Parasite prevalence, nutritionally-related blood metabolites and pre-slaughter stress response in Nguni, Bonsmara and Angus steers raised on veld

By

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DECLARATION

I, the undesigned declare that this dissertation submitted to the University of Fort Hare for the degree of Master of Science and work contained herein is my original work carried out under the supervision of Professors AI Okoh and M Chimonyo, and unless cited has not been submitted at any other University for any degree.

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Abstract

The effects of month on body weight, body condition scores, internal parasite prevalence and on nutritionally related blood metabolites were studied in Angus, Bonsmara and Nguni steers raised on sweet veld. Pre-slaughter stress was also determined using catecholamines, cortisol, dopamine, packed cell volume and serum creatinine levels. The blood chemical constituents evaluated included glucose, cholesterol, total protein, creatinine, urea, globulin, albumin, calcium, phosphorus, magnesium, aspartate amino transferase (AST), alkaline phosphatase (ALP) and creatinine kinase (CK). The Nguni steers maintained their body condition throughout the study period whereas Angus steers had the least body condition scores. Parasite levels were high during the rainy season and low during the dry season. The predominant internal parasites were *Haemonchus* (39.3%), *Trichostrongylus* (37.8%), *Cooperia pectinita* (25.5%), *Fasciola gigantica* (16.3%) and *Ostertagia ostertagi* (11.2%). The Nguni had the least parasite infestation levels and had high PCV levels. Nguni had higher levels of cholesterol and glucose (2.86 and 4mmol/l, respectively) than the other two breeds. Nguni and Bonsmara steers had higher ($P<0.05$) mineral levels. There were significant breed and month differences for glucose, cholesterol, creatinine, calcium, albumin, phosphorus, albumin-globulin ratio and ALP levels. Bonsmara was more susceptible to transport and pre-slaughter stress as it had the highest ($P<0.05$) levels of adrenalin (10.8nmol/mol), noradrenalin (9.7nmol/mol) and dopamine (14.8nmol/mol) levels, whereas the Nguni had the least levels of adrenalin (6.5nmol/mol), noradrenalin (4.6nmol/mol) and dopamine (4nmol/mol) levels. In conclusion, Nguni steers were better adapted to the local environmental conditions.

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List of original papers

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Chapter 1

General Introduction

1. Introduction

Although cattle provide diverse functions to farmers in Africa (Scoones and Graham, 1994), their productivity is generally low. Cattle provide draught animal power, social and cultural functions as well as serving as security and risk reduction in rural households (Corbet *et al*., 2005; Anderson, 2003). Cattle, therefore, contribute to subsistence farming and enhance the sustainability of smallholder farming systems. There is reduced cattle productivity as indicated by calving intervals which are way above the recommended 12 to 13 months (Chimonyo *et al*., 2000) and high calf mortality rates. There are various factors that reduce cattle productivity, chief of which is the low veld quality during the dry season, use of non-adapted exotic breeds and the general poor health management practices (Berry *et al*., 2006). It is possible that under nutrition is common during this period (Agenas *et al*., 2006). There is increasing interest in indigenous livestock as a possible solution to increased efficiency of production in harsh environmental conditions in southern Africa.

Indigenous cattle breeds are being threatened with disappearance largely due to indiscriminate crossbreeding (Otto *et al*., 2000) and institutional policies that promote the use of imported beef cattle breeds in rural areas. Efforts are now under way to repopulate the rural areas of South Africa with the indigenous Nguni cattle. The University of Fort Hare (UFH) Nguni Cattle Project aims not only to increase the number of the indigenous

Nguni cattle in the communal areas, but also to see through identification and creation of niche markets. The UFH model aims to increase the existing cattle population in the communal areas by eliminating all non-Nguni bulls within a community. The Nguni is increasingly attracting international interest, mainly due to its resistance to ticks and tick-borne diseases, high reproductive performance and good walking and foraging ability (Strydom *et al*., 2001; Muchenje *et al*., 2007). For efficient production and for improving their marketability, the nutritional and health status of the introduced animals needs to be closely monitored (Chimonyo *et al*., 2002).

Grazing animals are more prone to acquiring internal parasites as compared to feed supplemented animals. Breeds resistant to parasites must be adopted as they reduce the cost of rearing the animal and also produce organic meat where no chemicals have been used till animal slaughter. Besides immunomodulation, helminth parasites can also exert multiple deleterious clinical and sub-clinical effects on a host that indirectly may alter the outcome of vaccination. For example, parasitism causes inappetence (Krecek and Waller, 2000; Coop and Kyriazakis, 2001), alters feed utilisation and metabolism (Mahusoon *et al.,* 2004). Furthermore, protein losses occur during the course of parasitic infections (Coop and Kyriazakis, 2001), and the decreased protein levels may compromise proteinaceous components of the immune system. There is paucity of information on the susceptibility of Nguni cattle to internal parasites in the Eastern Cape Province. A study is therefore needed to provide a basis for implementing helpful adjustments in current cattle management practices so as to alleviate the constraints on productivity. Further research is also needed to provide information on the alterations in body metabolism which would account for the loss of body weight and condition in cattle due to the effects of internal parasites. Although body weights and body condition scoring have traditionally been used to assess nutritional status of cattle (Grunwaldt *et al*., 2005), blood metabolite concentrations provide more accurate assessments. Serum concentrations of metabolites such as glucose, cholesterol, non-esterified fatty acids, blood urea nitrogen, creatinine, total proteins albumin, globulin and minerals are commonly used to assess the nutritional status of cattle. Information on the effects that variations in breed and season of beef animals may have on serum concentrations of metabolites is scarce. Moreover, there is need to establish reference values for assessing nutritional status of animals, such as the indigenous Nguni cattle. Determination of blood metabolite concentrations for Nguni cattle will provide information that serves as the basis for the diagnosis, treatment, and prognosis of diseases that could affect these indigenous cattle breed (Yokus and Cakir, 2006). Information on blood metabolites in the Nguni cattle also increases an understanding of adaptability of cattle to local production conditions (Otto *et al*., 2000; Grunwaldt *et al*., 2005). There is paucity of information on the Nguni's adaptive value and their competitiveness against established breeds such as the Bonsmara and Angus assessed through the use of blood metabolites.

Besides adaptation to local conditions, responses of different breeds to stress is crucial. Transport and handling before slaughter affect both animal weight and meat quality (Leheska *et al.*, 2002). Poor handling causes economic losses to farmers, transporters and slaughterhouses (Gallo *et al.*, 2003). Stress occurs during common management practices in beef cattle production. Previous handling experiences may interact with genetic factors. The response of a breed to management stressors such as transportation and restraint may determine if that breed will be advantageous to rear. There is therefore need to compare the responsiveness of Nguni cattle to stress, as it affects not only growth performance, but also meat quality.

1.2 Objectives

The main objective of this study was to determine the parasite prevalence, nutritionally-related blood metabolites and pre-slaughter stress responses in Nguni, Bonsmara and Angus steers raised on veld. The specific objectives were:

1. To evaluate the effect of month on body condition scores, body weights and internal parasite levels in Nguni, Angus and Bonsmara steers;
2. To determine the effect of month and breed on nutritionally-related blood metabolites and;
3. To determine the influence of breed on pre-slaughter stress response.

1.3 Hypotheses

The experiments were designed to test the following null hypotheses:

1. Month does not have an effect on body condition scores, body weights and internal parasites levels in Nguni, Angus and Bonsmara steers;
2. Month and breed do not have an effect on nutritionally-related blood metabolites and;
3. Type of breed does not have an influence on pre-slaughter stress response.

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Chapter 2

Review of literature

2.1 Introduction

In order to increase cattle productivity and improve cattle management, nutritional and health status of cattle must be known. Body condition scores, body weights, internal parasites and blood metabolites are used to assess the nutritional and health status of animals. Stress hormones and their metabolites are also used to measure stress levels of animals in order to prevent reduced productivity as stressors affect the carcass quality and immune system of animals thus predisposing them to diseases and other parasitic infections. Determination of blood metabolite concentrations for Nguni cattle will provide information that serves as the basis for the diagnosis, treatment, and prognosis of diseases that could affect indigenous cattle breed (Yokus and Cakir, 2006). In this literature review, stress hormones and their metabolites, blood metabolites and pathological factors used for the assessment of the nutritional and health status of cattle are reviewed.

2.2. Traditional methods of assessing nutritional status of cattle

Determination of the nutritional status of cattle is useful in quantifying the extent to which cattle are affected by nutrition, disease or other environmental factors, especially where seasonal fluctuations in the quantity and quality of forages occur, as is common in dry tropical and subtropical areas. Body weights and body condition scoring are the traditional methods used to assess nutritional status of animals though they have several limitations or drawbacks.

2.2.1 Body condition scoring

Body condition scoring describes the systematic process of assessing the degree of fatness of an animal (Nicholson and Sayers, 1987; Roche *et al*., 2004). The score reflects the plane of nutrition on which an animal has been exposed over a reasonable length of time (Stuth *et al*., 1998). This information is important to appraise the adequacy of feeding programs, particularly in production systems where the availability of feeds are not constant such as those experienced by grazing animals (Roche *et al*., 2004) in southern Africa. The ability of ruminants to cope metabolically with the nutritional demands of additional production or seasonal changes in pasture yield and quality has made BCS an indispensable tool for the management of ruminants (Roche *et al*., 2004). Several authors have documented association between body condition scoring and fertility (Buckly *et al*., 2003; Roche *et al*., 2004) and health (Roche and Berry, 2006; Roche *et al*., 2004). The loin, ribs, tail head, brisket, flank, vulva and/or rectum and udder are the important parts of the body used in determining the score.

Initially, body condition scoring was conducted on a 5-point scale (Nicholson and Sayers, 1987). However, the 5-point scale has been deemed inappropriate for tropical breeds of cattle as it does not cover a wide enough range of animals in poor conditions that are commonly found in rural areas. In addition, it has been reported to be inappropriate for small-framed animals that predominate in the tropics. The 5-point scale is, however, quite popularly used by dairy producers. The 9-point scale is recommended for tropical cattle, such as *Bos indicus* (Nicholson and Butterworth, 1986; Eversole *et al*., 2000), the scores ranging from one (severely emaciated) to nine (obese). Body condition scoring is easy to apply and has been mostly used as a management tool largely in the dairy sector. Despite the reported repeatability estimates by experienced assessors (Ferguson *et al.*, 1994), the general subjective nature of body condition scoring makes it difficult for inexperienced herd managers, to correctly score the animals. Unlike body weight measurements, the automation of body condition scoring has, to date, been unsuccessful (Berry *et al*., 2006).

The amount of body fat cattle have is directly related to their body condition score. On the average BCS 1, 5, and 9 cows are approximately 3.77, 18.89, and 33.91% body fat respectively (National Research Council, 1996). Physiologically, the proportion of protein and water of the animal's bodyweight decrease as it gains body condition (National Research Council, 1996). Although there is a general consensus that the genes that influence body condition scores and body weights are either closely linked or could have pleiotropic effects on each other, Berry *et al*. (2006) observed a low correlation coefficient between body weight and condition scores.

2.2.2 Body weights

Typically, growth is measured as an increase in body weight and it includes not only cell multiplication (hyperplasia) but also cell enlargement (hypertrophy) and incorporation of specific components from the environment (e.g., apatite deposition) (Flier and Maratos-Flier, 2000**)**. Growth can be monitored by using body weights. Body weights are commonly used because they are easier and quicker to perform, not much expertise is required and they are not tedious to perform.

Body weights are mostly used for monitoring nutritional status and growth of animals (Chimonyo *et al*., 2000). However, the body weight of an animal *per se* does not reflect its nutritional status (Oulun, 2005). Animals with large frames may have higher body weights with low level of body reserves than small framed ones with abundant reserves. Changes in body weight, therefore, become more informative than body weights *per se*. Large variations in body weight may occur as a result of changes in gut fill and bladder fill, pregnancy and parturition (National Research Council, 1996). Moreover, weight changes may reflect tissue hydration rather than significant alterations in body protein or fat content (National Research Council, 1996). To minimize the effect of frame size of the animal, body weight measurements should be collected regularly, often on a monthly basis. The influence of gut fill on body weight measurements can be reduced by weighing the animals at a consistent time of day. This requirement is difficult to meet, since animals are usually presented to the handling facilities at different times. Calibration of weighing scales is also difficult.

2.3 Effects of internal parasites on cattle

It has been estimated that internal parasites cost the livestock industry in excess of US$2 billion per year in lost productivity and in increased operational expenses (Axford *et al.*, 2000). The fact that parasite infections affect the host immune system is well documented. The host has immune mechanisms that allow, under most cases, for the maintenance of the host-parasite relationship in equilibrium (Amarante, 2001). Gastrointestinal infections are characterized by the reduction of voluntary food intake and a decrease in the efficiency in the use of these feeds by the infected animals (Amarante, 2001; Muturi *et al.*, 2005; Keyyu *et al*., 2005). They, therefore, have a negative effect on animal growth and production (Kahn *et al*., 2000). Body condition scores and body weights can be used to monitor the effect of these internal parasites on animal growth and production (Keyyu *et al*., 2005).

The most common genera of nematodes causing reduced animal growth and production are *Trichostrongylus, Ostertagia* and *Haemonchus*. *Haemonchus contortus* is the most pathogenic nematode followed by *Ostertagia* and *Oesophagostomum* (Bowman and Georgi, 2002; Jackson and Miller, 2006). The pathogenicity of *Haemonchus contortus* could be explained by the large daily egg production of about 5000 to 15 000. Knowledge of when and where cattle become infected with internal parasites and information on the agricultural cycle and associated husbandry practices are needed in order to formulate rational policies for management that reduce the opportunity for infection. Many parasites exhibit dramatic seasonal variation in egg production. Weather conditions also affect larval availability thus indirectly affecting fecal exam results (Soun *et al*., 2006). Dry, hot weather decreases the availability of infective larvae, similar to sparse pasture conditions. In general, with all internal parasites, moist weather increases survivability of infective larvae in the vegetation and induces eggs to hatch (Jackson and Miller, 2006). There is paucity of information on the susceptibility and seasonal variation of internal parasites load in Nguni, Bonsmara and the Angus breeds raised on veld in the Eastern Cape province of South Africa.

Resistance traits are defined as those that influence the capacity to control infection and may be evaluated using the following parameters: number of cells in the intestinal mucosa membrane (eosinophil and mast cell), faecal egg count per gram, number of fourth and fifth stage larvae and number of male and female worms, as well as their length (Wiedosari *et al*., 2006). On the other hand, resilience is based on the ability of the animal to support the pathogenic consequences of an endoparasite infection, which may be evaluated observing the packed cell volume (PCV), haemoglobin, mean albumin concentration, food consumption and growth rate (Keyyu *et al*., 2005). For meat producing animals kept at pasture, which are subject to slower development rates due to endoparasite infection, it is important to observe the direct influence of diet type and helminth infection on the production process, as well as to use this information to reduce the number of worm treatments. Such parameters have not been determined for Nguni breed raised on veld.

In cattle and sheep, the reduction of weight gain and other adverse effects depend on the parasite burden (Coop and Kryziakis, 2001; Wiedosari *et al*., 2006). Ovine fasciolosis can result in significant blood losses with all associated consequences (Soun *et al*., 2006). *Fasciola hepatica*, also known as the liver fluke, migrates through the liver parenchyma and tissue. Adult flukes feed on the blood of the final host at a rate of 0.2 to 0.5 ml per day per fluke (Wiedosari *et al*., 2006) which leads to severe anaemia. Hypoalbuminaemia (reduction in albumin levels produced by the liver) and hyperglobulinaemia (increase in immunoglobulins synthesized by leucocytes) are common signs.

Efficient and welfare-friendly livestock production demands the control of nematode infection. Current control measures rely upon anthelmintic treatment but are threatened by the widespread evolution of drug-resistance in parasite populations. Several methods have been advocated to control nematodes without relying on anthelmintics. These include grazing management, biological control, nutritional supplementation, vaccination, and genetic approaches (Jackson and Miller, 2006; Wiedosari *et al*., 2006). Selecting lines of animals, either from within a breed or by introducing a 'resistant' breed to produce crosses that have an improved ability to regulate their endoparasite populations offers a means of increasing immunity against gastrointestinal nematodes at the herd level. Cattle studies have confirmed that at least part of the variation in an individual's ability to regulate worms is under genetic control and that it is a moderately heritable characteristic in cattle (Jackson and Miller, 2006). Selection is feasible using simple phenotypic markers such as faecal egg counts sometimes in conjunction with peripheral immunological markers such as antibodies or eosinophils in blood samples. No such markers have been carried out in Nguni, Bonsmara and Angus breeds to assess its tolerance to internal parasites.

2.4 Use of blood metabolites to complement traditional methods

Blood metabolite concentrations represent an integrated index of the adequacy of nutrient supply in relation to nutrient utilization of cattle (Chester-Jones *et al.*, 1990). They give an immediate indication of an animal's nutritional status at that point in time (Pambu-Gollah *et al.*, 2000). In the dairy industry, the use of metabolic profiles for assessing the nutritional and health status of cows is widespread (Doornenbal *et al.*, 1998; Grunwaldt *et al.,* 2005). Use of such metabolites in the management of beef cattle is still uncommon, as it is thought that blood parameters cannot predict the metabolic status of an animal without a characterization of its diet and phase and level of production (Roche *et al.*, 2004). Reference values for Nguni, Bonsmara and Angus cattle are not available, which makes it difficult to determine their nutritional status. There is, therefore, a need to develop reference values for such breeds for adequate characterization and to aid in their management. The major blood constituents that can be routinely assayed include haematology parameters, glucose, non-esterified fatty acids, β-hydroxyl-butyrate, cholesterol, total proteins, albumin, urea and minerals.

2.4.1 Blood metabolites related to energy metabolism

Blood glucose, β-hydroxy butyrate and non-esterified fatty acids are the most common metabolites used to assess the energy status of cattle. Blood glucose has a moderate diagnostic value in the assessment of nutritional status of cattle as it varies moderately in blood (Mayes, 2000). Insufficient nutrient intake can reduce circulatory glucose and cholesterol levels. In conditions of under-nutrition, the blood levels of propionate and other precursors derived from the diet decreases thus causing a reduction in the rate of glucose synthesis (Mayes, 2000; Reynolds *et al*., 2003). Age affects glucose levels in cattle as has been shown by Doornenbal *et al*. (1988) where glucose levels in calves were lower than those for mature animals. In growing animals, glucose requirement is determined by growth rate, which is set by metabolizable energy intake (Reynolds *et al*., 2003) whereas in mature animals only maintenance energy is required.

Grunwaldt *et al*. (2005) reported an effect of season in Agentina on glucose levels shown by a significant increase in blood glucose levels in autumn (February) as compared to summer (May) (Table 2.1). Glucose levels decrease with an increase in body temperature and respiration rate of animals normally experienced in hot summer season. Feed quality also affects blood glucose levels as poor grass quality reduces blood glucose levels. For example, Chimonyo *et al.* (2000) observed a significant reduction of the levels of plasma glucose in winter in cows. Glucose levels have also been shown to vary with breeds as shown in Table 2.2. Bonsmara steers had higher glucose levels as compared to Angus steers fed on rye grass overseeded with coastal Bermuda grass. However, no such data are available for Nguni steers in comparison with other breeds under natural pasture. Cholesterol has moderate variability in the blood thus giving a moderate diagnostic value. The reason for moderate variability of cholesterol is not clear but can probably be attributed to its metabolic variation with the blood glucose levels. Effect of season on cholesterol levels is also not clear. Elevated levels of cholesterol, triglycerides, and phospholipids are indicative of copper deficiency. The essential nature of copper is due to its co-factor role at the active site of a number of enzymes (Engle *et al*., 2000). Breed variation in serum cholesterol levels has also been shown by Hollenbeck *et al.* (2006) where Bonsmara steers had higher levels when compared to Angus steers (Table 2.2).

Table 2.1: Blood chemistry measurements from beef cattle in Mendoza plain, Brazil, in summer and autumn

Parameters	Sampling season			
	Summer		Autumn	
	Mean	SD	Mean	SD
Urea nitrogen (mmol/l)	6.7	1.8	6.2	1.5
Glucose (mol/l)	3.1	1.2	4.2	0.8
Calcium (mmol/l)	2.0	0.2	2.1	0.2
Inorganic phosphate (µmol/l)	1.5	0.3	1.3	0.4
Aspartate aminotransferase (U/l)	49.0	17.7	26.0	12.5
Creatinine(µmol/l)	88.0	20.3	133.0	21.1
Albumin (g/l)	47.0	5.7	40.0	4.3

Source: Grunwaldt *et al*. (2005)

Table 2.2: Blood chemistry measurements from Bonsmara and Angus steers fed on rye grass overseeded with coastal Bermuda grass

Parameter	Breed type		
	Angus	**Bonsmara**	**Significance**
Urea %/g	18.1	21.3	**
Cholesterol (mg/dl)	75.8	105.5	**
Glucose (mg/dl)	65.88	105.10	**
Albumin (g/dl)	3.56	3.5	***
Calcium (mg/dl)	9.64	9.86	***
Phosphorus (mg/dl)	5.89	6.69	***
Magnesium (meq/l)	1.86	1.72	***

** Significantly different (P < 0.05)

*** Not significantly different (P > 0.05)

Source: Hollenbeck *et al.*, 2006

2.4.2 Blood chemical constituents related to protein metabolism

Proteins perform unique functions in the body. At present there is no single metabolite that can be measured, which directly reflects protein status. As a result, a combination of parameters needs to be assessed, including blood urea nitrogen (BUN), creatinine, and total protein, albumin, and creatinine levels. Albumin and total protein have low variability in blood. As a result they both have a high diagnostic value in the assessment of nutritional status as compared to creatinine which has low diagnostic value due to its high variability in blood. Serum albumin is a very sensitive and early nutritional indicator of protein status (Agenas et *al*., 2006) because its turnover is only 16 days. Deficiency of protein impairs both humoral and cell mediated immunity, thus predisposing an animal to diseases (Titgemeyer and Loest, 2001).

Total protein levels are lower in young animals and higher in mature animals whilst albumin levels are lower at birth and then increase (Doornenbal *et al*., 1988; Otto *et al*., 2000). Malnutrition decreases albumin levels (Agenas *et al.,* 2006). Total protein and albumin reflect availability of protein and their concentrations decline in the face of protein deficiency.

Monitoring of blood urea levels can be used for measuring protein status in cattle from different feeding regimes and seasons (Hammond, 2006). Values for urea within the optimum range (<3.6mmol/l) in cattle indicate that the effective rumen degradable protein (ERDP) is adequate. High blood urea levels could indicate a high protein intake or the excessive mobilization of muscle (Chimonyo *et al.*, 2002). In ruminants a decrease in the blood urea concentration is related to low dietary intake of protein due to the recycling of urea from blood back to the rumen when dietary protein intake is low (Oulun, 2005). Grunwaldt *et al*. 2005 observed similar levels of urea nitrogen in summer and in autumn, as shown in Table 2.1. Breed differences in urea levels have been obtained as shown in Table 2.2, where Bonsmara steers had higher levels when compared with Angus steers.

The most common application of the use of blood urea nitrogen is as a retrospective diagnostic tool to analyze biological responses to protein or energy supplementation, changes in pasture or forage on offer, or change in pasture management (Hammond, 2006). Serum urea concentration may also increase despite low-protein feeding if energy intake is restricted, which is thought to reflect increased breakdown of endogenous proteins for energy production, a decrease in renal reabsorption of urea and/or haemoconcentration (Oulun, 2005).

Creatinine, a by-product of the breakdown of creatinine and phosphor-creatinine in muscle, is most commonly used as an indirect indicator of renal function and its impact on blood urea nitrogen. Serum creatinine concentrations vary due to an animal's diet, breed, muscle mass and sex (Otto *et al.*, 2000; Miller *et al*., 2004; Hammond, 2006). Grunwaldt *et al*. (2005) also showed lower creatinine levels during the summer than in autumn, as shown in Table 2.2. Reduced concentrations of creatinine indicate prolonged active tissue protein catabolism (Agenas *et al*., 2006). An increasing muscle mass from animal walking long distances in search of pasture can increase serum creatinine levels (Otto *et al*., 2000).

2.4.3 Packed cell volume

Packed cell volume (PCV) is the volume of erythrocytes expressed as a percentage of the volume of whole blood in a sample. It is the most accurate means of determining red blood cell volume and can be used to deduce total blood volume and haemoglobin levels. Several factors affect PCV levels. Higher haemoglobin and PCV values than the reference values could be attributed to the differences in ages of the animals (Grunwaldt *et al*., 2005). However Otto *et al*. (2000) reported no age effect on PCV values. No breed effects were observed between Angus and the Criollo Argentino on PCV values (Grunwaldt *et al*., 2005). Otto *et al*. (2000), however, observed a PCV value of 32% for Anguni cattle of Mozambique, which tended to be lower than Angus cattle thus showing breed differences. Reference levels for PCV and haemoglobin contents in indigenous cattle of Southern Africa raised under rural production conditions are largely unknown.

Packed cell volume could indicate anaemia, haemorrhage, bone marrow failure, destruction of erythrocytes, leukaemia, malnutrition or specific nutritional deficiency, multiple myeloma and rheumatoid arthritis (Jain, 1993; Farver, 1997). Packed cell volume values higher than the reference values could indicate dehydration due to diarrhoea, erythrosis and polycythermiavera (Thrall *et al*., 2004). Conditions that can result in a low haematocrit include vitamin or mineral deficiency, cirrhosis of the liver and malignances (Farver, 1997). Deficiency of iron decreases heme synthesis, causing hypochromic red blood cells (lacking the red hemoglobin pigment) and microcytic red blood cells (smaller than normal) (Kneipp *et al*., 2006).

2.4.4 Mineral metabolism

To promote normal tissue growth, homeostasis, enzyme function, cell regulation, and immune function, it is imperative that minerals be maintained within narrow concentrations within the body (Underwood and Suttle, 1999). Minerals play a vital role in forage digestion, reproductive performance, and the development of bones, muscle, and teeth. Sub-clinical trace mineral deficiencies occur more frequently than recognized by most livestock producers (Underwood and Suttle, 1999). Calcium, phosphorus and magnesium have a high diagnostic value in determining the nutritional status of animals due to their low variability in blood. Mahusoon *et al*. (2004) observed marked breed differences in mineral metabolism in goats. Mineral levels have been shown to vary with seasons (Yokus and Cakir, 2006) whereas Grunwaldt *et al*. (2005) showed no significant differences in autumn and summer for inorganic phosphate and calcium levels (Table 2.1).

Calcium is the most abundant mineral in the body; approximately 98 % functions as a structural component of bones and teeth. The remaining 2 % is distributed in extracellular fluids and soft tissues, and is involved in such vital functions as blood clotting, membrane permeability, muscle contraction, transmission of nerve impulses, cardiac regulation, secretion of certain hormones, and activation and stabilization of certain enzymes whereas phosphorus is involved in every metabolic reaction and energy transfer within the body (Invartsen and Andersen, 2000). Phosphorus is required for normal milk production, growth, and efficient use of feed and by the rumen microrganisms in the digestion of cellulose and synthesis of microbial protein. Mineral absorption increases in the gastrointestinal tract while mobilization is increased in the bones when an animal has phosphorus deficiency due to reduced levels in the feed consumed (Invartsen and Andersen, 2000). Season and breed, however, have been reported to have no effect on inorganic phosphate and calcium levels (Yokus and Cakir, 2006; Hollenbeck *et al*., 2006), as shown in Table 2.1 and Table 2.2 respectively.

Magnesium is an essential cation, involved in many enzymatic reactions, as a co-factor to adenosine triphosphatases. It is critical in energy-requiring metabolic processes, in protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, hormone secretion, and in intermediary metabolism (Laires *et al*., 2004). Serum magnesium concentration is maintained within a narrow range by the small intestine and kidney which both increase their fractional magnesium absorption under conditions of magnesium deprivation (Ghamdi *et al*., 1994). If magnesium depletion continues, the bone store helps to maintain serum magnesium concentration by exchanging part of its content with extracellular fluid (Laires *et al*., 2004). In dairy cows, magnesium levels are dependent on both physiological and seasonal variations (Yokus and Cakir, 2006). According to Hollenbeck *et al*., 2006, no breed differences in magnesium levels were found in Angus and Bonsmara steers. Serum magnesium levels reflect current daily intake rather than reserves, thus cattle are affected by low magnesium dietary content (Whitaker *et al*., 1999). Grass tetany occurs when the level of magnesium in blood falls below a critical threshold (below 1.2 mg per 100 ml) (Herdt *et al*., 2000).

2.4.5 Liver enzymes

Aspartate aminotransferase (AST) enzyme is present in many tissues; particularly liver, striated and cardiac muscle, making it a good marker of soft tissue damage (Otto *et al*., 2000). AST and alkaline Phosphatase (ALP) have low diagnostic value for nutritional status due to their high blood variability. These enzymes have a predominantly intracellular action and thus, under normal conditions, the serum enzyme activity is very low or absent; any increase in their activity would be evidence of damage in the tissues in which they are lodged (Grunwaldt *et al*., 2005). Red blood cells contain AST which can leak into plasma before there is any visual evidence of haemolysis (Abutarbush and Radostits, 2003). However, serum ALP has been reported to decrease with age, as a result of the faster growth rate in young animals, and leakage of the enzyme from the growing bones and intestines into the blood (Otto *et al*., 2000; Grunwaldt *et al.*, 2005). Many conditions that produce a significant rise in creatinine kinase (CK) activity will also produce elevated to high levels of AST (Abutarbush and Radostits, 2003). Vitamin E and selenium deficiency in the diet causes nutritional muscular dystrophy and diagnosis is usually based on elevated levels of muscle enzymes (CK and AST) (Abutarbush and Radostits, 2003). Vitamins C, E and selenium are important in the protection of cellular membranes from free radicals, which cause peroxidation of the membrane lipids (Abutarbush and Radostits, 2003; Karakilc *et al*., 2005). In healthy cows, the serum enzyme activity is low or absent. Neither seasonal nor physiological variations have been reported on AST levels (Yokus and Cakir, 2006). In contrast, there are higher AST levels during the rainy season (49U/L) than in the dry season (26U/L), as is shown in Table 2.1.

In practice, metabolite herd testing has a number of constraints that need to be overcome. The major challenges include high skilled labour required for blood sampling, availability of sampling ingredients, expertise in processing and storing blood and, perhaps, the most important, the high cost of analysing the samples (Grunwaldt *et al*., 2005). There is, for example, need to use friendly and appropriate techniques for restraining and bleeding that minimise distress. Appropriate infrastructure, such as strong cattle handling facilities, should be erected. Many rural communities in Southern Africa, lack such facilities in good operational order (Agenas *et al*., 2006). Farmers, thus, need to be educated on the need for determination and application of blood parameters as a tool to aid beef cattle management. This should destroy the generally held myth that animals are only handled when they are clinically sick or when they are ready for slaughter. Identifying animals for sampling should be done from different physiological states, sex, ages or production systems. These factors are crucial in the correct interpretation of haematological or serum chemistry status of the animals.

2.5 Pre-slaughter stress effects on cattle

Transportation unavoidably causes stress in animals. Some stress factors affecting welfare of transported animals include speed variations and vibrations of the truck, contact with strangers, high stocking density and animal commingling, establishment of new hierarchies, and weather conditions such as humidity and high temperatures (Gallo *et al.,* 2003). Although stress is not always an adverse experience and is a necessary and regular aspect of life, it causes changes in an animal's physiological status during transportation and for some period thereafter. Utilizing transported animals before their physiological status normalizes can have considerable and unintended effects on meat quality. Stress is defined as the response of the body to any threatening demand, and a resource-based trade-off between the immune system and costly behaviour that characterizes stress-induced immunosuppression (Mostl and Palme, 2002).

The hypothalamic-pituitary-adrenocortical (HPA) axis is a major component of the neuroendocrine response to stressful events (Mostl and Holme, 2002). Stress response results in production and secretion of glucocorticoids from the HPA axis and the catecholamines (adrenalin and noradrenalin) from the adrenal medulla. During short-term stress, glucocorticoids improve fitness by energy mobilisation and may change behaviour (Most and Palme, 2002). However, severe chronic stress (prolonged periods of high cortisol concentrations) may decrease individual fitness by inducing immunosuppression and atrophy of tissues.

Expression of receptors for the products of the nervous, endocrine, and immune systems and production of hormones in immune cells constitute the basis of immunoendocrine interactions. The function of lymphocytes is known to be modulated by the hormones of the pituitary gland (Most and Holme, 2002). Glucocorticoids are involved with glycogen deposition in liver and lipolysis, and are associated with the immune system, stress, and thermal regulation (Scope *et al*., 2002). Evidence exists that dopamine regulates cortisol secretion in cattle (Ahmadzadeh *et al*., 2006). Stress-induced enhancement of circulating concentrations of glucocorticoid hormones is a major cause of immune-suppression in animals subjected to stress (Stanger *et al*., 2005). A number of studies have noted that stress induced by transportation is detrimental to an animal's immune system by predisposing it to diseases and disturbances in the reproductive system (Grandin, 1997; Mostl and Holme, 2002). Hence, transportation of animals for long distances is a widely debated issue in animal welfare (Knowles and Warriss, 2000; Mostl and Holme, 2002).

Adrenocorticotropin release increased significantly from a mean level of 4.72pg/mL in lymphocytes harvested just before the start of the journey to a mean value of 8.24pg/mL in lymphocytes obtained from the same animals immediately after 14h of transportation (Scope *et al.*, 2002). In addition to the foregoing, a host of significant metabolic changes have also been observed in transported and handled cattle. These include an increase in blood enzymes such as creatine phosphokinase, lactate dehydrogenase, and aspartate aminotransferase (Scope *et al.,* 2002). Blood metabolite changes including increased concentrations of blood lactate and altered blood creatinine concentration have also been reported (Obernier and Baldwin, 2006).

Transport and handling stress have been observed to alter numerous blood cell components. In general, an increase in packed cell volume is commonly seen (Obernier and Baldwin, 2006) which likely reflects both a splenic response to stress and a degree of dehydration. Other authors have reported physiological cortisol concentrations in cattle to range from a baseline of 0.5 to 9.0 ng/ml (Grandin, 1997) to high levels of 120 ng/ml (Broom, 2003). Genetic differences have been reported at all levels of the HPA axis. These genetic differences indicate that heredity may play an important role in controlling the mechanisms that lead to different physiological responses to stress (Grandin, 1997).

Consequently, an animal's genotype or genetic make-up may affect its susceptibility and/or resistance to stressors.

The blood sampling procedure may itself elicit acute stress responses, thus interfering with the effect of the factors under study. Alternatives to plasma sampling include urine collection, which offers advantages for studying of HPA and sympathetic nervous system (SNS) activity: 1) urine is the main elimination route for catecholamines and glucocorticoids; 2) urine can be collected non-invasively when spontaneously voided; and 3) excretion products in urine accumulate over several hours. Thus, concentrations in urine can be considered as more integrative than those in plasma, and they may be more accurate for the detection of variations in stress hormonal activity (Hay *et al.*, 2000). Genetic factors such as temperament interact in complex ways with an animal's previous handling experiences and learning to determine how it will react during a particular handling procedure (Grandin, 1997). Data on blood metabolites, glucocorticoids and the excretion of catecholamines after exposure to transport and handling stress, are almost lacking so far in Nguni cattle and relatively few studies have reported concentrations in urine samples as compared to faecal material in other cattle breeds (Most and Palme, 2002). Absolute comparisons of cortisol levels between studies must be done with great caution as cortisol levels can vary greatly between individual animals (Grandin, 1994). It is therefore imperative to evaluate these parameters for different individual animals to assess breed variation.

2.6 Summary

To develop organized markets for promoting indigenous cattle products, there is need to develop parameters that objectively assess nutritional and health status of the animals while they are still growing. These are however, absent for Nguni cattle. Breed differences and genetic variation within breeds in rate and efficiency of growth, stress tolerant, disease resistance and tolerance can be assayed using blood metabolites so as to find genetically superior animals adapted to harsh environmental conditions. Stress levels are assessed in order to provide a basis for implementing helpful adjustments in current cattle management practices so as to alleviate the constraints on productivity.

Although body weight measurement and body condition scoring are easier to perform and are cheaper to determine, they have limitations that can be complemented by the use of blood metabolites and haematology. Metabolite profiling provides useful information such as the occurrence of negative energy balance, undernutrition and the presence of disease. Frequent monitoring of blood parameters, for example once in every season, assists in diagnosing metabolic problems and determining animals that are metabolically superior on veld or to identify animals that require supplementary feeding.

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Chapter 3

Effect of month on body condition scores, body weights and internal parasite prevalence in Nguni, Bonsmara and Angus steers raised on veld

Abstract

The objective of the study was to determine monthly changes in body condition scores, body weights and prevalence of internal parasites in Nguni, Bonsmara and Angus steers raised on veld from June 2005 until slaughter in March 2006. Body condition scores, body weights, blood samples and monthly faecal egg counts were carried out in 25 Nguni, 15 Bonsmara and 15 Angus steers. The Nguni managed to maintain its condition throughout the study period as compared to the other two breeds. Bonsmara had the highest body weight, whereas the Nguni had the highest PCV levels. PCV levels tended to decline during the rainy season for all the breeds. The predominant internal parasites were *Haemonchus* (39.3%), *Trichostrongylus* (37.8%), *Cooperia pectinita* (25.5%), *Fasciola gigantica* (16.3%) and *Ostertagia ostertagi* (11.2%). Faecal egg counts (FEC) were generally low. Of the three breeds, Nguni steers had the lowest parasites infestation, with the Angus being more susceptible than the other two breeds. The internal parasite prevalence was high during from October until March. Rearing of the Nguni breed will, therefore, greatly reduce production costs as it has been shown to habour less internal parasites as compared to Bonsmara and Angus steers.

Key words: body condition scoring, steers, internal parasites, sweetveld

3.1 Introduction

Parasite infections are one of the greatest causes of disease and loss of productivity in livestock (Ploeger *et al.*, 1990; Mahusoon *et al.*, 2004). Grazing ruminants are always exposed to internal parasite in which the infections can be clinical or sub-clinical. The greatest economic losses associated with parasite infections are sub-clinical (Dimander *et al*., 2000; Belem *et al*., 2001). The typically sub-clinical nature of parasitoses necessitates the aid of quantitative diagnostic tools to monitor worm burdens or the level of immunity. The effects of helminths are increased when there are nutritional deficiencies (Chimonyo *et al*., 2002; Mahusoon *et al*., 2004). Control and prophylaxis of parasite infections can be improved by comprehensive epidemiological studies within the particular management area and season; and their interaction with the host in a specific climate (Barger, 1999; Vercruysse and Claerebout, 2001). Selecting cattle with enhanced resistance to parasites, as an alternative control strategy, has a huge potential to reduce dependence on anthelmintics and the consequent reduced use of anthelmintics should have a positive impact by reducing selection for drug resistance (Baker, 1995; 1998; Krecek and Waller, 2006).

There is now evidence for genetic variation in resistance to gastrointestinal nematode parasites both between and within cattle breeds (Coles, 2005). In addition, selection for parasite resistance has been effective in *H. contortus* resistance in cattle (Krecek and Waller, 2006). Mature, healthy cattle develop resistance to internal parasites. However, cattle in sub-optimal body condition may have diminished capacity to resist parasite infection (Krecek and Waller, 2006). Gastrointestinal infections are characterized by a reduction of voluntary food intake and increased feed conversion efficiency in infected animals (Dimander *et al*., 2000; Vercruysse and Claerebout, 2001). Adapted breeds are able to thrive in their local environments and to tolerate, or even resist, infections. Ability of the animal to tolerate parasites can be evaluated using changes in body condition score, body weights and PCV (Mulcahy *et al*., 2004). The PCV values, for example, have been used when evaluating blood sucking parasites (Urquhart *et al*., 1996; Crompton and Nesheim, 2002).

Most reports on parasite tolerance in beef cattle are under controlled conditions with a specific level of infection in cattle breeds, which do not reflect the true effect of these internal parasites on the animal under natural conditions. No information is available on the adaptation of indigenous Nguni cattle to internal parasites raised on veld. Relationships among worm egg counts, body condition, body weight and PCV in the indigenous Nguni, Angus and Bonsmara steers under veld conditions are also not available. The main objective of this study was, therefore, to determine the effect of month on body condition, body weight and internal parasites prevalence in Nguni, Bonsmara and Angus steers. The hypothesis tested was that month and breed of steer did not affect body condition scores, body weights and the prevalence of internal parasites.

3.2 Materials and Methods

3.2.1 Description of the study site

The study was conducted at the University of Fort Hare Research Farm, in the false thornveld vegetation of the Eastern Cape. The site is 520m above sea level and is 32.8^{o}

latitude and 26.9^o longitude. The vegetation is composed of several tree, shrub and grass species. *Acacia karroo, Themeda triandra, Panicum maximum, Digitaria erientha, Eragrostis* species, *Cynodon dactilon* and *Penisetum clandestinum* are dominant. The topography of the area is generally flat with a few steep slopes. The climate is semi-arid with the annual rainfall about 480mm, most of which occurs in summer, and the annual average temperature is 18.7°C.

3.2.2 Animals and their management

Twenty-five Nguni, 15 Angus and 15 Bonsmara eight-month old steers were maintained in one herd from June 2005 until slaughter in March 2006. The animals were raised under natural pasture in paddocks. Veld condition in July was poor. The pasture quantity then improved and lush pasture was available from the end of October to the beginning of January, which then started to decline until March when the animals were slaughtered. No supplementary feeding was provided. The average initial body weights of Angus, Bonsmara and Nguni steers were 183 ± 11.7, 203 ± 6.8 and 190 ± 5.2 kg, respectively. The average initial body condition score was 5.0, and was similar among the three breeds.

3.2.3 Experimental procedures

Body weights, body condition scores (scale 1-9) (Nicholson and Butterworth, 1986; Wagner *et al*., 1988) and faecal samples were collected once a month in the morning from July to March 2007. The faecal egg output was estimated using a modified McMaster technique (Whitlock, 1948) and multiplied by 50 to convert to egg/gram. About 10 ml of blood was also collected by jugular veni-puncture from beef steers every month into vacutainer tubes for determination of PCV levels.

3.2.4 Statistical analysis

Body condition scores were square root transformed, whereas the faecal egg counts were transformed using $\log_{10}(x + 1)$. All data were analyzed as repeated measures using the MIXED procedures of SAS (SAS, 2003). The effects of month, breed and the interaction between month and breed were incorporated into the linear model for repeated measures. The occurrence of nematodes was estimated using the PROC FREQ procedure (SAS, 2003) and the correlation between parasite levels and season was also analysed. The association between breed and occurrence of nematodes was assessed using the chi-square test.

3.3 Results

3.3.1 Body condition score and weight changes

Figure 3.1 shows the effects of month on body condition scores in the Angus, Bonsmara and Nguni steers. There was month x breed interaction ($P<0.05$) on BCS levels. The BCS for Nguni steers were higher ($P<0.05$) than the other two breeds across the 9-month period. The peak BCS for Nguni was in March. The BCS for Angus declined from September until January. The BCS for Bonsmara increased similarly with the ones for the Nguni, but in February, the Bonsmara began to lose their body condition.

The effect of month on body weights on the three breeds is shown in Figure 3.2. There was no month x breed interaction ($P>0.05$) in body weights. Bonsmara had the highest body weights followed by Nguni and Angus had the least body weights. The body weights for the three breeds decreased in September and then increased until March. In March, the body weight for Bonsmara was high despite the drop in its body condition score. Angus had the least body weight and body condition scores.

3.3.2 Packed cell volume

Changes in the PCV levels for the breeds over the 9-month period are shown in Figure 3.3. The PCV levels declined beginning October and were highest in September. Angus steers had the lowest PCV levels (P<0.05) throughout the study period.

3.3.3 Occurrence of helminths

The frequencies of helminths, expressed as percentages, are shown in Table 3.1. The common helminths that were found across the three breeds were *Haemonchus contortus, Trichostrongylus spp*, *Cooperia pectinita* and *Fasciola gigantica*. The frequencies of other pathogenic internal parasites, for example, *Toxocara vitulorum* and *Marshallagia marshalli* were low.

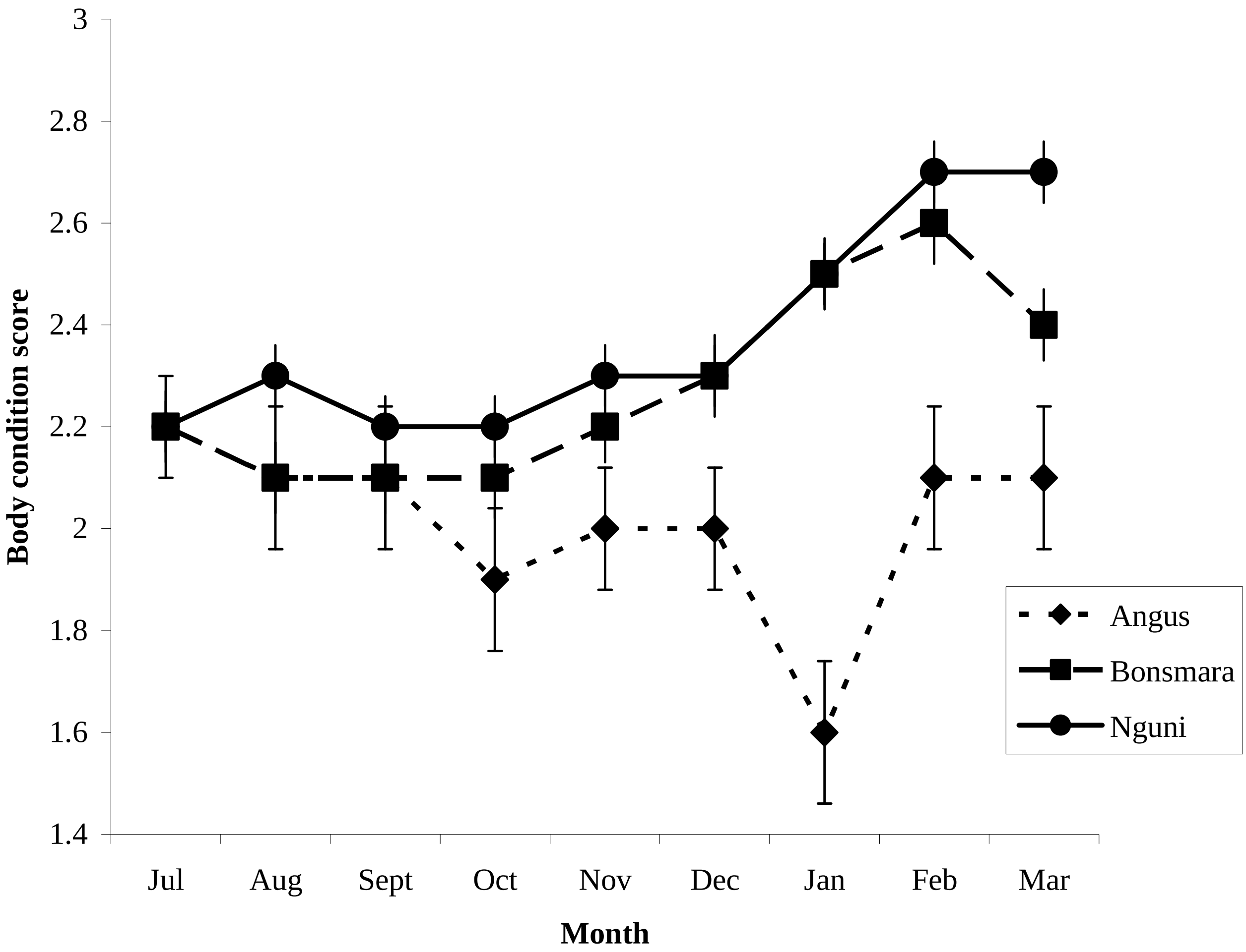

Figure 3.1: Influence of month on body condition scores in the Angus, Bonsmara and Nguni steers

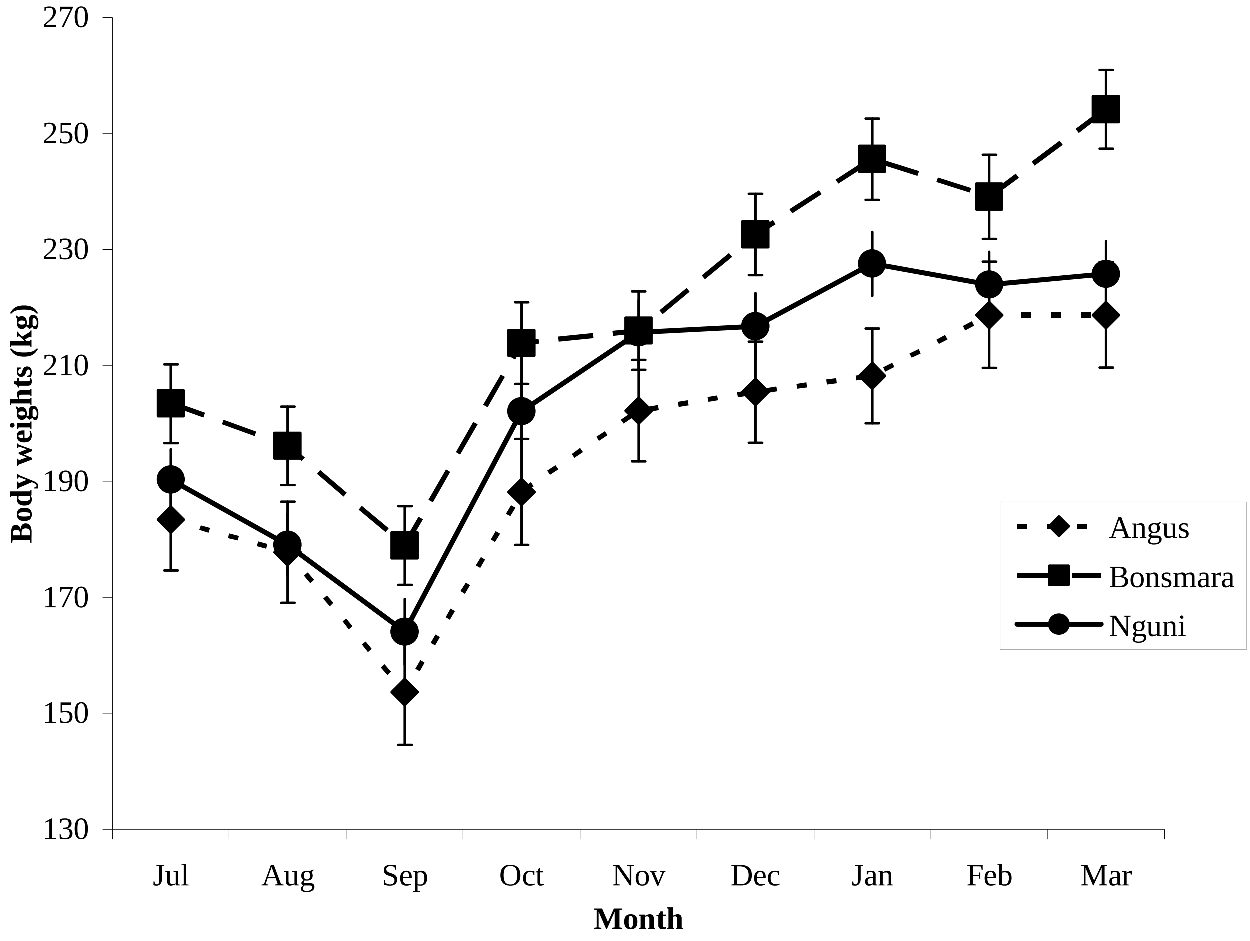

Figure 3.2: Monthly body weight changes in Angus, Bonsmara and Nguni steers

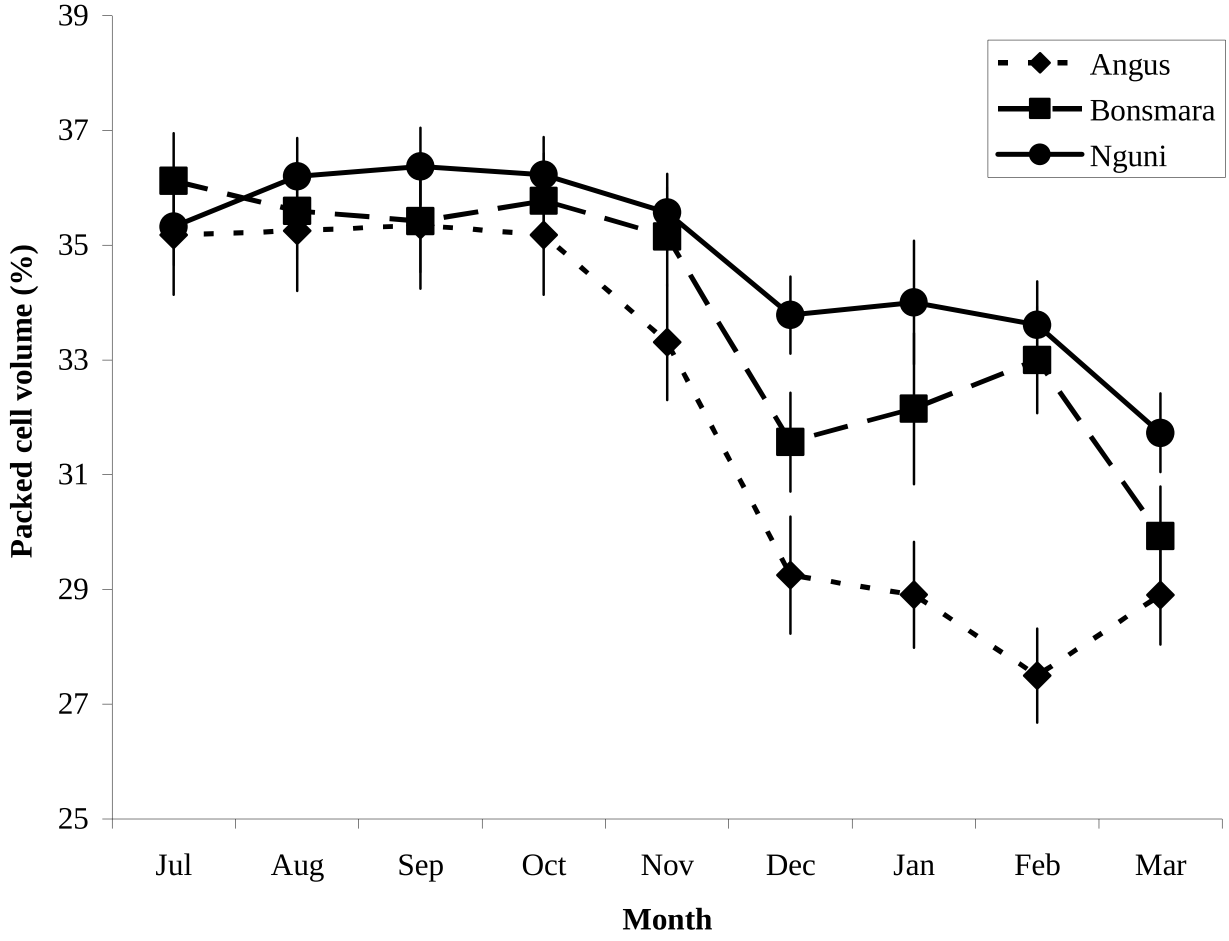

Figure 3.3: Packed cell volume changes in Angus, Bonsmara and Nguni steers

Table 3.1: Frequencies of nematodes in Nguni, Bonsmara and Angus steers

Nematodes	Frequency (%)	Number
Haemonchus contortus	39.32	138
Trychostrongylus spp	37.82	132
Cooperia pectinita	25.5	89
Fasciola spp	16.33	57
Ostertagia circumcincta	11.17	39
Bunostomum spp	9.14	32
Nematodius spathiger	8.02	28
Oesophagostomum radiatum	8.02	28
Schistosoma spp	6.88	24
Mecistocirrus digitatus	3.44	12
Buxtonella sulcata	2.58	9
Paramphistomum	1.72	6
Monienza expansa	1.43	5
Marshallagia marshalli	0.86	3
Toxocara vitulorum	0.86	3

Breed of steer had an effect on the parasite load ($P<0.05$). As shown in Table 3.2, there was no association between breed and occurrence of *Haemonchus contortus*, whereas a significant association was found between breed and occurrence of *Trichostrongylus*, *Cooperia pectinita* and *Fasciola* spp. Month variations of the most prevalent parasites are shown in Figure 3.4. There was a general increase in *Cooperia* spp, *Haemonchus contortus*, *Fasciola* and *Trichostrongylus* species from October, reaching a peak in December or January. The month patterns of infection with *Cooperia pectinita*, *Fasciola spp.*, *Ostertagia circumcincta*, *Bunostomum spp.*, *Nematodius spathiger*, *Oesophagostomum radiatum*, *Schistosoma spp.*, *Mecistocirrus digitatus*, *Buxtonella sulcata*, *Paramphistomum*, *Monienza expansa*, *Marshallagia marshalli*, *Haemonchus*, *Oesophagostomum spp*., *O. ostertagi*, *Toxocara vitulorum* and *Trichostrongylus* were all similar and were negatively correlated to dry season (July to September) and wet season (October to March).

Table 3.2: Occurrence of nematodes in Angus, Bonsmara and Nguni steers

		Breeds			
Type of nematodes	Angus	Bonsmara	Nguni		
Haemonchus contortus				$\chi 2$	P value
% positive samples[2]	41.03 (16)[1]	44.35 (55)	35.64 (67)	0.297	0.296
Trichostrongylus spp.					
% positive samples	43.59 (17)	45.16 (56)	31.72 (59)	0.042	0.042
Cooperia pectinita					
% positive samples	20.51 (8)	35.48 (44)	19.89 (37)	0.007	0.006
Fasciola spp					
% positive samples	58.97 (23)	14.52 (18)	8.6 (16)	0.0001	0.0001
Ostertagia circumcincta					
% positive samples	20.51 (8)	14.52 (18)	6.99 (13)	0.020	0.017

[1]Values in parentheses indicate the number of samples that were observed.

[2]Positive %samples indicate the percentage of faecal samples with the type of nematode species indicated.

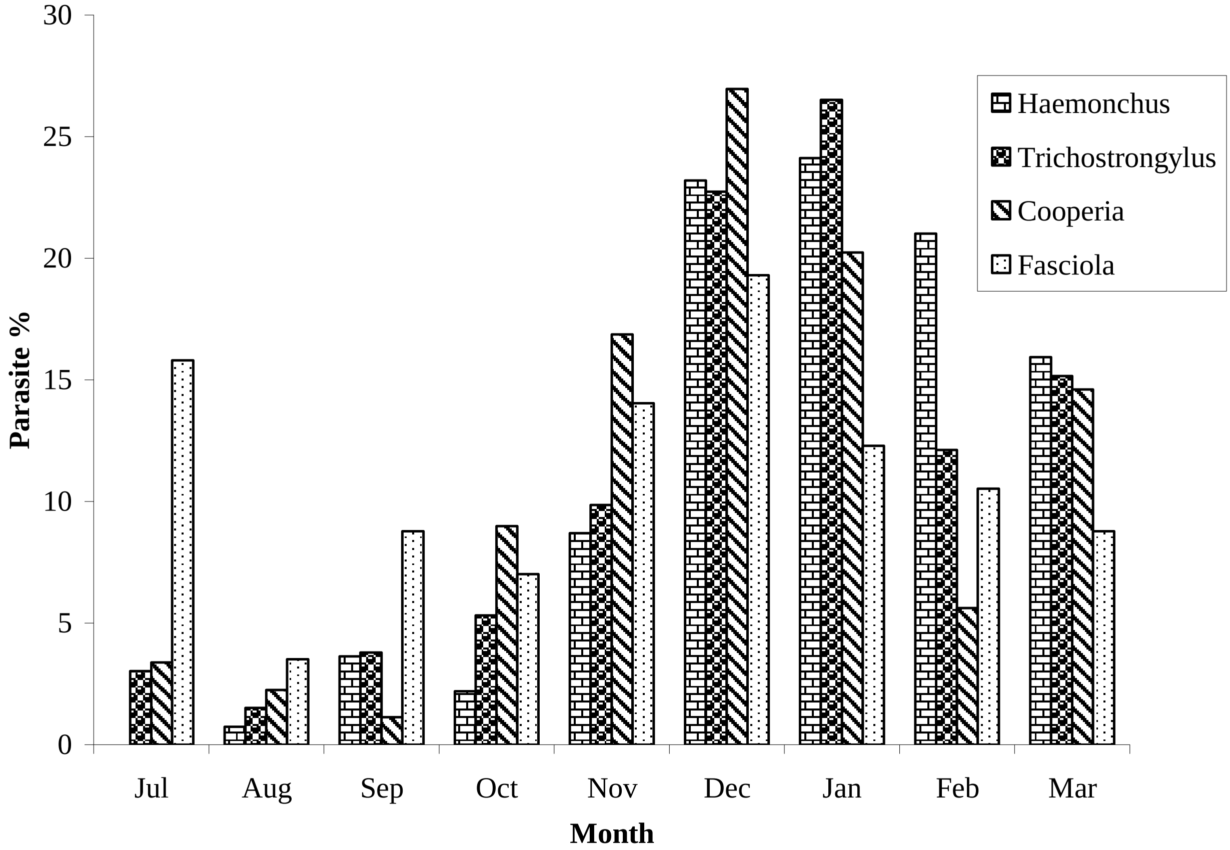

Figure 3.4: Influence of month on *Haemonchus*, *Trichostrongylus*, *Cooperia spp.* and *Fasciola spp.*

3.4 Discussion

Nguni steers had the highest body condition scores as compared to Bonsmara and Angus steers indicating high energy reserves in Nguni, most likely as an adaptation to the local production conditions. The adaptation of animals to different levels of nutrition is a powerful tool that livestock breeders and producers have been exploiting with evident advantages (Chilliard *et al*., 2000; Blanc *et al*., 2004). Rural farmers prefer animals that store fat reserves in periods when grass availability is high and mobilize those reserves to cope with production needs during the dry season, since they do not afford dietary protein supplements. Body condition scoring is one of the practical indicators of the nutritional status of cattle (Ortigues, 1991). Though body weights are traditionally used to assess nutritional status of animals (Chimonyo *et al*., 2002), they vary with breed size, gut fill and tissue hydration (NRC, 1996). Bonsmara had the highest body weights due to its big breed size. Bonsmara began to lose condition in March despite its high body weights possibly due to decreased quality of feed in post-rainy season and hydrolysis of body reserves such as lipids to maintain its body energy needs. Muchenje *et al.* (2007) obtained higher losses in daily weight in Angus (872g/day) and Bonsmara (991g/day) whereas for Nguni the weight loss was 566g/day on steers raised on natural veld during the dry season. Angus steers had the least body weights, probably due to poor adaptation to local environmental conditions. Angus is a large sized breed and was, therefore, expected to have higher body weights as compared to medium and small sized breeds. Although body size is genetically determined, it can be altered by nutritional and hormonal factors (Corbet *et al*., 2005; Pryce *et al*., 2006).

The observed low parasite prevalence obtained could be due to the low rainfall received in the Eastern Cape Province in South Africa (Belem *et al.,* 2001; Bowman and Georgi, 2002). Results of faecal egg counts (FEC) have indicated that gastro-intestinal nematode infection in the province has a seasonal pattern. The highest FEC during the rainy/late rainy season and lowest during the dry season concur with studies in other tropical countries with a distinct rainy and dry season (Moyo *et al*., 1996; Waruiru *et al.,* 2001; Keyyu *et al.,* 2005). The conditions that favour grass growth also favour parasite larvae growth. This could explain the increase in parasite frequencies from October, the beginning of the wet season. Moisture is essential for larval survival and transport. The low helminth prevalence could also be due to the age of animals (Loyacano *et al*., 2002). Sheep and cattle develop high worm burdens during the first months of the animals' life (Halvorsen *et al*., 1999). Thereafter, acquired immunity is considered to reduce the rate of nematode establishment.

Of the three breeds, Angus steers were more susceptible to internal parasites as shown by high infestation levels and and low PCV levels at the beginning of the rainy season. This calls for strategic deworming of Angus steers if raised on natural pasture to prevent the reduction in production performance. In cattle, the reduction of weight gain and other adverse effects depend on the parasite burden (Baker *et al*., 1991, Stear *et al*., 1995, Loyacano *et al.,* 2002). Larvae and adults of other species cause abomasitis and enteritis which further interferes with nutrient absorption (Keyyu *et al.,* 2005)**.** Fascioliasis has been correlated with depressed appetite and weight gain, increased mortality, and liver condemnation resulting in economic losses in several countries (Thiodoropaulos *et al*., 2002). This could explain the reduced body condition and weights for Angus steers.

Nguni steers had lower levels of *Cooperia pectinita, Fasciola* and *Trichostrongylus spp.* as compared to other breeds showing its tolerance to internal parasites. Exposure to parasites leads to the development of acquired resistance against infection which, although not clearly established, enables the satisfactory co-existence of host and parasite. Immune competence develops with age and with experience of infection (Barger, 1988). The immune response is generally strong for *Nematodirus spp.* and *Trichostrongylus spp.* but more labile in the case of *Ostertagia spp.* and *H. contortus* (Anderson *et al*., 1978). This could explain the observed lack of breed differences on *Haemonchus* infestation level. The effect of immunity is to prevent the continuing accumulation of worm burdens as a simple consequence of ingesting larvae. The genetically influenced acquired resistance is expressed through prevention of establishment of ingested larvae (immune exclusion), through depression of the size and fecundity of adult worms that do establish and, possibly, through increased rates of expulsion of L4 and adult worms (Barger, 1998) which is the most probable mechanism in Nguni cattle.

Several reports have concluded that adequately nourished animals are better able to withstand the detrimental effects of nematode parasite infection than those less adequately nourished (van Houtert and Sykes, 1996; Knox, 2000). The findings in this study could suggest that the Nguni steers efficiently utilized the available feed resources as compared to Bonsmara and Angus cattle.

Higher prevalence for *Haemonchus placei, Cooperia spp., Trichostrongylus, Cooperia pectinita***,** *Fasciola* and *Oesophagostomum radiatum* were observed when compared tp other internal parasites such as *Mecistocirrus digitatus*, *Buxtonella sulcata*, *Paramphistomum*. *Haemonchus* feeds on blood directly and if large numbers are present, blood loss can be acute leading to death (Bowman and Georgi, 2002). *Haemonchus* peak burdens were observed in December and January as also observed by Horak (1978). Horak (1978) reported marked inhibition in larval development from April-July. *Cooperia spp*. peak burdens occurred during December which is similar to what was observed in this study. Horak *et al*. (2004) observed infection with *Ostertagia ostertagi* as the most intense and prevalent in the Eastern Cape, followed by *Cooperia oncophora* in bull calves.

In December, low PCV levels were observed in Angus, which coincided with high levels of increased *Haemonchus* levels. *Haemonchus* feeds on blood directly and if large numbers are present, such as from 100 to 1000, seepage of blood into the abomasum can occur (Begum *et al*., 2004). The rise in PCV percentage in January for all the breeds is difficult to explain but could possibly be due to dehydration or increased parasite infestation (Keyyu *et al*., 2005). However, there was a decrease in PCV levels at the beginning of the rainy season which coincided with an increase in helminthes levels. Nematode parasites can affect an animals' ability to maintain erythropoesis (Molina *et al*., 2006). Nguni had higher PCV levels than Angus and Bonsmara, suggesting its resilience to internal parasites (Mulcahy *et al*., 2004).

3.5 Conclusions

Nguni steers managed to maintain their body condition scores throughout the study period and whereas Bonsmara began to loose condition in March. Angus steers had the least body condition scores indicating less energy reserves and its poor performance under local environmental conditions. Angus steers had the least body weights whereas Bonsmara had the highest body weights. Low prevalences of helminthes were generally observed. *Haemonchus contortus, Cooperia pectinita, Trichostrongylus, Fasciola* and *Oesophagostomum radiatum* predominate in the Eastern Cape of South Africa. Nguni cattle had lower levels of internal parasites and higher PCV levels as compared to Bonsmara and Angus. Farmers can greatly save money through the rearing of the Nguni cattle. Besides using BCS and body weights, there is also a need to evaluate the use of nutritionally related blood metabolites in beef animals, as more accurate and objective indicator of their nutritional status.

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Chapter 4

A comparison of nutritionally-related blood metabolites among Nguni, Bonsmara and Angus steers raised on veld

Abstract

The objective of the study was to determine monthly variations in serum glucose, cholesterol, total protein (TP), urea, albumin, globulin, albumin-globulin ratio, creatinine, aspartate aminotransferase (AST), creatinine kinase (CK), alkaline phosphatase (ALP), calcium, phosphorus and magnesium in Nguni, Bonsmara and Angus beef steers raised on veld. Twenty-five Nguni, 15 Aberdeen Angus and 15 Bonsmara 8-month old steers were studied for nine months. Over the nine months, Nguni had higher concentrations of glucose ($P<0.05$) and cholesterol ($P<0.05$) than the other two breeds. The overall glucose and cholesterol concentrations in the Nguni were 4 and 2.86 mmol/L, respectively. There was a breed × month interaction on glucose, cholesterol, creatinine, calcium, albumin and phosphorus concentrations. Breed had no effect on TP, urea, globulin, and AST concentrations. In conclusion, Nguni and Bonsmara cattle performed well under veld.

Key words: Nguni cattle, exotic breeds, veld, serum chemistry

4.1 Introduction

Natural selection in stressful climates can create populations of animals that have specific adaptations to certain conditions. Because of their long history in southern Africa, Nguni cattle are reported to be less susceptible to droughts, parasites, insects and diseases when compared to the European breeds such as the Aberdeen Angus and Hereford (Durkin *et al*., 1982; Otto *et al*., 2000; Bester *et al.,* 2003). Efforts have been made to produce a fast-growing beef breed that is adapted to the tropical hot conditions. As a result, the Bonsmara, a synthetic breed, has been developed for use under commercial conditions in South Africa (Strydom *et al*., 2001). Information on the adaptive value of indigenous livestock, such as the Nguni and their competitiveness against European breeds, is limited. In sweetveld areas of southern Africa, rainfall is low and uncertain, making plant growth erratic. In these conditions, the grasses tend to maintain high nutritional quality throughout the year, relative to the grasses in mixed- and sourveld areas (Dean and McDonald, 1994).

Blood metabolite concentrations are commonly used in monitoring herd health and nutritional status. Various studies have demonstrated temperature-season and breed differences in blood parameters (Doornenbal *et al*., 1988; Thrall *et al*., 2004; Grunwaldt *et al*., 2005). Monitoring metabolite concentrations across seasons assists in diagnosing metabolic problems (Verheyen *et al.*, 2007) and determining breeds that are metabolically superior under veld conditions (Ruane, 1999; Grunwaldt *et al.,* 2005) and to design management interventions to improve beef cattle production (Brown and Adjei, 2001; Agenas *et al.*, 2006). Adapted breeds are expected to be raised with minimal external inputs, such as the need for dietary supplementation and veterinary medicines.

To date, little effort has been put into determining the variation in nutritionally-related blood constituents of indigenous cattle of South Africa. The objective of the current study was to compare the concentration of nutritionally-related blood metabolites of Nguni to established beef breeds in the Eastern Cape Province. The null hypothesis tested was that the concentration of the metabolites in Nguni is similar to the established beef breeds under the local environmental conditions.

4.2 Materials and Methods

4.2.1 Description of study site

The description of the study site was given in Section 3.2.1.

4.2.2 Experimental animals and their management

Twenty-five Nguni, 15 Angus and 15 Bonsmara eight-month old steers were maintained in one herd from June 2006 until when they were slaughtered in March 2007. All the steers were clinically healthy throughout the trial. The animals were raised under natural pasture in paddocks. All the animals were not housed. The quantities of grass were low in July. The pasture quantity then improved and lush pasture was available from the end of October to the beginning of January, which then declined in quantities until March when the animals were slaughtered. No supplementary feeding was provided. The average initial body weights of Angus, Bonsmara and Nguni steers were 183 ± 11.7, 203 ± 6.8

and 190 ± 5.2 kg, respectively. The average initial body condition score was 5 using a 1-9 scale, and was similar among the three breeds.

4.2.3 Blood sample collection and analysis

Blood was taken from the jugular vein into plain tubes. The samples were collected once every month. They were centrifuged within two hours of collection at 2500rpm for 10 minutes to obtain serum. The serum was stored at -20^{0}C pending analysis. Serum samples were analyzed using a Chexcks machine (Next/Vetex Alfa Wasseman Analyser, Woerden, Netherlands) and commercially purchased kits (Siemens, South Africa). The serum was analyzed spectrophotometrically for total proteins (TP) (Wechselbaum, 1946), albumin (Doumas, 1972), creatinine (Tietz, 1995), alkaline phosphatase (ALP) (Tietz *et al*., 1983), calcium (Cali *et al*., 1972), inorganic phosphorus (Young, 1990) and magnesium (Tietz, 1976) using colorimetric methods. For glucose (Gotchman and Schmitc, 1972) and urea (Tietz, 1995) analysis, enzymatic methods were used while ultraviolet methods were used for aspartate aminotransferase (AST) (Bergmeyer *et al.*, 1986) and creatinine kinase (CK) determinations (Horder *et al*., 1991). Globulin levels were calculated as the difference between TP and albumin.

4.2.4 Statistical analysis

All data were analyzed using the PROC MIXED procedures of SAS (2003). The effects of month, breed and their interaction on body condition scores and levels of blood metabolites were incorporated into the linear model for repeated measures. The initial body weight was incorporated as a covariate.

4.3 Results

4.3.1 Body condition scores

The changes in body condition scores are described in Section 3.3.1.

4.3.2 Effect of breed and month on glucose and cholesterol levels

Table 4.1 summarises the levels of significance of month, breed and the month × breed interaction on blood parameters. Figure 4.1 shows changes in serum glucose and cholesterol concentrations during the nine month period. Both the breed ($P<0.05$) and month ($P<0.05$) affected glucose concentrations but no month × breed interactions ($P>0.05$) were observed. Peak glucose concentrations for all the breeds were observed in October and started to decline until January/ February. The trend for the glucose concentrations was similar among the three breeds. Overall, Bonsmara and Nguni steers had similar glucose concentrations, but were greater than for the Angus steers (Table 2). Cholesterol concentrations were affected by both breed and month ($P<0.05$). No month × breed interaction was observed for cholesterol ($P>0.05$). The peak for all the three breeds was observed in March. As also shown in Table 4.2, Nguni had the highest concentrations of cholesterol throughout the study period followed by Bonsmara, across the nine months. Angus steers had the least cholesterol concentrations.

Table 4.1: Levels of significance for breed, month and breed × month interaction on metabolite concentrations and enzyme activities

	Breed	Month	Breed x Month
Glucose (mmol/L)	**	***	NS
Cholesterol (mmol/L)	***	*	NS
Total protein (g/L)	NS	***	**
Urea (mmol/L)	NS	***	**
Creatinine (μmol/L)	***	***	**
Globulin (g/L)	NS	***	**
Albumin ((g/L)	**	***	*
Albumin/Globulin ratio	NS	***	*
Magnesium (mmol/L)	***	***	*
Calcium (mmol/L)	NS	**	***
Phosphorus (mmol/L)	***	***	NS
CK (U/L)	NS	NS	NS
AST (U/L)	NS	*	NS
ALP (U/L)	***	***	NS

*P<0.05; **P<0.01; ***P<0.001; NS-no significant difference (P>0.05).

CK- creatinine kinase, AST-aspartate aminotransferase, ALP-alkaline phosphatise

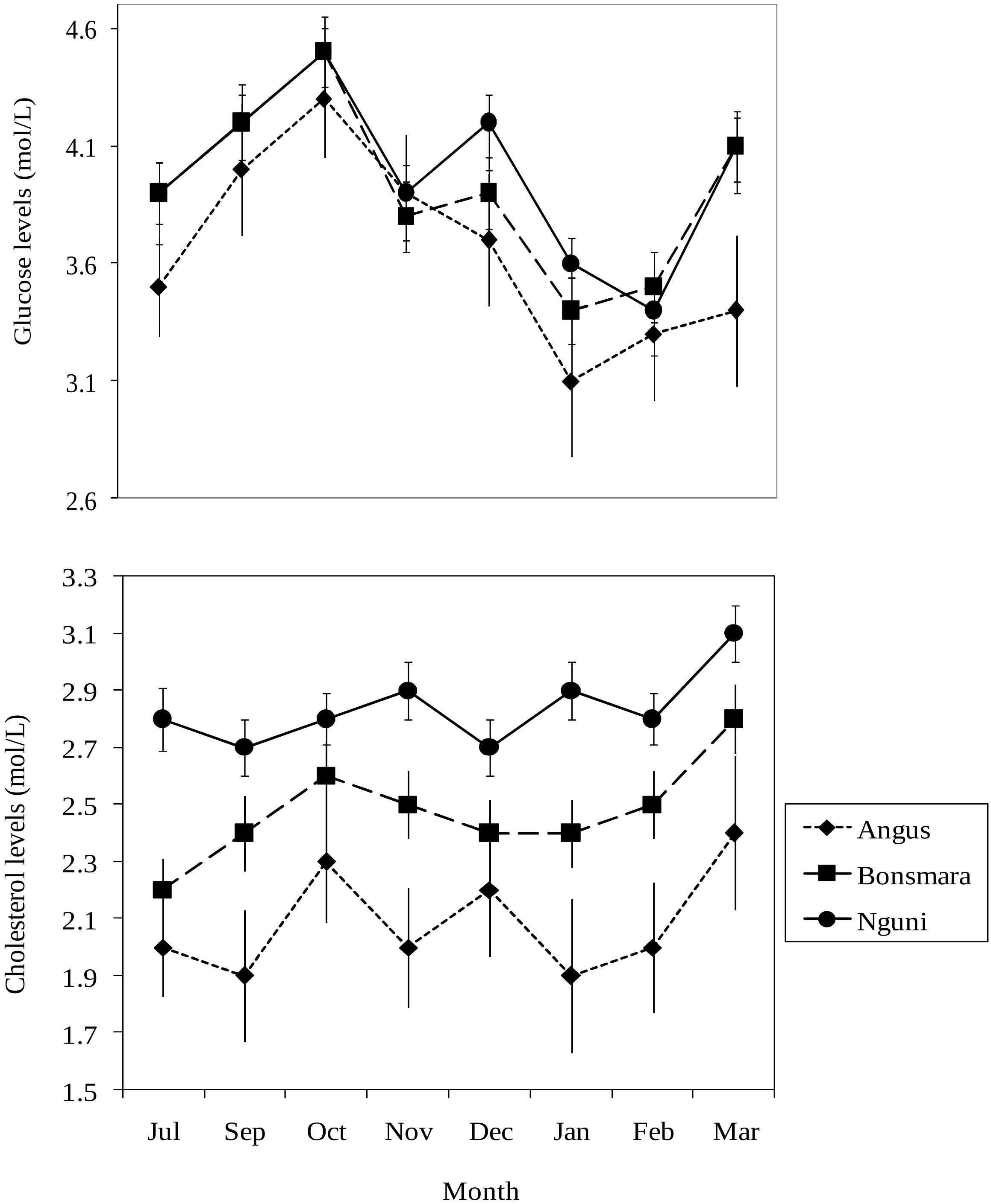

Figure 4.1: Influence of month and breed on glucose and cholesterol levels.

Table 4.2: Influence of breed on nutritionally related blood metabolites

Parameter	RR	Angus	Bonsmara	Nguni	Significance
Urea (mmol/l)	3.6-10.7	3.4 ± 0.209	3.17 ± 0.110	3.27 ± 0.099	NS
Creatinine (µmol/l)	10-133	112.11 ± 2.361[b]	113.48 ± 1.286[c]	107.56 ± 1.041[a]	**
Globulin (g/l)	28-42	43.17 ± 0.996	43.22 ± 0.543	44.56 ± 0.431	NS
Albumin ((g/l)	28-37	31.92 ± 0.443	32.24 ± 0.241	32.62 ± 0.235	NS
A/G Ratio	0.9-1.4	0.77 ± 0.020	0.76 ± 0.012	0.74 ± 0.010	NS
Magnesium (mmol/l)	0.6-1.2	0.70 ± 0.015[a]	0.73 ± 0.008[b]	0.80 ± 0.008[c]	***
Calcium (mmol/l)	2-2.9	2.36 ± 0.037	2.44 ± 0.020	2.43 ± 0.016	NS
Phosphorus (mmol/l)	1.2-2.3	1.77 ± 0.060 [a]	1.94 ± 0.033[b]	2.05 ± 0.026[b]	***
CK (U/l)	12-146	159.60 ± 50.017	158.89 ± 27.241	204.27 ± 25.388	NS
AST (U/l)	21-167	81.88 ± 4.227	79.68 ± 2.302	74.237 ± 2.196	NS
ALP (U/l)	33-328	73.5 ± 9.155[a]	99.09 ± 4.986[b]	144.18 ± 4.577[c]	***

[a,c] Values with different superscripts within each row are significantly different ($P < 0.05$)

* $P<0.05$, ** $P<0.01$, *** $P<0.001$. NS: $P>0.05$.

CK- creatinine kinase, AST-aspartate aminotransferase, A/G ratio-albumin/globulin ratio

ALP-alkaline phosphatase

RR: Reference range (Farver, 1997)

4.3.2 Effect of breed and month on total protein, urea, creatinine levels, albumin, globulin and albumin/globulin ratio

There was breed × month interaction ($P<0.05$) on TP. Although month affected ($P<0.05$) TP concentrations, the breed of steer did not ($P>0.05$). The peak concentrations for Bonsmara and Nguni were observed in December whilst for Angus the peak was observed in January (Figure 4.2). Serum urea concentrations are also shown in Figure 4.2 for the 9-month period. There was a month × breed interaction ($P<0.05$) on serum concentrations. Month also had an effect ($P<0.05$) on urea concentrations, while breed had not (P=0.181). Urea concentrations fluctuated markedly with each month in all the breeds. Month, breed and the interaction between the two ($P<0.05$) affected creatinine concentrations. Peak concentrations for creatinine were observed in July for all the breeds (Figure 4.2).

As shown in Figure 4.3, month × breed interaction ($P<0.05$) was observed for albumin concentrations. The albumin concentrations also varied with breed ($P<0.05$) and month ($P<0.05$). Month affected ($P<0.05$) the globulin concentrations while breed did not ($P>0.05$). There was, however, breed × month interaction ($P<0.05$). The peak albumin concentrations for Bonsmara and Nguni breeds were observed in December, whereas the peak for Angus was in January. The globulin concentrations increased from July to December. There was however breed x month interaction ($P<0.05$). Month affected ($P<0.05$) the albumin: globulin ratio, while breed did not ($P>0.05$). The albumin: globulin ratio decreased from July until November and then became constant for Nguni and Bonsmara. For the Angus, the concentrations continued to decrease.

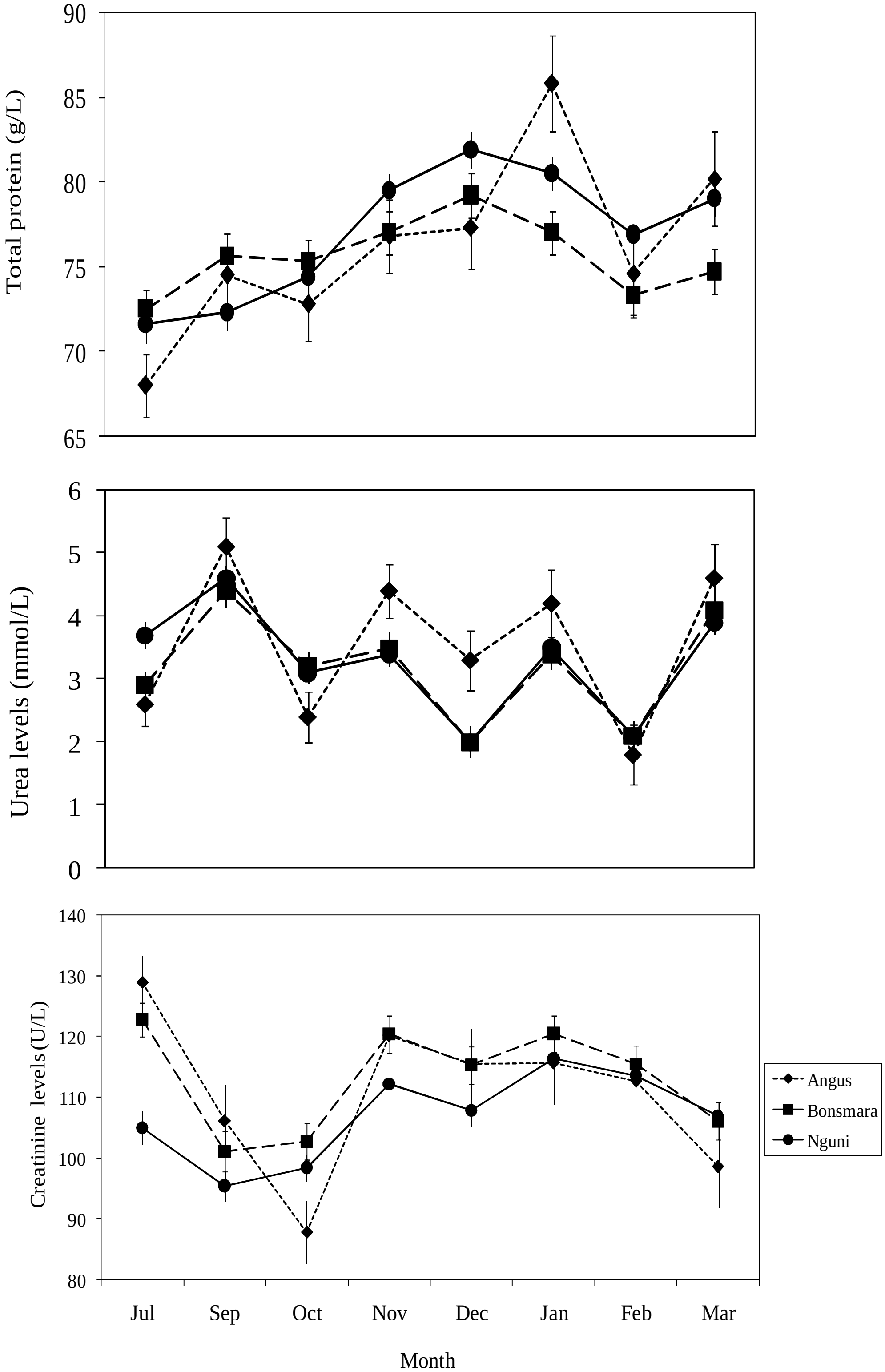

Figure 4.2: Effect of month and breed on TP, urea and creatinine concentrations.

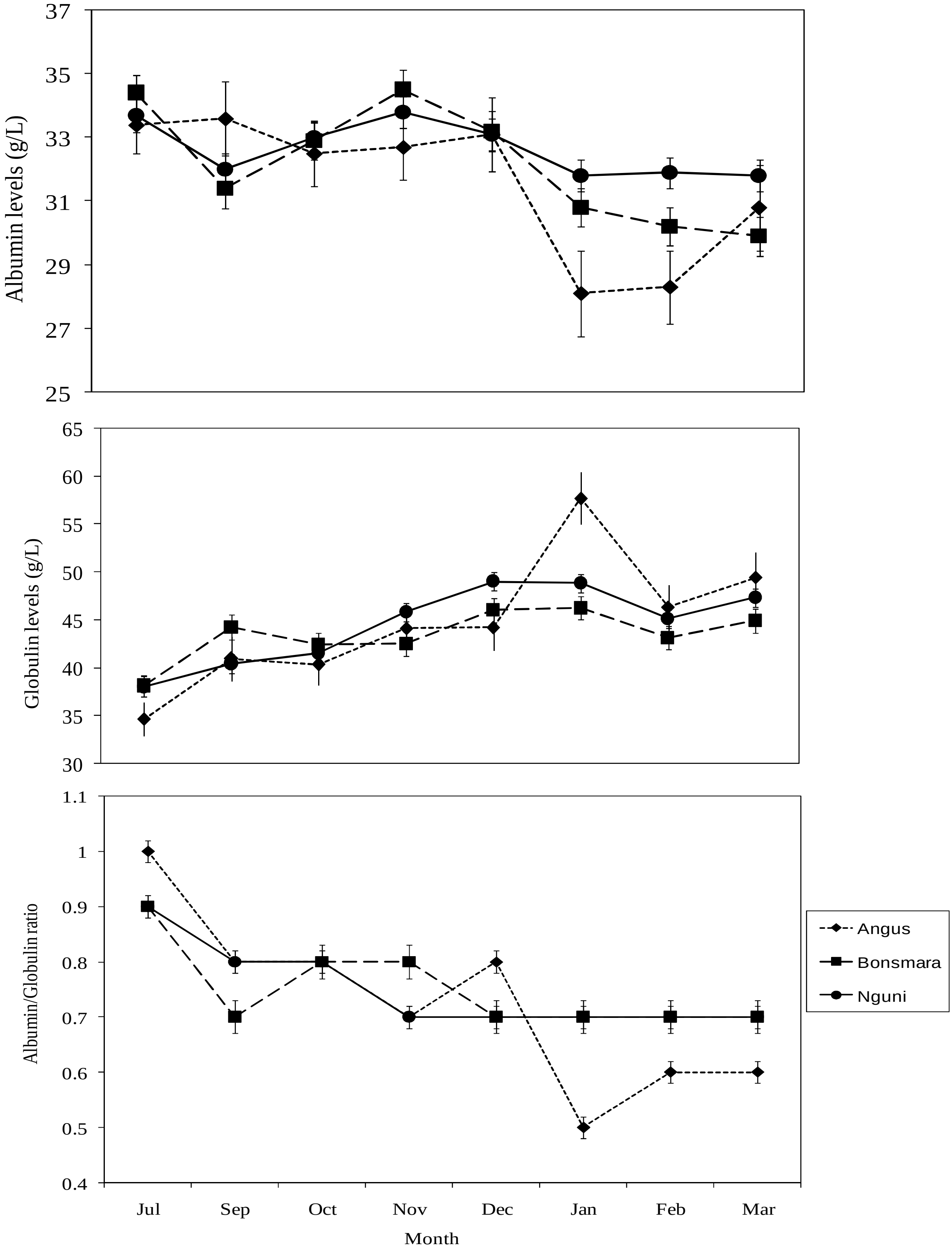

Figure 4.3: Effect of month and breed on albumin, globulin concentrations and A/G ratio

4.3.3 Effect of breed and month on calcium, phosphorus and magnesium concentrations

Figure 4.4 shows the changes in calcium, phosphorus and magnesium concentrations in the Nguni, Bonsmara and Angus steers. Significant month × breed interactions ($P<0.05$) were observed for calcium concentrations. Although month was significant ($P<0.05$), breed, however, had no effect ($P>0.05$) on calcium concentrations. The peak calcium level for Bonsmara was in November while for Nguni and Angus was observed in March. As also shown in Figure 4.4, both the breed and month affected ($P=0.001$) phosphorus concentrations. No month × breed interactions ($P>0.05$) were observed. Peak phosphorus concentrations for all the breeds were observed in February. The phosphorus concentrations decreased from October until December and peaked in February. Bonsmara and the Angus had similar values across the months but greater than for the Nguni steers (Figure 4.4). Both the breed and month affected ($P<0.05$) magnesium concentrations and month × breed interactions ($P<0.05$) were also observed. The Bonsmara, Angus and Nguni steers had different magnesium least square means over the nine month period (Table 2). The Nguni had the same magnesium concentrations between October and January most probably due to the same mineral levels in grass during that period. Bonsmara and Angus steers had similar magnesium concentrations from July to October.

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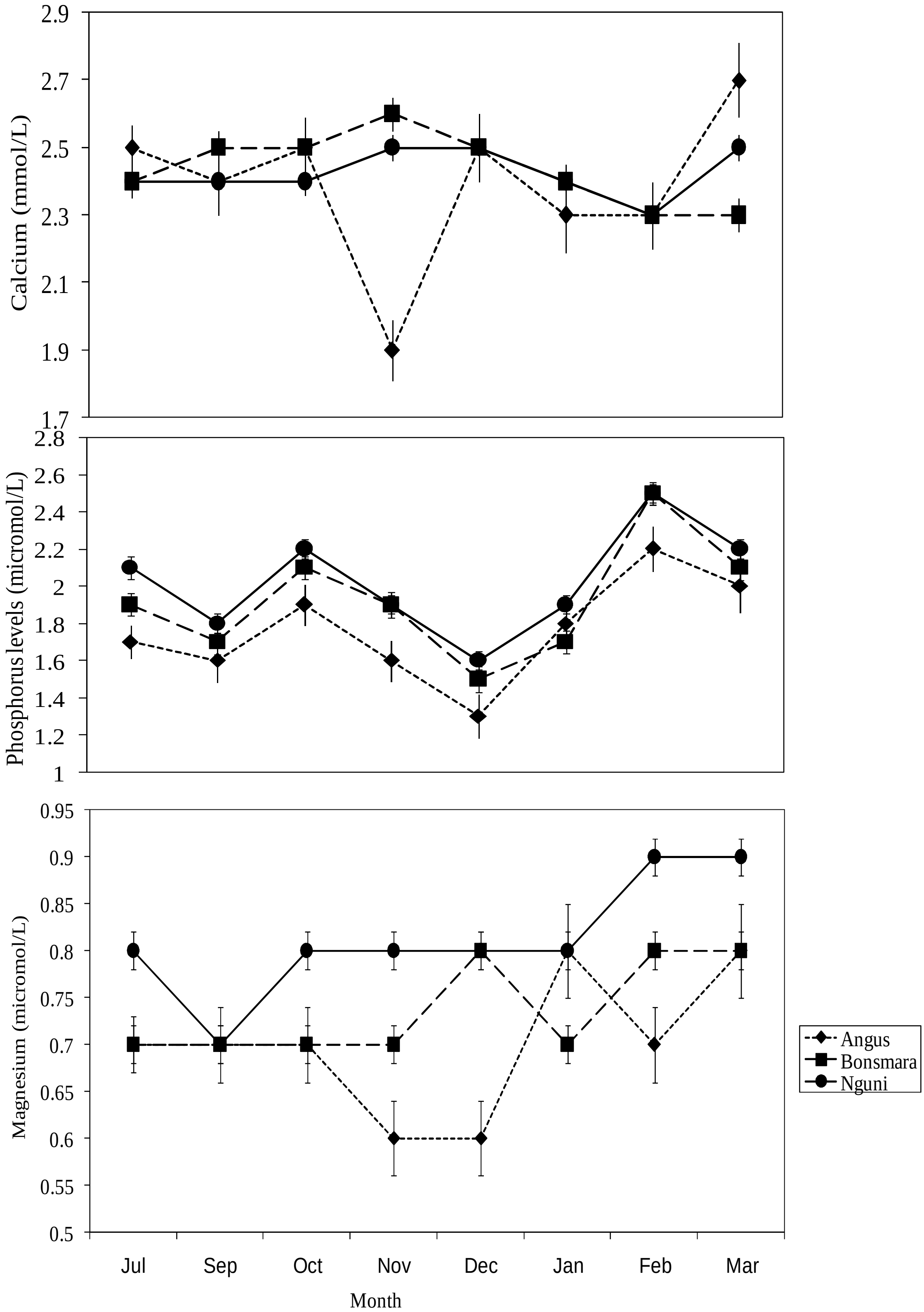

Figure 4.4: Month and breed influence on Ca, P and Mg concentrations

4.3.4 Effect of breed and month on creatinine kinase, aspartate aminotransferase and alkaline phosphatase concentrations

Both the breed and month ($P>0.05$) had no significant effect on CK activity. No month × breed interactions ($P>0.05$) were observed. Month affected ($P<0.05$) AST activity while breed did not ($P>0.05$). There was no month × breed interaction ($P>0.05$) observed for AST activity. The ALP activities in the three breeds are shown in Figure 4.5. Both the breed and month affected ($P<0.05$) ALP activity, but there was no month × breed interaction ($P>0.05$). The trend for the ALP activity was similar for all the breeds, with the peak being observed in March. Angus had the highest ALP activity, followed by Bonsmara (Table 4.2). Nguni steers had the least ALP activity.

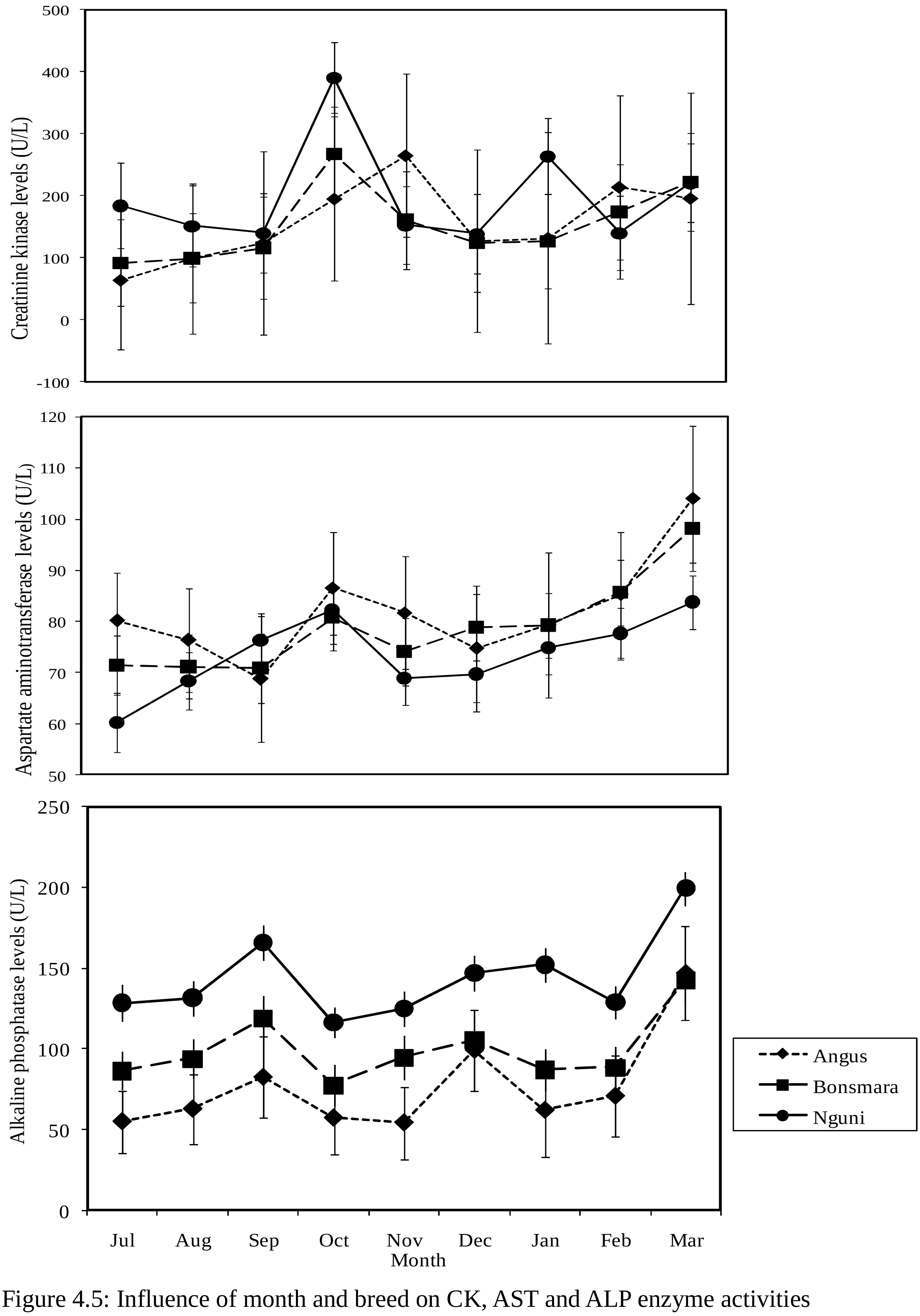

Figure 4.5: Influence of month and breed on CK, AST and ALP enzyme activities

4.4 Discussion

All the steers used in this study were clinically healthy and the parameters reported could, therefore, represent 'normal' values for each breed. The observation that glucose concentrations varied with month agrees with Grunwaldt *et al*. (2005), who reported that glucose varied with season in Angus steers and Criollo Argentino steers raised on veld. Insufficient nutrient intake can reduce circulatory glucose concentrations (Reynolds *et al*., 2003). Poor quality of the feed, which is usually experienced during the dry season, has been reported to affect blood glucose concentrations. For example, Chimonyo *et al.* (2000) observed a significant reduction of the concentrations of plasma glucose in winter in cows. In this study, glucose concentrations peaked in October, although no possible explanations could be obtained. Blood glucose, however, has a moderate diagnostic value in the assessment of nutritional status of cattle as it varies considerably (Coppo *et al*., 2002). The moderate diagnostic value of glucose is also shown by the large standard error bars for glucose.

Glucose concentrations obtained in the current study for all the breeds are similar to those reported by Otto *et al.* (2000) for Angoni cattle of Mozambique, which were maintained on extensive rangelands. In an experiment where ryegrass was overseeded on Coastal Bermuda grass, Hollenbeck *et al.* (2006), however, observed higher glucose concentrations than those obtained in this study (4.77 g/L for Angus and 6.37 g/L for Bonsmara steers). The observation that Bonsmara steers had higher glucose concentrations than Angus agrees with Hollenbeck *et al.* (2006). The finding that glucose concentrations were low during the hot months agrees with literature (Berne and Levy, 1993; Grunwaldt *et al.*, 2005). The absence of breed × month interaction for glucose concentrations indicates that all the breeds responded similarly to seasonal temperature and rainfall changes. The higher glucose concentrations observed in Bonsmara and Nguni steers than the Angus could indicate the adaptation of the two breeds to higher temperatures. Hollenbeck *et al.* (2006) reported higher respiration rates in the Angus steers, which could explain the lower glucose concentrations in these breeds than the Nguni. More work, however, is required to determine the respiration rates and the effects of thermal stress in Nguni cattle.

The pattern for the changes in cholesterol concentrations was similar to that obtained for glucose. Nguni steers had higher serum cholesterol concentrations than the other two breeds. The cholesterol concentrations observed in the Nguni cattle were, however, lower than those reported by Otto *et al.* (2000) for Angoni cattle. In ruminants, cholesterol is mainly used as an energy source (Adachi *et al.,* 1997). However, high glucose concentrations promote the secretion of insulin (Reynolds *et al.,* 2001), which, in turn, decreases cyclic adenosine monophosphate (cAMP) concentrations, thus stimulating cholesterol synthesis. Peak cholesterol concentrations for all the three breeds were observed when the BCS were also highest. The higher cholesterol and glucose concentrations for the Nguni could also be related to the physiological adaptation of Nguni cattle for their energy needs (Nazifi *et al.*, 2003) when searching for feed (Farver, 1997; Otto *et al.,* 2000). Thus, besides volatile fatty acids, glucose provides a considerable amount of energy in intramuscular adipose tissue and skeletal muscles in cattle (Overton and Waldron, 2004). The high cholesterol concentrations in the Nguni could suggest that it has more energy reserves than the other two breeds (Thrall *et al.,* 2004). Ruegg *et al.* (1992) reported a linear relationship between cholesterol concentration and the body condition. As an indication of lipolysis and utilization of body fat for energy, the serum cholesterol concentration was high for all the breeds from July, where feed quality was at its lowest.

The observed differences in TP concentration with month could be explained by the variation in grass quality (Chimonyo *et al.,* 2000). The TP concentrations observed among the three breeds were similar to those obtained by Otto *et al.* (2000) for the Angoni cattle raised on veld. Various factors have been reported to influence total protein concentrations. These include dietary protein intake and rumen degradability (Slobodianik *et al.*, 1999), dietary amino acid composition, protein intake, liver and kidney function, muscle tissue breakdown and dietary carbohydrate content (Brown and Adjei, 2001; Nazi *et al.,* 2003; Thrall *et al.*, 2004). However these factors did not vary within breeds as observed in our findings. Since the trend for CK was different from that of urea, the increased production of urea is not likely to be due to muscle catabolism. Blood urea concentrations reflect short-term changes in protein metabolism and, thus, could be used to explain TP and albumin concentrations (Payne, 1987).

Since TP and albumin concentrations were within the reference ranges, the urea concentrations below reference range are difficult to explain. Hollenbeck *et al.* (2006) reported low urea concentrations for Angus steers (1.0 mmol/L) and (1.36 mmol/L) for Bonsmara steers fed on ryegrass overseeded on Coastal Bermuda grass as compared to those observed in the current study. Urea concentrations observed in this study were, however, lower than those obtained by Otto *et al.* (2000) (4.1 ± 1.1 mmol/L) in Angoni cattle of Mozambique. Our findings could suggest that the effective rumen degradable protein from the veld was inadequate (Grunwaldt *et al*., 2005). There is need to determine the efficiency with which nitrogenous substances are converted to amino acids and muscle in these steers.

The marked increase in plasma creatinine concentration in July could be explained by the recycling of urea, which increases when dietary protein intake is low. When protein intake is adequate or high, creatinine excretion into urine increases (Thrall *et al.*, 2004). Plasma creatinine concentrations have also been reported during times of nutritional deprivation, when protein is used as an energy source (Braun *et al.*, 2003; Thrall *et al.*, 2004). Bonsmara steers had the highest creatinine concentrations, perhaps because of their large striated muscle size (Strydom *et al*., 2001; Thrall *et al.*, 2004).

Month × breed interactions observed for globulin concentrations indicate differences in breed susceptibility to common diseases endemic in the area. The high globulin concentrations in Angus, indicates it is more susceptible to parasites than the Nguni and Bonsmara (Grunwaldt *et al.,* 2005). Elevated globulin concentrations also suggest a chronic inflammatory response (Whitaker *et al*., 1999; Krecek and Waller, 2006). The reduced albumin concentrations in the Angus, especially from December to March, further suggests the presence of a chronic disease, such as helminthosis (Van Hutert and Sykes, 1996). The month effect on albumin concentrations could possibly be due to the seasonal differences in feed quality (Chimonyo *et al.,* 2000) as serum albumin is a sensitive early nutritional indicator of protein status (Doornenbal *et al.,* 1988; Slobodianik *et al.,* 1999), because its plasma mean life (turnover) is only 16 days. The high albumin concentrations observed in Nguni, especially from November to March, could, therefore, indicate their high protein status, possibly suggesting the higher efficiency with which Nguni cattle utilize dietary nitrogen. The concentrations of albumin in all the steers were within the expected ranges, indicating normal liver function (Grunwaldt *et al.,* 2005). Pyne *et al.* (1987) reported variations in albumin concentrations could be used to assess the liver function. The reduction of albumin: globulin ratio from December to March in Angus could be explained by the high globulin concentrations, which could have been caused by a relatively high prevalence of internal parasites (Thrall *et al.,* 2004; Grunwaldt *et al.,* 2005). A decreasing albumin/globulin ratio could be associated with low protein uptake (Fischbach, 2000; Wallach, 2000; Thrall *et al.,* 2004).

The finding that month affected calcium concentrations contradicts reports on the lack of month differences in beef cattle (Abdelrahman *et al.,* 2002; Grunwaldt *et al.,* 2005; Yokus and Cakir, 2006). All the breeds had calcium concentrations within the reference range. Our findings agree with McDowell *et al.* (2005), who observed seasonal changes in calcium concentrations in sheep raised on veld. In November and December, phosphorus and magnesium concentrations were low in Angus steers (Goff, 2000; Overton and Waldran, 2004). Subsequent increases in magnesium concentrations from December to January were accompanied with increases in calcium concentrations, as also reported earlier (Ghamdi *et al.,* 1994; Goff, 2000; Thrall *et al.,* 2004). In the current study, phosphorus concentrations were high when glucose, TP and urea were low. Low energy intake has been reported to increase serum phosphorus concentration (Thrall *et al.* 2004).

All the steers had normal AST activities, indicating that the animals exhibited similar levels of muscular activity (Otto *et al.,* 2000), as they were managed as a single herd. However, Otto *et al*. (2000) found higher AST activity in Angoni cattle raised under veld conditions than those we observed, indicating that the steers in the current study were not travelling long distances in search for food. At most, the furthest paddock was 2 km from the cattle handling facility, where they were collected for sampling once in two weeks.

The high ALP activity in the Nguni, possibly, suggests that their carcasses have a higher bone: muscle ratio. It is expected that high ALP activity indicate rapid skeletal growth (Doornenbal *et al*., 1988**;** Otto *et al.,* 2000**;** Grunwaldt *et al*., 2005). There is a need to evaluate carcass performance of Nguni animals and the benefit of higher skeletal growth as an adaptation mechanism (Strydom *et al.,* 2001; Muchenje *et al.,* 2007). All the breeds had ALP activity falling within the reference range (Farver *et al*., 1997). In addition to skeletal growth, serum ALP indicates the zinc status in animals (Adachi *et al*., 1997).

4.5 Conclusions

The Nguni had high BCS, whereas Angus could not maintain its condition throughout the study period. Nguni steers had a higher serum glucose and cholesterol, probably as an adaptive measure to survive times of poor feed quality. Angus had high globulin concentrations during the rainy season, indicating its high susceptibility to chronic infection. Nguni and Bonsmara had higher concentrations of phosphorus. Angus had the least calcium and magnesium levels from October until December. Further research is needed to investigate differences in glucose and cholesterol concentrations between the Nguni and imported breeds and to determine concentrations of cholesterol in the meat from Nguni steers. Rearing an adapted breed that can efficiently utilize veld has great economic benefits. There is, however, need to assess breed effects on the response to pre-slaughter handling stress.

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Chapter 5

Effect of breed on pre-slaughter stress response in Angus, Nguni and Bonsmara steers

Abstract

The effect of breed on the level of stress hormones at slaughter was determined in forty five 18-month old Angus, Bonsmara and Nguni steers. The steers were slaughtered at the East London abattoir, which is about 120km from the University of Fort Hare farm, where the animals had been raised. Serum creatinine and PCV levels were determined before cattle transportation. Blood was also sampled along the slaughter line soon after throat slitting for the determination of cortisol, creatinine and PCV. Urine samples were collected for the measurement of creatinine, adrenalin, noradrenalin and dopamine levels. Estimated glomerular filtration rate was also estimated. Bonsmara had highest levels of adrenalin (10.8nmol/mol), noradrenalin (9.7nmol/mol) and dopamine (14.8) levels, whereas the Nguni had the least levels of adrenalin (6.5nmol/mol), noradrenalin (4.6nmol/mol) and dopamine (4nmol/mol) levels. The Nguni had the highest serum cortisol levels (216.65nmol/l) and Angus had the least levels (93.75nmol/l). There were no breed differences in serum and urea creatinine levels, and on estimated glomerular filtration rate (eGFR). Nguni steers were, therefore, least susceptible to transport and handling stress than the other two breeds. Further research on the effect of transport and handling stress on meat quality is required.

Key words: Packed cell volume, cortisol, dopamine, adrenalin

5.1 Introduction

There is increasing interest in the measurement of stress as an indicator of animal welfare, nutritional status and disease. Domestic animals are transported for various reasons including breeding and slaughter (Arthington *et al*., 2003; Fazio and Ferlazzo, 2003). Transportation can lead to distress, deleterious effects on health, wellfare and, ultimately, meat product quality (Tarrant and Grandin, 2000; Borell, 2001). Whether a stressor can be considered as harmful depends on the way an organism is able to cope with a threatening situation as it regains homeostasis (Sheridan *et al*., 1994; Knowles and Warriss 2000; von Borell, 2001) which can vary with breeds (Grandin, 1997). The age, health status, genotype, and previous experiences of animals can influence the way an animal copes with stress (Warriss 2000; Wurtman, 2002). Rough handling may be more detrimental and stressful to animals with an excitable temperament compared to animals with a more placid temperament. Dopamine, adrenalin and noradrenalin have been shown to be good indicators of stress in animals (Mcguiness *et al*., 1997; Oczenski, 1999; Wurtman, 2002). Cortisol is the most commonly measured glucocorticoid released in stressed animals (Tyler and Cummins, 2003; Yagi *et al.,* 2004) as elevated levels of cortisol have been shown to have a significant impact on the immune system, increase blood glucose levels (Kannan *et al.,* 2000; Sapolsky *et al.* 2000).

Transportation of animals for long distances is a widely accepted as an animal welfare issue (Knowles, 1999; Most and Holme, 2002). There are both economic and benevolent reasons to attenuate transport and handling stress (Stokes, 2000; Arthington *et al.*, 2003). Temperament in cattle, which is heritable, may affect the animal's reaction to handling

(Le Neindre *et al*., 1995). Studies on the heritability of temperament in beef cattle estimate heritability as 0.48 (Stricklin *et al*., 1980). Urine is the main elimination route for catecholamines and glucocorticoids and excretion products in urine accumulate over several hours. Thus, concentrations in urine can be considered as more indicative of stress levels than those in plasma (Hay *et al*., 2000; Mostl and Palme, 2002). Therefore, urine samples were used, in this current study, for the assay of stress hormones.

Transport and handling stress have been observed to increase PCV (Fenwick and Green, 1986; Scope *et al.*, 2002). The increase reflects both a splenic response to stress and, to some extent, dehydration. Creatinine has also been used to assess the stress effect on the functioning of kidneys (NRC, 2006; Scope *et al*., 2002). There is no information on how Nguni, Bonsmara and Angus cattle respond to handling and transport stress. A stress tolerant breed, as indicated by low hormone levels, is more economic to rear than a non-adapted one. Therefore, the objective of the current study was to determine the effect of breed on level of hormones at slaughter. The hypothesis tested was that breed had no effect on level of hormones.

5.2 Materials and Methods

5.2.1 Study site and animal management

The study site and animal management are described in sections 3.2.1 and 3.2.2, respectively.

5.2.1 Animals and sample collection

Blood samples were collected into red topped vacutainer tubes with no anticoagulant and into EDTA containing tubes by jugular-venipuncture from 45 steers at the farm 24 hours before transportation of the animals to the abattoir. The steers were transported to East London which is about 120 km away from Honeydale farm. On the day of transportation, the minimum temperature was 13.5 ^{o}C and the maximum was 24.0 ^{o}C. The steers were kept overnight at the abattoir holding pens without food for 24 hours. Water was available at all times. Cattle were slaughtered after stunning with a captive bolt suspended by a hind leg and exsanguinated.

Blood was sampled along the slaughter line into vacutainer tubes containing EDTA anticoagulant and the other one with no anticoagulant and soon after the throat was slit. Urine samples were collected from the slaughter line from the bladder of each animal using a syringe into sampling bottles. The sample bottles contained 6M hydrochloric acid to stabilize the catecholamines. The samples were then frozen at -20 ^{o}C awaiting analysis.

5.2.2 Hormone and metabolite analysis

Creatinine levels were analyzed using a colorimetric quantitative reaction (Boehringer PAP method). Packed cell volume for each steer was measured by the standard micro-haematocrit method with a haematocrit centrifuge at 12,000 rpm for 5 min. Serum concentrations of cortisol were quantified using an immunoassay, as previously described (Meunier-Salaun *et al*., 1991). The interassay coefficient of variation (CV) was 4.6%. Urinary dopamine and free catecholamines (noradrenalin and adrenalin) were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection, as previously described (Hay and Mormede, 1997; Ruis *et al.*, 2002). The interassay CV was 5.2, 4.7 and 4.1% for dopamine, noradrenalin and adrenalin, respectively.

Estimated glomerular filtration rate was determined in accordance with the method of Liu *et al*. (1999). Creatinine was used to correct for urine dilution (Hay *et al.*, 2000; Klante *et al.*, 1997) therefore, catecholamine levels were expressed as ratios to creatinine concentrations.

5.2.3 Statistical analysis

The effects of breed on PCV, creatinine, cortisol, catecholamines and dopamine were analyzed using Generalised Linear Models procedures of SAS (2003). Pair-wise comparisons between least-square means were compared using the PDIFF test of SAS (2003).

5.3 Results

Table 5.1 shows PCV and serum creatinine levels of Angus, Bonsmara and Nguni steers before and after transport and handling stress. There was an increase ($P<0.05$) in PCV levels before and after transportation in all steers while no significant differences ($P> 0.05$) were obtained in serum creatinine levels before and after transportation of steers. There were no breed differences ($P>0.05$) in creatinine and PCV levels before and after transportation and handling the animals in preparation for slaughter.

Figure 5.1 shows the breed differences in adrenalin, noradrenalin and dopamine levels. Bonsmara had the highest ($P<0.05$) levels of adrenalin, noradrenalin and dopamine levels whereas the Nguni had the least ($P<0.05$) levels.

Figure 5.2 shows the breed influence on dopamine levels. The Nguni had the highest ($P<0.05$) serum cortisol levels (Figure 5.2) and Angus had the least ($P<0.05$) levels. As shown in Figure 5.3, there were no breed differences ($P>0.05$) in estimated glomerular filtration rate (eGFR).

Table 5.1: Packed cell volume and serum creatinine levels of Angus, Bonsmara and Nguni steers before and after transport and handling stress

Parameter	Before transportation	After transportation	P value
PCV (%)			
Angus	33.33 (1.202)	36.00 (1.354)	P= 0.021
Bonsmara	29.93 (1.087)	33.36 (0.935)	
Nguni	31.73 (0.826)	35.71 (1.031)	
Serum creatinine (µmol/L)			
Angus	98.60 (3.528)	87.50 (2.784)	P= 0.130
Bonsmara	106.10 (1.878)	91.29 (2.782)	
Nguni	106.90 (2.075)	92.87 (2.834)	

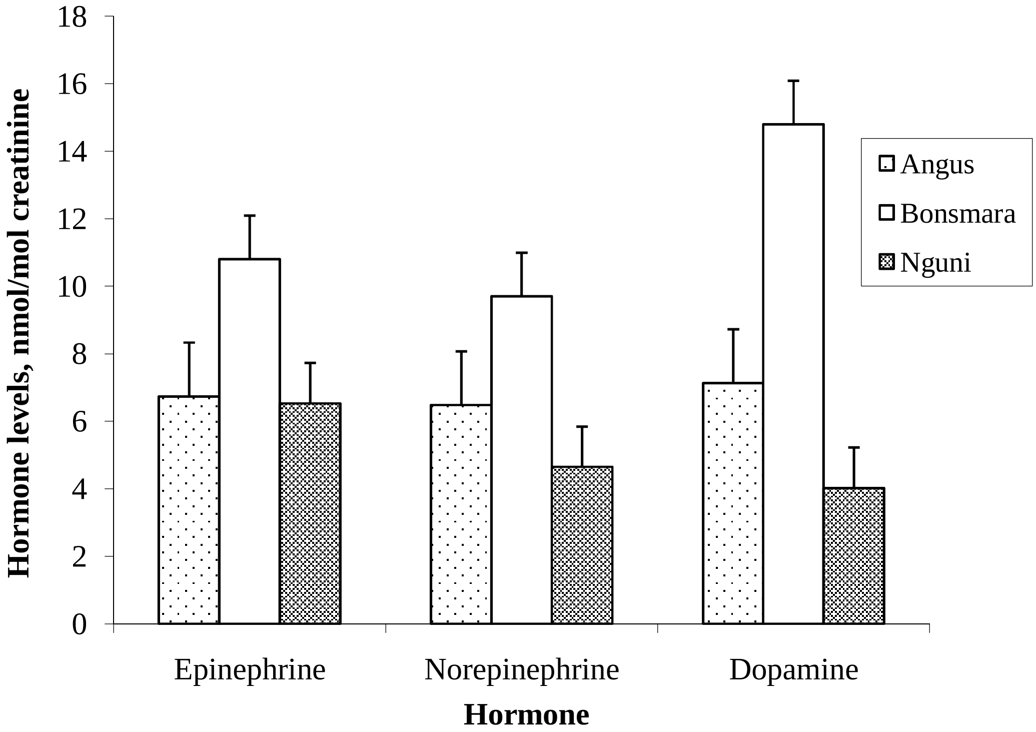

Figure 5.1: Urine adrenalin, noradrenalin and dopamine levels of Angus, Bonsmara and Nguni steers after transport and handling stress

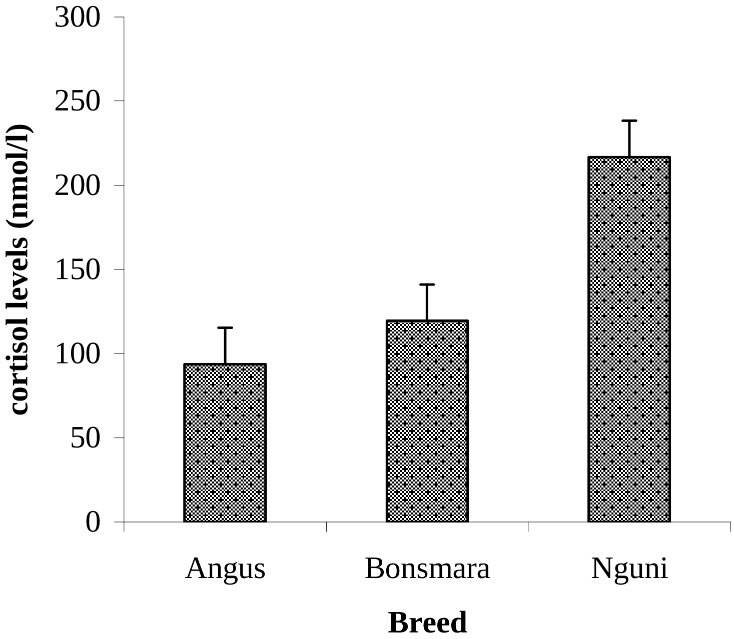

Figure 5.2: Serum cortisol levels for Angus, Bonsmara and Nguni steers after transportation and handling stress

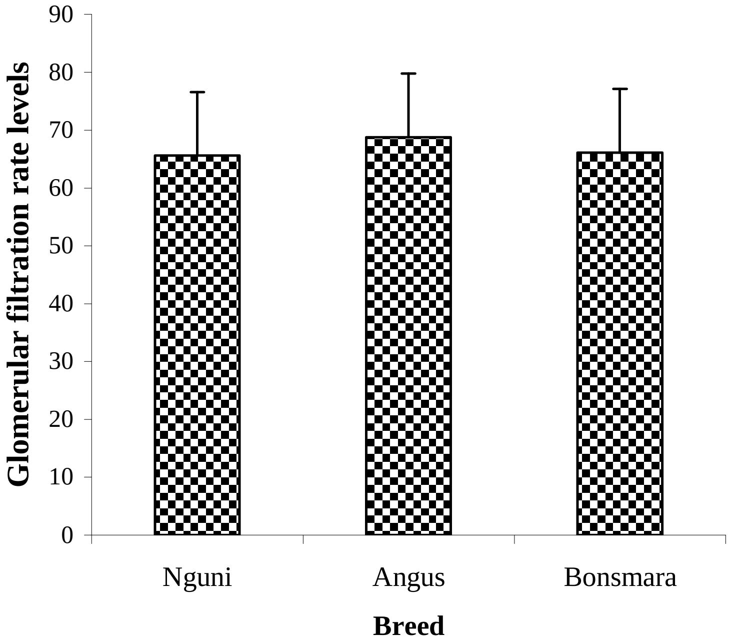

Figure 5.3: Estimated glomerular filtration rate for Angus, Bonsmara and Nguni steers after exposure to transport and handling stress

5.4 Discussion

Predicting secondary physiological outcomes resulting from transportation is a challenge because multiple stressors occur, often in combinations unique to a particular transportation scenario or species. Nguni steers had high levels of cortisol possibly due to pre-slaughter stress. Transport and handling are reported to evoke an increase in circulating cortisol levels (Cockram and Corley, 1991; Scott *et al*., 1993). It has been documented that dopamine regulates cortisol secretion in ewes (Sowers *et al*., 1983), yearling steers and heifers (Browning *et al*., 2000) and cows (Ahmadzadeh *et al.*, 2006). Glucocorticoid secretion is, therefore, regulated, in part, by the dopaminergic system and via dopamine receptors. With this argument, cortisol and dopamine levels were both expected to be high in Nguni. However according to Grandin (1997), absolute comparisons of cortisol levels between studies should be done with caution, as cortisol levels can vary greatly between individual animals and breed. For example, cortisol levels have not been found to correlate with stress in swine during transport (Brown *et al.,* 1999; Hicks *et al.* 1998).

The low cortisol levels in Angus and Bonsmara breeds are difficult to explain. However in a study conducted by Koch (2004), Bonsmara had lower plasma cortisol concentration following 30 minutes transportation compared to the plasma cortisol concentrations prior to transportation. Blecha *et al.* (1984) reported similar findings in that Angus feeder steers also had decreased cortisol concentrations following 10 hours of transportation. These authors suggest that it was likely that this was due to the animals acclimating to the trailer. Warriss *et al*. (1995) reported that cattle transported 10 or 15 h had an increase in plasma cortisol concentrations but the increases were slight and decreased with longer duration of transportation. As the transportation time of these steers in the current study was less than three hours, it is difficult to conclude that the Angus and Bonsmara had acclimated to transportation as acclimation to occur during a short trip is unlikely.

Bonsmara had high levels of adrenalin and noradrenalin as compared to Nguni and Bonsmara thus confirming findings by Grandin, (1997) and Gonyou, (2000) who observed that genotype can also influence the physiological changes. The effect of genetics is also well documented and an example of stress susceptibility in swine is where sudden death following stress, is inherited by a single recessive gene (*Hal*n). When subjected to transport stress, the three Hal genotypes (*Hal*N/N, *Hal*N/n, *Hal*n/n) had different cortisol concentrations immediately after transport (Nyberg *et al*. 1988). Catecholamine levels (adrenalin and noradrenalin), heart rate, respiratory rate and packed cell volume have been shown to respond immediately to transport and handling stress (Harris *et al*., 1999; Bonacic *et al*., 2006). Bonsmara is more susceptible to transport and handling stress as compared to Angus and Nguni as it had high adrenalin and noradrenalin levels.

Estimated glomerular filtration rate was between 60 and 89ml/min thus indicating possibly mild renal function impairment in all the steers. However this finding is not conclusive as the creatinine levels were within reference ranges (Farver, 1997). Serum creatinine levels can be used to assess renal function. Creatinine concentration alteration in stressed animals have been reported (Cole and Hutcheson, 1985), though in this study no significant differences were found between the levels of creatinine before transportation and after transportation. However, an increased creatinine concentration due to muscular activity and a decrease in renal excretion because of vasospasm in the kidney produced by catecholamines has been described (Harthoorn, 1976). Adrenalin (40%) and noradrenalin (20%) cause a decrease in renal blood flow, thus predisposing to renal hypoxia (Guyton and Hall, 1996). In the present study, it is difficult to quantify the adrenalin and noradrenalin percentile increase as no urine was sampled prior to slaughter for detection of basal adrenalin and noradrenalin levels.

The increase in PCV levels observed in this study after transport and handling of animals agrees with observations made by Ganong (2002). The first step in stress response is the activation of the sympathetic nervous system, stimulating the adrenal medulla and releasing catecholamines (Montane *et al*., 2003). Increases in PCV is associated with splenic contraction caused by the effect of catecholamines on adrenergic receptors located in the splenic capsule (Ganong, 2002), and partly to a reduction in plasma volume (Wesson *et al*., 1979; Cross *et al*., 1988). Scope *et al.* (2002), however, found no significant differences in PCV levels on the influence of stress from transport and handling on PCV levels of racing pigeons.

5.4 Conclusions

Bonsmara had highest levels of adrenalin, noradrenalin and dopamine levels whereas the Nguni had the least levels. The Nguni had the highest serum cortisol levels and Angus had the least levels. There were no breed differences in serum and urea creatinine levels, and on estimated glomerular filtration rate (eGFR). There was no difference in creatinine levels in all the breeds whereas PCV increased after transport and handling slaughter. From the results it can be concluded that Nguni is more stress tolerant of the three breeds and Bonsmara is more susceptible to stress. Further research needs to be carried out to determine the high cortisol levels and low dopamine levels obtained in Nguni, and the relationship between stress hormones and meat quality. In future studies, pre- transport and handling physiological events must be recorded in order to demonstrate and make a comparison on non steady-state condition experienced by cattle while being transported and handled.

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Chapter 6

General discussion, conclusions and recommendations

6.1 General discussion

Optimal productivity of beef enterprises can be significantly enhanced by using appropriate breeds for the local environment (Corbet *et al*., 2005; Anderson, 2003). In sub-Saharan Africa, the appropriate breed should cope with variable feed supply, frequent occurrences of droughts, high ambient temperatures and high disease and parasite pressures. Adapted breeds are also able to utilize these otherwise unproductive resources (Otto *et al*., 2000). The main objective of this study was to assess the nutritional and health status of Nguni, Bonsmara and Angus steers raised on natural veld in the Eastern Cape Province of South Africa. The null hypothesis that there were no breed differences in nutritional and health status of Nguni, Bonsmara and Angus steers was tested. However breed differences were found.

In Chapter 3, body condition scores, body weights and faecal samples were collected monthly. The BCS for Nguni were high despite the poor forage quality during the dry season and post-rainy seasons when grass quality would be reduced. This, therefore, indicated the adaptation by Nguni to the local environmental conditions. The BCS and body weight for Angus declined from September until January. A common feature of infections with gastrointestinal nematodes and trematodes is a reduction in voluntary feed intake, and in moderate to heavy infections food intake can be reduced by 20% or more (Coop and Kyriazakis, 2001). As described in Chapter 3, parasite infestation varied seasonally. High levels of internal parasites were observed during the rainy season, which could be due to the moist conditions favouring the hatching of eggs (Axford *et al.*, 2000). *Haemonchus placei, Cooperia pectinita Trichostrongylus, Cooperia pectinita, Fasciola* and *Oesophagostomum radiatum* were the predominant internal parasites observed. Internal parasites use dietary nutrients from the host to multiply and survive (Kadima *et al*., 2000) while some parasites such as *Haemonchus* spp. suck blood from the animal for their nutrition. In December, low PCV levels were observed in the Angus, which coincided with high levels of increased *Haemonchus* levels. Nguni had higher PCV levels and lower parasite infestation levels than Angus and Bonsmara. The high PCV confirms the resilience of the Nguni to internal parasites (Mulcahy *et al.,* 2004). Of the three breeds, Angus was more susceptible to internal parasites shown by its high infestation levels.

Besides using body weights and condition scores in assessing the nutritional status of cattle, haematology and blood metabolites have been increasingly used as a management tool in beef cattle production (Grunwaldt *et al.*, 2005; Agenas *et al.*, 2006). Breed differences on these parameters were described in Chapter 4. Blood samples were collected monthly for the assessment of nutritional status of Nguni, Angus and Bonsmara. Albumin levels decreased from December whereas globulin levels increased, which could, most likely, have been due to parasite infestation (Parkins and Holmes, 1989). Similar to the trends in body condition scores, changes in blood constituents related to protein, carbohydrate, lipid and mineral metabolism varied with each breed and monthly. Nguni steers had higher serum glucose and cholesterol levels than the other two breeds. The higher cholesterol and glucose levels for the Nguni cattle could also be related to the physiological adaptation of Nguni cattle for their energy needs (Nazi *et al*., 2003) during winter where forage quality is low. Nguni had the highest magnesium and phosphorus levels, probably indicating the efficient utilization of feed by the Nguni steers. Minerals are essential for livestock as sub-clinical deficiency may go unrecognized thus causing a reduction in growth and efficiency (Grunwaldt *et al*, 2001).

When steers used in the study were transported to the abattoir for slaughter, breed differences in handling and transportation stress were determined. Chapter 5 described the breed differences in urine adrenalin, noradrenalin, dopamine and serum cortisol, creatinine and PCV levels. Bonsmara steers were more susceptible to transport and handling stress as they had the highest levels of adrenalin, noradrenalin and dopamine levels followed by the Angus. Fear responses in a particular situation are difficult to predict because they depend on how the animal perceives the handling or transport experience. The animal’s reactions are governed by a complex interaction of genetic factors and previous experiences (Grandin, 1997). In this study, previous experiences of the steers was assumed to be the same as these steers were raised under one herd until slaughter at 18 months. Rough handling may be more detrimental and stressful to animals with an excitable temperament as was shown by the behaviour of Bonsmara steers compared to animals with a more placid temperament such as Nguni (Tarrant and Grandin, 2000).

6.2 Conclusions

The BCS for Bonsmara increased similarly with the ones for the Nguni, but the Bonsmara began to lose their body condition in the post-rainy season. The prevalence of helminth parasites was low. *Haemonchus placei, Cooperia pectinita Trichostrongylus, Cooperia pectinita***,** *Fasciola* and *Oesophagostomum radiatum* were the predominant parasites. Nguni cattle had lower levels of internal parasites and higher PCV levels as compared to Bonsmara and Angus. Nguni steers had higher serum glucose and cholesterol as compared to Bonsmara and Angus. Nguni and Bonsmara had higher levels of magnesium, phosphorus and calcium. Nguni cattle, therefore, utilize available feed efficiently. Bonsmara steers had the highest catecholamine and dopamine levels whereas the Nguni steers had the least levels, suggesting that Bonsmara cattle were more susceptible to stress whereas Nguni cattle were the least stress tolerant of the three breeds.

6.3 Recommendations

In the current study, Nguni has been shown to out-perform Bonsmara and Angus steers when raised on natural pasture. Farmers can therefore rear the Nguni adapted breed to improve their livelihood as rearing this breed requires low inputs such as feed and veterinary medicine. Rearing a stress tolerant breed such as the Nguni has several merits for farmers as cattle which are more susceptible to transport and handling stress have been shown to produce meat of poor quality which is undesirable for human consumption.

The following aspects, however, requires further investigation:

1. Seasonal variation of nutritionally-related blood metabolites of Nguni cattle in the communal areas should be determined. In addition, the effect of age, sex and physiological status on the levels of these blood metabolites needs to be assessed.
2. Since energy is the most important nutrient affecting animal performance, there is need to evaluate the levels of energy-related blood metabolites, such as NEFA and BHB in Nguni cattle.
3. Further research is required to investigate resistance/ tolerance of the Nguni to diseases and droughts, which occur frequently in Southern Africa. The mechanisms of inheritance patterns for resistance to internal parasites, such as *Haemonchus* spp. are not fully understood. The Nguni Major Histocompatibility Complex (MHC), which may be involved in conferring increased resistance or tolerance to internal parasites, also needs further investigation.
4. Further research is needed to investigate the potential of safe organic meat production from the Nguni cattle for the benefit of the consumers and the rural farmers.

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