply for pregnancy to reduce the risks of calving problems and post-calving complications. Therefore, identifying factors leading to the incidence of reproductive conditions is essential to help prevent and reduce production losses.

3.2. Methodology

3.2.1. Description of the Study area

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 encountered between August and March with an annual rainfall which averages from 200 to 500 mm (Materechera, 2011). 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 (Balarane & Oladele, 2012). The Mafikeng area, in total size, is approximated to be about 3 703 square kilometres. This area is known to be divided into 28 wards and 102 suburbs and villages according to Matsane and Oyekale (2014). This study area is found in the District of Ngaka Modiri Molema. Agriculture is the major driving force of the economy, producing mainly cattle and crops.

3.2.2. Study tool

A sample size of 135 was determined using a Raosoft calculator. Convenient sampling method was used to generate the sample size. Questionnaires were distributed to farmers who reported cow reproductive disorders (dystocia, downer cow syndrome, retained placenta, vaginal prolapse and abortion) at the NWU (Dale Beighle) Animal Hospital. A structured questionnaire was developed to capture data on a wide range of variables such as the farmer's demographics, cow production and reproduction, herd health and management factors. The data collection tool was tested (pilot-testing) prior to the commencement of the study. The pretesting was to evaluate the questionnaire strengths, precision and weaknesses and to test whether it would bring out the anticipated responses. The pilot-run revealed that some items in the questionnaire were unclear and needed to be rephrased. The questionnaire was reviewed to make necessary improvements and to eliminate vague questions before being administered to the farmers. Some 135 questionnaires were then distributed to farmers who reported cases of reproductive

conditions in communal areas around Mafikeng. The data collected included farmer demographics, cow information and medical history, incidences of reproductive conditions (dystocia, downer cow syndrome, retained placenta, vaginal prolapse and abortion) as well as management practices (veterinary check-ups, feeding systems, vaccination protocols, disease control programs, breeding practices) under which cows are reared and the farmer's knowledge about Brucellosis in their herd was also recorded. To assure good quality of data collection the lead researcher self-administered the questionnaires.

3.3. Statistical considerations

The data were analysed using SPSS version 25. SPSS was preferred for its higher analysis abilities and also because of its capacity to handle the multiple arrangements of data obtained from the survey. SPSS was therefore used to calculate all frequencies and descriptive statistics. Pie charts were used to summarise the responses for each variable in the questionnaire. The chi-square test of association was used to determine the association between the occurrence of the reproductive conditions and the attributes of the cows as well as the farm management practices. For the variables showing significant associations, cross-tabulations were used to determine the probability of the occurrence of the reproductive conditions given the cow attributes or farm management variables. The two-stage cluster analysis was used to segment the animals based on the variables associated with the reproductive conditions as identified by the chi-square test of association. A further Chi-square test of association was performed to confirm the association between the reproductive condition and the cluster to which the cow was allocated.

3.4. Results

The results for the farmer's demographics and information about the cows experiencing reproductive conditions are represented below.

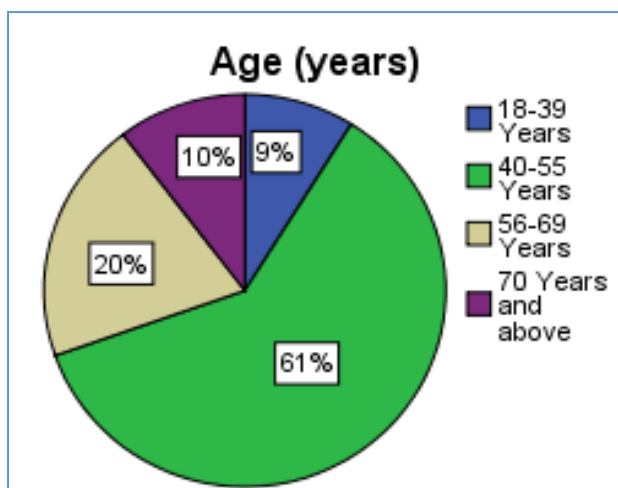


Figure 3.1: The age groups of the farmers

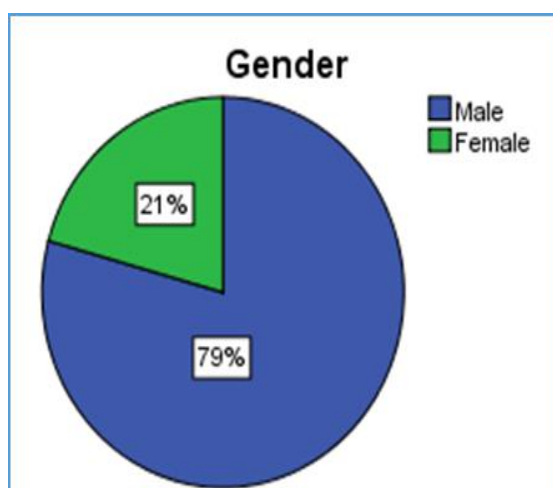


Figure 3.2: Gender of famers in communal area

Figure 3.1 shows that most of the communal farmers were in age group between 40-55 years (61%), while 20% of the farmers were found in the age groups of 56-69 years; subsequently only 10% of the farmers were 70 years of age and above, and the least proportion were of farmers between 18-39 years (9%). The results of this study show that most of the farmers involved in the small scale farming system were males (79%) while female farmers represented only 21% of the communal farmers (Figure 3.2).

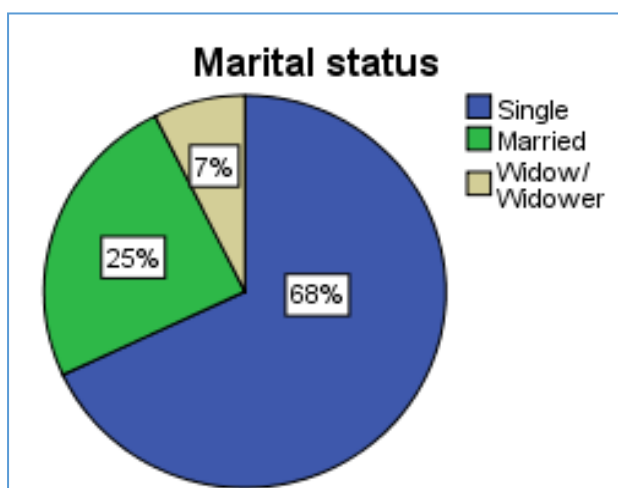


Figure 3.3: Marital status of farmers

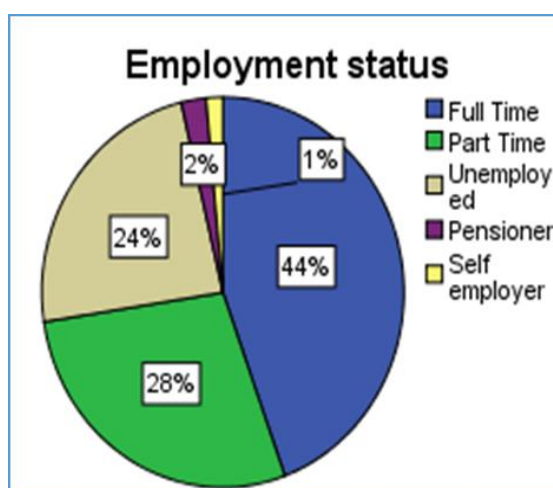


Figure 3.4: Farmers employment

Figure 3.3 shows that only 25% of the farmers were married, while most of the farmers were single (68%). The study further indicated that the fewest of farmers were widowed (7%). The data showed that 44% of the farmers have full time employment, whereas those with part-time jobs made up to 28% of the farmers and only about 24% were unemployed farmers (Figure 3.4).

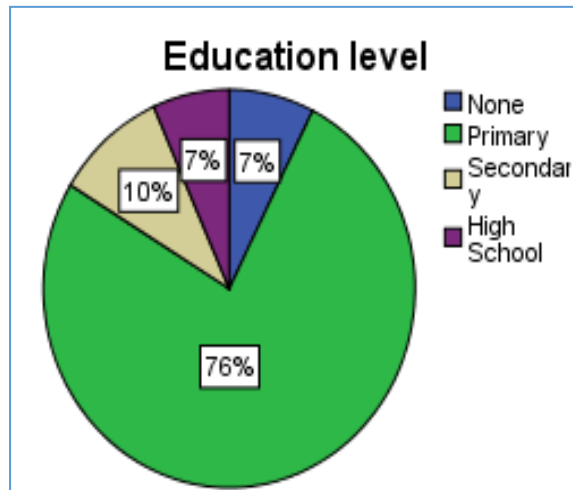


Figure 3.5: Levels of education of the farmers.

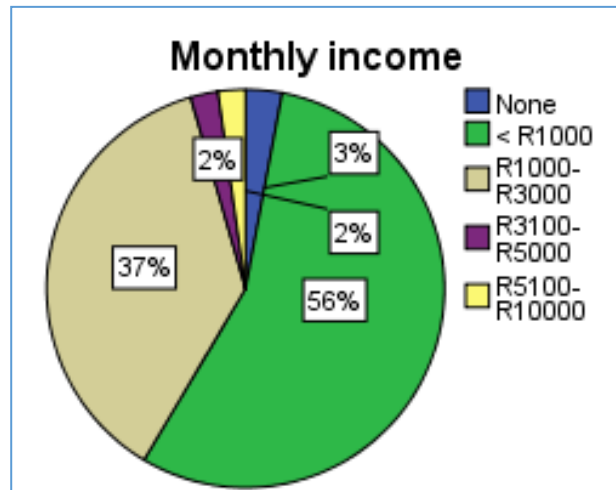


Figure 3.6: Levels of income amongst farmers in this study.

The present study indicated that most farmers had at least primary level (76%) education, followed by (10%) secondary level, high school (7%) and those with no education (7%) as indicated in Figure 3.5. Those who had no income were about 3% of the farmers. A total of 56% of the farmers earned less than one thousand rand. Those with between R1000–R3000 were 37% of the population. Only 2% of the farmers had income above R3100 and R10 000 (Figure 3.6).

Table 3.1: Test of association between reproductive conditions encountered

Cow attributes	Chi-Square	Asymp. Sig. (2-sided)
Age of the cow	Likelihood Ratio	0.349
Body condition score	Likelihood Ratio	0.11
Breed	Likelihood Ratio	0.002**
Parity	Likelihood Ratio	0.003**
Number of occurrences of the condition	Likelihood Ratio	0.816
Feeding system	Likelihood Ratio	0.026*
Feed type	Likelihood Ratio	0.136
Whether the cows are supplemented	Likelihood Ratio	0.817
Supplements given to the cows	Likelihood Ratio	0.559
Type of supplements	Likelihood Ratio	0.124
Whether treatment was for the current condition before	Pearson Chi-Square	0.114
Whether the cow was given anti-parasitic medication	Pearson Chi-Square	0.448
Whether the cow was vaccinated	Pearson Chi-Square	0.546
Vaccine administered to the cow	Likelihood Ratio	0.453
Whether the farmer has heard about Brucellosis	Pearson Chi-Square	0.001**
Any other disease/s encountered	Pearson Chi-Square	0.353
The frequency of getting animals checked by a veterinarian	Likelihood Ratio	0.005**

*Significance ($P < 0.05$); **=Significance ($P < 0.01$).

The Chi-square tests indicate that the reproductive condition encountered is significantly associated ($p < 0.05$) with the following variables: Breed, Parity, Feeding system, whether the farmer had heard of Brucellosis and the frequency of getting animals checked by a veterinarian (Table 3.1).

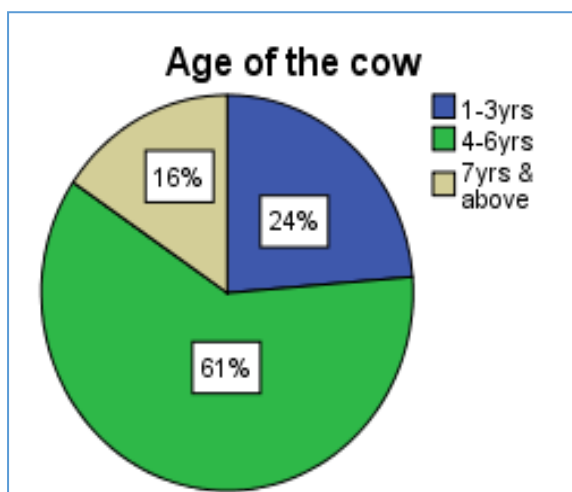


Figure 3.7: Ages of cows presenting with reproductive conditions.

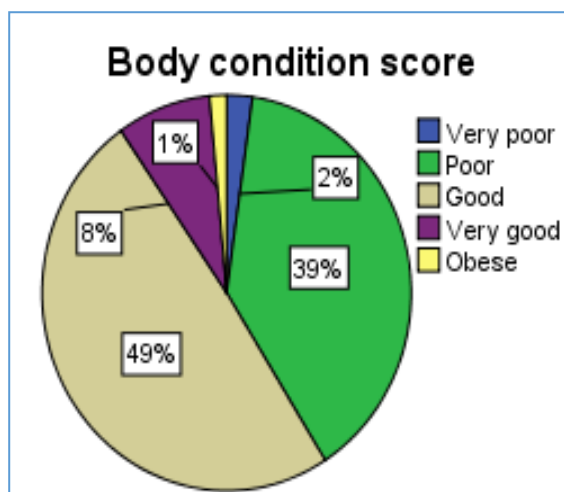


Figure 3.8: Body condition scores of the cows experiencing reproductive conditions.

Most of the conditions were seen in cows in the 4-6-year age range (61%), while 24% of the cows were of age between 1 and 3 years and only 16% of the cows were 7 years and above (Figure 3.7). About 49% were in a good body condition. The study also showed that 39% were in a poor body condition. Additionally, the least proportions of cows were in a very good (8%), very poor (2%) and the obese (1%) (Figure 3.8).

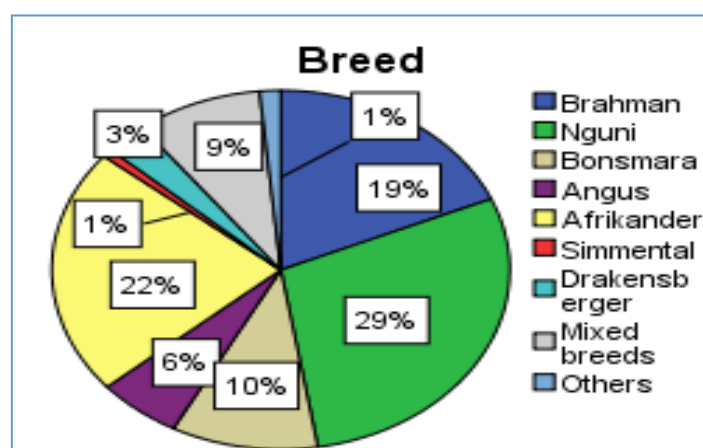


Figure 3.9: Breed types among the cows which experienced reproductive condition.

The data shows a decrease in proportion of cows among different breed type from Nguni (29%), Afrikaner (22%), Brahman (19%), Bonsmara (10%), mixed breed (9%), Drakensberger (3%) and Simmental (1%) seen in figure 3.9.

Table 3.2 Association between the incidence of reproductive conditions and the cow breed

		Breed * Reproductive condition encountered				
		Condition encountered				
		Downer cow	Abortion	Retained placenta	Vaginal prolapse	Dystocia
Breed						
	Brahman	12.0%	20.0%	28.0%	24.0%	16.0%
	Nguni	25.6%	38.5%	17.9%	12.8%	5.1%
	Bonsmara	21.4%	42.9%	7.1%	14.3%	14.3%
	Angus	-	37.5%	62.5%	-	-
	Afrikaner	20.0%	6.7%	23.3%	23.3%	26.7%
	Simmental	100.0%	-	-	-	-
	Drakensberger	-	50.0%	-	50.0%	-
	Mixed breeds	16.7%	-	33.3%	8.3%	41.7%
	Others	50.0%	-	-	-	50.0%

Others = unidentifiable breeds

The result of the present study revealed that all (100%) Simmental cows in the study were affected by downer cow syndrome. Moreover, among the breeds Drakensberger cows had a relatively high chance of experiencing abortion (50%), while Angus cows stand a relatively high chance of experiencing retained placenta (62.5%), Drakensberger also stand a relatively high chance of experiencing vaginal prolapse (50%) whereas Other breeds have a higher chance of experiencing Dystocia (Table 3.2).

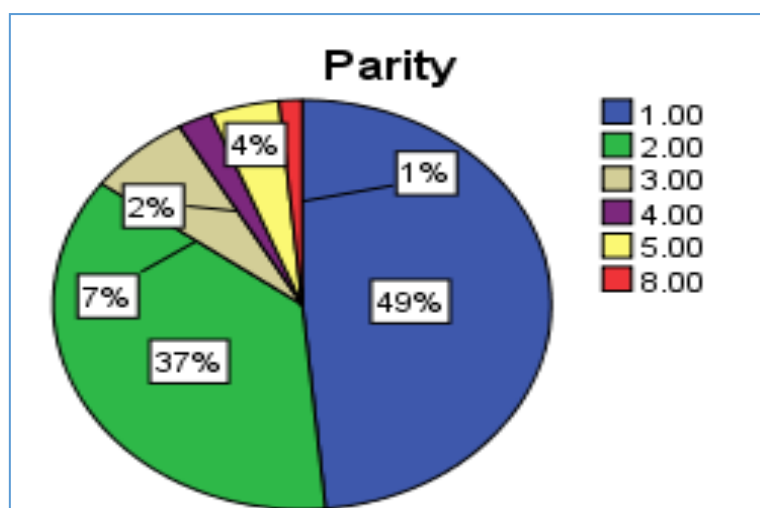


Figure 3.7: Parity of the cows which encountered different reproductive conditions.

About 49% of the cows were in the first parity and were the majority (Figure 3.10). The second largest group of cows were in their second parity (37%). Only 7% of the cows were in the third parity and about 4% have calved five times (Figure 3.10). The least proportion was of the cows in their 8th parity (1%) (Figure 3.10).

Table 3.3 Association between the incidence of reproductive conditions and the number of parity

Parity * Reproductive condition encountered						
		Reproductive condition encountered				
		Downer cow syndrome	Abortion	Retained placenta	Vaginal prolapse	Dystocia
Parity	1	23.1%	21.5%	18.5%	23.1%	13.8%
	2	14.3%	38.8%	26.5%	6.1%	14.3%
	3	22.2%	-	22.2%	22.2%	33.3%
	4	-	-	-	-	100.0%
	5	16.7%	-	33.3%	50.0%	-
	8	-	-	100.0%	-	-

The cows with parity 1 have a high chance (23.1%) of having downer cow syndrome, the ones with parity 2 stand a high chance of experiencing abortion whereas 100% of the cows with

parity 8 are highly likely to experience retained placenta. In addition, cows of parity 5 stand a high chance (50%) of experiencing vaginal prolapses whereas the cows with parity 4 stand the highest (100%) of having dystocia.

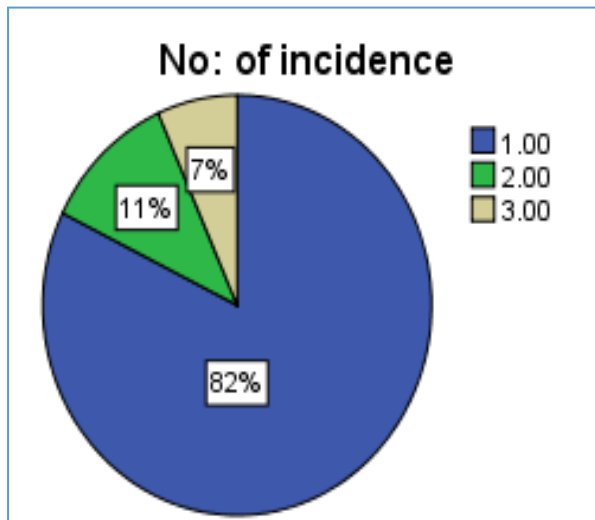


Figure 3.8: Frequency of condition encountered

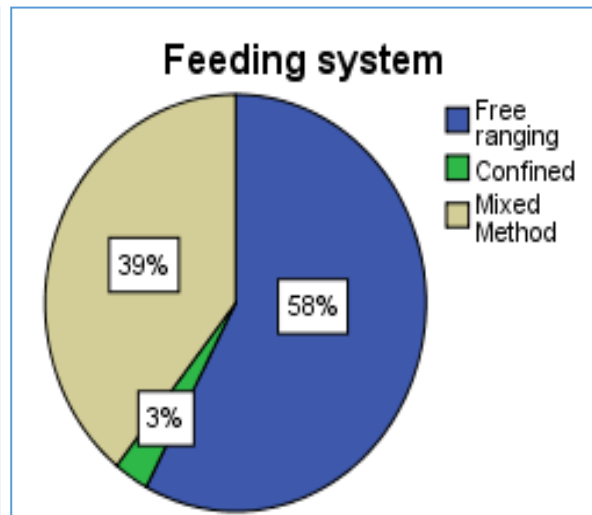


Figure 3.9: Most common feeding system in communal farming

Most of the cows encountered the condition for the first time (82%) while only 11% of the cows had repeated incidence of the same condition and only 7% of the cows had the conditions for the third time (Figure 3.11). Free range cattle rearing was the most practiced method of feeding (58%). On the other hand, mixed feeding methods (39%) were the second most common feeding method and only 3% used confined feeding method (Figure 3.12).

Table 3.4: Association between the incidence of reproductive conditions and feeding system

Feeding system * Reproductive condition encountered						
		Condition encountered				
Feeding systems		Downer	Abortion	Retained	Vaginal	Dystocia
		cow		placenta	prolapse	
	Free ranging	20.5%	16.7%	29.5%	17.9%	15.4%
	Confined	-	100.0%	-	-	-
	Mixed	18.9%	30.2%	15.1%	17.0%	18.9%
	Method					

The cows grazing from a free-range farm, stand a relatively higher chance (20.5%) of experiencing downer syndrome. All (100%) of the cows in confined feeding experienced abortion. The free range cows stand a relatively higher chance (29.5%) of experiencing retained placenta. The chance of experiencing vaginal prolapse was almost equal across the three feeding systems. Cows fed through the mixed method stand a relatively higher chance of experiencing dystocia.

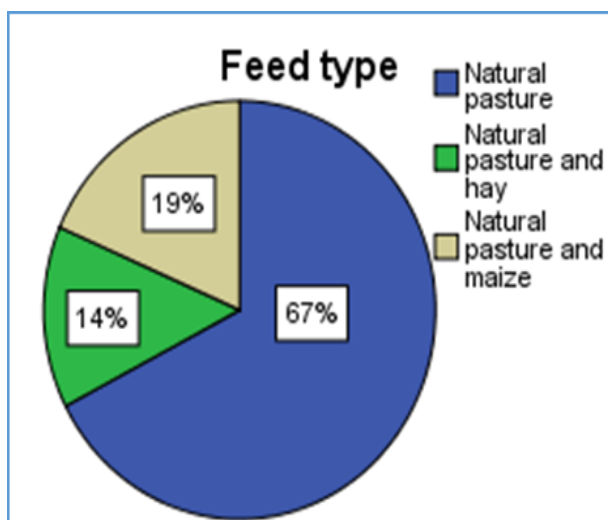


Figure 3.10: Feed type given to cows in communal farming

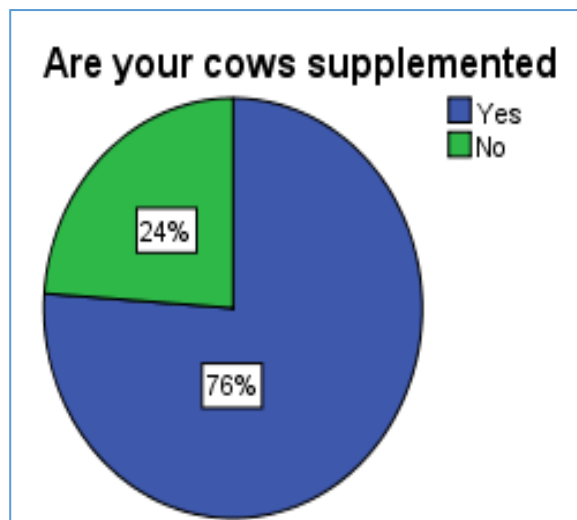


Figure 3.11: Whether the cows were supplemented

The different proportions of feed types represented in the study included natural pasture (67%), Natural pastures and maize (19%) and Natural pastures plus hay (14%) (Figure 3.13). The study shows that 76% of the cows were supplemented and only 24% were not supplemented (Figure 3.14).

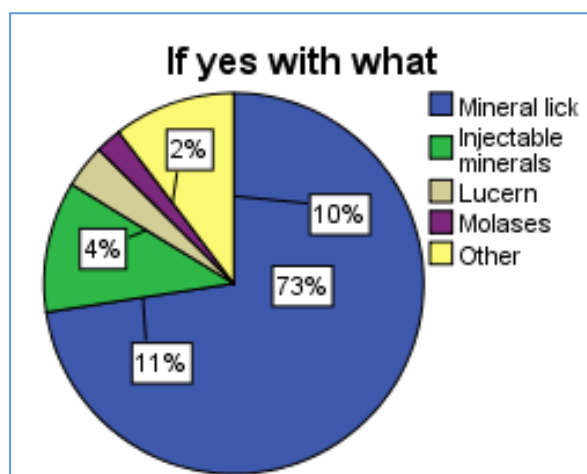


Figure 3.12: Types of supplements

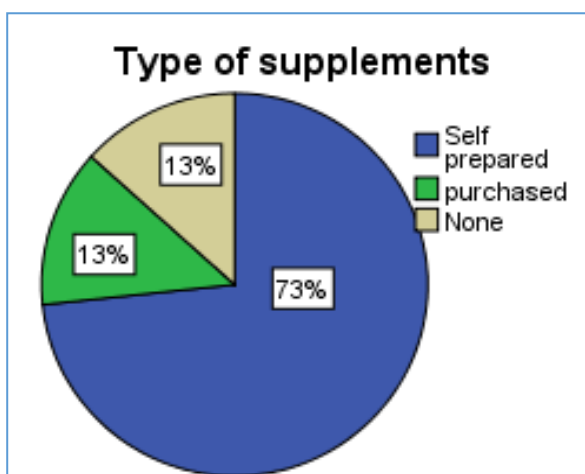


Figure 3.13: Types of supplements the cows were given

A large proportion of the cows were given mineral lick (73%), injectable minerals (11%) and other supplements (10%) as is indicated in figure 3.15. The results show that most of the

farmers prepared/mixed (73%) the supplement they were giving to the cow and those who bought the supplements were about 13% (Figure 3.16).

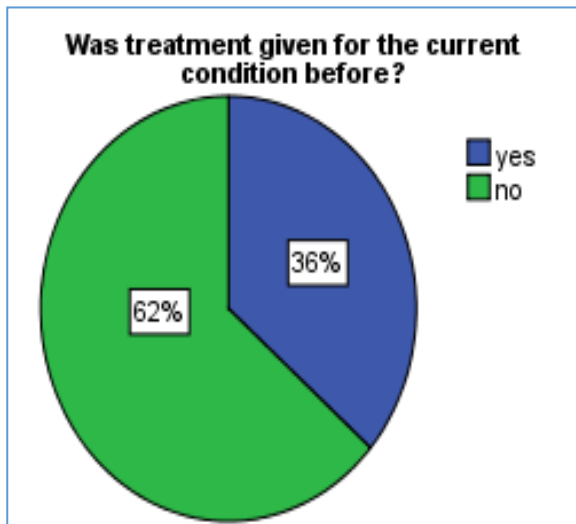


Figure 3.14: Cows treated for the same condition before.

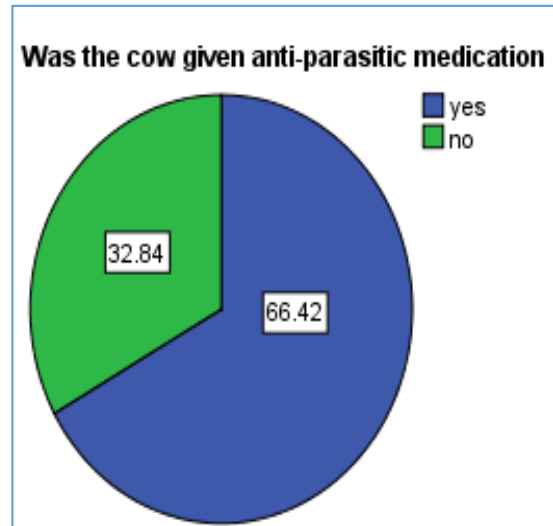


Figure 3.18: Cows given anti- parasitic medication.

A relatively large proportion of cows were not given treatment on their previous encounter of the reproductive condition, whereas about 36% were previously treated (Figure 3.17). It was shown that in most (66%) cases the cows were treated for parasite infestation-preventing medication while 35% were not (Figure 3.18).

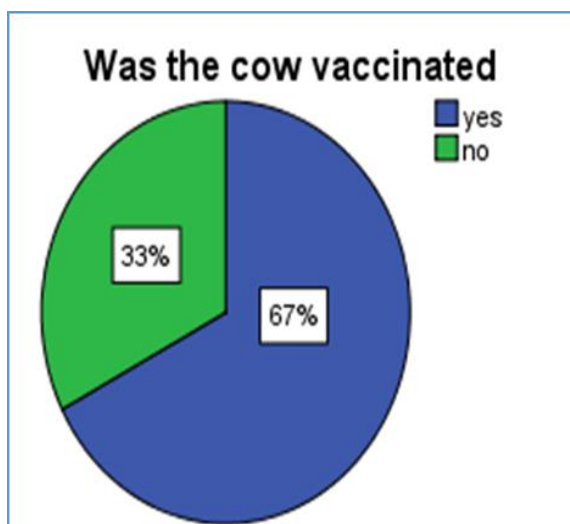


Figure 3.19: Vaccination status of the cows.

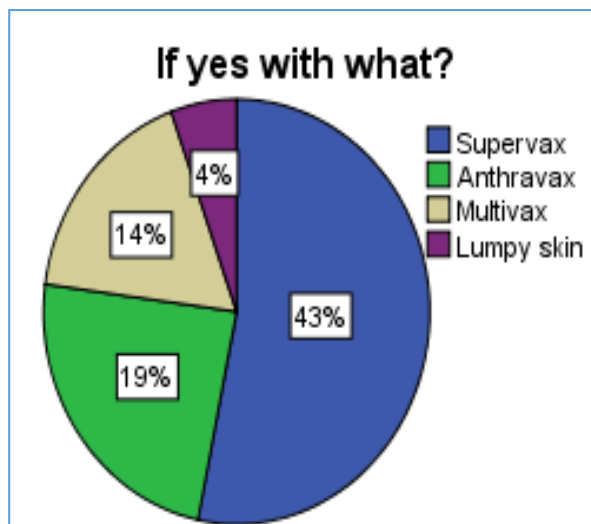


Figure 3.15: Commonly used vaccines

The data reveals that most of the cows (67%) were vaccinated and those not vaccinated were about 33% (Figure 3.19). Figure 3.20 shows the types of vaccines the communal farmers commonly use. Supervax (43%) was the most commonly used, followed by Anthrax (19%), Multivax (14%) and the least frequently used the lumpy skin vaccine (Figure 20).

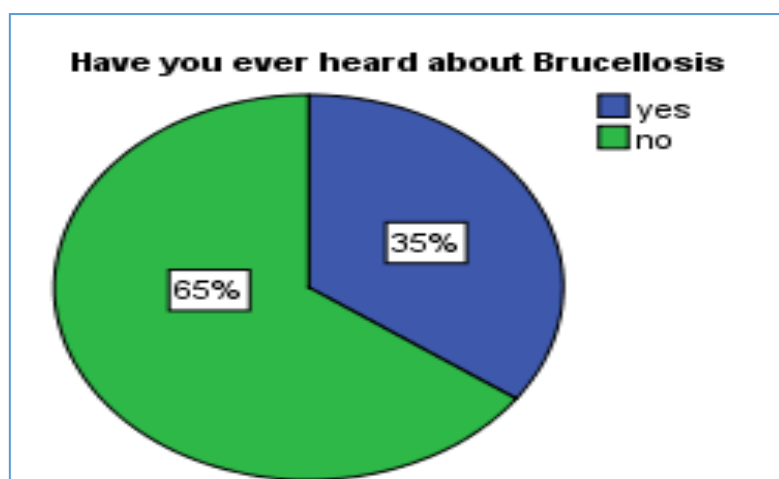


Figure 3.16: Farmers have heard of Brucellosis or not

The results as shown in figure 3.21 indicate that most farmers (65%) have not heard of Brucellosis. Moreover, the study also showed that only 35% of the farmers have heard about the Brucella infection.

Table 3.5 Association between the incidence of reproductive conditions and farmer's knowledge of brucellosis

Have you ever heard about Brucellosis * Reproductive condition encountered						
		Reproductive condition encountered				
		Downer	Abortion	Retained	Vaginal	Dystocia
		cow		placenta	prolapse	
		syndrome				
Have you ever heard about	yes	34.0%	6.4%	27.7%	17.0%	14.9%
Brucellosis						
	no	11.4%	34.1%	20.5%	17.0%	17.0%

The cows from the farms whose farmers had heard about Brucellosis reflected a high (34%) chance of experiencing Downer syndrome and a relatively high chance of experiencing retained placenta (Table 3.5). However, the cows from farms whose farmers had never heard of Brucellosis stand a relatively high chance of experiencing abortion (34.1%) and dystocia

(17%). On the other hand, the chance of experiencing vaginal prolapse was equal for both farmers (those who have heard and those who have not heard about Brucellosis).

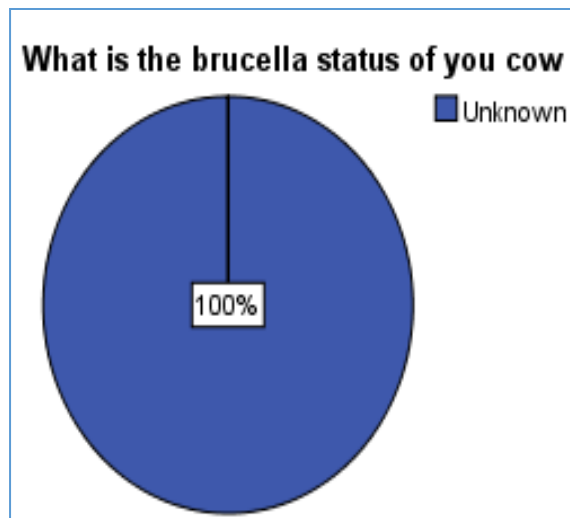


Figure 3.17: Brucella status of the farms in the study area.

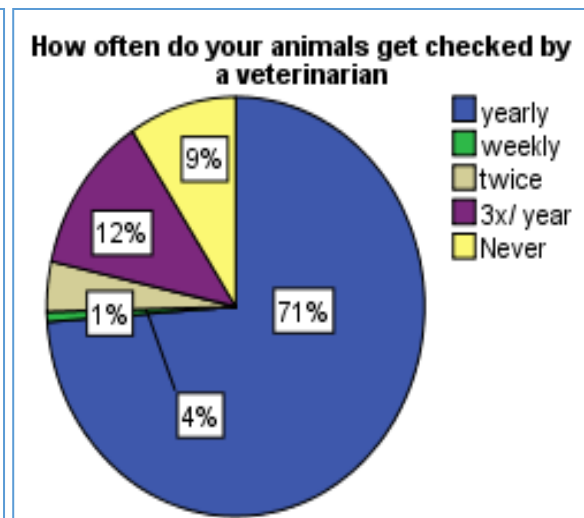


Figure 3.18: Veterinary check-up in communal farms.

The results revealed that all the farms had an unknown Brucella status (Figure 3.22). The frequency of veterinary check-ups was largely done yearly (71%) in most farms, only 3 times a year (12%) and 9% of the farmers never get their cows checked by a veterinarian (Figure 3.23).

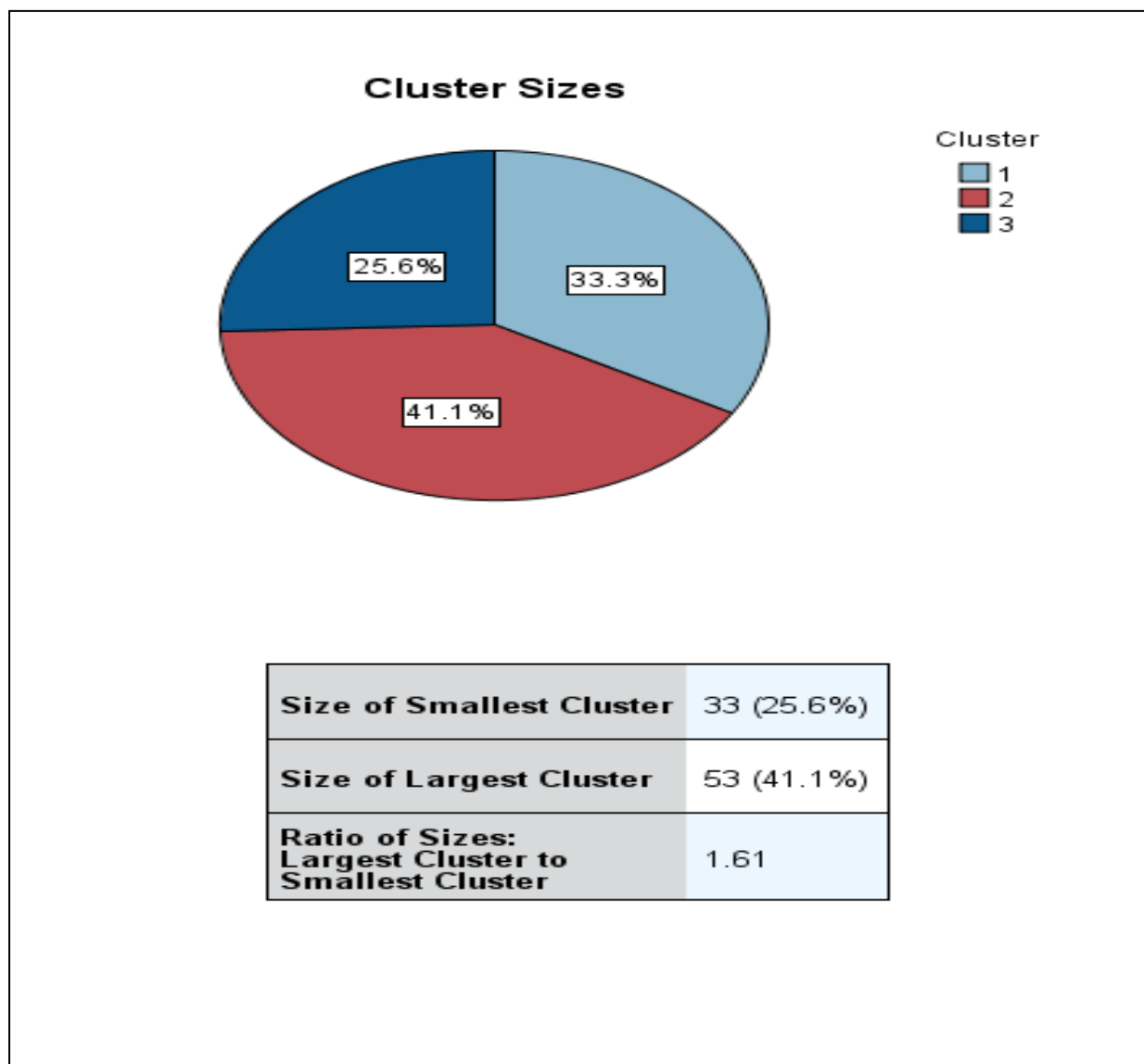


Figure 3.19: Segmentation of the animals based on the variables associated with the reproductive conditions.

The animals can be classified into three groups (cluster 1-3) (Figure 3.24) with cluster 2 having the highest percentage of animals (41.1%), cluster 1 has the second highest percentage of animals (33.3%) whereas cluster 3 has slightly more than a quarter of the animals allocated to it (25.6%) (Figure 3.24).

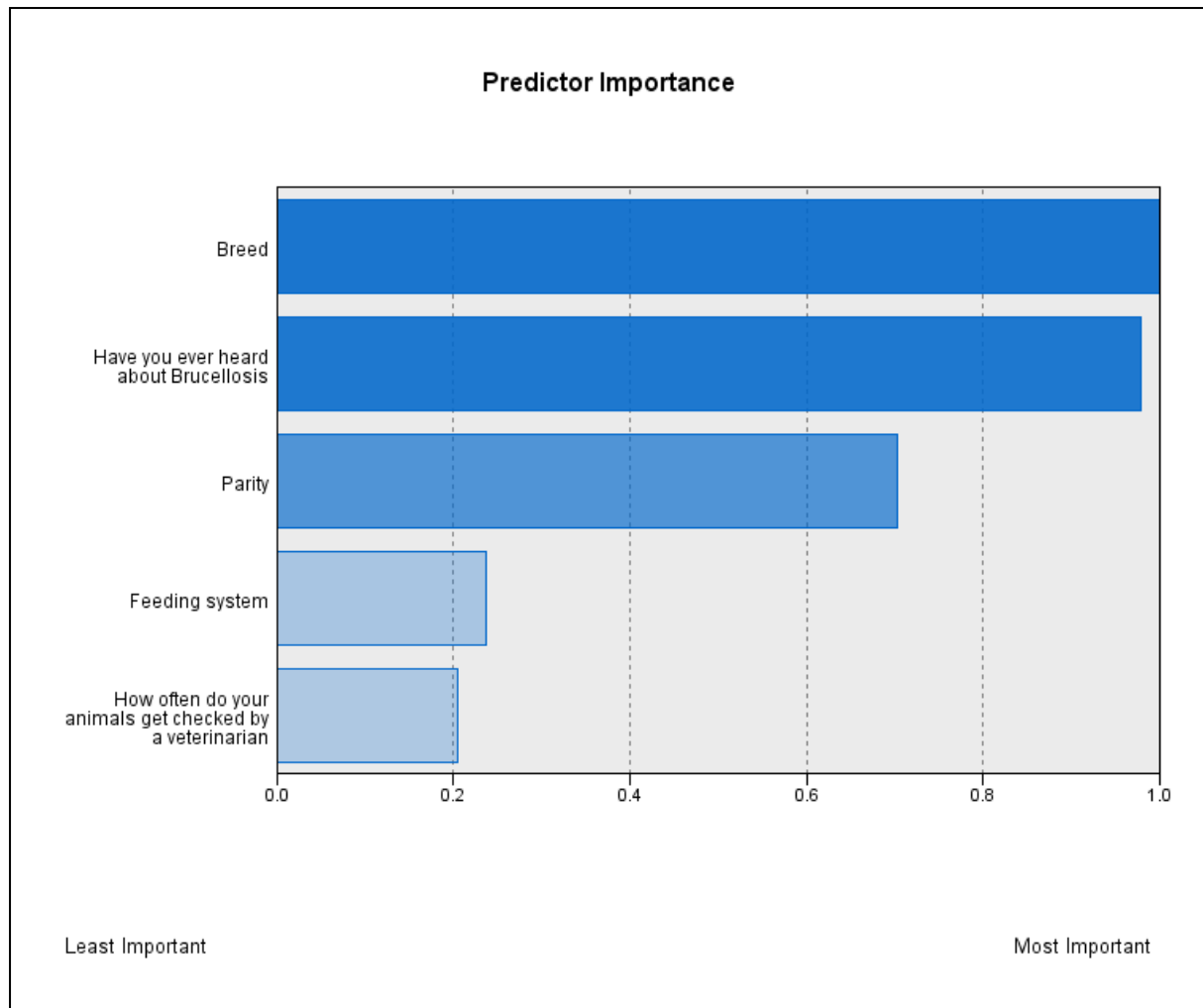


Figure 3.20: Reproductive condition predictor significance in term of association.

The results of this study revealed that the breed contributes more to the segmentation of the animals, followed by whether the farmer has heard about Brucellosis, then the parity (Figure 3.25). The feeding system is the fourth most important variable in the segmentation of the animals and least important variable is the frequency of having one's animals checked by a veterinarian. Each cluster is described next (Figure 3.25).



Figure 3.21: Cluster 1 of factors associated with the incidence of reproductive condition.

Cluster 1 (33.3% of the animals) comprised of animals in the breed “Other (any none identifiable breed)” or Bonsmara, the farmers keeping these animals had never heard of Brucellosis, their cows were of parity 2, the animals were subjected to the mixed method of feeding and were checked by a veterinarian only once a year (Figure 3.26).

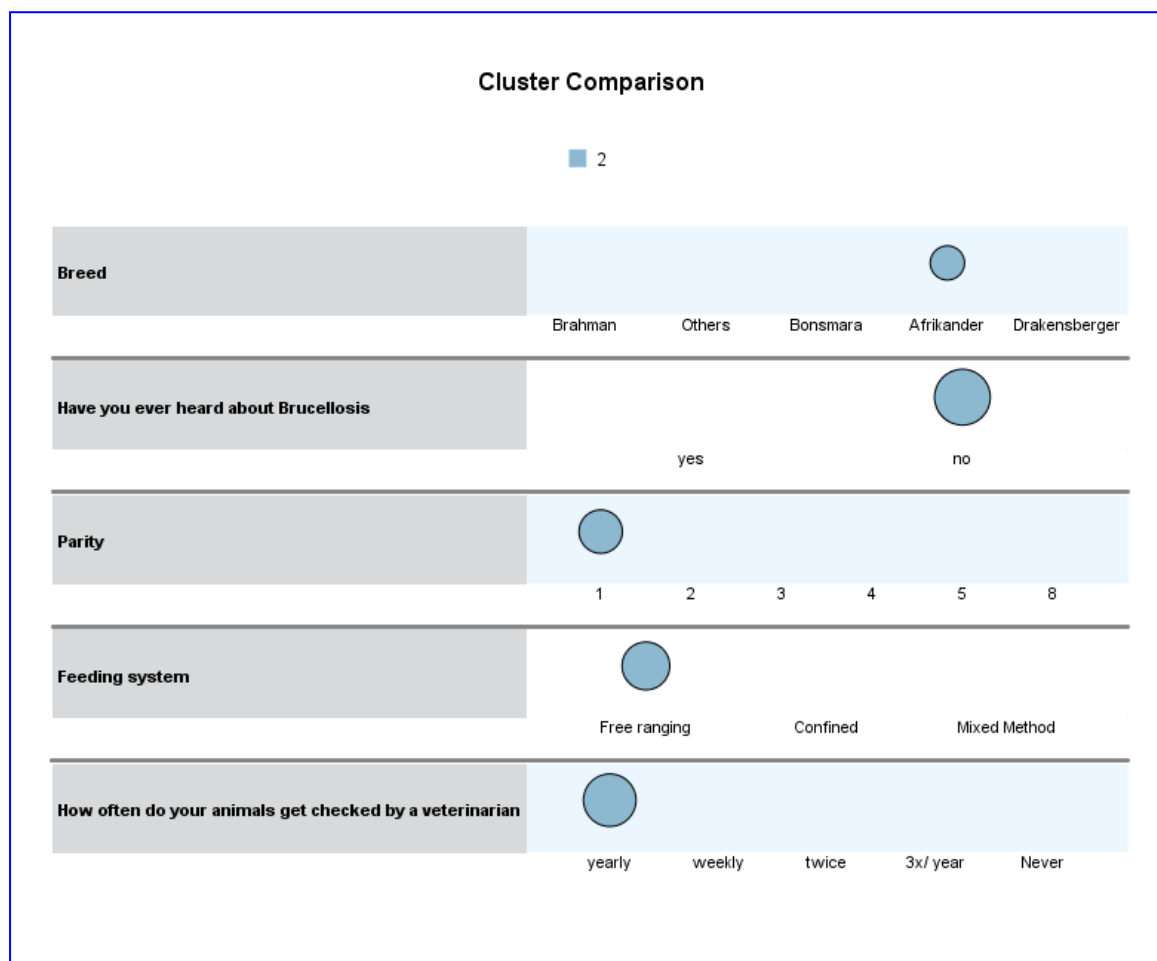


Figure 3.22: Cluster 2 of factors associated with the incidence of reproductive condition.

Cluster 2 (41% of the animals) comprised of animals of the Afrikaner breed. The farmers keeping these animals had never heard of Brucellosis, their cows were of parity 1, the animals were allowed the free ranging method of feeding and were checked by a veterinarian only once a year (Figure 3.27). Therefore, the difference between cluster 1 and cluster 2 was the breed, parity and feeding system. (Figure 3.27).



Figure 3.23: Cluster 3 of factors associated with the incidence of reproductive condition.

Cluster 3 (25.6% of the animals) comprised of Brahman breed animals, the farmers keeping these animals had heard about Brucellosis, their cows were of parity 1, the animals were fed on the free ranging method of feeding and they were checked by a veterinarian only once a year (Figure 3.28). Therefore, the difference between cluster 1 and cluster 3 is the breed, whether the farmer has heard about Brucellosis, parity and feeding system whereas the difference between cluster 2 and cluster 3 is the breed and whether the farmer has heard about Brucellosis (Figure 3.28). Next the study determined the association between the derived clusters and the presence of the reproductive conditions.

Table 3.6 Cluster association significance value (p-value < 0.05)

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	18.838	8	0.016*

The reproductive condition is significantly associated with the cluster to which the cow is allocated (p-value < 0.05) (Table 3.6).

Table 3.7 Cluster allocation to the incidence of reproductive condition

Twostep Cluster Number * Reproductive condition encountered						
		Conditions encountered				
		Downer	Abortion	Retained	Vaginal	Dystocia
		cow		placenta	prolapse	
Two-step Cluster Number	1	23.3%	39.5%	18.6%	9.3%	9.3%
	2	11.3%	24.5%	24.5%	20.8%	18.9%
	3	18.2%	3.0%	30.3%	24.2%	24.2%

Animals allocated to Cluster 1 (33.3% of the animals) which comprise animals with the breed “other” or Bonsmara, the farmers keeping these animals had never heard of Brucellosis, their cows were of parity 2, the animals were allowed the mixed method of feeding and were checked by a veterinarian only once a year 23.3% of them had experienced downer cow syndrome and 39.5% of them had an abortion (Table 3.7). About 41% of Afrikaner breed cows were allocated to Cluster 2, the farmers in this cluster had never heard of Brucellosis, their cows were of parity 1, the animals enjoyed the free range method of feeding and they were checked

by a veterinarian only once a year stand a higher chance of experiencing abortions (24.5%) than the ones allocated to cluster 3 (Table 3.7).

For animals allocated to Cluster 3 (25.6% of the animals) which comprised of Brahman breed of animals, the farmers keeping these animals had heard about Brucellosis, their cows were of parity 1, the animals enjoyed the free range method of feeding and they were checked by a veterinarian only once a year stood a higher chance of experiencing downer syndrome (18.2%) than the ones belonging to cluster 2, and they stood a high chance of experiencing retained placenta (30.3%), vaginal prolapse (24.2%) and dystocia (24.2%) than the ones allocated to the other two clusters (Table 3.7).

3.5. Discussion

The aim of this study was to identify cattle production management practices that influence the occurrence of reproductive conditions in communal farming. The results show that most of the farmers were male (79%) while female farmers represented 21% of all farmers (Figure 3.2). A similar report by Balarane & Oladele (2012) demonstrates that the state of farming in communal areas remains a male-dominated sector. Despite the emphasis on gender equality concepts and the attempts to redistribute resources, females are still at a disadvantage (Moloi *et al.*, 2008). Participation of female farmers in communal farming still is still low. Research shows that other contributing factors restricting women's participation in farming include culture, social and economic structures (Lwoga *et al.*, 2010). There is still a need to improve the different programs put in place to make them more gender accommodative and sustainable.

This study has revealed that most of the farmers were in ages between 40-55 years (61%), followed by those of ages 56-69 years (20%), then 10% were of farmers aged 70 years and above, while the smallest proportion was of farmers between 18-39 years (9%) (Figure 3.1). Similar observations were reported in a study by Balarane and Oladele (2012) which indicated

that the communal farmers were most commonly older (51-60 years). The results of this study suggest that older people are the most participating group in livestock production. This could be due to the perception of young people that farming is for old people, not a reliable source of income or lack of interest in agriculture. These outcomes are also in line with reports stating that age influences adaptation to farming methods and technologies (Gwala *et al.*, 2016; StatsSA, 2016). Consequently, this fact greatly disadvantages production as the youth in rural areas most commonly have a higher level of education compared to older people.

The results indicate that the fewest farmers were widowed (7%) as is depicted in figure 3.3. A study by Gwala *et al.* (2016) also reported similar findings indicating that communal farmers were widowed or unmarried (7.1%). The study further revealed that 25% of the farmers were married, while most of the farmers were single (68%) (Figure 3.3). However, previous reports by Balarane and Oladele (2012) and Gwala *et al.* (2016) showed that most of the farmers in the communal areas were married.

The data showed that 44% of the farmers have full time employment, those with part-time jobs made up 28% of the farmers and 24% were unemployed (Figure 3.4). However previous studies have shown that most farmers rely mostly on pensions (StatsSA, 2016; Molefi & Mbajorgu, 2017). The difference in the results could be due to the method of sampling which was a convenient sampling which relied on farmers reporting reproductive condition cases in their farms and their willingness to participate.

The study showed that most farmers had primary level of education (76%), followed by secondary level (10%), high school (7%) and those with no education (7%) indicated in figure 3.5. Studies by Oladele and Mabe (2010) and Modirwa and Oladele (2012) similarly reported that most communal farmers of North West Province have low level of education. Other research by Gwala *et al.* (2016) and Statistics South Africa (2016) showed that farmers in rural

areas have at-least primary to secondary education with frequencies of about 57% and 60%, respectively.

Even more, reports of Molefi & Mbajorgu (2017) showed that higher proportions of farmer have low education. These points to the fact that improved farming can be hindered due to the level of education of the farmers. Adoption of new farming innovations and technologies could take longer due to the low level of farmer's education since highly educated people are more likely to improve technological and managerial skills in relation to correct designing and implementation of improvement programmes, and obtaining advanced material to increase marketing capacities (Oladele & Mabe, 2010; Molefi & Mbajorgu, 2017).

Farmers with no income were found to make up to 3%, while most of the farmers (56%) earned less than one thousand rand (Figure 3.6). The results also showed that farmers with an income between R1000–R3000 were approximately 37% of the population, with only 2% of the farmers having an income between three thousand and ten thousand Rand (Figure 3.6). A study by Schwalbach *et al.* (2001) showed that most communal farms have a low income. Another study by Sikhweni and Hassan (2014) indicated that the reliance on livestock as a source of income was about 99% for communal farmers. This could explain the low levels of income reported by the present study, since most of the rural farmers are still not significantly involved in the commercial marketing of livestock. The results of this study also highlight a great potential for development held by the communal farming sector, provided agricultural resources and support are easily made accessible to the farmers.

The data shows significant variation in proportions of cows among different breed types: Nguni (29%), Afrikaner (22%), Brahman (19%), Bonsmara (10%), mixed breed (9%), Drakensberger (3%) and Simental (1%) shown in Figure 3.10. The results imply that Nguni, Afrikaner, Brahman breeds were most susceptible to reproductive conditions. Furthermore, Table 3.2 also

shows that the incidence of downer cow syndrome mostly reported in Simmental cows, while retained placentas were mostly seen in Angus breed, whereas abortions and vaginal prolapses were noted mainly in Drakensberger cows. Additionally, mixed breed cows and the other cows which farmers could not identify the breed were mostly prone to dystocia. These outcomes are in agreement with a study of Meher *et al.* (2017) who observed high incidence of reproductive conditions in cross breed cows. Lack of homogeneity in the number of cows among different breeds in this study could explain the differences in these findings.

In about 49% of the cows the reproductive condition occurred in their first parity while 37% of the cases were seen in the cow's second parity (Figure 3.11). Additionally, the smallest fraction of cases reported were of cows in the eighth parity (1%) as indicated in figure 3.11. The result signifies that the reproductive conditions are more prevalent in primiparous cows. In table 3.3 it was demonstrated that downer cow syndrome and vaginal prolapse occurred mostly in first parity cows. It also indicated that the incidence of abortions was high mostly in cows of the second parity. It was also noticed that cows in their eighth parity mostly suffered retained placenta. The significance of parity in reproduction has been emphasised in previous studies (Kanuya *et al.*, 2006; Matiko *et al.*, 2008). Parity remains a substantial predictor of cow reproductive conditions in most communal farms. Farmer's knowledge about the contribution of calving history of the cows have an essential part in relation to reproductive capacity of the cows, as well as in the decision-making about keeping older animals in their herds as that can negatively impact on the reproductive profit.

This study also showed that a total of 58% reported cases were mostly in cows under free ranging method/system of feeding (Figure 3.13). These results imply that in communal areas cows are in most instances reared on natural grassland alone. Table 3.4 shows that the most prevalent reproductive condition under free range feeding system is retained placenta. It also indicated that in both confined and mixed feeding methods cows are most likely to abort.

Similar observations were noted by Mazimpaka *et al.* (2017) stating that natural pastures are the main feed resource in local farming. The results imply that in communal farming the most prominent feeding system is free ranging as compared to other methods of feeding. Natural pastures lose nutritional value during the dry period (Ndlovu *et al.*, 2007). This could explain the increase in incidence of reproductive conditions as the nutrient content of feed has a direct impact on reproductive health of the cows.

It was as well seen that the frequency of veterinary check-ups was largely done yearly (71%) in most communal farms. Additionally, the study also showed that some cows were checked only 3 times a year (12%) and in about 9% of the farms cows never got checked by a veterinarian (Figure 23). These results illustrate that the use of veterinary assistance is not a common practice amongst communal farmers as most indicated to only have had a veterinary visit at least once in a year. The outcomes of the current study are in agreement with reports of Mumba *et al.* (2018) indicating that the use of veterinary services by communal farmers was low. The reason for this could be that most of the farmers cannot afford the services and the number of state veterinarians is low compared to the number of farmers.

3.6. Conclusion

Livestock farming in communal areas is still a male dominated agricultural sector, with most of the farmers being elderly, having low levels of education, generating less monthly income, and included full time employees and unemployed farmers. The incidence of reproductive conditions can be influenced by the cattle of breed type used, the parity of the cow, knowledge of other diseases posing a threat to the animals, the farm feeding system and the frequency of using veterinary services on a farm. The occurrences of abortion, downer cow syndrome, vaginal prolapses, retained placenta and dystocia cases in rural farms are directly related to farm management and practices. Brahman breeds in communal farming are at a predisposition to encounter increased incidence of reproductive condition. Brucellosis is still a concern as

most of the communal farms have an unknown Brucella status in their herds. Lack of frequent interactions between communal farmers and veterinary practitioners does limit cattle production capacity. The development of properly designed disease control and feeding programmes, proper breed selection and constant monitoring of the cow production status in communal farms are necessary to ensure improved production and reduce losses.

3.7. Recommendations

1. Farmer education on the management practices which could help improve herd productivity.
2. Further studying of the role of breed in reproduction of cattle reared in communal farming is necessary as a means to improve the state of production.
3. The relationship between Brucellosis and other reproductive disorders encountered in cattle needs to be further investigated.
4. The studying of the association between farmer education and training, extension work, and herd health management can help reduce the risk of reproductive disorders in communal farms.

3.8 References

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CHAPTER 4

EFFECTS OF REPRODUCTIVE CONDITIONS ON MINERAL LEVELS IN COWS REARED ON COMMUNAL FARMS OF MAFIKENG AREA IN THE NORTH WEST PROVINCE OF SOUTH AFRICA

Abstract

Minerals play a significant role in cattle production and reproduction. Minerals are also known for their contribution to several physiological processes such as cell formation and they also participate in activities of vitamins, enzymes and hormones. The aim of this study was to determine mineral concentrations in cows with dystocia, downer cow syndrome, abortion, retained placenta and vaginal prolapse. Mineral concentrations of cows from communal areas of Mafikeng were determined. A total of 179 blood samples were collected from cases of cows 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 University (NWU) Animal Hospital at the Mafikeng Campus. A convenient sampling method was employed where samples were collected following reports of reproductive cases. Concentrations of minerals such as phosphorus, magnesium, selenium, zinc, iron, iodine and copper were determined. Cows with deficiencies of zinc were most likely to experience abortions and dystocia. An excess of iron, magnesium and phosphorus in cows increased the probability for downer cow syndrome. Phosphorus concentrations were reduced in cases of vaginal prolapse. Reduced iodine concentrations were linked to incidence of downer cow syndromes, vaginal prolapse, retained placenta, abortion and dystocia. The mean concentrations of magnesium (Mg), phosphorus (P), copper (Cu), zinc, iodine (I) and selenium (Se) were affected by reproductive conditions in a semi-arid environment such as Mafikeng

and surroundings. Therefore, minerals may be used as predictors for the incidence of reproductive conditions in cows.

Key words: Abortions, downer cow syndrome, dystocia, minerals, vaginal prolapse and retained placenta

4.1. Introduction

Minerals are significant contributors in cattle reproduction. In animal health, mineral imbalances have been implicated in the increase of an animal's susceptibility to infections and the risk of reproductive conditions (Yatoo *et al.*, 2013). Mineral irregularities such as deficiencies or toxicities can adversely influence the animal's health (Balamurugan *et al.*, 2017). Deficiencies and toxicities of certain mineral elements may cause reproductive disorders as minerals play an important role in the health and reproduction of livestock (Sharma *et al.*, 2007). Animal reproduction, health and growth can be affected by immunological or physiological disorders which arise as a result of mineral deficiency (Velladurai *et al.*, 2016).

Generally, the incidence of reproductive disorders depends largely on the cow's immune responses (Azawi, 2008). Goff *et al.* (2002) stated that the cow's immune system is severely challenged during the peri-partum period. Other reviews have also revealed that the prevalence of reproductive condition may increase during post-partum period and subsequently reproductive performance will be affected (Santos *et al.*, 2009). Reports have shown that a lack of data and inadequacy of study sites where diagnosis of reproductive conditions is done hinders assessment of impact (Thornton, 2010). As a result, the status of reproductive conditions and cattle reproductive capacity in communal farming is not clearly defined, but only presumed to be low (Ndlovu *et al.*, 2007).

In South Africa, the livelihoods of many communal farmers largely relies on livestock production (Ainslie *et al.*, 2002). It has been estimated that communal farmers in South Africa

own almost half of the 14.1 million cattle in the country (Mapiye *et al.*, 2007). A lack of knowledge of the innovative agricultural practices limits cattle production (Menbere, 2014). The variation of different mineral concentrations in cattle have been determined, however their variation and comparison among different reproductive disorders have not been studied. Therefore, this study aimed at examining the mineral profile in cows affected by reproductive conditions.

4.2. Materials and methods

4.2.1 Study site

This study was conducted in Mafikeng areas in the North West Province of South Africa. The study location was as previously indicated in Section 3.2.1 of Chapter 3.

4.2.2. Sample collection and preparation

A convenient sampling method was employed, and samples were collected during ambulatory farm visits. 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.

4.2.3. 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) by adding 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 (Ndou & Dlamini, 2012).

4.2.4. 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 (HNO₃, Merck) was used to digest the samples. Standard stock solutions of Arsenic and Mercury containing 10µg/mL in 2% HNO₃ were prepared as per the procedure of Uluozlu *et al.* (2007). Certified reference materials (CRM) (National Institute of Standard Technology NIST-8436) were used for the standardization and validation of the method.

4.3. 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 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 (De Muth, 2014).

4.4. Results

This study aimed at assessing the possible effects of the levels of minerals in affected animals and the type of reproductive condition. Mineral analysis was done on blood samples of cows with retained placenta, dystocia, vaginal prolapse, downer cow syndrome and abortion. The study hypothesized that the types of reproductive condition does have an effect on the mean levels of minerals.

Table 4.1: Multivariate Tests of mineral concentrations in different reproductive conditions

	Value	F	Hypothesis df	Error df	Sig.
Pillai's Trace	1.638	14.744	32	680.000	0.000
Wilks' Lambda	0.085	18.308	32	617.461	0.000
Hotelling's Trace	4.204	21.742	32	662.000	0.000

Table 4.1 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 influenced at least one of the mean levels of the minerals of the affected animals.

Table 4.2 identifies the exact minerals whose mean levels were affected or not affected by reproductive conditions.

Table 4.2: Tests of Between-Subjects Effects showing overall proportions of minerals and significance levels in relation to reproductive conditions

Source	Dependent Variable	Df	F- test	P-Value
Reproductive Conditions	Magnesium (Mg)	4	18.178	0.000
	Phosphorus (P)	4	17.519	0.000
	Iron (Fe)	4	13.706	0.000
	Copper (Cu)	4	18.393	0.000
	Zinc (Zn)	4	31.005	0.000
	Iodine (I)	4	1.570	0.184
	Selenium (Se)	4	12.592	0.000

Df =degrees of freedom; F=F-test /F-statistics (Variation between sample means/variation within the samples)

Table 4.2 shows that all individual ANOVA tests for each mineral were significant at 1% ($P < 0.01$) except for Iodine which was not significant. This implies that reproductive conditions had a significant effect on the mean mineral concentration being either low or higher.

Table 4.3 shows the reference ranges of minerals in healthy cows which were used to compare the normal mineral levels and the means from unhealthy cows (affected by reproductive conditions). The units of the results report after sample analysis were mg/L hence the reference values were converted from different units to mg/L as represented in the table below.

Table 4.3: Mineral reference ranges in cows

Mineral	Normal range (Djokovic <i>et al.</i>, 2014)	In mg/L
Inorganic phosphorus (P)	0.720-6.400 mmol/L	81.446-723.968
Magnesium (Mg)	0.4945-1.506 mmol/L	12.019-36.603
Copper (Cu)	7.801-23.621 μ mol/L	0.882-2.669
Iron (Fe)	12.500-44.811 μ mol/l	1.414-5.068
Zinc (Zn)	10.710-19.890 μ mol/L	1.212-2.250
Selenium (Se)	0.024-3.000 μ g/dL	0.003-0.339
Iodine (I)	4.960-19.850 μ g/L	4.960-19.850

The reference ranges were used to determine whether the levels of minerals were high, low or normal.

Table 4.4 shows how each mineral (indicated in Table 4.2) differs across the type of reproductive condition and its statistical significance based on their comparison with normal mineral ranges (indicated in Table 4.3).

Table 4.4: 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)
<i>Downer cow syndrome</i>	46.945**	42.751**	0.988	1.165	22.865**	2.789**	12.688**
<i>Retained placenta</i>	40.606**	37.365**	1.441**	1.818	26.614**	6.792	5.383**
<i>Vaginal prolapse</i>	26.194	27.076**	1.024	1.443	25.638**	0.335**	6.674**
<i>Abortion</i>	29.051	27.248**	1.720	0.642**	18.717**	1.489**	5.119**
<i>Dystocia</i>	25.007	18.782**	0.449**	0.440**	11.423**	2.245**	6.701**

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

Table 4.4 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 4.3). Selenium (Se) levels were significantly higher than the upper limit of normal range (0.003-0.339 mg/L) across all the conditions. Significantly lower zinc concentrations were observed in abortion and dystocia cases since the normal range is 1.2115-2.25 mg/L.

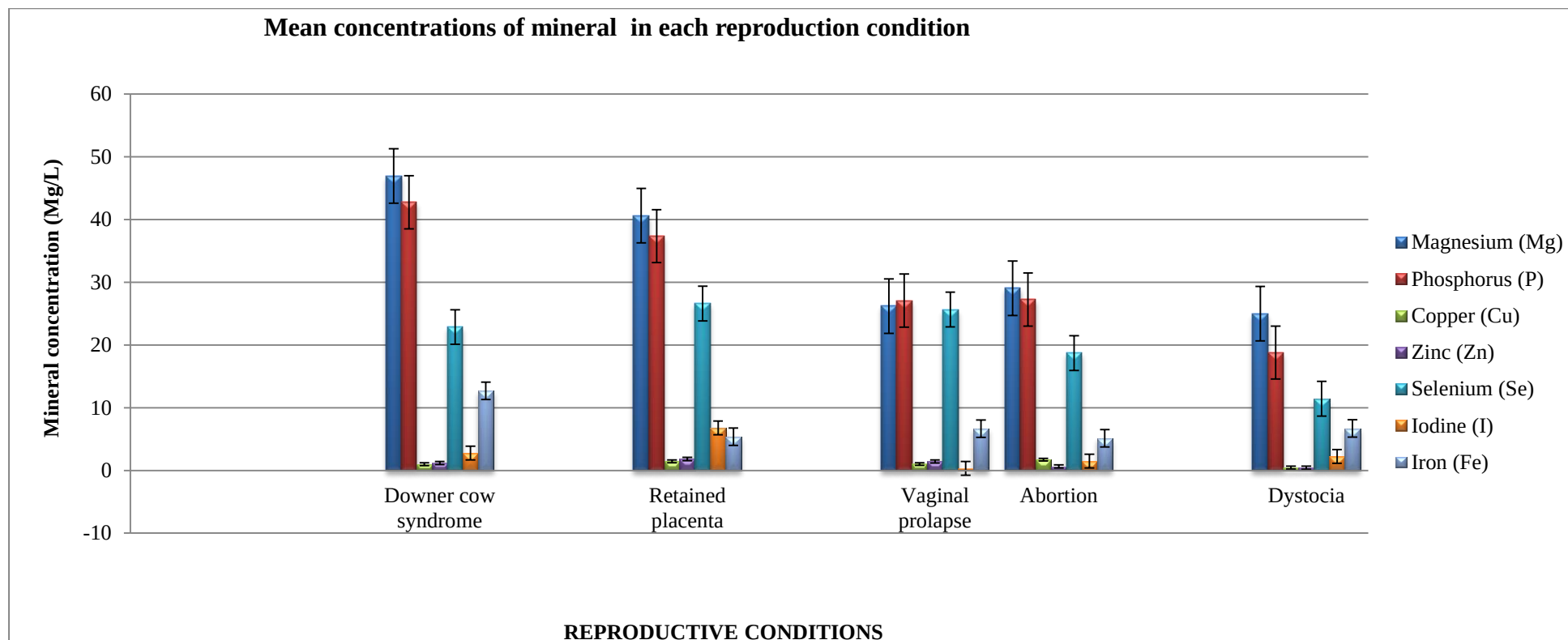


Figure 4.1: Mineral mean concentrations across different reproductive condition

Magnesium and phosphorus mean concentrations were the highest in downer cow syndrome and the least in dystocia across the conditions (Figure 4.1). Retained placenta cases exhibited highest selenium mean concentrations while the least amounts were seen in cases of dystocia. Iron mean concentrations were the highest in downer cow syndrome cases with its lowest level seen in abortion cases.

Table 4.5: Multiple comparisons of serum metabolites among the reproductive condition

Dependent Variable	(I) Reproductive Condition	(J) Reproductive Condition	Mean Difference (I-J) in mg/L
Magnesium (Mg)	Downer cow syndrome	<i>Vaginal prolapse</i>	20.751*
	Retained placenta	<i>Vaginal prolapse</i>	18.412*
	Downer cow syndrome	<i>Vaginal prolapse</i>	15.675*
Phosphorus (P)	Retained placenta	<i>Dystocia</i>	18.583*
	Vaginal prolapse	<i>Dystocia</i>	8.293*
	Abortion	<i>Dystocia</i>	9.466*
Iron (Fe)	Downer cow syndrome	<i>Retained placenta</i>	7.306*
		<i>Vaginal prolapse</i>	6.014*
		<i>Abortion</i>	7.291*
Copper (Cu)	Downer cow syndrome	<i>Dystocia</i>	5.987*
		<i>Abortion</i>	-0.731*
		<i>Dystocia</i>	0.993*
Zinc	Downer cow syndrome	<i>Abortion</i>	-0.653*
	Retained placenta	<i>Retained placenta</i>	1.379*
Selenium (Se)	Downer cow syndrome	<i>Dystocia</i>	11.443*
	Retained placenta	<i>Abortion</i>	6.764*
	Abortion	<i>Dystocia</i>	8.427*
	Vaginal prolapse	<i>Dystocia</i>	14.215*

Table 4.5 shows mean differences in mg/L which were significantly different across the reproductive conditions. The mean magnesium level in cows with retained placenta exceeds {Mean Difference (I-J) is positive} that in aborting cows. The study indicated that phosphorus in aborting cows was significantly higher than in dystocia cases. The results also show that the mean copper (Cu) level from cows with downer cow syndrome was significantly less than (Mean Difference (I-J) is negative) the one from animals which suffered an abortion. Similarly,

the level of zinc from cows with downer cow syndrome was significantly less than that in retained placenta cases.

4.5. Discussion

The implications of mineral disproportions in cows have long been associated with reduced reproductive capacity and efficiency (Kumar, 2015). Several reproductive conditions may occur due to a lack of consideration for physiological functions of minerals required by the cows during the peri-partum period (Bindari *et al.*, 2013). Minerals are required to sustain numerous metabolic processes in the body, increase productivity and optimise the health status of the animal by boosting the immune system (Velladurai *et al.*, 2016). This study aimed to determine the profile of minerals in cows with abortions, retained placenta, vaginal prolapse, dystocia and downer cow syndrome. Significant variations were observed showing that reproductive conditions do have effects on the level of minerals in the animal (Table 4.1).

In particular, the mean concentrations of magnesium (Mg), phosphorus (P), copper (Cu), zinc and selenium (Se) were significantly different in cases of reproductive disorders (Table 4.2). Disproportion of any sort in minerals greatly impacts the animal's reproductive health, as the animal's immune function can be compromised and its disease resistance reduces (Fulton, 2016). Although the causes of downer cow syndrome may be musculoskeletal, metabolic, toxic, neoplastic, neurologic, inflammatory, or infectious (Kincaid, 2008), the current study shows that magnesium concentrations were elevated in downer cows (Table 4.4 and figure 4.1). Previous studies have indicated that magnesium increase in downer cows is typically due to lowered cellular uptake, bone metabolization and lowered urinary loss of magnesium (Fikadu *et al.*, 2016). This elevation of Mg suggests a linkage between downer cow syndrome in the present study with nutritional disproportion.

The study found that the mean phosphorus concentration was significantly lower in vaginal prolapses, abortion and dystocia when compared to downer cow syndrome (Table 4.5 and Figure 4.1). Low phosphorus increases the occurrence of downer cow syndrome and retained placenta (Kachhwaha *et al.*, 2010). In cows with dystocia the phosphorus mean concentrations were lower than in condition of retained placenta, vaginal prolapse and abortion (Table 4.5 and Figure 4.1). According to Zaborski *et al.* (2009) the incidence of dystocia are influenced by nutrient insufficiencies.

The mean iron concentration in retained placenta, vaginal prolapse, abortions and dystocia were all significantly lower than the concentration in downer cow syndrome (Table 4.5 and figure 4.1). An imbalance of iron levels has been associated with the incidence of retained placenta (Sheetal *et al.*, 2014; Kumar, 2014). Mineral level disproportions in animals have been associated with impaired immune functions (Fulton, 2016), this could explain the imbalanced iron concentrations in vaginal prolapse, abortions and dystocia (Table 4.5 and figure 4.1).

Copper deficiencies have been linked to increased incidence of embryonic death and foetal placental membrane retention (Velladurai *et al.*, 2016). However, such associations were not seen in the present study, as copper concentrations were higher in retained placenta and abortion cases (Table 4.4 and figure 4.1). Reduced zinc mean concentrations were typically evident in aborting cow than in non-aborting cows (Akar & Yildiz, 2005), and the authors emphasised that dietary zinc deficiency can lead to abortions. Zinc concentrations are normally lower during placental membrane retention in cows which increases the risk of abortion during subsequent pregnancies (Waldner, 2014). The lowered zinc concentrations may also be as a result of high calcium and phosphorus which decrease intestinal zinc absorption (Kreplin & Yaremicio, 2009). The current study has as well shown significantly low mean concentration of Zn in retained placenta and aborting cows (Table 4.4 and figure 4.1).

Selenium deficiency has been associated with the incidence of retained placenta and abortions in cows (Waldner & Van De Weyer, 2011; Kumar, 2015). However, the present study found that the mean selenium concentration was significantly higher in retained placenta, abortions, vaginal prolapse and downer cow syndrome condition (Table 4.4 and figure 4.1). The results were in line with reports indicating that high selenium may cause pregnant cows to abort due to toxicity; this is attributable to selenium having a narrow safety margin leading to prominence in toxicities than deficiencies (Balamurugan *et al.*, 2017).

Significantly lower mean concentrations of iodine were seen in cows with abortions, dystocia, downer cow syndrome with the lowest level being seen in vaginal prolapse (Table 4.4 and figure 4.1). A decrease in iodine concentrations may cause abortions (Kumar, 2015). 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 (Abuom *et al.*, 2012). The incidence of downer cow syndrome may also be seen in times of nutrient insufficiencies (Hansen *et al.*, 2003). Iodine deficiency has functional characteristics of influencing reproductive sufficiency in cows (Balamurugan *et al.*, 2017).

4.6. Conclusion

Mineral imbalances are a hindrance to reproductive success of cows in rural areas. The novelty of this study was that it showed that the mean concentrations of magnesium (Mg), phosphorus (P), copper (Cu), zinc, iodine (I) and selenium (Se) were affected by reproductive conditions. Magnesium levels are higher 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 were lower than in retained placenta, vaginal prolapse and abortion. In domestic animals, adequate amounts of minerals in their biologically active forms are essentially required for optimum production and reproduction. Mineral needs for cow immunity and reproduction are normally

high, nonetheless necessary to reduce the risk of disorders of reproduction. Proper understanding of mineral interaction and relationships with other nutrients, and knowledge about mineral sources and bioavailability are essential for proper supplementation because any imbalance can hinder reproductive performance. It is important to regularly obtain the latest data with regards to mineral consumption of the animals as well as mineral availability in different feed stuff to correctly determine mineral needs for the cows. Trace mineral evaluation is a very important management practice in livestock farming.

4.7. Recommendations

1. Further studying of the interaction between several other causes (such as bacterial agents) of reproductive conditions, their connection and effects on minerals is necessary to reduce the risk of losses and increase production output.
2. Initiation of monitoring programmes for communal farms to assist farmers in the implementations of improved farming practices such as supplementation.
3. Assist farmers to properly design feeding programs on their farms.
4. In addition it will be important to follow pregnant cows during the last trimester and assess the level of minerals in order to predict any condition.

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CHAPTER 5

SERUM BIOCHEMICAL PARAMETERS AND POSSIBLE CORRELATIONS BETWEEN DIFFERENT COW REPRODUCTIVE CONDITIONS

Abstract

Measures of serum metabolites have been used as indicators in disease diagnosis and nutritional status of animals. The aim of this study was to determine cow serum metabolites in cases of vaginal prolapse, downer cow syndrome, abortion, retained placenta and dystocia. The study was conducted in the Mafikeng area of the North West Province of South Africa. A total of 179 blood samples were collected from cows during the visits to the North West University (NWU) veterinary clinic at the Mafikeng Campus. Samples were conveniently collected following reports of reproductive condition cases. Biochemical metabolites evaluated in this study were total protein, aspartate amino-transferase (AST), urea, chloride, potassium, sodium, lipase, cholesterol, ammonia, uric acid, triglycerides and creatinine kinase, calcium. The data were analysed using SPSS version 25. The results were significantly different at 5% (p-values <0.05). Significant differences were seen in the concentrations of urea/BUN, total bilirubin, aspartate amino-transferase (AST), ammonia and lipase. In an event of abortion, retained placenta, vaginal prolapse, dystocia and downer cow syndrome the serum biochemical constituents were significantly altered (from the normal). The present study also suggests that adequate energy supply during and after gestation is important in reducing the incidences of reproductive conditions.

Key words: Serum metabolic profile, dystocia, downer cow syndrome, vaginal prolapse and abortions

5.1. Introduction

The examination of blood chemistry plays a significant role in animal health as part of metabolic profiling (Oetzel, 2004). 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 (Zhang *et al.*, 2018). The significance of blood biochemical parameters in the assessment of physiological, nutritional and health status of cows has also been emphasised by many researchers (Nozad *et al.*, 2011; Ashmawy, 2015).

Abnormalities in various biochemical components have been held responsible for reproductive failures (Bazzano *et al.*, 2016). The significance of energy balance has been associated more with reproductive failure and fertility (Gomez *et al.*, 2018). During the transition period (late pregnancy and early lactation) cow's immune strength is reduced and about 50% of the cows are affected by disease and disorders which can typically be of nutritional, infectious or metabolic nature (Dubuc *et al.*, 2010; Onasanya *et al.*, 2015).

Alteration in metabolic status may subsequently lead to negative energy balance (NEB) due to reduced feed intake experienced in the peri-parturum period and ultimately affect the general reproductive health (Sheehy *et al.*, 2017). Disturbed metabolic processes are related to an increase in reproductive conditions (Jeong *et al.*, 2018). Pathophysiological reactions and the health status of the animal can be indicated by alterations in serum metabolites (Onasanya *et al.*, 2015). Any deficiency or excess in serum biochemical parameters can be an indication of poor nutritional status. Therefore, this study sought to assess the effects of reproductive conditions on the serum biochemistry of cows raised on natural pastures.

5.2. Materials and methods

5.2.1 Study site

This study was conducted in Mafikeng areas in the North West Province of South Africa. The study location has previously been described in Section 3.2.1 of Chapter 3.

5.2.2. Sample collection and preparation

Sample collection and preparation were done as described in chapter 4 section 4.2.2.

5.2.3. Method Validation

Samples were analysed using the IDEXX Catalyst Chemistry analyser according to the manufacturer's instructions. The Catalyst Dx* chemistry analyzer uses internal standards; the machine performs a self-check every time it is powered on, when it is initialized, as well as when a test is performed.

5.2.4. Statistical considerations

Analysis of data was conducted using Statistical Software for Social Sciences (SPSS) version 25. The effect of the reproductive conditions on the serum metabolic profile of affected animals was estimated by means of the Multivariate Analysis of Variance (MANOVA). Further tests of individual analysis of variance (ANOVA) were subsequently used to examine the effects of each reproductive condition on the serum metabolite composition of affected cows. A *post-hoc* test (Tamhane T²) was used for the significant ANOVA tests, to determine the statistical significance and the magnitude of the differences in the mean levels of each serum metabolite 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 T² is a *post-hoc* test for determining the significance and the magnitude of the difference in means based on the treatments. Since the cows 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 (De Muth, 2014).

5.3. Results

In this section the results show the effect of the type of reproductive condition on the level of serum metabolites in the affected animals. The hypothesis was that the type of reproductive condition has an effect on the mean levels of the serum metabolites.

Table 5.1: Multivariate Tests for serum metabolites in reproductive conditions

	Value	F	Hypothesis df	Error df	Sig.
Pillai's Trace	0.725	2.479	60	672.000	0.000
Wilks' Lambda	0.432	2.584	60	646.305	0.000
Hotelling's Trace	0.987	2.690	60	654.000	0.000

Table 5.1 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 serum metabolites of the affected animals. Table 5.2 identifies the exact serum metabolites whose mean levels are affected by the types of reproductive condition.

Table 5.2: Tests of Between-Subjects Effects showing proportions of serum metabolites with reproductive conditions

Source	Serum metabolites	Mean Square	Df	Sig.	Reference ranges
Reproductive Condition	Urea/BUN	127.890	4	0.000	2.50–6.7 mmol/L*
	Phosphates (PHOS)	229.811	4	0.297	1.38–2.55 mmol/L**
	Calcium (Ca)	2753.079	4	0.079	2.2–2.62 mmol/L*
	Total Protein (TP)	235.573	4	0.062	8–80 g/L**
	AST	71363.263	4	0.000	0–91 U/L**
	GGT	2616.061	4	0.051	0–80 U/L**
	Total bilirubin (TBIL)	91.370	4	0.007	2.8–9.3 µmol/L*
	Cholesterol (CHOL)	2148.928	4	0.805	1.96–5.86 mmol/L**
	Ammonia (NH ₃)	362915.479	4	0.005	0–90 µmol/L**
	Triglycerides (TRIG)	1.451	4	0.236	0.08–0.20 mmol/L*
	Lipase (LIPA)	29260.567	4	0.000	30–400 U/L**
	Creatinine kinase (CK)	303707.387	4	0.285	0–110 U/L**

Reference ranges sources: (*Cozzi *et al.*, 2011; ** IDEXX VetTest* Chemistry Analyzer-instruction manual)

Mean squares -in ANOVA are used to determine whether factors (treatments) were significant; Df= degrees of freedom. ALT = Alanine Transaminase; GGT= Gamma-glutamyltransferase; AST = aspartate transaminase; U/L = units/litre; g/L = gram/litre; mmol/l = millimoles /litre; µmol/L= micromole/litre.

The results in Table 5.3 show that in the individual ANOVA tests the means of the following serum metabolites were significantly different across the reproductive conditions at 5% (p-values <0.05): Urea/BUN, Uric acid, AST, Total bilirubin (TBIL), Ammonia (NH₃) and Lipase (LIPA).

Table 5.3: Variations of means concentrations of each of these serum metabolites differ across the type of reproductive condition in cows

Reproductive Conditions	Serum metabolites mean ($\bar{x}$) concentration											
	BUN (mmol/L)	PHOS (mmol/L)	CA (mmol/L)	TP (g/L)	AST (U/L)	GGT (U/L)	TBIL (μ mol/L)	CHOL (mmol/L)	NH3 (μ mol/L)	TRIG (mmol/L)	LIPA (U/L)	CK (U/L)
<i>Retained placenta</i>	4.919	7.328	1.994**	80.143	114.643**	15.857	7.357	2.768	898.571**	0.172	93.143	74.00
<i>Downer cow syndrome</i>	6.891**	2.128	2.284	74.941	167.029**	23.441	7.074	2.829	822.912**	0.561	74.00	320.294**
<i>Abortion</i>	4.12	1.932	18.179**	73.754	113.493**	18.942	6.472	16.234	686.51**	0.327	74.71	395.058**
<i>Dystocia</i>	7.731**	5.285	2.33	71.757	201.1**	36.56	9.92**	2.946	629.38**	0.438	132.92	325.40**
<i>Vaginal prolapse</i>	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, $\bar{x}$ = Sample mean.

The results showed a significant correlation between the retained placenta cases registered and the levels of TP ($\bar{x}$ =80.143 g/L), AST ($\bar{x}$ =114.643 U/L), CHOL ($\bar{x}$ =2.768 mmol/L), NH₃ ($\bar{x}$ =898.571 μ mol/L), LIPA ($\bar{x}$ =93.143 U/L). Significantly different levels of BUN ($\bar{x}$ =93.143 mmol/L), TP ($\bar{x}$ =93.143 g/L), AST ($\bar{x}$ =93.143 U/L) and CHOL ($\bar{x}$ =93.143 768 mmol/L) were seen. The study also showed significant differences in serum creatinine kinase, triglyceride and lipase levels on abortion, dystocia and vaginal prolapse respectively.

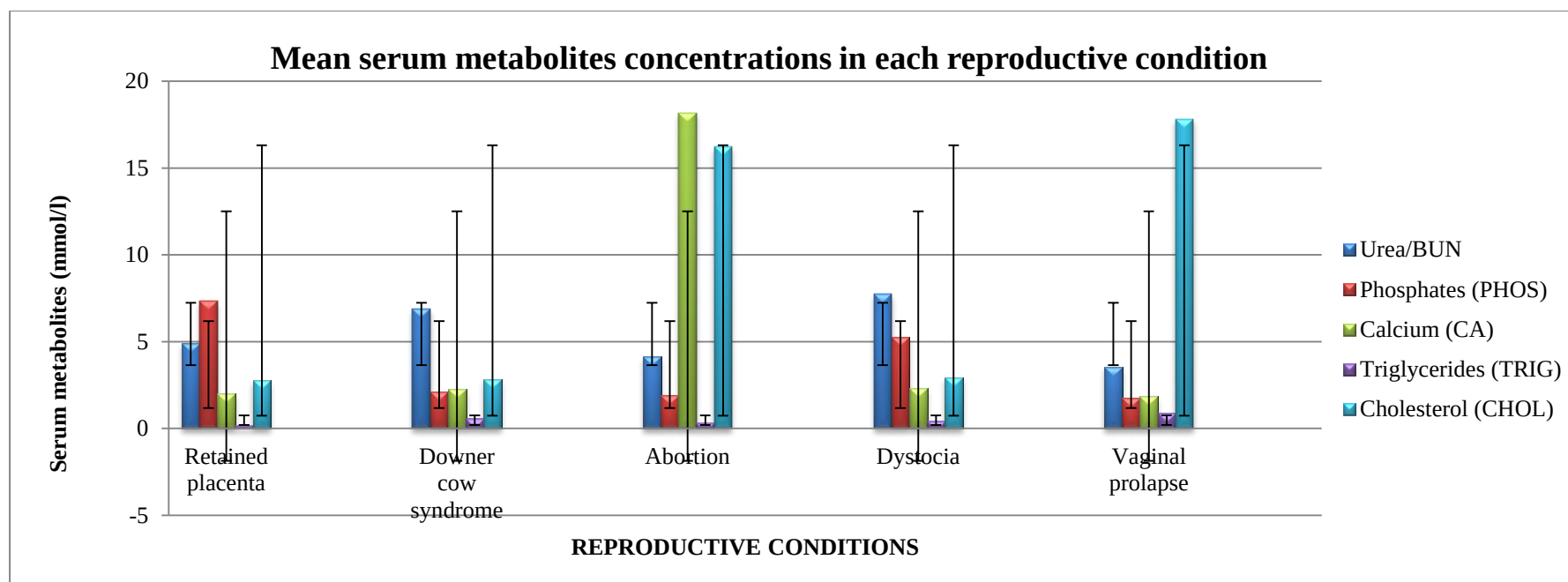


Figure 5. 1: Mean concentrations of Urea, phosphates, calcium, triglycerides and cholesterol in cows.

The highest level of cholesterol was seen in vaginal prolapse, with the least of its measure being seen in cows with retained placenta. Figure 5.1 shows the urea concentrations were higher in animals presenting with dystocia (7.731 mmol/L) while the minimum was observed in animals presenting with abortion cases (4.12 mmol/L). It was also observed that phosphate levels (7.328 mmol/L) were found to be the highest in retained placenta cases while they were very low (1.754 mmol/L) in vaginal prolapses (Figure 5.1). The results also show that triglycerides were the least

in abortion cases and high in cows with vaginal prolapses. The calcium mean concentration was higher in downer cow syndrome and dystocia as compared to vaginal prolapse (Figure 5.1).

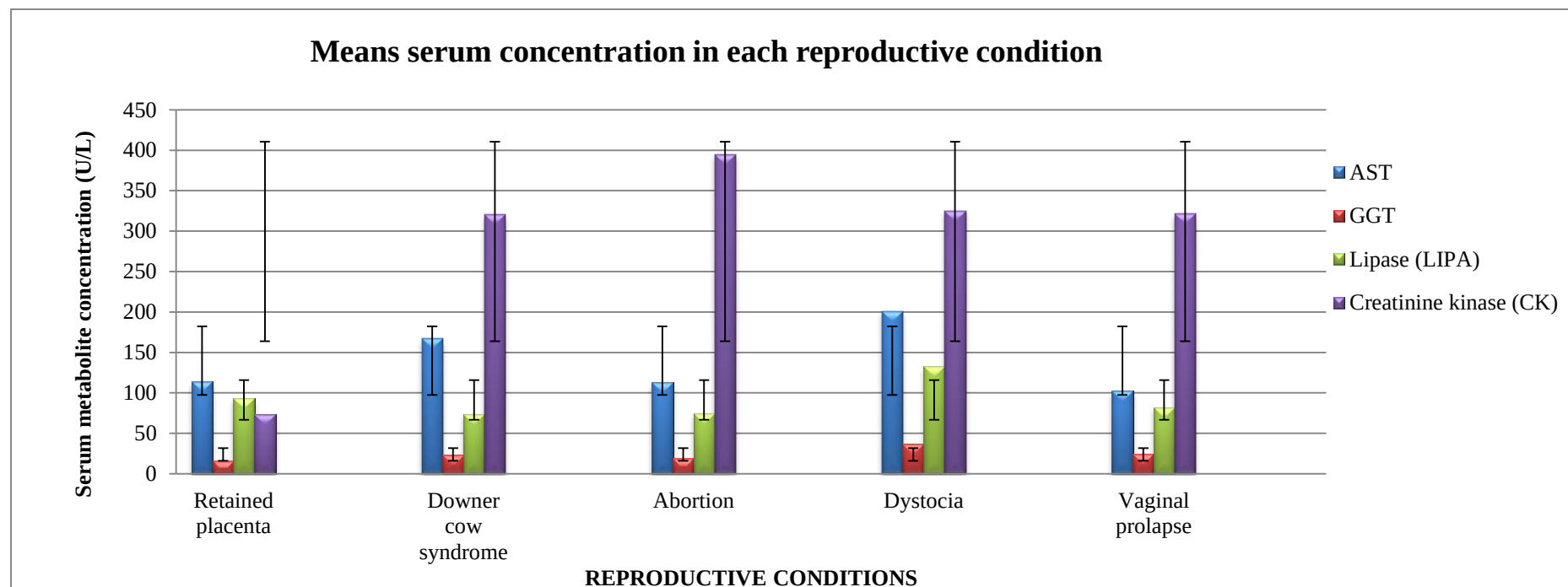


Figure 5. 2: Mean concentration of serum AST, GGT, LIPA and CK in different condition.

Serum AST was seen to be highest among dystocia cows and was the least in vaginal prolapse compared to all other conditions (Figure 5.2). Dystocia cows had elevated concentration of GGT than other conditions and its lowest concentration was in retained placenta cases (Figure 5.2). Serum lipase concentration of nearly 150 U/L in dystocia cases was the highest among all the reproductive conditions and was low in downer cow

syndrome. Creatinine kinase was highest (close to 400 U/L) among aborting cows and was above 300 U/L among downer cow syndrome, dystocia and vaginal prolapse cows and its lowest values were in retained placenta cases (Figure 5.2).

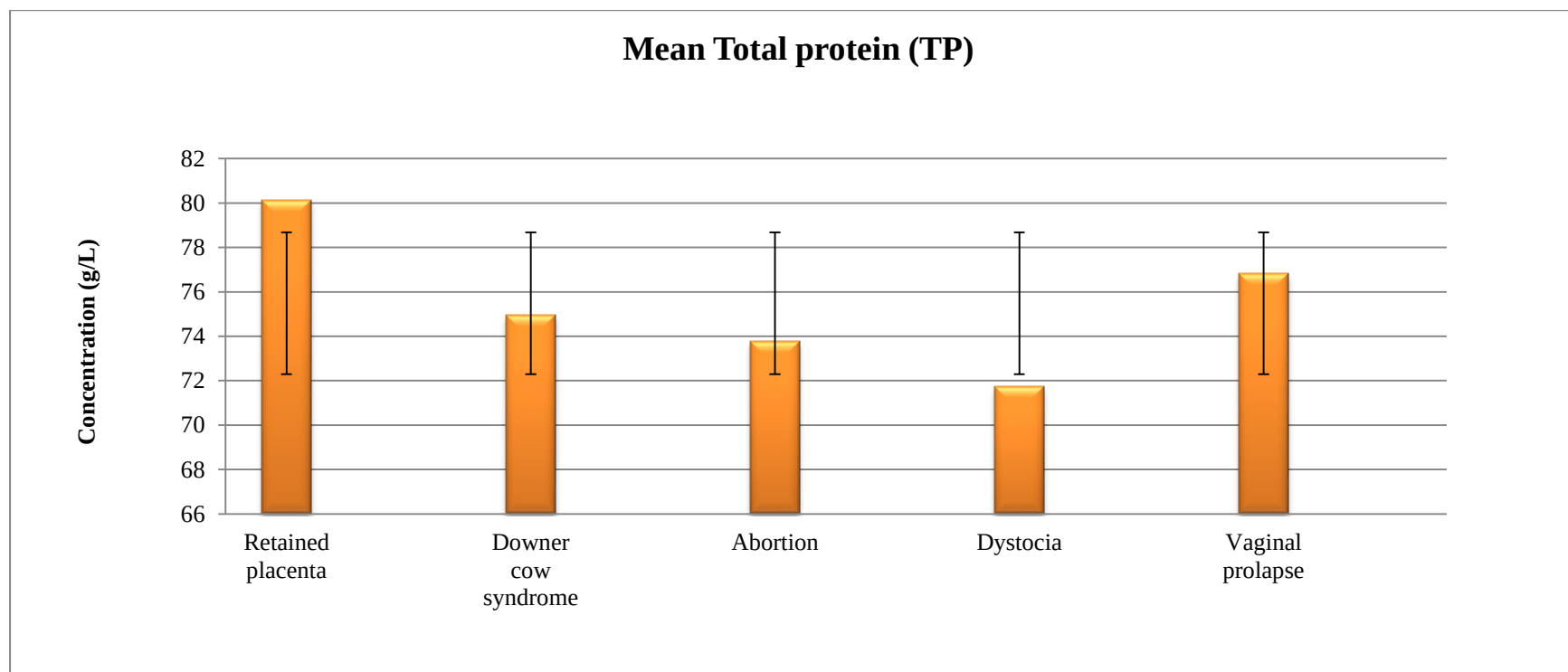


Figure 5. 3: Total protein mean concentration across different reproductive conditions

The serum metabolite distribution in figure 5.3 shows that the highest concentration of total protein (TP) in cows affected with retained placenta cases with measures slightly above 80 g/L and was very low in the other reproductive conditions, measuring the least in dystocia which was nearly 72 g/L.

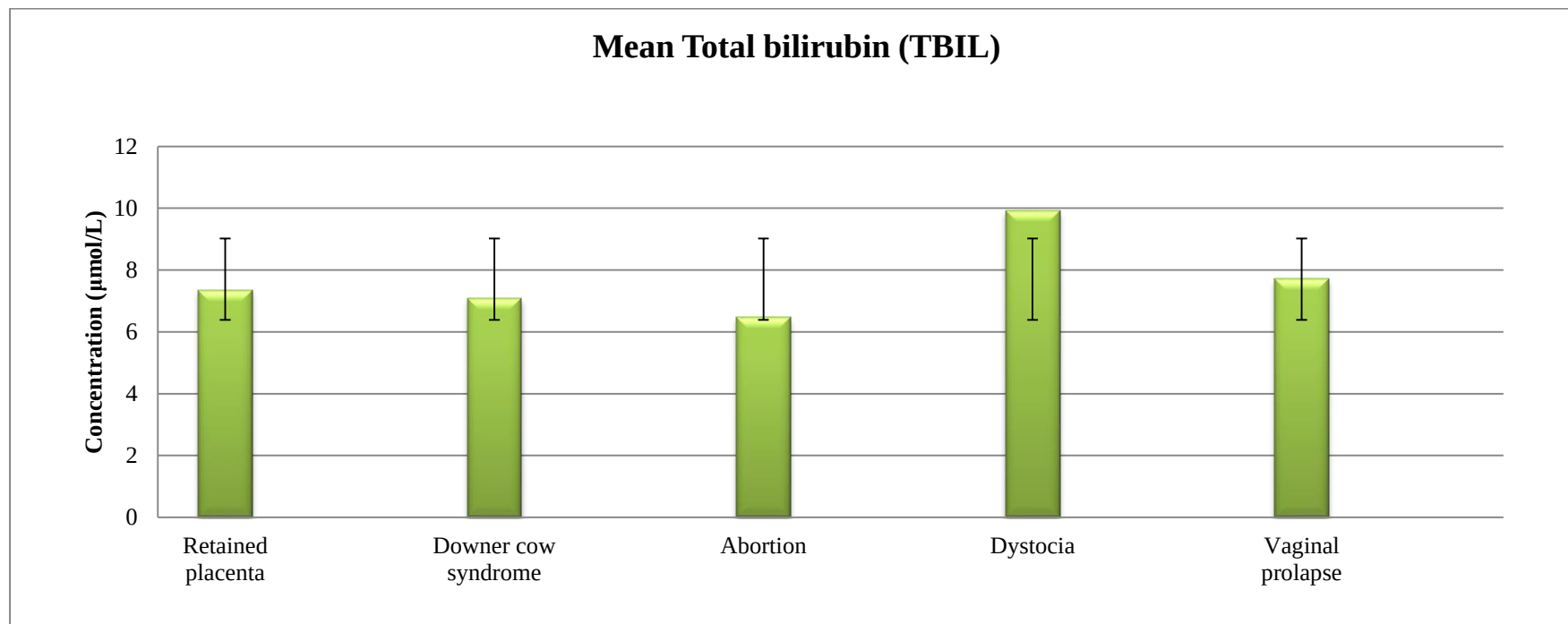


Figure 5. 4: Mean concentration of Total bilirubin across different reproductive conditions

The results revealed that of all the conditions the concentration of total bilirubin was highest in dystocia at about 10 mmol/L while its concentration was about 7mmol/L in most of the other conditions and was the lowest in abortion cases (slightly above 6 mmol/L).

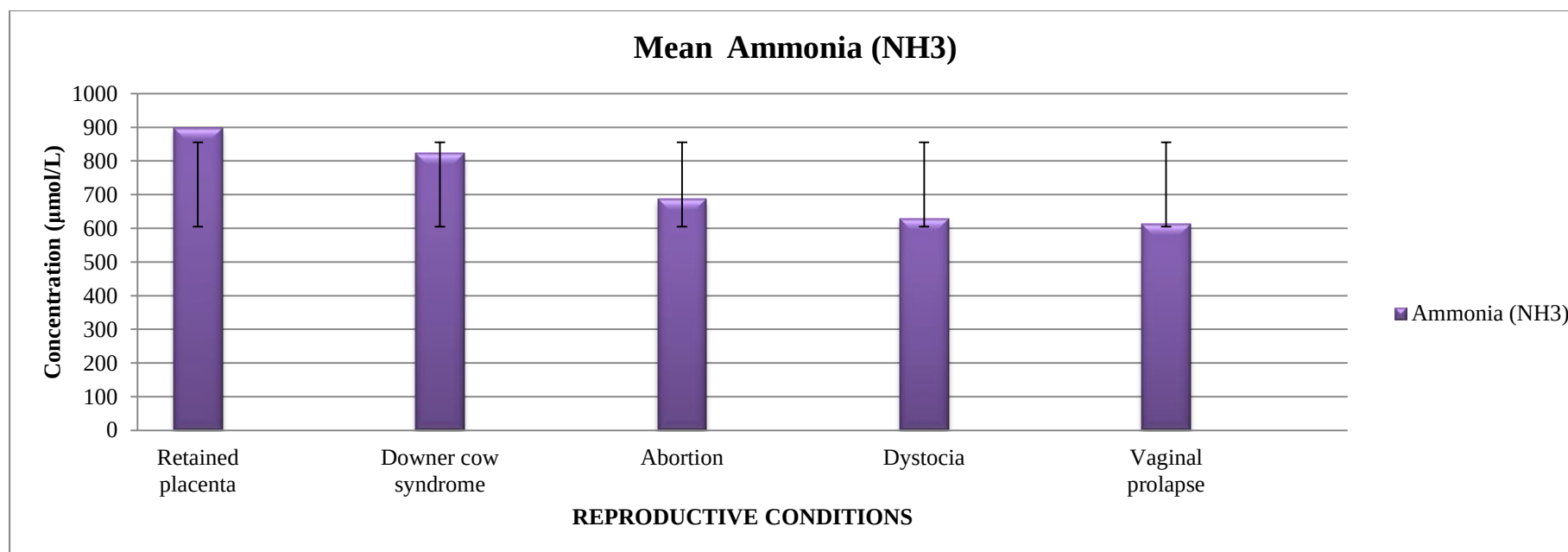


Figure 5. 5: Mean concentration of Ammonia across different reproductive conditions

The highest observed level of ammonia was seen in cows with placental membrane retention which was approximately 900µmol/L and the least amongst the conditions was seen in cases of birth difficulty (dystocia) measuring slightly above 600 µmol/L.

Table 5.4 Multiple Comparisons of serum metabolites among reproductive conditions

Dependent Variable	(I) Reproductive Condition	(J) Reproductive Condition	Mean Difference (I-J)	Std. Error	Sig.
UREA/BUN	Retained placenta	<i>Dystocia</i>	-2.812*	0.804	0.010
	Abortion	<i>Dystocia</i>	-3.610*	0.681	0.000
	<i>Dystocia</i>	<i>Vaginal prolapse</i>	4.166*	0.896	0.000
Uric Acid	Downer cow syndrome	<i>Abortion</i>	14.476*	4.063	0.006
	Abortion	<i>Dystocia</i>	-16.139*	4.988	0.016
AST	Retained placenta	<i>Dystocia</i>	-86.457*	25.658	0.013
	Downer cow syndrome	<i>Abortion</i>	53.537*	12.606	0.001
	Downer cow syndrome	<i>Vaginal prolapse</i>	64.382*	16.595	0.004
	Abortion	<i>Dystocia</i>	-87.607*	22.389	0.002
	<i>Dystocia</i>	<i>Vaginal prolapse</i>	98.453*	24.855	0.002
Total bilirubin (TBIL)	Abortion	<i>Dystocia</i>	-3.448*	0.960	0.005
Ammonia (NH3)	Retained placenta	<i>Abortion</i>	212.521*	64.439	0.025
	Retained placenta	<i>Dystocia</i>	269.191*	69.309	0.004
	Downer cow syndrome	<i>Dystocia</i>	193.532*	61.136	0.022
Lipase (LIPA)	Downer cow syndrome	<i>Dystocia</i>	-58.920*	13.627	0.001
	Abortion	<i>Dystocia</i>	-58.209*	14.755	0.002

*Significant at 1% level of significance ($P < 0.001$)

Table 5.4 shows only the serum metabolites which were significantly different across the reproductive conditions. For example the table shows that the mean UREA/BUN level in animals with retained placenta was significantly less than (Mean Difference (I-J) is negative) that of the animals with dystocia by 2.812 mmol/L. The study also revealed that the AST mean level from animals which presented with downer cow syndrome was significantly more than (Mean Difference (I-J) is positive) the one from animals affected by abortion by 53.537 mmol/L. Other results from Table 5.6 may be interpreted in a similar fashion.

5.4. Discussion

Measures of serum metabolites have been used as predictive tools to precisely identify animal's nutritional status by indicating nutrient metabolism in the body (Herath *et al.*, 2018). Another study indicated that serum metabolites are also indicators for energy deficit (Shin *et al.*, 2018). The results showed that all individual ANOVA tests for urea/BUN, aspartate amino-transferase (AST), total bilirubin (TBIL), ammonia (NH₃) and lipase (LIPA) were significantly different across the reproductive conditions at 5% (p-values <0.05) as indicated in Table 5.2.

Urea /BUN (Blood urea nitrogen) is a good metabolic biomarker for protein metabolism as well as kidney function (Dampney *et al.*, 2014). A study by Tshuma *et al.* (2014) linked high urea concentrations leading to pH reduction in the uterus which can be harmful to 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 5.3.

Other studies have associated urea concentrations with the incidence of dystocia in cows (Kruip *et al.*, 2001; Taylor *et al.*, 2003). Similarly, the present study found serum urea concentration to be higher in dystocia cases (Figure 5.1). This association could be due to high urea levels known to lower calcium which is responsible for maintaining adequate contraction during parturition (Ok *et al.*, 2005). For that reason also, we could suggest that since in most cases downer cow syndrome may be seen in hypocalcaemia cows, that could explain the significantly high urea concentrations in downer cow syndrome cases (Table 5.3) as it has previously been reported (Fleischer *et al.*, 2001).

Elevated serum urea concentration can also be seen during transamination of amino acids derived from skeletal muscles (Jorritsma *et al.*, 2003).

The multivariate association between retained placenta and dystocia, abortion, dystocia, dystocia and vaginal prolapse interactions, the mean differences of urea across all the conditions was significantly ($P>0.01$) lower than it was in dystocia (Table 5.4). Even with the known chances of the concurrent occurrence of these reproductive conditions, there was variation in serum urea concentration among the conditions. Studies supportive of the present findings have indicated that the incidences of dystocia increase the probability for other reproductive conditions such as retained placenta and metritis which can also induce abortions in subsequent pregnancies due to impaired fertility and infections (McDougall, 2001; Benzaquen *et al.*, 2015).

Changes in metabolism can elevate AST levels in blood as a result of excess fat accumulation in the liver as the cow transitions during the peri-partum period (Baimishev *et al.*, 2018). AST enzymes are also significant predictors for soft tissue damage in various organs, primarily the liver and cardiac muscle (Cozzi *et al.*, 2011). Furthermore, Shin *et al.* (2018) associated high AST concentration with dystocia, retained placenta, metritis, endometritis and poor reproductive performance in cows. This was in agreement with the findings of the current study which indicated that serum AST concentrations were significantly high in dystocia and low in vaginal prolapse (Figure 5.1). Proper muscular function is important to allow smooth parturition and avoid difficulty during calf birth (Baimishev *et al.*, 2018). This could explain AST imbalances seen in dystocia cases.

Figure 5.2 depicted high serum AST concentrations in dystocia cows with the lowest measures found in vaginal prolapse compared to all the other conditions in the current study. The MANOVA interrelation association revealed that retained placenta and dystocia, as well as downer cow syndrome and vaginal prolapse interactions showed significant differences indicating that the AST concentrations were higher in dystocia and downer cow syndrome (Table 5.4). Previous researchers have related high levels of AST with muscle damage and

complications during parturition which could predispose cows to both the incidence of dystocia and downer cow syndrome observed in the present study (Cozzi *et al.*, 2011; Baimishev *et al.*, 2018).

High total bilirubin concentrations is associated with dystocia incidence as well as impaired liver function in postnatal cows (Shin *et al.*, 2018). This is in line with current findings showing significantly high serum total bilirubin concentrations in cows with calving difficulties (Table 5.3). Figure 5.2 shows the highest measure of TBIL cows with dystocia. Multiple comparisons of TBIL 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 5.4). 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 (Farid *et al.*, 2013).

Intensive energy deficit is related to high circulatory ammonia concentration which is brought about by the increased energy requirements during amino acid transamination necessary for excess NH_3 excretion (NRC, 2001). Significantly high values of ammonia were evident in the present study in the cases of abortion. This is in agreement with previous reports showing that high systemic NH_3 compromises initial embryo growth in the oviduct which could lead to embryo mortality (Kenny *et al.*, 2002). Elevated ammonia concentration has also been linked to impaired fertility and poor reproductive health (Mudron *et al.*, 2004). Serum ammonia concentrations were highest in cows with dystocia and they were the least in those with retained placenta (Figure 5.1). This could be linked to the excessive energy deficiency which has been recognised as a cause of dystocia in cows (Hoff & Duffield, 2003). Excess ammonia in the blood is toxic; this may be a failure to convert ammonia to a less toxic form for excretion as a result of liver disorders (Kenny *et al.*, 2002).

The individual ANOVA tests showed non-significant variations in serum calcium, triglycerides, cholesterol, GGT and creatinine kinase (Table 5.3). However, the multivariate associations indicated significant variations in the conditional interrelation for each serum metabolite. The animal's immunity may be suppressed during parturition due to negative energy balance (NEB), and subsequently increase the incidence of reproductive conditions (Herath *et al.*, 2018). Early identification of factors predisposing cows to reproductive conditions is essential in monitoring programs for herd health management.

5.5. Conclusion

The hypothesis of this study was that the incidence of reproductive conditions affects the level of serum metabolites. This study aimed to determine the profiles of serum metabolites in cows which were afflicted by abortions, retained placenta, vaginal prolapse, dystocia and downer cow syndrome. The serum metabolic profiles of urea/BUN, total bilirubin, aspartate aminotransferase (AST), ammonia and lipase were affected by reproductive conditions. Urea/BUN concentrations in downer cow syndrome and dystocia were higher than normal. In the 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. The incidences of reproductive conditions in communal areas are linked to poor nutrition during the transition phase on the abnormal serum metabolite assessment of the current study. Several studies have produced data on the poor nutritional status of communally reared cattle; however, whether or not actions to implement suggested corrective measures are taken is still questionable. Studies on the implementation processes and disease monitoring programs

should be looked in to. This could help change the state of livestock production in communal areas and reduce unnecessary economic losses.

5.6. Recommendations

1. Adoption of a regular metabolic profiling can be a useful method that can assist to alter the status of reproductive performance in rural farms by enabling early detection of possible calving and post calving disorders.
2. Implementation of metabolic profile testing to monitor the health and nutritional status of production animals prior to and during the gestation period to reduce calving complications.

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CHAPTER 6

SERUM HORMONE CONCENTRATIONS IN COWS WITH VARIOUS REPRODUCTIVE CONDITONS

Abstract

The role of hormones in cows requires careful consideration prior to and during pregnancy, as any imbalance can negatively impact the cow's overall reproductive health. The aim of this study was to determine the hormonal profiles in cows with certain reproductive conditions. Blood samples were collected from cows in different communal areas of Mafikeng. The convenient sampling method was employed for sample collection. Blood samples were collected from cases of cows experiencing dystocia (n=50), retained placenta (n=13), downer cow syndrome (n=34), vaginal prolapse (n=16) and abortions (n=69) following cases reported at the North West University Animal Hospital at the Mafikeng Campus. The data were coded and analysed using the Statistical Software for Social Scientists (SPSS) version 25. Descriptive statistics such as mean, standard deviation and skewness were used to describe the distribution of the hormone levels across the reproductive conditions. Estradiol (pg/ml) concentrations were significantly higher in cases of abortion (1122.99 ± 71.99), downer cow syndrome (781.32 ± 135.7) and dystocia (862.09 ± 123.44), while the lowest concentrations of estradiol were noted in cases of vaginal prolapse (187.94 ± 91.44) and retained placenta (312.39 ± 112.37). Oxytocin (pg/ml) concentrations differed significantly in cows with the higher mean of 370.50 ± 71.66 (Dystocia) and 574.73 ± 60.65 (Aborting cows). Mean Progesterone (ng/ml) concentrations in cows with abortion (2.45 ± 1.509), dystocia (8.59 ± 0.402), vaginal prolapse (4.67 ± 0.301), downer cow syndrome (4.40 ± 1.222) and retained placenta (3.78 ± 0.151) were significantly low. Abortions were seen in situations of elevated serum estradiol and oxytocin, with progesterone levels reduced. In vaginal prolapses and retained placenta, serum

progesterone and estradiol concentrations were low. Cows with dystocia had low progesterone and elevated concentrations of estradiol and oxytocin. Downer cow syndrome was associated with high estradiol and progesterone concentrations. Increased prostaglandin alpha was seen in cases of vaginal prolapse and abortion. Serum hormonal profiling is an essential management tool useful in the assessment of a cow's reproductive health. This study provides useful data which can aid in the valuation of herd health which would help reduce animal losses and infertility in farms.

Keywords: Cows, hormones, reproductive conditions.

6.1. Introduction

Gestation period in cows induces oxidative stress which results in immune dysfunctions and high incidence of peri-partum disorders (Sordillo & Aitken, 2009; Abdisa, 2018). Abortions, vaginal prolapse, dystocia and retained placenta are among some of the main reproductive disorders (Tagesu, 2018). Normal mechanical contraction of the uterus depends on the release of prostaglandin and oxytocin at the onset of labour necessary for foetal delivery (Abdisa, 2018). In preparation for the new pregnancy the cow has to return to the non-pregnant state in the postpartum period (Kaya *et al.*, 2012). However, uterine involution may occur due to several post-parturient reproductive conditions such as abortion, retained placenta, uterine prolapse, dystocia, subclinical/clinical endometritis and pyometra which delay the preparation for the subsequent prenatal period and consequently decrease conception rate (Sheldon *et al.*, 2006).

Progesterone, oestrogen, oxytocin and prostaglandins have both direct and indirect effects on the contraction of the myometrium in cows (Heppelmann *et al.*, 2018b). Pituitary oxytocin release is influenced by ovarian oestrogen and is inhibited by progesterone (Chen *et al.*, 2014). Strong utero-muscular contractions may occur through oxytocin receptor regulation in the

myometrium triggering prostaglandin F2 alpha (PGF2 α) secretion through an increase in oestrogen (Fuchs *et al.*, 2001; Abdisa, 2018). Myometrial contractions initiated by prostaglandin cause corpus luteal lysis which secrete relaxin and decrease progesterone concentrations (Abdisa, 2018).

Progesterone (Pg) is a steroid hormone primarily involved in receptor coordination of female reproductive processes such as pregnancy initiation and maintenance (De Amicis *et al.*, 2012). Pg is produced by the ovaries before pregnancy and by the placenta in the course of pregnancy during which its concentration is elevated (Martin *et al.*, 2013). The functional properties of progesterone include preparation of the uterus for pregnancy and maintenance of the pregnancy (Blavy *et al.*, 2016). There is a significant alteration in progesterone concentration during abortions in cows (Kindahl *et al.*, 2004).

Increased progesterone due to stress stimulates immunosuppressive protein accumulation in the lumen of the uterus causing susceptibility to persistent bacterial infections (Kaczmarowski *et al.*, 2006; Azawi, 2008; Abdisa, 2018). An increase in serum progesterone and lowered estradiol are seen in cows with retained placental membrane (Kaczmarowski *et al.*, 2006). Changes in progesterone profile and less oestrone sulphate production have been linked to the incidence of dystocia (calving difficulty) in cows (Kindahl *et al.*, 2004).

Oestrogen is an intra-ovarian reproductive hormone characteristically known to affect calcium homeostasis, the skeleton, the liver, hypothalamus, pituitary gland and it influences growth, development, maturation, and sexual differentiation in animals (Balthazart *et al.*, 2009). There are three forms of oestrogen namely estrone (E₁), estradiol (E₂) and estriol (E₃) of which estradiol oestrogens are produced by the ovaries while estriol is secreted by the placenta (Çiftci, 2013). The production of estradiol (E₂) and prostaglandin F2 alpha are lowered in cases of placental membrane retention (Abdisa, 2018). Elevation in gene expression during parturition

is stimulated by oestrogen which contracts and excites the myometrium, as well as increasing the oxytocin sensitivity in the uterus throughout the period of gestation (Abdisa, 2018)

A nonapeptide hormone oxytocin (OT), is functionally recognised for its ability to regulate reproductive processes such as pregnancy, milk let down and stimulating influx of calcium in cows (Arrowsmith & Wray, 2014). Other functions of OT include contraction of the uterus during labour, stimulation of the uterine muscle contraction and also aiding in the increase of prostaglandin production which moreover elevates contractions (Blanks & Thornton, 2003). OT is inhibited by progesterone during pregnancy (Heppelmann *et al.*, 2018b). It is also known to stimulate the synthesis of prostaglandins in the tissue endometrium (Kubo *et al.*, 2018). Monitoring of OT is done during the peri-partum period and is beneficial as a preventive and corrective measure for reproductive disorders.

Prostaglandins (PGF2 α) are physiological lipid compounds having hormone-like effects in animals and their functional properties include the regulation of smooth muscle tissue (Sauls *et al.*, 2018). PGF2 α are uterotonic mediators involved in increasing uterine contractility and can as well be used for treatment of metabolic disorders such as retained placenta (Heppelmann *et al.*, 2018b). Other functions of prostaglandins include labour induction, oestrus synchronisation and also they stimulate the production of luteinising hormone (Sauls *et al.*, 2018). Prostaglandin increase has been associated with the occurrence of abortions in cows (Kindahl *et al.*, 2004). Hormones are essential and require much consideration in cows during the production stage. Any excess or deficiency of reproductive hormones during the peri-partum period produces harmful effects on the health of both the cow and the calf.

6.2. Methods and materials

6.2.1. Study area

This study was conducted in Mafikeng areas in the North West Province of South Africa. The study location has previously been described in Section 3.2.1 of Chapter 3.

6.2.2. Sampling

In this study sampling was done in different communal farms in Mafikeng local municipality. Samples were conveniently collected when reproductive cases were reported to the North West University Animal Hospital. A total of 182 blood samples were collected from cows with dystocia (n=50), retained placenta (n=13), downer cow syndrome (n=34), vaginal prolapse (n=16) and abortions (n=69). Blood samples were collected through venepuncture using double pointed vacutainer 18 gauge needles into red capped tubes, then placed on ice in a cooler box and taken for analysis at the North West University Laboratories. Serum was harvested by centrifuging the blood for 15 minutes at 1000×g at 2~8°C. Serum was then frozen at -20°C pending examination (Picht *et al.*, 2007).

6.2.3. Estradiol analysis

Serum was analysed using the E₂ (Estradiol) ELISA Kit (Elaboscience-USA) following the manufacturer's instructions. Sample preparation and assay were as follows: Blood samples were allowed to clot for 2 hours at room temperature or overnight at 4 °C before centrifugation for 15 min at 1000×g at 2~8 °C. The supernatant was collected to perform the assay. Blood collection tubes should be disposable and non-endotoxin. Some 50µL of standard or sample was added to each well of the Elisa plate. Immediately about 50µL of HRP-labeled Estradiol was added to each well. Some 50 µl of detection Ab was added to each well. The tubes were incubated for 1 hour at 37 °C. The tubes were then aspirated and washed 3 times. About 50µL of substrate A and substrate B were added to each well. Incubate for about 15 minutes at 37 °C

with shading light. About 50µL Stop Solution was added. The sample was read at 450 nm immediately. Using a micro-plate reader results were then calculated.

6.2.4. Progesterone analysis

Serum progesterone concentrations were analyzed using Pg (Progesterone) ELISA (Enzyme immunoassay) Kit (Elaboscience-USA) as previously described by Vasconcelos *et al.* (2003). The procedure is similar as described in section 6.2.3 in chapter 6 for estradiol assay.

6.2.5. Prostaglandins analysis

The samples were analysed using the Human PGF2 α (Prostaglandin) Elisa kit following the manufacturer's instructions (Elabscience[®], USA). Briefly, about 50µL standard or sample was added to each well of the Elisa plate. Immediately 50µL biotinylated detection Ab was added to each well. The plate was incubated for 45 minutes at 37°C. The supernatant was aspirated and washed 3 times. Then 100µL HRP Conjugate was added to each well. The plate was incubated for 30 min at 37°C. The supernatant was aspirated and washed 5 times. About 90µL substrate Reagent was added. The preparation was incubated for 15 minute at 37°C. Thereafter 50µL Stop Solution was added. Immediately after, a reading was done at 450 nm, the results were read using a micro-plate reader.

6.2.6. Oxytocin analysis

Samples were examined using OT (Oxytocin) ELISA Kit Elabscience[®] (USA). Similar procedure for prostaglandin as described in the above section 6.2.5 in chapter 6 was followed for serum preparation and oxytocin analysis according to the manufacturer's instructions.

6.3. Statistical considerations

In this study sample readings were recorded in Microsoft excel (2010). The data were analysed using the Statistical Software for Social Scientists (SPSS) version 25. This study used descriptive statistics such as mean, standard deviation and skewness to describe the distribution of the hormone levels across the reproductive conditions. The multivariate analysis of variance (MANOVA) was used to compare the hormone levels across the reproductive conditions. The MANOVA was also useful in determining whether there is a significant difference in the hormone levels according to the different reproductive conditions. The test statistic (F) for Wilk's MANOVA test was used. A significant p-value of the F-statistic of the unbalance factorial design (A p-value less than the significance level of 5%) indicates that the independent variable has an impact on the level of hormones.

6.4. Results

The table below shows that the mean and standard deviations of the levels of hormones were different across the reproductive conditions.

Table 6. 1: Mean $\pm$ Standard deviation of hormones in cows with reproductive conditions

REPRODUCTIVE CONDITIONS	HORMONES		
	Estradiol (pg/ml)	Prostaglandins (pg/ml)	Oxytocin (pg/ml)
Abortion	1122.99 $\pm$ 71.99*	300.41 $\pm$ 24.48**	574.73 $\pm$ 60.65*
Dystocia	862.09 $\pm$ 123.44*	153.06 $\pm$ 43.61 ^{ns}	370.50 $\pm$ 71.66*
Vaginal prolapse	187.94 $\pm$ 91.44*	241.84 $\pm$ 28.35**	433.53 $\pm$ 14.07 ^{ns}
Downer cow syndrome	781.32 $\pm$ 135.70*	205.13 $\pm$ 25.08**	602.73 $\pm$ 0.15 ^{ns}
Retained placenta	312.39 $\pm$ 112.37*	92.06 $\pm$ 45.57**	598.66 $\pm$ 0.10 ^{ns}

P<0.001(*)

P<0.05 (**)

^{ns} =Not significant

Pg/ml=Picogram/milliliter

The table shows that the mean levels of progesterone, estradiol, prostaglandin, and oxytocin differed significantly across the reproductive conditions (P<0.05) (Table 6.1). The following table 6.2 determines how these hormones are affected by reproductive conditions. That is, the levels of hormones are seemingly dependent on the reproductive conditions.

Table 6. 2: MANOVA test used to estimate the significance of the relationship between reproductive conditions and hormones (Estradiol, Progesterone, Prostaglandin and Oxytocin)

MULTIVARIATE TESTS					
	Value	F	Hypothesis df	Error df	Sig.
Pillai's Trace	0.767	9.966	16.000	672.000	.000
Wilks' Lambda	0.358	12.613	16.000	504.721	.000
Hotelling's Trace	1.462	14.938	16.000	654.000	.000

The multivariate analysis of variance (MANOVA) tests were significant at 5% ($P < 0.05$); therefore, the mean levels of hormones differ across the reproductive conditions (Table 6.2). The following table determines the specific hormones whose levels differ significantly across the reproductive conditions (Table 6.2).

Table 6. 3 Tests of Between-Subjects Effects showing significant values of hormones in reproductive conditions (abortion, retained placenta, vaginal prolapse, dystocia and downer cow syndrome)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
REPRODUCTIVE CONDITION	Progesterone(ng/ml)	2034.520	4	508.630	8.145	.000
	Estradiol (pg/ml)	16347109.821	4	4086777.455	17.968	.000
	Prostaglandin (pg/ml)	1414698.459	4	353674.615	24.654	.000
	Oxytocin (pg/ml)	1505616.169	4	376404.042	3.693	.007

The table above shows that the mean levels of Progesterone, Estradiol, Prostaglandin, and Oxytocin differ significantly across the reproductive conditions ($P < 0.05$) (Table 6.3).

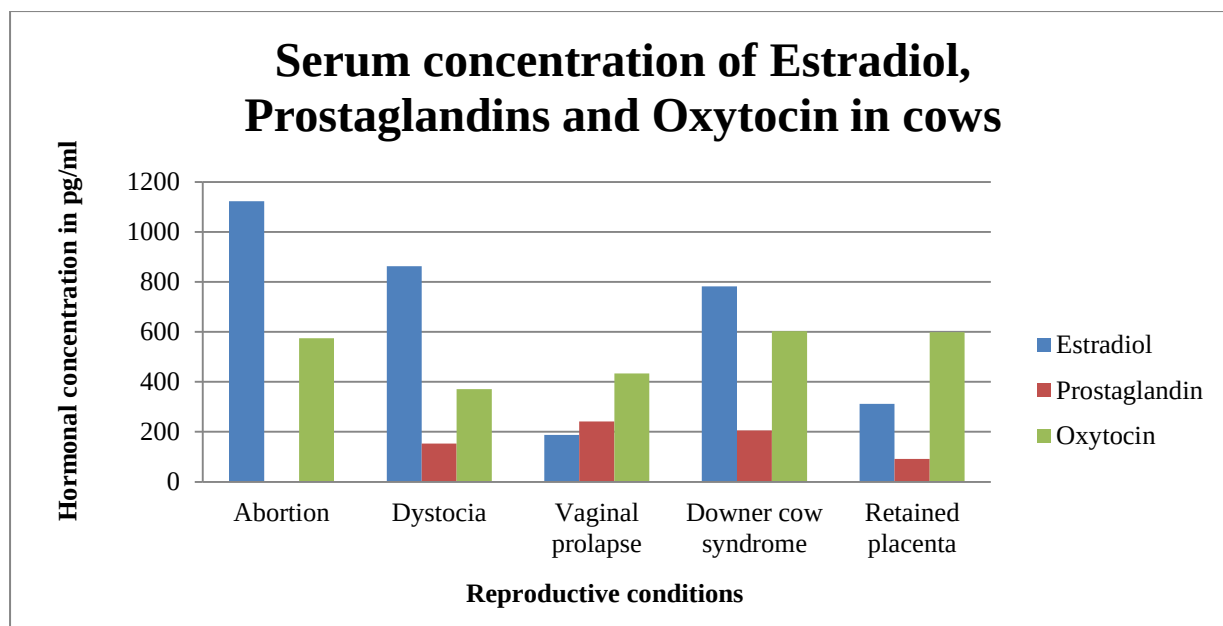


Figure 6. 1: Serum concentrations Estradiol, prostaglandins and oxytocin in cows with different reproductive conditions.

The highest concentrations of estradiol were found in abortion cases, followed by dystocia and downer cow syndrome and the least concentration was observed in vaginal prolapse cases (Figure 6.1). Prostaglandin was higher in vaginal prolapses and the least in retained placenta. Oxytocin concentrations were equally high in retained placenta and downer cow syndrome (Figure 6.1).

Table 6. 4: Hormonal values obtained in studies for pregnant cow

Reproductive hormones	Reference values (During pregnancy)	Reported in a study of:
Progesterone (ng/ml)	Low (6-15 ng/ml) high (20-50 ng/ml)	(Purohit <i>et al.</i> , 2012)
Estradiol (pg/ml)	115.7-858.5 pg/ml	(Opara <i>et al.</i> , 2006)
Prostaglandin (pg/ml)	6.4 ng/ml	(Jerome & Srivastava, 2012).
Oxytocin (pg/ml)	8.7- 51.2 pg/ml	(Chen <i>et al.</i> , 2014)

Reference values for hormone in cows reported in previous studies

Table 6.4 shows the reference values used to compare normal and abnormal levels found in the cows having different reproductive conditions.

Table 6. 5: Mean $\pm$ Standard Error of progesterone in different reproductive conditions

Reproductive conditions	Progesterone (ng/ml)
Abortion	2.45 $\pm$ 1.509*
Dystocia	8.59 $\pm$ 0.402*
Vaginal prolapse	4.67 $\pm$ 0.301*
Downer cow syndrome	4.40 $\pm$ 1.222*
Retained placenta	3.78 $\pm$ 0.151*

***P<0.01; ng/mg=** Nanogram/milliliter

The mean concentration of progesterone (ng/ml) seen in all reproductive conditions were significantly lower than normal (Table 6.5).

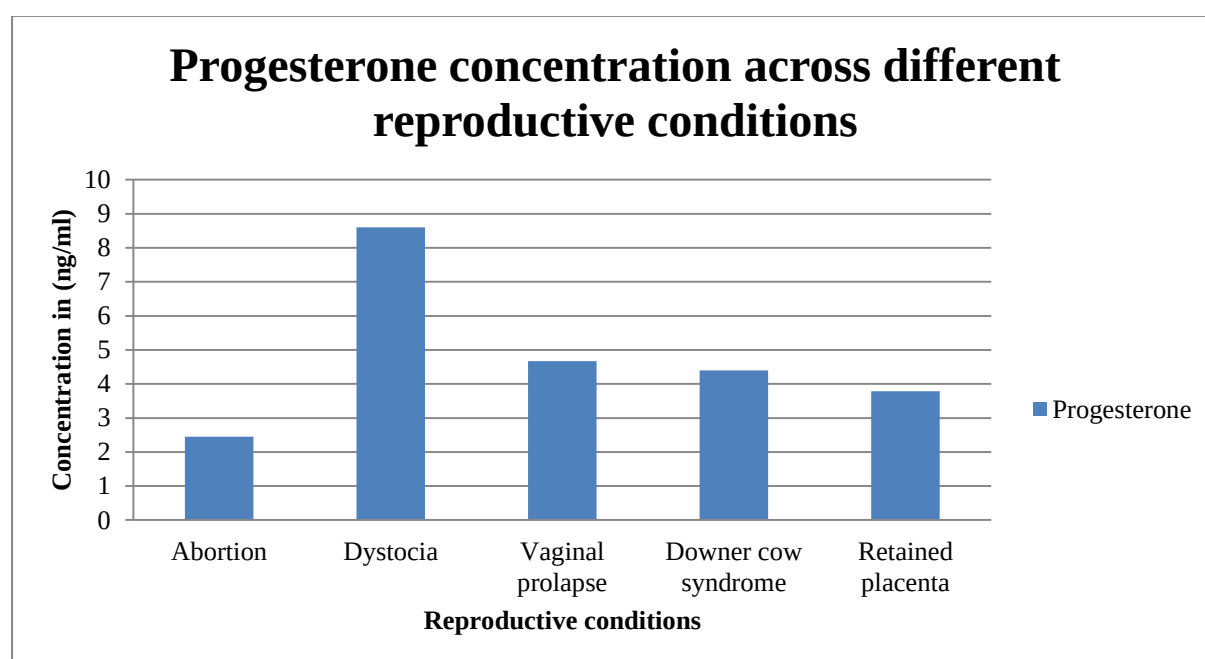


Figure 6. 2: Progesterone concentrations in different reproductive conditions.

The concentration of progesterone was highest in dystocia cases, followed by vaginal prolapse, downer cow syndrome, retained placenta and the least in aborting cows (Figure 6.2).

6.5 Discussion

Hormonal equilibrium during pregnancy is essential to ensure positive reproductive performance. The regulation of prostaglandin (PGF2 α) secretion in ruminants is through estradiol (E₂), progesterone (Pg) and oxytocin (OT) (Jerome & Srivastava, 2012). Through a positive feedback loop, PGF2 α 's primary release triggers corpus luteal production of OT and E₂ increases PGF2 α secretion through epithelial cell oxytocin stimulation in the cow uterus (Jerome & Srivastava, 2012). The results of the current study indicated significantly ($P < 0.001$) different hormonal levels in cows suffering from various reproductive conditions (Table 6.2).

Estradiol (E₂) is one of the three forms of oestrogen (Çiftci, 2013). The mean estradiol (pg/ml) concentrations were significantly higher in cases of abortion (1122.99 ± 71.99), downer cow syndrome (781.32 ± 135.7) and dystocia (862.09 ± 123.44), while the lowest concentrations of estradiol were noted in cases of vaginal prolapse (187.94 ± 91.44) and retained placenta (312.39 ± 112.37) seen in Table 6.1 and Figure 6.1. The values obtained in the present study were above the highest and below lowest values reported in a study of Opara *et al.* (2006) indicating estradiol concentrations of 115.7 ± 1.2 pg/ml and 858.5 ± 6.8 pg/ml in pregnant cows, respectively. The results are in line with a study which indicated a relationship between lowered E₂ and vaginal prolapse in cows (Purohit *et al.*, 2012).

The findings of the current study were similar to those of previous works, with reports indicating that significantly higher levels of circulating oestrogen are typically seen during gestation (Opara *et al.*, 2006). Another study of Mehrvar *et al.* (2017) reported that the high incidence of calf mortality was due to relaxation deficiency of the pelvic ligaments as a result of insufficient estradiol. A deficiency of oestrogen which is responsible for connective tissue movements such as relaxation and contractions predisposes cows to vaginal prolapse (Criado & Esteban, 2001).

Prostaglandins (PGF2 α) are uterotonic mediators responsible for muscle contractility (Heppelmann *et al.*, 2018b). The current study showed that the mean PGF2 α (pg/ml) values were significantly higher in vaginal prolapse (241.84 $\pm$ 28.35) cases and abortion (300.41 $\pm$ 24.48) seen in Table 6.1. Prostaglandins released due to protozoal invasion of the placenta and the heart can cause abortion in cows, whereby embryo tissue is destroyed due to oxygen insufficiency and a lack of nutrients (Khajeh & Khaj, 2018). Additionally, reduced maternal immunity in the foetus can result in placental inflammation which can induce abortion through the production of harmful inflammatory substances (Alexandre *et al.*, 2011).

Changes in luteal blood causing luteal lysis induced by prostaglandins is usually followed by lowered progesterone production which then leads to abortion (Jerome & Srivastava, 2012). Several other studies have reported negative effects (embryo mortalities, deformities and stillbirths) due to elevated prostaglandin alpha (PGF2 α) concentrations on the survival of the embryo (Lemaster *et al.*, 1999; Moon *et al.*, 2004 ; Scenna *et al.*, 2005; Jerome & Srivastava, 2012).

Oxytocin (OT) is a paraventricular and supraoptic nuclei pituitary-secreted hormone known to positively influence uterine contractility, uterine involution, uterine motility, calving, expulsion of uterine contaminants and stimulation of Prostaglandins (PGF2) synthesis (Uvnäs-Moberg *et al.*, 2001). Oxytocin (pg/ml) concentrations differed significantly being higher in cows with the mean range of 370.50 $\pm$ 71.66 (Dystocia) and 574.73 $\pm$ 60.65 (Abortions) as seen in Table 6.1 and Figure 6.1. The values are higher than the mean concentrations obtained in a study by Chen *et al.* (2014) which indicated an OT range of 8.7- 51.2 pg/ml in pregnant cows.

In the present study OT concentrations (pg/ml) were also high in incidence of vaginal prolapse (433.53 $\pm$ 14.07), retained placenta (598.66 $\pm$ 0.10) and downer cow syndrome (602.73 $\pm$ 0.15) but not significantly different (Table 6.1). There are myometrial contractility surges due to OT

production (Purohit *et al.*, 2012). Oxytocin stimulation of milk let down could explain the high values of OT in cows with downer cow syndrome which may occur due to loss of calcium ions through the milk production causing cows to be downer. Progesterone (Pg) is an important regulator of several reproductive processes such as pregnancy maintenance, ovulation and menstrual cycle (De Amicis *et al.*, 2012). A high concentration of Pg is sustained during gestation and it is the best biomarker for evaluation of reproductive status (Valadez-Cosmes *et al.*, 2016). The result of this study indicated that mean progesterone (ng/ml) mean concentrations in cows with abortion (2.45 ± 1.509), dystocia (8.59 ± 0.402), vaginal prolapse (4.67 ± 0.301), downer cow syndrome (4.40 ± 1.222) and retained placenta (3.78 ± 0.151) were significantly lower than the value reported in a study of Purohit *et al.* (2012) indicated in table 6.4 and figure 6.1.

Marginal progesterone concentration in cows fluctuate between 6 and 15 ng/mL throughout pregnancy, whereas the highest mean values range from 20-50 ng/ml throughout gestation (Purohit *et al.*, 2012). Low Pg levels have been linked to foetal mortalities due to its relationship with poor nutrition which results in negative energy (NEB) balance which therefore interferes with the microenvironment in the uterus causing abortion in cows (Jerome & Srivastava, 2012). Any hormonal imbalance in cows can negatively affect productivity through cow/calf losses and impaired reproductive health with both direct and indirect effects.

6.6: Conclusion

Variations in hormonal profiles can significantly influence reproductive conditions in cows. The study established a positive relationship between abortion and high estradiol, elevated oxytocin and lowered progesterone concentrations. The incidence of vaginal prolapse and retained placenta are likely to occur when estradiol and progesterone levels are low. Dystocia incidences in cows have been linked to high estradiol, an increase in oxytocin and low progesterone serum concentrations. Cases of downer cow syndrome were significantly

associated with high serum progesterone and estradiol. Elevated concentrations of prostaglandin alpha are related to cases of abortions and vaginal prolapse. Cows undergo severe alteration as the transition from late gestation to early lactation sets in. Hence neuro-hormonal balance becomes such an important factor in reducing foetal and cow mortalities, and incidence of reproductive conditions which have negative economic implications in livestock agriculture.

6.7. Recommendation

1. Further study of the relationship between incidence of reproductive conditions and the level of hormones in a controlled environment is necessary for definitive results confirmation.

6.8. References

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CHAPTER 7

EFFECTS OF MINERAL SUPPLEMENTATION ON REPRODUCTION IN CROSS BRED BONSMARA COWS: AN EXPERIMENTAL STUDY

Abstract

The presence of minerals in animal feed occurs 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 utilisation in metabolism. The aim of this study was to evaluate the impact of mineral supplementation on reproduction in communally grazed cross bred Bonsmara cows. A farm was selected for the experiment from the client list of the Dale Beighle Animal Hospital in the North West University, Mafikeng Campus. The selected farm comprised of cross bred cows with Bonsmara predominance. Twelve primiparous and multiparous pregnant cows were selected based on age, weight and parity. The group of 12 cows was then sub-divided into two groups (an experimental and a control) of 6 cows in each. Cows in the experimental group were given mineral supplements at normal dose from mid to late pregnancy and the control group did not receive any supplements throughout the pregnancy. Cows were given MULTIMINTM + Se +Cu at a dosage of 1 ml/45 kg BW and Calci 50 p.i. at the dosage 100-150 ml/500kg BW at 6 weeks interval from 27 July 2017- 6 October 2017. Both groups of cows were all allowed to graze in the veld as per farmer's routine. Blood samples were collected before every injection, stored on ice, and transported to the North West University Animal Health Laboratory for analysis with the IDEXX Catalyst DX^{*} Chemistry Analyser. The study used the t-test to relate the mean weight gain and mean serum metabolite between the experimental and control group. This study revealed that natural pastures lack phosphorus, zinc, copper and iodine. The cows in the experimental group all calved normally.

The study showed that 16% of cows that did not receive supplements (those in the control group) had 1 case of retained placenta and dystocia. Serum analysis revealed that supplemented cows had significantly ($P < 0.05$) high levels of triglycerides and creatinine kinase. The body weight increase was also significantly higher in the experimental group. Mineral supplementation could improve productivity and reduce calving disorders.

Key words: Cows, communal rearing, minerals, reproductive condition, supplementation.

7.1. Introduction

Globally, livestock production plays an essential role in many communal areas (Randolph *et al.*, 2007). In South Africa and other developing countries, there is a great reliance on livestock agriculture for food security and better livelihood (finances and insurance) for those embarking on small-scale farming (Feldt *et al.*, 2016). Communal farmers mostly, struggle to access veterinary resources and important aspects such as primary animal health care, disease control and surveillance are generally neglected (Hangara *et al.*, 2011). The incidence of reproductive conditions hinders cattle production, negatively affects profitability and ultimately limits communal farmers' contribution to the broader economy.

Nutrient requirements increase as pregnancy progresses and failure to account for the nutritional demands during this period can affect reproductive performance and foetal growth (Caldow & Riddell, 2015). Minerals are critically essential as any alteration in supply during gestation can predispose cows to reproductive failure (Griffiths *et al.*, 2007; Andrieu, 2008). Deficiencies of calcium, magnesium, phosphorus, copper, selenium, zinc and manganese have been associated with the occurrence of hypocalcaemia, retained placenta, abortion, dystocia, vaginal prolapse, downer cow syndrome and overall depressed reproductive performance in cows (Sepúlveda-Varas *et al.*, 2015; Amen & Muhammad, 2016; Musa *et al.*, 2016; Velladurai *et al.*, 2016; Yatoo *et al.*, 2018)

In mammals, reproductive needs for minerals are commonly consistent with the foetal and conception product (i.e. foetal fluid, uterus, placenta) mineral content (Suttle, 2010). Most cattle rely on natural pastures to obtain their nutritional needs (Hangara *et al.*, 2011). The soil or plant characteristics affecting the primary amount of the various fodders include: species of the plant, maturity stage of the plant, drainage system, pH, breeding practices and the interrelations among different minerals (Al-Ghareebawi *et al.*, 2017). Cattle naturally obtain minerals from consuming forages and feeds. Therefore, supplementation in cattle reared on natural pasture feeding system is necessary. Nonetheless, it is equally essential to ensure a balanced nutrient intake as any deficiency or excess can result in impaired reproductive performance.

The content of minerals in fodder relies mainly on the genetic identity of the plant, soil properties (physical, biological and chemical factors), climatic conditions and the plant's stage of development (Suttle, 2010). Whenever natural grasslands are the main or only source of nutrition which the animals rely on, it is essential to determine the nutritional content of the pastures, to quantify and measure different elements in order to account for any deficiencies and improve the feed to enhance production and reproduction (Al-Ghareebawi *et al.*, 2017). Procedures for addressing mineral insufficiencies have been established; some of which include indirect inclusion of minerals in pasture and feeds as they grow as well as supply of minerals to animals. However, most communal farmers have not extensively benefited from such innovation.

For optimum benefits, the use of mineral supplementation should be employed only when natural forage cannot supply adequate amounts of minerals to the animals. The increased attempts to reduce mineral deficiencies have as well increased the risk of toxicity. It is a common practice to offer cow mineral licks to balance the requirements, but this is not achievable unless the concentrations of minerals in the supplements are exactly the amount

required. Therefore, proper supplementation is necessary to reduce the incidence of reproductive problems and animal losses due to nutritional imbalances. Hence this study aimed at examining the role of mineral supplementation on reproduction in pregnant cows reared on natural pastures.

7.2. Methodology

7.2.1 Study site

This study was conducted in Mogosane village found in Mafikeng area of the North West Province of South Africa.

7.2.2 Sample collection and preparation

Sample collection and preparation for analysis were conducted as indicated in section 5.2.2 of Chapter 5.

7.2.1. Study animals selection

A communal farm in Mogosane village of Mafikeng area in the North West Province, South Africa was selected from previous encounters of bovine reproductive conditions. The Mafikeng area is a semi-arid area which comprises of both rural and commercial farms; however, the majority are communal farms. The selected farm comprised of cross bred cows with Bonsmara predominance and it was selected based on having cows with previously encountered cases of dystocia, retained placenta, vaginal prolapses, downer cow syndrome, and abortions. Some of the samples from this farm were among those analysed in this study. Breeding history (as per what the farmers observed) for all the cows selected in the experiment were recorded.

7.2.2. Study design

Starting from June 2017 to October 2017, twelve pregnant cows of about 3- 4.5 months were identified and grouped by age, weight and parity. Both the treatment and control cows were

examined for pregnancy by manual rectal palpation. They were then randomly allocated to one of two treatments: A trace mineral supplemented (TMS) or control group (without mineral supplementation). A randomly selected sample of primiparous and multiparous (3 to 5 years old, 347-540 kg initial body weight and parity of 1-2) were assigned to control (no injection) or supplement group given three injections at the recommended dose rate of chelated mineral supplement (NRC 2001): Manganese (Mn), Zinc (Zn), Copper (Cu), Selenium (Se), Calcium (Ca), Magnesium (Mg) and Phosphorus (P) at 6-week intervals in mid- to late gestation. Pregnant cows were then tagged according to the group (treatment or control) allocation.

The animals were subcutaneously injected with MULTIMIN™ +Se+ Cu at a dose of 1 ml/45 kg BW and Calci 50 p.i. at the dosage 100-150 ml/500kg BW. 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), and 5 mg Se/mL (as sodium selenite) and Calci 50 p.i. contains calcium 45, 6 mg; Magnesium 7, 8 mg and phosphorus 1, 32 mg.

All cows were allowed to graze in the veld and offered injectable mineral supplements: one group (n=6 cows) were given normal dosage and the control group (n= 6 cows) were not given any supplements. The amount of minerals offered were calculated according to the NRC (2001) recommendations. This supplementation experiment continued until all the cows gave birth.

7.2.3. Mineral analysis

Blood samples were collected before the commencement of the experiment and three times (before the first, second and last injections of minerals were given) at a 6 weeks interval from June 2017. Blood was analysed for zinc (Zn), copper (Cu), iron (Fe), iodine (I) and selenium (Se) using NexION 300X ICP-MS (Perkin-Elmer, Waltham, USA), according to the procedure described by Ndou & Dlamini (2012). Briefly, about 0.7 ml of serum was transferred into a sterile centrifuge tube: About 6.650 ml of the prepared TCA (50g TCA into 500ml) was added and the tubes were covered and shaken individually on an electric stirrer (vortex machine), left

for 10 minutes on the bench, then centrifuged at 2500 rpm (revolutions per minute) for 10 minutes. Then for each 5 ml of supernatant fluid was transferred to the clean test tubes. Afterwards, about 5ml of TCA was added to the 5ml of supernatant fluid to make 10 ml, this was left overnight, then samples were filtered and 13 ml of samples was placed into 15 ml centrifuge tube. Analysis using the ICP-MS (Inductively coupled plasma mass spectrometry) 300Q machine was carried out (Ndou & Dlamini, 2012).

7.2.4. Serum metabolites analyses

Samples were analysed for serum biochemical parameters such as calcium (Ca), 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 (NH₃) using IDEXX Catalyst chemistry analyser according to the manufacturer manual's instructions.

7.2.5. Grass sampling

Pasture samples around the area (Mogosane village) where the cows were grazing were harvested according to species and analysed for 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 collected included *Eragrostis rigidior* (Curly grass), *Bothriochloa radicans* (stinking grass), *Eragrostis lehmanniana* (Lehmann's Love Grass), *Panicum maximum* (White buffalo grass), *Aristida congesta* Subsp. *barbicollis* (Spreading Three-awn), *Eragrostis rotifer* (Pearly Love Grass), *Urochloa oligotricha* (Perennial Signal Grass), *Themeda triandra* (Red grass), *Heteropogon contortus* (Spear grass), *Eragrostis superba* (Saw-tooth Love grass) and *Brachiaria nigropedata* (Black footed grass). The pastures were picked at the stage of maturity and about 10 cm from the ground. Grasses were then labelled according to different species in separate collection paper bags. After harvesting, the grasses were taken to the NWU Animal Health laboratories for examination, and then the samples were placed on benches to air dry.

Then they were ground using Polymix PX-MFC 90 D. Powdered samples were subsequently placed in sample containers and stored until analysis.

7.2.4. Grass sample preparation

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 sixteen hours (at 60°C) in a hot air oven to dry. After drying, the crucibles were placed in a desiccator for six hours to allow cooling, and then they were weighed to obtain crucible weight before adding the samples. Grass samples were sun dried and grounded. An analytical balance calibrated to four decimal places was used for grass sample weighing. The difference between mass of the crucible with the fresh grass sample and the empty crucible weight were used to calculate the fresh weight {fresh weight= (crucible+ fresh sample weight) – (empty crucible weight)}. Exactly 1 gram of the powdered grass sample was weighed into the clean crucible. Crucibles containing ground grass samples were then placed in the oven for drying at 106 °C for sixteen hours. The samples were then placed into the oven to remove excess moisture. After removing the crucible from the oven, samples were then placed in a desiccator to cool and reweighed after six hours. The difference between the crucible weight with the dry sample and empty crucible weight was recorded as the dry weight of the sample {Dry weight= (crucible +dry sample weight) - (empty crucible weight)}. After weighing the samples were ashed in a muffle furnace at 800°C for 16 hours.

The ash was removed from the furnace and cooled, then 1ml Nitric acid and 9 ml hydrochloric acid were added to the crucible, the mixture was 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 100ml mark using a clean glass funnel. The solution was left overnight on the bench. The following

day the sample was filtered using the Whatman filter papers into sterile centrifuge tubes. Then analysis of digested samples was performed by using the ICP-MS (Inductively coupled plasma mass spectrometry) 300Q machine.

7.2.5. Instrument conditions for the ICP-MS (Inductively Coupled Plasma Mass Spectrometry)

Blood samples and grasses were analysed using the ICP-MS. Instrument standardization and validation of the method were conducted as described in Section 4.2.5 of Chapter 4.

7.2.5. Statistical considerations

The data were captured in Excel and analysed using Statistical Package for Social Science (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) group. The level of significance was set at $P < 0.05$. MedCalc's online Odds Ratio (OR) statistical calculator was used to calculate chances of occurrence of the incidence of reproductive conditions. Odds ratios (OR) is used to calculate the incidence of reproductive condition.

This was calculated using the following formula:

$$OR = \frac{\text{odds of condition for exposed}}{\text{odds of condition for unexposed}} = \frac{(ad)}{(bc)}$$

Where:

a = number of cows having reproductive condition and are not supplemented

b = number of cows not supplemented but having the reproductive condition

c = number of cows supplemented and are experiencing reproductive condition

d = number of cows supplemented and do not experience reproductive condition

7.3. Results

The following tables and figure compare body weight and the measurements of serum metabolites between the supplemented pregnant cows and those that were not given minerals supplements. The aim was to evaluate the possible role of mineral supplementation in cow reproduction.

Table 7. 1: Experimental mean weight gain in pregnant cows given mineral supplements (Mn, Zn, Cu, Se, Ca, Mg and P) compared to those not supplemented

Group Statistics					
Months	Cow ID	N	Mean weight gain/loss	Std. Deviation	Std. Error Mean
June-July	Experimental	6	61.500	58.037	23.694
	Control	6	-19.500	46.561	19.008
July-August	Experimental	6	-13.500	75.931	30.999
	Control	6	17.500	22.314	9.110
August-September	Experimental	6	-20.000	30.835	12.588
	Control	6	-12.667	18.811	7.680
September-October	Experimental	6	19.000	53.051	21.658
	Control	6	-51.500	33.441	13.652

The above table shows that there was some variation in the descriptive statistics of weight gain between the control and experimental group (Table 7.1). The mean values indicate both positive and negative weight gain (i.e. for June -July the experimental group gained average weight of about 61.5 kg while in the control group there was weight loss of 19.5 kg) (Table 7.1).

Table 7. 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

The results of the t-test (Norušis, 2006) show that the mean Weight Gain (June-July) (Table 7.2) differs significantly ($P < 0.05$) between the experimental and the control group with the former exceeding the later by 81. The results of the t-test (Norušis, 2006) also show that the mean Weight Gain (September-October) differs significantly ($P < 0.05$) between the experimental and the control group with the former exceeding the later by 70.5 (Table 7.2).

Table 7. 3: Mean differences of the experimental (supplemented) and the control (non-supplemented) group within a particular age and parities

	Age in years	Parity	Mean± Std.	Std. Error	Sig.
	(no: of cows)	(no: of cows)	Deviation	Mean	
Weight Gain (June- July)	3.00 (4)	1 st (5)	42.750±89.574	44.787	0.538
	4.00 (8)	2 nd (7)	10.125±54.057	19.112	
Weight Gain (July- August)	3.00 (4)	1 st (5)	-33.500±81.847	40.924	0.287
	4.00 (8)	2 nd (7)	19.750±30.570	10.808	
Weight Gain (August-September)	3.00 (4)	1 st (5)	-12.750±18.839	9.420	0.704
	4.00 (8)	2 nd (7)	-18.125±28.140	9.949	
Weight Gain (September-October)	3.00 (4)	1 st (5)	-6.500±76.857	38.429	0.745
	4.00 (8)	2 nd (7)	-21.125±48.230	17.052	

Significantly different ($P > 0.05$) across the ages and parities of the animals

The results of the t-test (Norusis, 2006) show that the means of weight gain (June-July) are not significantly different ($P > 0.05$) across the ages and parities of the animals (Table 7.3).

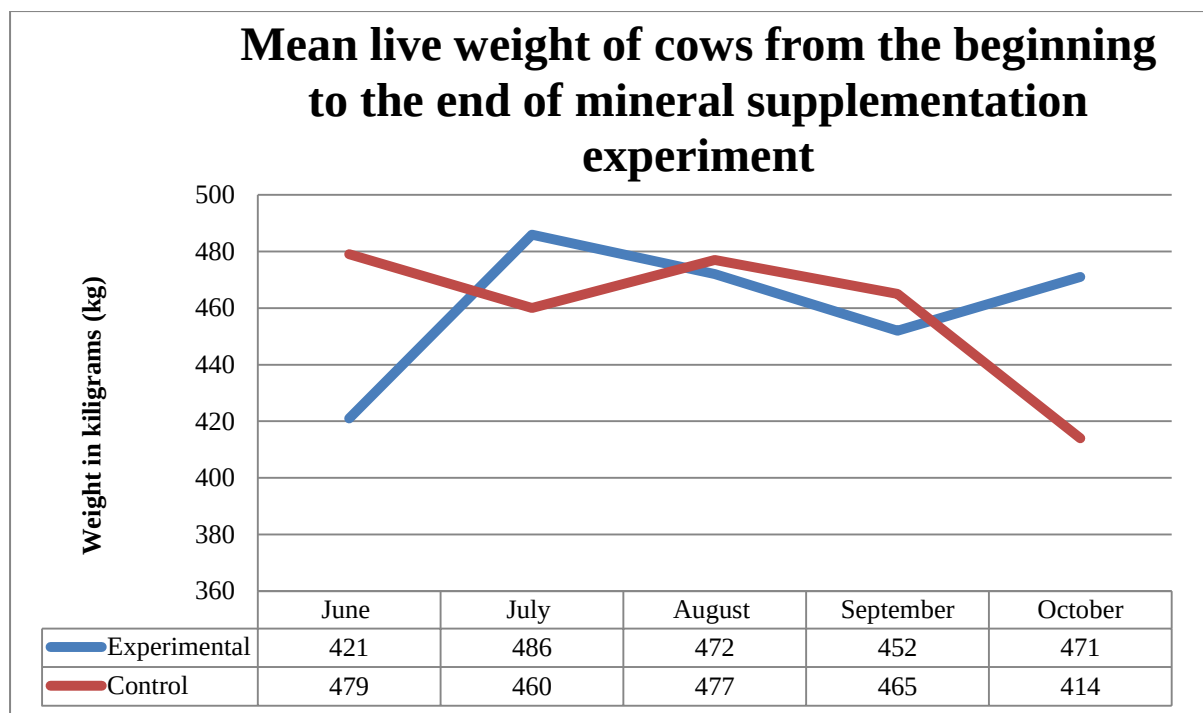


Figure 7. 1: Weight gain comparison between supplemented and non-supplemented pregnant cows.

In cows given injectable mineral supplements, weight gains were higher a month after first supplementation as compared to the control group (Figure 7.1). At the end of the experiment (between September and October) the weight gains were significantly higher in the control group (Figure 7.1).

Table 7. 4: Serum metabolite comparison between the experimental (supplemented) and the control (non-supplemented) group

Period	Serum metabolites	t	df	Sig. (2-tailed)	Mean Difference
10- June- 2017	UREA/BUN	-0.955	10	0.362	-0.433 mmol/L
(Before the first injection of the experiment)	Phosphates (PHOS)	-0.114	10	0.911	-0.018 mmol/L
	URIC Acid	2.983*	10	0.014	17.667 μ mol/L
	Calcium (CA)	0.200	10	0.845	0.010 mmol/L
	Magnesium (MG)	1.583	10	0.144	0.072 mmol/L
	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 μ mol/L
	Cholesterol (CHOL)	-0.029	10	0.978	-0.012 mmol/L
	Ammonia (NH ₃)	1.270	10	0.233	86.167 μ mol/L
	Triglycerides (TRIG)	-0.791	10	0.448	-0.003 mmol/L
	Lipase (LIPA)	0.285	10	0.781	3.667 U/L

*Sig. =significant differences ($P \leq 0.001$); ALT=Alanine Aminotransferase; AST=Aspartate aminotransferase; GGT= Gamma-glutamyl transferase.

Serum mean concentrations of uric acid differed significantly between the experimental and control (Table 7.4). The mean difference for uric acid (17.667 μ mol/L) showed that the experimental mean was greater than the control mean concentration (Table 7.4).

Table 7. 5: Serum metabolite comparison between the experimental (supplemented) and the control (non-supplemented) group after the second supplementation

Period	Serum metabolites	t	df	Sig. (2-tailed)	Mean Difference
6-October-2017 (before the last injection of minerals)	UREA/BUN	1.916	10	0.084	0.917 mmol/L
	Phosphates (PHOS)	-0.025	10	0.981	-0.008 mmol/L
	URIC Acid	2.072	10	0.065	12.500 μ mol/L
	Calcium (CA)	-1.118	10	0.290	-0.238 mmol/L
	Magnesium (MG)	-2.208	10	0.052	-0.153 mmol/L
	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 μ mol/L
	Cholesterol (CHOL)	-0.451	10	0.662	-0.195 mmol/L
	Ammonia (NH3)	-1.083	10	0.304	-99.167 μ mol/L
	Triglycerides (TRIG)	-4.661*	10	0.001	-1.800 mmol/L
	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 ($P \leq 0.001$).

Serum triglycerides (TRIG) and creatinine kinase (CK) were significantly different between the experimental (supplemented) and control (non- supplemented) groups (Table 7.5). The negative mean differences (calculated as = experiment mean – control mean) for both TRIG (-45.833) and CK (-110.333) indicate that the mean concentrations in the control groups were higher than those in the experimental group (Table 7.5). The other serum metabolites did not show any significant differences when compared with the supplemented and the non-supplemented groups ($P > 0.001$) (Table 7.5).

Table 7. 6: Serum metabolites Mean±Standard Error of cows given mineral supplements from 3-4.5 months of pregnancy

	Serum metabolites Means±SE		
	Uric acid	Triglyceride	Creatinine kinase
Experimental groups			
Treatment	52.167±3.986	0.217±0.034	69.500±6.312
Control	34.500±4.379	2.017±0.384	179.833±22.015
Normal ranges	2.810-3.930 mg/dL	0.080–0.200 mmol/L	0–110 U/L

Normal ranges for uric acid, triglycerides and creatinine kinase were sourced from Mamun et al., 2013, and Cozzi et al., 2011.

Table 7.6 shows that the mean uric acid recorded on 10-June-2017 (before first injection) was higher than normal in both the treatment and control groups, and the means of triglycerides (TRIG) and creatinine kinase (CK) were lower for the supplemented cows.

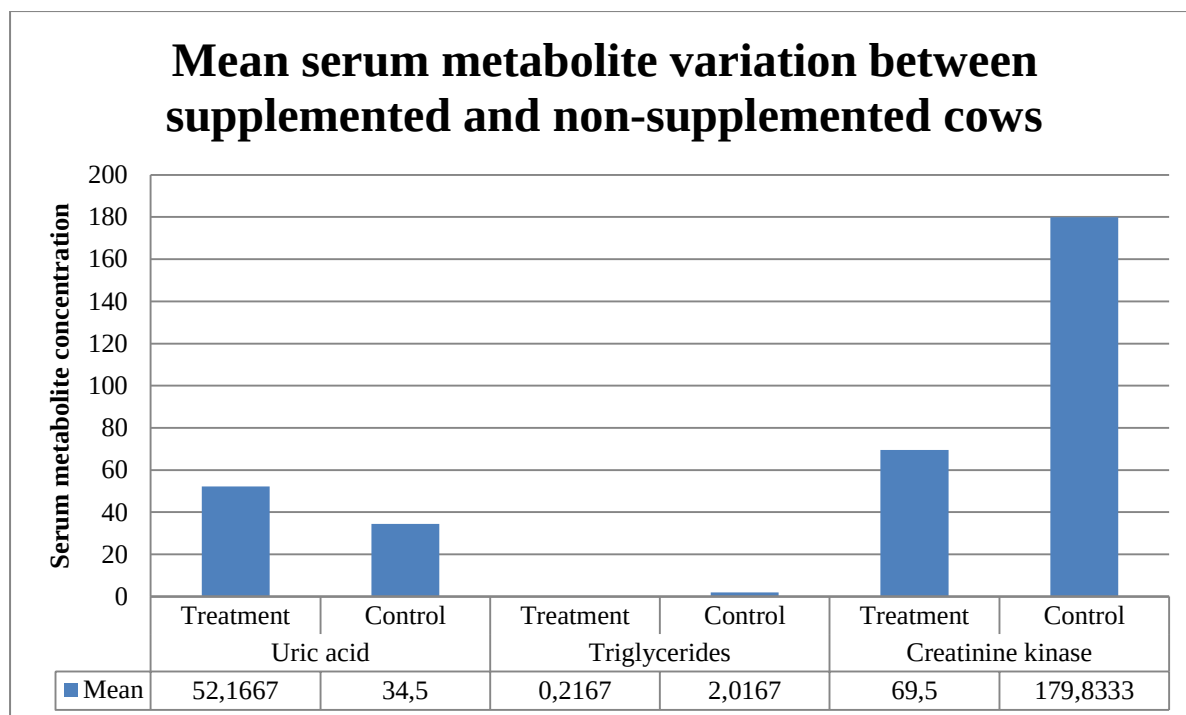


Figure 7. 2: Mean serum metabolites in the supplemented and non-supplemented cows

The mean uric acid was significantly higher in the treatment than in the control group. The triglycerides mean concentration in the control group significantly exceeded that in the treatment group. The creatinine mean concentration in the control group was higher than that in the treatment group (Figure 7.2).

Table 7. 7: Odds ratio for incidence of reproductive conditions in cows given injectable mineral supplements

Variables	% Control cows (Non-supplemented)	%Treatment cows (supplemented)	Adjusted odd ratio (95% C.I.)	P-value
Retained placenta and Dystocia	16.6%	0%	0.015	0.144

The odds ratio was used to estimate the probability of abortion, retained placenta, dystocia, vaginal prolapse, and downer cow syndrome. Only two cases (retained placenta and dystocia) were seen in the control group (16%) and all cows in the supplemented experimental group

calved normally with no negative effects (incidence of reproductive conditions). The odds ratio was not significantly different ($P = 0.144$).

Table 7. 8: Grass nutrient composition, mean standard deviation and reference ranges

Nutrients	Mean $\pm$ std (ug/ml)	Reference ranges	Level of concentration
Phosphorus (P)	9.412 $\pm$ 1.622	81.4464- 723.968 mg/L*	L
Magnesium (Mg)	17.946 $\pm$ 2.279	12.0188-36.6033 mg/L*	N
Calcium (Ca)	106.00 $\pm$ 10.728	88.172–90.576 ug/mL **	H
Manganese (Mn)	4.97 $\pm$ 4.135	0.18-0.19 mg/L***	H
Iron (Fe)	8.827 $\pm$ 1.6118	1-2 ug/ml***	H
Zinc (Zn)	0.209 $\pm$ 0.125	0.8-1.2 ug/mL***	L
Copper (Cu)	0.389 $\pm$ 0.125	0.57-0.96 ug/mL***	L
Selenium (Se)	10.66 $\pm$ 9.832	0.7-1.3 ug/mL ***	H
Iodine (I)	2.858 $\pm$ 1.943	4.96-19.85 mg/L**	L

Reference ranges sources: * Djokovic *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: ug/ml =mg/L

Table 7.8 shows the mean $\pm$ standard deviation values for nutrients content of grasses in the grazing area of the experimental farm chosen for the mineral supplementation experiment. The results indicated that the concentration of P, Zn, Cu and I in the grass were lower than the normal range. The concentration of Mg was found to be within the normal range. The study also found the concentrations of Ca, Fe, Mn and Se to be higher than the normal values in the grass.

7.4. Discussion

The aim of this study was to evaluate the effects of mineral supplementation on the incidence of reproductive conditions (abortion, dystocia, retained placenta, vaginal prolapse and downer cow syndrome) in communally grazed cows. Reproductive conditions are commonly seen in communal farms almost every calving season. This study was conducted on twelve pregnant cows which were divided into two groups of six. The role of supplementary trace minerals during pregnancy has shown inconclusive (positive, negative and sometimes neutral) results on reproductive performance (Joksimović-Todorović *et al.*, 2016).

The report by Sales *et al.* (2011) indicated that mineral supplementation reduced probabilities of abortions (embryo mortality) in crossbred heifers. Mineral deficiencies have also been associated with an increase in the incidence of retained placenta and dystocia (Musa *et al.*, 2016; Tucho & Ahmed, 2017). The present study showed cases of retained placenta (1 case) and dystocia (1 case) in the non-supplemented group and no cases of reproductive conditions in supplemented group; the study also revealed a lack of significant differences between mineral supplementation during pregnancy and the incidence of reproductive conditions with the odds ratio of 0.015 (Table 7.7). The findings are similar to previous studies which reported no significant variation of mineral supplementation on cows reproductive performance during pregnancy (Sales *et al.*, 2011; Joksimović-Todorović *et al.*, 2016).

Pasture samples were also collected in the grazing areas surrounding the selected experimental farm. All cows were allowed to graze according to the farmer's normal programme (morning to afternoon); feed and water consumption were not controlled in this experiment. In semi-arid areas, patterns of rainfall mostly affect the quality and availability of pastures during the dry season and improvements in quality and availability are seen as more rainfall is experienced (Bezabih *et al.*, 2014). Consequently animals reared under such conditions are prone to

nutritional imbalance due to fluctuating nutrient consumption which ultimately result in poor reproductive performance (Velladurai *et al.*, 2016).

The current study indicated that the concentrations of phosphorus (9.412 ± 1.622), zinc (0.209 ± 0.125), copper (0.389 ± 0.125) and iodine (2.858 ± 1.943) in the grass were lower than the normal range (Table 6.8). Natural pastures are generally low in phosphorus during the dry season which predisposes cows to impaired muscle function and has been associated with the incidence of retained placenta and downer cow syndrome (Ate *et al.*, 2009). Zinc deficiency impairs reproduction, influences the immune function, retards embryonic development (Velladurai *et al.*, 2016). Shortage of copper may lead to the occurrence of repeat breeding and retained placenta (Kumar, 2014). Only the grass concentration of Mg (17.946 ± 2.279) was found to be within the normal range. This study also found that the concentrations of calcium (106.00 ± 10.728), iron (8.827 ± 1.6118), manganese (4.97 ± 4.135) and selenium (10.66 ± 9.832) were higher than the normal values in the grass. The evidence of mineral imbalances in communal pastures could explain the annual incidence of reproductive conditions. This situation is a threat to reproductive performance and overall cattle productivity. Although the mineral supplementation did not show any significant influence on the incidence of reproductive conditions, the indication of deficiencies and excess of mineral in the grass suggests that further investigation should be done on supplementation and other factors influencing reproduction to obtain substantial data and that could help prevent animal losses in communal farms.

Nonetheless, significant differences in body mass and serum metabolites were noted between pregnant cows given injectable mineral supplements (Mn, Zn, Cu, Se, Ca, Mg and P) and those that were not. It is difficult to measure the state of nutrition in cows traditionally reared on natural pasture, therefore live-body weight measurements become a very useful management tool for monitoring nutritional status during and after pregnancy (Dhakal *et al.*, 2013).

The use of changes in body mass and metabolic profile measures in cows simplifies the interpretations of reproduction and nutrition interactions (Caldow & Riddell, 2015). In the current results, the t-test shows that the mean weight gain (June-July) differs significantly ($P < 0.05$) between the experimental and the control group with the former exceeding the latter by 81 (Table 6.2). The current study indicates that during winter (June –July) pregnant supplemented cows (61.5 ± 58.037 kg) gained more weight than the non-supplemented cows which showed average weight loss of 19.5 ± 46.56 kg (Table 7.1 and Figure 7.1).

The results also revealed that the mean weight gain in spring (September-October) differs significantly ($P < 0.05$) between the experimental and the control group with the former exceeding the latter by 70.5 (Table 7.2 and Figure 7.1). Changes 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 *et al.*, 2016). The observations of this study indicate that a lack of supplementation of minerals can bring about production losses in animals relying only on natural pasture (Dhakal *et al.*, 2013). Losses in body mass have been associated with poor productivity and incidence of reproductive conditions (Van Saun, 2008). The results imply that supplementation does help improve animal production during the dry season and helps maintain a good body condition.

Serum metabolites can be 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 the supplementation. The concentrations of uric acid, triglycerides and creatinine significantly varied between the supplemented (treatment) and non-supplemented (control) cows. (Table 7.6 and figure 7.2). In the current study, the serum mean concentrations of uric acid were significantly above the normal range in both the experimental and control groups before the commencement of the supplementation (Table 7.6). Additionally, the mean

difference for uric acid (17.667 mg/dL) showed that the experimental mean was greater than the control mean concentration (Table 7.4).

Serum triglycerides (TRIG) and creatinine kinase (CK) were significantly different between the experimental (supplemented) and controls (non-supplemented) groups (Table 7.5). The concentration of CK (179.833 ± 22.015 U/L) was significantly higher in the control group in which cases of dystocia and retained placenta occurred (Table 7.6). Previous reports have shown that elevated CK concentrations may be seen due to the impairment or exertion of skeletal muscles as a consequence of difficult parturition (Murray *et al.*, 2015). It is therefore rational for cows in the control which experienced difficult birth to show high creatinine kinase levels.

The results of this study showed that the mean TRIG (2.0167 ± 0.384 mmol/L) was above normal in the non-supplemented cows (Table 7.6). Negative energy feedback has been associated with low triglycerides levels (Alves *et al.*, 2014). In situations of positive energy balance triglycerides are likely to be high (Sevinç *et al.*, 2003). High nutritional requirement is associated with lowered triglyceride levels (Petkova *et al.*, 2008). This could explain the lower body mass seen in the control group after supplementation had started. Fatty liver syndrome is also related to a decrease in TRIG concentrations which is also directly proportional to the level of cholesterol (Qureshi *et al.*, 2016). The other serum metabolites (urea, phosphates, total protein, AST, GGT, TBIL, CHOL, NH₃, and LIPA) did not show significant differences when comparing the supplemented and the non-supplemented groups ($P > 0.001$) throughout the experiment (Table 7.4).

7.5. Conclusion

The major findings of this study revealed that in semi-arid areas such as Mafikeng, during the dry periods the concentration of minerals in forage gets depleted and animals solely dependent on natural grasses experience nutrient insufficiencies which could impair reproduction. In addition, the results showed low phosphorus, zinc, copper and iodine in the natural pasture consumed by the animals. All cows given mineral supplements calved normally. Mineral supplementation during pregnancy increases weight gain. The study also indicated that significantly high concentrations of triglycerides and creatinine kinase were observed in pregnant cows given injectable minerals. The data found in this study can be useful in future studies on minerals and reproductive interactions as well as in development of farm supplementation programs.

7.6. Recommendations

1. This study recommends the use of mineral supplements as part of feeding plans in communal farms as natural pastures have deficiencies of some essential minerals.
2. Further studies on mineral and reproduction interactions should be conducted to increase data which could be used by cattle producer for strategic planning.
3. Further study on the nutritional benefits of different grass species preferred by the cows in the semi-arid condition and their roles on reproductive performance should be conducted.

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CHAPTER 8

GENERAL CONCLUSION AND RECOMMENDATIONS

The poor cow reproductive status of cows in the rural communities has kept most communal farmers struggling financially, limiting their contribution to the broader livestock agricultural production and from economically benefiting in the formal market. Good reproductive health is necessary for optimum calf production and in subsequent reproductive performance. The multifaceted nature of reproductive conditions creates a huge challenge in the precise identification of causative factors related to their occurrence. Hence it was necessary to initiate this study with the aim of determining the possible effects of mineral supplementation on the incidence of reproductive conditions, with specific objectives to look at the managerial factors, minerals, serum metabolites, hormonal profiles and mineral supplementation during pregnancy in cows reared on pastoral tenure. The concurrent occurrence of reproductive conditions in communal areas severely affects communal farmers' economic stability and their contribution to the larger economy. The findings of this study suggest that females rarely participate in livestock farming as males still form a greater part of contributing group in the agricultural sector. The general profile of farmers actively involved in livestock production in communal farming has been shown to be older males, having low educational levels, mostly unemployed and earning a subsistence income, at most. Cow attributes such as breed, parity, feeding system and access to veterinary services were also found to influence reproductive performance. In most cases, farmers kept cows with unknown Brucellosis status, which is an important disease in cow reproduction as it can result in substantial animal losses. Mineral imbalance has been associated with reproductive failure due to its role in several metabolic processes and immune function. In this study, cows with dystocia showed low concentrations of phosphorus, copper, zinc selenium and iodine, with only the iron level being high. In aborting cows, the levels of

phosphorus and iodine were low, whereas the concentration of selenium was elevated. In cases of downer cow syndrome, the serum concentrations of magnesium and selenium were higher than normal while concentrations of phosphorus and iodine were lower. Cows with retained placenta had high concentrations of magnesium, selenium, iron and low phosphorus. The concentrations of phosphorus and iodine were low in vaginal prolapse cases while selenium and iron were high. Serum metabolic profile testing has been used in the assessments of animal health, nutritional and reproductive status. The present study revealed that cows with dystocia and downer cow syndrome have high concentrations of urea/ BUN. Calcium concentrations were low in cows that presented with retained placenta, abortions and vaginal prolapse. AST concentrations were high in incidences of retained placenta, vaginal prolapses, abortion, dystocia and downer cow syndrome. Lack of supplementation in most rural farms is the cause of low reproductive performances and severe losses on profit. This study also revealed that due to the low nutrient content in pastures, communally reared cows should be supplemented as a measure to prevent production losses as a result of peri-partum nutrient deficiencies.

Variations in hormone concentrations during pregnancy can highly influences the cow's reproductive ability due to their significant roles on the reproductive system as well as cow and foetal survivability. Low estradiol and progesterone concentrations were seen in cows with retained placenta and vaginal prolapses. Concentrations of oxytocin, and estradiol were higher than normal in dystocia cows while progesterone concentrations were low. Moreover, progesterone and estradiol concentrations were higher in downer cows. In cows with vaginal prolapse and abortions the concentrations of prostaglandins was higher than normal. It is therefore vital to ensure optimal hormonal balance during the transition period to help reduce complication which may hinder good reproductive performance. The study recommends that farmers should consider supplementation to help provide additional nutrients required during pregnancy which will reduce the reproductive conditions. Farmer education on different breeds

and their benefits, as well as implementation of proper farm management can be put in place as measures to lower the impact of reproductive conditions on productivity. Increased collaborations between farmers, researchers and government will bring about sustainable strategies which will intensify farm management aimed at reducing production losses. Further study of other non-nutritional factors that may lead to mineral and hormonal imbalances which hinder reproductive performance should be explored. Proper mineral supplementations for cows during pregnancy to improve reproductive performance and reduce the incidences of reproductive conditions in cows reared under semi-arid condition should be explored.

This study aimed to establish physiological profiles of minerals, serum metabolites and hormonal levels for each specific reproductive condition (downer cow syndrome, dystocia, retained placenta, vaginal prolapse and abortion) and to study possible correlations between these profiles and each condition for all cases. This was done with the aim of developing a reference tool as a means to predict the chances for each condition to occur that would assist animal care practitioners to detect or identify earlier the risk of the occurrence of these conditions and be able to prevent them or reduce the risk by supplementing with the missing elements. Table 8.1 below summarises the conclusion of the study. It shows the levels of indicative serum metabolites, minerals and hormones in cows presenting with different reproductive conditions. It highlights the abnormal parameters found and shows the reference value of normal levels of serum metabolites, minerals and hormones.

Table 8.1: Summary of predictive characteristics which could increase the risk of cows experiencing reproductive conditions

	SERUM METABOLITE	Reference values	MINERALS	Reference values	HORMONE	Reference values
Dystocia	- High AST -High urea/ BUN	Urea/BUN- (2.50–6.7 mmol/L) Phosphates (PHOS)- (1.38– 2.55 mmol/L) Calcium (Ca)- (2.2–2.62 mmol/L) Total Protein (TP)- (8–80 g/L)	-Low phosphorus, copper, zinc selenium and iodine -High Iron	Phosphorus (P)- (0.720-6.400 mmol/L) Magnesium (Mg)- (0.4945-1.506 mmol/L) Copper (Cu)- (7.801-23.621 µmol/L)	-High oxytocin and estradiol -Low progesterone Low estradiol and progesterone	Progesterone (ng/ml)- Low (6-15 ng/ml) high (20-50 ng/ml) Estradiol (pg/ml)- (115.7-858.5 pg/ml) Prostaglandin (pg/ml)- (6.4 ng/ml)
Retained placenta	- High AST -Low calcium	Aspartate amino-transferase (AST)- (0–91 U/L) Gamma-glutamyl Transferase (GGT)- (0–80 U/L)	-Low phosphorus and iodine -High selenium and iron were high	Iron (Fe)- (12.500-44.811 µmol/l) Zinc (Zn)- (10.710-19.890 µmol/L)	Low estradiol and progesterone	Oxytocin (pg/ml)- (8.7- 51.2 pg/ml)
Vaginal Prolapse	- High AST -Low calcium	Total bilirubin (TBIL)- (2.8- 9.3 µmol/L)	-Low phosphorus and iodine -High selenium	Selenium (Se)- (0.024-3.000 µg/dL)	-High prostaglandins	
Abortion	- High AST -Low calcium	Cholesterol (CHOL)- (1.96–5.86 mmol/L) Ammonia (NH3)- (0–90 µmol/L) Triglycerides (TRIG)- (0.08–0.20 mmol/L)	-High magnesium, selenium, copper and Iron -Low phosphorus and iodine are lower	Iodine (I)- (4.960-19.850 µg/L)	-High progesterone and estradiol	
Downer cow syndrome	- High AST -High urea/ BUN					

		Lipase (LIPA)-(30–400 U/L)				
		Creatinine kinase (CK)-(0– 110 U/L)				

APPENDICES

1. Publication (Chapter three)

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Cattle Production Management Practices Predisposing Animals to the Incidences of Reproductive Failures in Small Scale Farming

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Abstract: Preparing for an effective animal reproduction requires proper management practices before parturition. This study aimed to identify cattle production management practices that predispose cows to the incidences of reproductive conditions in small scale farming. One hundred and thirty-five (135) structured questionnaires were used to obtain information from the farmers following reported cases of dystocia, downer cow syndrome, retained placenta, vaginal prolapse and abortion in cows at the North West University animal hospital. The data included information such as cow age, parity, breed, number of incidence of the conditions, feeding system, feed type, whether or not the animals were supplemented, supplement type, condition experienced by the cow, vaccination status, cow's Brucellosis status and access to veterinary services were also assessed. The data were analysed in Statistical Product and Service Solutions (SPSS) version 25 using descriptive statistics, Chi square and Stage-Two Cluster analysis. The results showed that the factors significantly related ($p < 0.05$) to the incidences of reproductive conditions were cow breed, parity, feeding system, whether the farmer has heard about Brucellosis and the frequency of getting animals checked by a veterinarian. The study found that in communal areas, herds with Afrikaner breeds, when farmers have not heard about Brucellosis, when cows are the first parity, while they are in free ranging method of feeding and with an annual veterinary check-up mostly experience abortions. Implementation of management strategies for the improvements of farming methods in communal farming is necessary to change the state of livestock agriculture through the reduction of reproductive failures in small scale farms.

2. Questionnaire

QUESTIONNAIRE



TOPIC: Evaluation of cow production management in relation to the incidence of bovine reproductive conditions in communal areas of Mafikeng of the North West Province

FARMER'S DEMOGRAPHICS

1. Gender

Male ☐ Female ☐

2. Age

18-39 years ☐ 40-55 years ☐ 56-69 years ☐ 70-80 years ☐

3. Marital status

Married ☐ Divorced or separation ☐ Widow or widower ☐ Single ☐

4. Employment status

Pensioner ☐ Part-time ☐ Full time ☐ Unemployed ☐

5. Educational levels

Primary ☐ Secondary ☐ High school ☐ Tertiary ☐

6. Monthly income

<R1000 ☐ R1000-R3000 ☐ R3000-R5000 ☐ R5000-R10000 ☐ >R10000 ☐

ANIMAL INFORMATION

7. Cow identity (tag number).....

8. Age of the cow

1-3 years ☐ 1 4-6 years ☐ 2 7-9 years ☐ 3

9. Body condition score

1-very poor ☐ 2-poor ☐ 3-good ☐

4-very good ☐ 5-obese ☐

10. Breed

Brahman ☐ 1 Nguni ☐ 2 Bonsmara ☐ 3 Angus ☐ 4
Friesland ☐ 5 Afrikaner ☐ 6 Simmental ☐ 7 Dromedary ☐ 8
Charolaise ☐ 9 Mixed breeds others ☐ 10 ☐ 11

11. Parity

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
8 ☐ 9 ☐ 10+ ☐

12. Number of incidences if the condition

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
☐ ☐ ☐ ☐

7 8 9 10+

13. Type of feeding system

Free ranging ☐

Confined ☐

Mixed method ☐

14. What type of feed you give to your animals

.....

.....

.....

.....

15. Are the cows supplemented?

Yes ☐

No ☐

16. If yes with what?

.....

.....

.....

17. Reproductive condition encountered

	2	3
Downer Cow Syndrome	1	Abortion
Dystocia	4	Vaginal Prolapses
		5
		Retained Placenta

18. Medical history of the same condition

Yes ☐

No ☐

19. Was treatment given for the same condition before?

Yes ☐ No ☐

20. Are the animals dewormed?

Yes ☐ No ☐

21. Do you vaccinate your animals?

Yes ☐ No ☐

22. If yes, against what?

☐ ☐ ☐ ☐

23. What is the Brucella status of your herd?

Positive ☐

Negative ☐

Unknown ☐

24. Any other disease/s encountered by the cow

.....
.....
.....

25. How often do your animals get checked up by the veterinarian?

Never ☐

Regularly ☐

Annually ☐

Thank you very much for your participation in this study.

Yours: Miss Molefe Keitiretse

Under supervision of Dr M. Mwanza

3. Consent form



INTERVIEW CONSENT FORM

Research Project title: *Evaluation of cow production management in relation to the incidence of bovine reproductive conditions in communal areas of Mafikeng in the North West University.*

Research investigator: Ms Keitiretse Molefe

Institution: North West University (Mafikeng Campus)

Participant's name:

Date:

The interview will take at-least 15 minutes. We don't expect that there are any risks associated with your participation, but you have the right to stop the interview or withdraw from the

research at any time. Thank you for agreeing to be interviewed as part of the above research project. Ethical procedures for academic research undertaken from North West University institution require that interviewees explicitly agree to being interviewed and how the information contained in the interview will be used. This consent form is necessary for us to ensure that you understand the purpose of your involvement and that you agree to the conditions of your participation.

I will read and explain the questions to you and then you can sign this form to certify that you approve the following:

1. The interview will be recorded and a transcript will be produced.
2. The transcript of the interview will be analysed by Ms Keitiretse Molefe as a research investigator.
3. Access to the interview transcript will be limited to Ms Keitiretse Molefe, academic colleagues and the researchers with whom he might be collaboration with as a part of the research process.
4. Any summary interview content, or direct quotations from the interview, that are made available through academic publication or other academic outlets will be anonymized so that you cannot be identified, and care will be taken to ensure that other information in the interview that could identify yourself is not revealed.
5. The actual recording will be kept until the completion of the study

All part of the content of your interview may be used;

1. In academic paper, policy papers or news articles.
2. On our websites and in other media that we may produce such as spoken presentations.
3. On other feedback events.
4. In an archive of the project as noted above.

By signing this form I agree that;

1. I am voluntarily taking part in this project. I understand that I don't have to take part, and I can stop the interview at any time.
2. The transcribed interview or extracts from it may be used as described above.
3. I have read the information sheet.
4. I don't expect to receive any benefit or payment for my participation.
5. I can request a copy of the transcript of my interview and may make edits I feel necessary to ensure the effectiveness of any agreement made about confidentiality.
6. I have been able to ask questions I might have and I understand that I am free to contact the researcher with any questions I may have in the future.

Farmer's consent

I agree to participate in this study freely without pressure. I declare that information provided in this questionnaire is correct.

Signed.....at (area).....on (date).....

