

**Seasonal variations in tick loads, coat characteristics, temperature-humidity index and
blood metabolite profiles of extensively raised Boran cows**

By

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Declaration

I, Wonga John Ntilini, proclaim that this Dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof V. Muchenje. All assistance towards the production of this work and all the references contained herein have been fully acknowledged.

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Wonga John Ntilini

.....

Date

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Prof Voster Muchenje

Abstract

Seasonal variations in tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of extensively raised Boran cows

BY

Wonga John Ntilini

This study sought to determine the seasonal variations in tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of Boran cows reared in arid rangelands of South Africa. A total of 20 Boran cows of different age groups were used in this study. Tick counts, hair length, coat score, average temperature and relative humidity readings were measured twice (beginning and end) in each season (post-rainy, cold-dry, hot-dry, hot-wet). Blood samples were taken seasonally (beginning and end of the season) for the analysis of creatine kinase (CK) activity and haematological parameters (HP). Ticks were observed and counted from different anatomical sites, namely anterior (neck, head, ears, and around eyes), ventral (belly, udder, and limbs) and posterior (back and under the tail). The tick species observed from the Boran cows were *Rhipicephalus evertsi evertsi* (34.58%), *Amblyomma hebraeum* (43.35%), *Rhipicephalus simus* (4.48%) and *Rhipicephalus (Boophilus) decoloratus* (17.59%). Significant differences were observed in tick burdens at various anatomical sites, with posterior position having the highest tick loads during the post-rainy, cold-dry and hot-dry season. While the ventral position recorded the highest tick loads during the hot-wet season. It was also observed that the cattle had significantly long hair lengths during the cold-dry season. Furthermore, coat score and hair length were observed to be negatively ($P < 0.05$) correlated with tick counts, while coat scores were positively ($P < 0.05$) correlated with hair length. Significant differences were observed in CK activity across season, with higher levels during the hot-dry and hot-wet seasons compared to the post-rainy and cold-dry seasons. Boran cows were exposed to a

mild stress condition during the hot-dry and hot-wet season when the THI values were high (>70). Seasonal variations had an effect ($P<0.05$) on some HP (HB, HCT, MCV, MCH, EOS, and BAS). Neutrophils (-0.267) and basophils (0.268) were significantly correlated with THI. In conclusion, the Boran cows were more susceptible to tick loads during the hot seasons. Likewise, the levels of creatine kinase activity and temperature-humidity index were high during the hot-dry and hot-wet season. Therefore, seasonal variations had an effect on tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of extensively raised Boran cows.

Keywords: *Amblyomma*, haematological parameters, *Rhipicephalus*, temperature-humidity index

Dedication

A special dedication to my late brother (Mzimasi Ntilini), may your soul continue to rest in peace.

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List of Abbreviations

ASAL – Arid and Semi-Arid Land

ATP – Adenosine tryphosphate

BAS – Basophils

BCS – Body condition score

BW – Body weight

CK – Creatine kinase

CS – Coat score

EOS – Eosinophils

HB – Haemoglobin

HCT – Haematocrit

HL – Hair length

HP – Haematological parameters

LYMP – Lymphocytes

MCH – Mean concentration haemoglobin

MCH – Mean corpuscular haemoglobin

MCHC – Mean corpuscular haemoglobin concentration

MCV – Mean cell volume

MON – Monocyte

NEU – Neutrophils

PCV – Packed cell volume

RCC – Red cell count

SAS – Statistical Analysis System

TBD – Tick-borne disease

TC- Tick count

THI – Temperature-humidity index

USMARC – United States Meat Research Centre

WCC – White cell count

1. General Introduction

1.1 Background

Boran cattle are large Zebu (*Bos indicus*) breed that have diverse herds of unimproved and improved herds (Domestic Animal Genetic Resource Information System, 2007). The Boran is known to have originated from Southern Ethiopia, where it was developed by the Borana people (Mpofu, 2002). The breed was then exported to Kenya by the Ethiopian and Somalian nomads (Mpofu, 2002), where they were, and are still being exploited in subsistence and semi-commercial systems of production. Among the East African breeds, Boran cattle were the most favored by commercial farmers because they easily adapt to the tropical production environment (Mpofu, 2002; Okomo-Adhiambo, 2002; Kahi, 2015). This important adaptation trait has resulted in the breed being crossed with other breeds in order to improve their productivity (Wasike *et al.*, 2009). Moreover, their adaptability facilitates greater ability of the animals to survive, grow fast to attain high weights and reproduce in the semi-arid conditions (Moyo, 1995). Ojango *et al.* (2006) reported that the most commercially viable *B. indicus* breed is the Boran because of its resilience to heat stress. It is additionally resistant to the most prevailing diseases in tropical regions, and has the ability to withstand extended periods of water and feed deficiency. These attributes have been accomplished after several years of natural selection in state of increased temperatures, poor quality of feed and increased pathogen occurrences that characterize Arid and Semi-Arid Lands (ASALs) (Okomo-Adhiambo, 2002; Ojango *et al.*, 2006).

In ASALs, cattle are exposed to different parasites, such as ticks, and to tick-borne diseases, which decrease their performance (Mpofu, 2002). There are approximately 879 tick species

worldwide (Mapholi *et al.*, 2014) and nearly each nation has one or more species of ticks. Rony *et al.* (2010) noted that ticks affected an estimated 1.4 billion cattle worldwide. Consequently, such challenges may be due to poor cattle health management, lack of drug knowledge (Dold and Cocks, 2001), and the use of unsuitable cattle breeds (Marufu *et al.*, 2010, 2011). According to Muchenje *et al.* (2008), ticks reduce live weight gain, milk production, fertility and often cause death in cattle. Moreover, ticks reduce cattle productivity as a consequence of lessened growth (Scwalbach *et al.*, 2003), and death connected with tick-borne diseases (TBD's) (Mugisha *et al.*, 2005; Kivaria, 2006). They also pose a health problem since they transmit diseases such as anaplasmosis, theileriosis, gallsickness, redwater, heartwater, brucellosis and sweating diseases in calves.

Approximately 70% of the total world's beef cattle are produced in environments with the highest tick burdens. Prayaga (2003) and Jonsson (2006) reported that in those regions where ticks exist, they pose an adverse impact on beef quality. Additionally, Foster *et al.* (2008) suggested that coat characteristics such as hair length, skin thickness, and coat scores influence tick counts and are significantly related to tick resistance in cattle on rangelands. Animals with shorter hairs and smoother coats are likely to have lower tick counts contrasted to those with longer hairs and woolier coats (Martinez *et al.*, 2006). Similar findings were outlined by Verissimo *et al.* (2002) and Katiyatiya *et al.* (2014), who reported the importance of establishing tick loads by determining coat characteristics. Additional research is required to establish the tick burdens, and to offer information on the modifications in body metabolism which would account for the loss of body weight and condition in cattle due to the effects of ectoparasites. Although body weights and body condition scoring have typically been utilized to assess the health status of animals (Grunwaldt *et al.*, 2005), blood metabolite concentrations such as creatine kinase

(CK) activity and full blood counts provide extra precise assessment. However, these blood parameters may vary due to environmental changes (Gashaw, 2005).

1.2 Problem statement

Ticks are one of the major challenges in cattle production because they limit animal productivity (FAO, 2011). Heavy tick burdens on cattle promote high economic losses (Alim *et al.*, 2012) by causing stress and discomfort, blood damage, decline in production, injury to rawhides and transmission of pathogens such as *Babesia bigemina*, *Babesi bovis* and *Anaplasma marginale* (Valente *et al.*, 2014). Moreover, a rising need for breeders in most countries is to make use of appropriate cattle breeds, which have features such as hardiness, ability to withstand high temperatures, disease and tick resistance, non-selective grazing and good quality beef.

The major tick control method which is based on the use of acaricides is becoming increasingly expensive and its effects are not always complete because of the advancement of resistance by ticks. Tick resistance to acaricides is a major problem in their control, largely because most resistance arises from the *Boophilus* species, as well as many multi-tick hosts, which may have five generations per year (Gertenbach, 2001). In addition, several researchers reported that consistent dipping to avoid tick infestation is an expensive activity for most farmers as it results in expanded imperviousness to ticks, cattle movement and management (Marufu *et al.*, 2011; Katiyatiya *et al.*, 2015).

1.3 Justification of study

In order to raise cattle productivity and advance cattle management, the nutritional and health status of cattle must be well-known. Information on body weights, body condition scores, external parasites and blood metabolites can be utilized to evaluate the nutritional and health

status of animals. The capability of cattle to adapt in local production environments can also be increased by the information on blood metabolites (Otto *et al.*, 2000). Selection of adaptive breeds such as Boran, which exhibit increased overall resistance, high reproductive potential and maximum performance can minimize the use of acaricides and uplift the production rate of the emerging farmers. Furthermore, Katiyatiya *et al.* (2014) reported that the selection of animals and their survivability competences can be assisted by knowledge of the relationship of their coat characteristics. Therefore, through advanced management and selection, performance of the Boran cattle can be vague progressed. Likewise, the selection of highly adaptive cattle breeds can allow producers to meet the expected increase in demand for high-quality beef (DAGRIS, 2007). Hereditary variations for tick numbers in cows has been accounted within and across breeds, hence, Silva *et al.* (2007) demonstrated the *Bos indicus* cattle breeds to be more resistant to ticks compared to *Bos taurus* (Fraga *et al.*, 2003; Regitano *et al.*, 2008).

1.4 Objectives

The broader aim of this study was to determine seasonal variations in tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of extensively raised Boran cows. The specific objectives were:

- To determine the physical and production traits on tick loads at different anatomical positions of Boran cows reared in a semi-arid rangeland of South Africa
- To determine the seasonal variations in creatine kinase activity, temperature-humidity index and haematological parameters of Boran cows reared in a semi-arid rangeland of South Africa

1.5 Null hypothesis

It was hypothesized that there are no seasonal variations in tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of extensively raised Boran cows.

The following specific null hypotheses were tested:

- ❖ Physical and production traits have no effect on tick loads at different anatomical position of Boran cows reared in a semi-arid rangeland of South Africa
- ❖ There are no seasonal variations in creatine kinase activity, temperature-humidity index and haematological parameters of Boran cows reared in a semi-arid rangeland of South Africa

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Chapter 2: Literature review

2.1 Introduction

South Africa produces a large number of livestock, with different breeds and species, varying according to environmental conditions, rangeland type and farming systems (Commercial, communal or small-scale) (Meisner *et al.*, 2013). Collectively, South Africa has approximately 13.9 million cattle (DAFF, 2013); of which the majority of these cattle originate in the Eastern Cape Province of South Africa (DAFF, 2012). About 60% of the cattle populace in South Africa is contributed by commercial farmers and 40% by emerging and subsistence communal farmers (DAFF, 2014). Emerging local farmers keep their cattle on communal rangelands (Muchenje *et al.*, 2008a), where they extensively graze with other cattle from different communities (Tada *et al.*, 2013). Conversely, Foster *et al.* (2008) and Marufu *et al.* (2011) noted that in these rangelands, cattle are affected by external parasites such as ticks, and tick-borne diseases (TBD) which negatively impact on their performance. Cattle infested with ticks have been reported to have reduced live weight gain, milk production and fertility, and in severe cases may also result to death (Muchenje *et al.*, 2008b). Ticks have a negatively influence in the productivity of animals by reducing their growth (Scwalbach *et al.*, 2003), as well as death allied with TBD's (Mugisha *et al.*, 2005; Kivaria, 2006; Marufu *et al.*, 2011). Hence, there is a need to utilize the cattle breeds, such as indigenous cattle breeds, that are well adapted to the hostile environments (Muchenje *et al.*, 2008a). Due to its tolerance to prevailing diseases in the arid areas and its capability to withstand long draught periods, the Boran breed is one of the viable indigenous cattle breeds (Ojango *et al.*, 2006). Therefore, this review focuses on the factors that influence tick loads, coat characteristics and some blood metabolite profiles of pure and crossbred Boran cattle. It also seeks to review some of the major tick species and TBD's of cattle in South Africa.

2.2 The potential of the Boran cattle

2.2.1 Physical characteristics and adaptation of the Boran cattle

Rege (2001) reported that the Boran cattle are predominantly white in colour; however, they may also possess light gray, fawn or light brown colour with gray, black or dark brown shading on head, neck, shoulders and hindquarters (Haile *et al.*, 2011). Their bulls normally have the darker skin pigment which is vital for heat tolerance (KBCBS, 2010). While Rege (2001) defined these cattle as large-sized, Kahi (2015) defined them as medium-sized cattle breed, with a body weight ranging from 318 to 680 kg in males and 225 to 454 kg in females (Haile *et al.*, 2011). In addition, they have a well-developed hump, dewlap and a dumpy strong neck (Kios, 2012). Their legs are solid and square shaped with medium length (KBCBS, 2010).

Boran cattle are known to be more docile, highly productive and generally calm as compared to other Zebu breeds (Zander *et al.*, 2008; Haile *et al.*, 2011). Thus, a large number of Boran bulls can be kept in one herd without injuries among them. In addition, Boran cattle also possess a good herd instinct (Haile-Mariam *et al.*, 2006). They usually move together as one herd and by doing so they protect themselves and their calves from predators, ensuring better calf survival rates (Rewe *et al.*, 2008). Their heifers and cows are not difficult to handle (KBCBS, 2010). However, cows with newly-born calves might be normally aggressive while ensuring the safety of their new-borns (Rewe *et al.*, 2008). Furthermore, the cows are known of their simplicity of calving, which is an advantage in farming systems where poor calving management is present (KBCBS, 2010). Mpofo (2002) and Kios *et al.* (2012) reported that Boran cows are intermediate performer when compared to other tropical breeds for weaning weight. In a study conducted by Lunstra and Cundiff (2003), Boran cattle breed had faster average daily gain than Tuli and this resulted in their sired bulls having heavier 12-month weight than Tuli sired bulls.

There is evidence that Boran cows have versatile traits of essential significance to aid in their continued survival in areas with extreme temperatures and poor veld quality (Demeke *et al.*, 2004). Such traits include; capability to be drought tolerant or to survive long periods of water and feed shortage, and also the ability to travel long distances for grazing (Haile-Mariam *et al.*, 2006). In order for animals to be regarded as adaptive, they must possess a potential to grow, reproduce and remain productive under given production condition (Silva *et al.*, 2007). According to Said *et al.* (2001), Boran cattle and their crosses can adapt well to tropical conditions. In addition, Boran cattle are well-adapted to arid and semi-arid environments, which are characterized by high disease and parasite challenges as well as poor feed quality (Kahi, 2015). Kabubo-Mariara (2009) reported that this cattle breed is mostly reared in semi-dry terrains, which are dominated by defective rainfall, with poor vegetation quality. In a study conducted by Bradley *et al.* (1996), comparing the African and European cattle breeds, the Boran breed was credited to have great heat resilience; with numerous sweat glands that are bigger compared to those of *Bos taurus* and a skin surface is through existence of additional folds.

2.2.2 Growth performance of the Boran cattle

The significance of growth in farm animals has received an adequate attention from many researchers (Arthur *et al.*, 2001; Lawrence *et al.*, 2002). Growth performance of cattle is of great economic importance to farmers (Bures and Barton, 2012). Growth traits directly influence carcass characteristics, reproduction and milk production traits (Burrow, 2001). Haile *et al.* (2011) reported that, Ethiopian Boran has better growth performance than other breeds. Several researchers reported that Boran cattle have better growth and weaning weight, average daily gain and yearling weight compared to many breeds as shown in Table 2.1. In addition, DAGRIS

(2007) reported that advancement of management and viable selection, the performance of Boran cattle has been considerably developed.

Table 2.1: Comparison of growth performance traits among different cattle breeds

Breeds	BTW (kg)	WW (kg)	ADG (g)	YWT (kg)	BW (kg)
Boran	22.9	95.2	401.4	129.3	304
Fogera	21.9	92.9	-	125.2	-
Begait	22.6	92.0	385.3	124.5	-
Horro	19.9	88.0	377.6	123.0	-
Brahman	30	162	-	-	-

BW= birth weight; WW= weaning weight; ADG= pre-weaning average daily gain; YWT= yearling weight; BW= body weight.

Source: Plasse *et al.* (2002); Demeke *et al.* (2003); Demeke *et al.* (2004)

It has been pointed out that Boran cattle have high reproductive performance when compared with other indigenous cattle breeds (Haile *et al.*, 2011). In addition, this breed has been credited of its hardiness, well adapted and under marginal conditions it has a potential to produce a calf every year (Okeyo *et al.*, 1998). On the other hand, Wasike *et al.* (2007) reported that the Boran cattle have extremely good mothering ability. It can provide good quality and adequate milk for its calf until weaning (Ouda *et al.*, 2001).

2.2.3 Maturity

Boran heifers are known to reach puberty at an earlier stage as compared to breeds such as Bonsmara (Maiwashe *et al.*, 2002) and Nguni (Carvalho *et al.*, 1995). Haile *et al.* (2011) reported that under normal circumstances, Boran heifers take 385 days to reach the stage of puberty. In addition, Freetly and Cundiff (1997) stated that crossbred heifers conveying half of the Boran genes, achieved early puberty and had better calving and weaning rates as compared to Brahman sired heifers. The maturity of cattle depends ordinarily on the quality and quantity of feed available (Cardoso *et al.*, 2015), which in turn can determine the market weight and sale of the cattle (Wasike *et al.*, 2009). However, this poses less threat to the Boran cattle since this breed require a comparatively low maintenance need. Likewise, such potential was also validated in a modern experiment which was carried out at the United States Meat Animal Research Centre (USMARC) in Nebraska (Haile-Mariam *et al.*, 2006).

2.2.4 Feed utilization

National Research Council (2001) reported that the Boran cattle have a tremendous rumen capacity and excellent complexity of the body that encourages it to be effectively fattened from the natural pastures, without any additional feed supplements. In addition, Okomo-Adhiambo (2002) and Ojango *et al.* (2006) reported that Boran cattle is a good browser and is capable to survive and produce under environments with low quality feed. Such reports were substantiated by the records kept in the European and the Boran cattle feedlot at Lanet in Kenya. It was noted that crossbred Boran cattle grew 31% faster on the high concentrate proportions (76% concentrate), however had no advantage compared to the pure Boran under low (33% concentrate) and medium (50% concentrate) rations (Haile-Mariam *et al.*, 2006).

2.3 Tick loads and Tick counts

Ticks are widely distributed around the world (Magnarelli, 2009; Navas *et al.*, 2009), but, they are usually more prevalent in countries with warm, humid or moist climates, since they need a certain amount of vapor in the air in order to undertake transformation. More or less 80% of the world's fauna are ixodid ticks (683 species), Argasidae ticks (183 species) and Nuttallidae (1 species) (Horak *et al.*, 2002). Of these ixodid ticks, approximately 75 are present in South Africa (Horack *et al.*, 2002). According to Moyo and Masika (2009), the conditions that are more conducive for tick multiplication are mostly in the spring and summer seasons, hence, cattle experience the high tick infestation during this period.

Mans and Neitz (2004) reported that ixodids have a potential to remain on the soil and vegetation for a longer periods, and actively pursue the hosts when the season is appropriate. Argasids are known to be very resistant to starvation (Sonenshine *et al.*, 2002; Vial, 2009) and are capable of survival for numerous years without feeding (Mans and Neitz, 2004). It is, however, also reported that besides the two major families, there is a monotypic third tick family (Nuttallidae), which possesses features of both soft (Argasidae) and hard ticks (Ixodidae) (Latif *et al.*, 2012; Manzano-Román *et al.*, 2012).

Several studies have been conducted to determine tick loads of various cattle breeds in different provinces of South Africa (Baker, 1989; Flourie and Horack, 1991; Hlatswayo *et al.*, 2002; Mbatia *et al.*, 2002, Muchenje *et al.*, 2008a; Moyo and Masika, 2009; Marufu *et al.*, 2011; Katiyatiya *et al.*, 2015). In a study conducted by Moyo and Masika (2009), it was reported that the most widely recognized tick species in the provincial regions of the Eastern Cape are *Amblyomma hebraeum*. These observations concur with Muchenje *et al.* (2008b) who reported *Amblyomma hebraeum* and *Boophilus annulatus* as the predominant tick species in the Eastern

Cape Province. Furthermore, Muchenje *et al.* (2008b) highlighted that the least prevalent tick species were *Hyalomma spp.* These findings correspond with results later reported by Katiyatiya *et al.* (2015) who also found the same tick species in their study. Horack *et al.* (2000) reported that each tick species has its own favored hosts, geographical distribution and seasonal existence.

2.3.1 Seasonal abundance of ticks on cattle

Danger elements that have been recognized to impact the event of tick borne diseases (TBDs) includes the appropriation and abundance of tick vectors, and also the movement of cattle populace (Bakheit and Latif, 2002; Salih *et al.*, 2007). The understanding of the abundance of ticks is based on the tick's life cycle, which interchanges between the grounds (Osegboka, 2012). However, it has not yet been fully addressed how the long-term pattern of a variable would affect the complete life cycle of ticks in the field and their capacity to endure in the field as permanent populaces (Estreada-Peña and de la Fuente, 2014). On the other hand, Estrada-Peña and de la Fuente (2014) reported that climate affects the seasonal action and ticks survival. Furthermore, the microclimate in the layers of vegetation populated with ticks is a critical variable directing the abundance of their populace.

According to Hoffmann (2010), climatic conditions vary from one environment to the other and these conditions differ from one season to another. In addition, Katiyatiya *et al.* (2014) reported that environmental variations caused by seasonal changes leads to both desirable and undesirable influences of tick prevalence in an area. Similarly, Gashaw (2005) reported that climatic factors such as temperature, rainfall and relative humidity influences tick loads in an area. The existence and the multiplication of ticks depends on the climatic conditions in an environment (Austin, 2002), hence many tick species are affected by seasonal differences of climate within their habitats (Walker *et al.*, 2003; Estrada-Pena, 2008).

2.4 The effect of ticks on body condition, body weight and coat characteristics on cattle

2.4.1 Body condition score and body weight

Body condition scoring (BCS) portrays the efficient procedure of surveying the level of fatness of animals. This information is vital to evaluate the availability of feed, especially under production systems where the accessibility of feed is not constant, such as those experienced by free ranging animals (Roche *et al.*, 2004). Several authors have documented the association between BCS, body weights (BW) and health (Roche *et al.*, 2004; Roche and Berr, 2006). While, Ndlovu (2007) noted that the body weights are normally utilized on the grounds that they are less demanding and faster to perform, Rossi and Wilson (2006) reported that BW can also be used as an alternative tool for cattle farmers who cannot weigh their cattle.

However, BCS and BW of animals can be affected by various factors. Ticks and tick-borne diseases are one of the factors that alter the BCS and BW within different seasons (Norval *et al.*, 1997). Mapiye *et al.* (2007) and Mirkena *et al.* (2010) reported that there is a rapid weight loss related to parasitic abundance of ticks on cattle, which may result in death of some animals depending on the severity of the infestation. Likewise, Marufu *et al.* (2010) reported lower weights during the hot-dry season, when the tick infestation in cattle was very high.

2.4.2 Coat characteristics

Coat characteristics include coat smoothness, coat colour, coat thickness and hair length; which are reported to affect tick infestation, especially in cattle reared on range-lands (Foster *et al.*, 2007; Marufu *et al.*, 2011; Ibelli *et al.*, 2012). According to Mapholi *et al.* (2014), tick loads are affected by numerous coat characteristics, and many of them are heritable (Regitano and Prayaga, 2010). Gasparin *et al.* (2007) and Machado *et al.* (2010) also reported that cattle with

smooth, short and light coloured coats have fewer ticks than cattle with rough, long and dark coloured coats. Similar findings were reported by Verissimo *et al.* (2002), who stated that smooth coated animals have lower tick infestation as compared to rough coated animals. Short and smooth coat expose ticks to the external environment and makes it difficult for ticks to stay for longer periods (Martinez *et al.*, 2006). Bernabucci *et al.* (2010) and Abdalla and Hassan (2010) reported that dark-skinned animals retain more heat compared to lighter-skinned animals. In addition, Fraga *et al.* (2003) reported that animals with warm skins tend to have high tick loads.

2.5 Common tick species in South Africa

According to Jongejan (2007), ticks transmit several pathogenic micro-organisms from diseased cattle to healthy ones. They draw blood (Gates and Wescott, 2000), damage hides and skins (Mtshali *et al.*, 2004), and most importantly ticks transmit disease from one cattle herd to another (Jonsson, 2006; Moyo and Masika, 2009). The common types of ticks in South Africa are summarized in Table 2.2.

Table 2.2: Tick species, their descriptions, the pathogens transmitted in cattle and the diseases they cause

Tick species	Description	Pathogens transmitted	Disease caused
<i>Rhipicephalus (Boophilus) spp.</i>	Bluish ticks with hexagonal basis capitulum, short compressed and ridged palps, faint/absent anal groove	<i>Babesia bigemina</i>	Babesiosis
<i>Rhipicephalus appendiculatus</i>	Brownish, reddish-brown or dark ticks with short palps and reddish-brown legs	<i>Theileria parva</i>	Theileriosis
<i>Rhipicephalus evertsi evertsi</i>	Medium sized, beady-eyed, dark brown ticks with reddish-orange legs	<i>Anaplasma marginale</i>	Anaplasmosis
<i>Hyalomma spp.</i>	Dark-brown-bodied ticks with numerous punctations on the scutum and long, banded legs	<i>Anaplasma marginale</i>	Anaplasmosis
<i>Amblyomma hebraeum</i>	Brightly ornamented ticks, with eyes and long, robust mouthparts	<i>Ehrlichia ruminantium</i>	Erlichiosis

Source: Coetzer *et al.* (1994), Makala *et al.* (2003), Iseki *et al.* (2007) and Kocan *et al.* (2010)

2.6 Attachment sites of ticks in cattle

Ticks have their own preferences (in terms of where they attach in animals) like any other living organisms. They prefer certain positions on animal's body to secure as their natural habitats (Marufu *et al.*, 2011; Katiyatiya *et al.*, 2015). Animals are usual hosts of ticks (Horak *et al.*, 2002) and according to Muchenje *et al.* (2008a), they favor warm, humid and concealed spots in an animal's body. On the other hand, Web and David (2002) observed high tick burdens on the belly which commonly has longer hairs. This clearly indicates that ticks attach easily in sites with longer hairs compared to shorter hairs. In addition, Marufu *et al.* (2011) also observed high tick loads in the perineum and belly regions. This supports the fact that ticks are more comfortable in areas that are not fully exposed to the external environment. Furthermore, Stachurski (2008) conducted a study on tick counts and he noticed high tick loads on the limb ends and near the hoof tips.

2.7 Tick-induced stress

The definition of stress in natural terms was first introduced by Selye (1936). According to Moberg and Mench (2000), Tolleson (2007), Kim *et al.* (2011), and Lobão (2011) the concept of stress is risk to homeostasis. Furthermore, Lobão (2011) and Chulayo *et al.* (2012) reported that such hazards can result in high secretions of enzymes and hormones to the circulatory system and consequently, lead to poor performance and meat quality. Similarly, tick bites are reported to be one of the major problems which result in loss of production since they cause discomfort (stress) (Shaw, 2002; Ghosh *et al.*, 2007) and irritation for the animal (Department of Agriculture, 2008).

Many scientists reported that stress results to a comparative increase in stress hormone levels (Grandin, 1997; Moolchandani *et al.*, 2008; Singh, 2010; Comin *et al.*, 2012; Moya *et al.*, 2013)

and simultaneous changes in numerous aspects of immunity (Tolleson, 2007). Moreover, Venkatraman and Pendergast (2002) stated that stress result in high cortisol; neutrophilia; lymphopenia; decrease in granulocyte oxidative burst, nasal mucociliary clearance, natural killer cell activity, lymphocyte proliferation, the delayed-type sensitivity response, the production of cytokinines in response to mitogens, and nasal and salivary immunoglobulin A levels, and increases in blood granulocyte and monocyte phagocytosis and pro- and anti-inflammatory cytokines.

2.7.1 Animal stress-related bio-chemical compounds

2.7.1.1 Cortisol

According to Choi *et al.* (2012), cortisol (also known as the steroid hormone) is a file of mental anxiety, which is delivered in the cortex of the adrenal organs situated on top of every kidney. Cortisol is usually released when an animal is stressed (Chulayo *et al.*, 2012; Hart, 2012; Jama *et al.*, 2015), hence it is known as a physiological stress indicator (Moya *et al.*, 2013). Aggarwal and Singh (2010) highlighted that stress markers increases when an animal is exposed to temporal stressful conditions and decreases over continuing occurrences. Furthermore, they reported that these conditions results in reduced body heat production in an animal's body. Levels of cortisol differ within and across breeds and the way stress is induced (Moolchandani *et al.*, 2008). There are basically four ways in which stress can be induced in animals as noted by Bayazit (2009), those are: physiologically, physical, environmentally and chemically or emotionally. In addition, these all influences production rates of cortisol (Moolchandani *et al.*, 2008).

2.7.1.2 Creatine Kinase

Stress in animals results in stimulation of biochemical body responses (Melesse *et al.*, 2011). Creatine Kinase (CK) is a biochemical body response that is caused by the tissue damage and poor muscular tissue reperfusion (Vojtic, 2000). Its classic effect is to generate Adenosine Triphosphate to maintain energy homeostasis (Diene and Storey, 2009; Chulayo and Muchenje, 2013). In animal production CK is generally utilized as an indicator of physical stress (Mpakama *et al.*, 2014). Its increase is mainly caused by an increased demand of ATP in the blood that is used to accelerate respiration and metabolism during stressful conditions (Chulayo and Muchenje, 2013). The CK activity alters with seasons, depending on the stressors an animal is exposed to. Several authors have reported that CK levels increases during the summer season, when the temperatures are high (Kannan *et al.*, 2000; Miranda-de la Lama *et al.*, 2012). Tadich *et al.* (2005) reported high CK levels in steers transported during the summer season, when the temperatures were high. Likewise, Melesse *et al.* (2011) reported high CK levels during the hot-wet season, when the high physical activity that leads to muscular damage was experienced.

2.7.1.3 Haematological parameters

Haematological parameters such as Red Blood Cells (RBC) (Koubkova *et al.*, 2002), White Blood Cells (WBC), Haemoglobin (HB) (Sature *et al.*, 2009), Haematocrit (HCT) (Aengwanich *et al.*, 2009), Mean Cell Volume (MCV) (Vengust *et al.*, 2002), Neutrophils (NEU), Lymphocytes (LYMP), Monocytes (MON), Eosinophils (EOS) and Basophils (BAS) are turning to be progressively imperative diagnostic apparatuses for numerous illnesses caused by parasites (Aengwanich *et al.*, 2009; Çetin *et al.*, 2009). In addition, Anderson *et al.* (1999) reported that these parameters are commonly used as stress and welfare indicators of animals during different management processes. Moreover, these HP also indicates the adaptability of animals to hostile

environmental conditions (Koubkova *et al.*, 2002). However, there are various components that can influence the Haematological parameters in animals. These include breed, sex, age and seasonal changes (Çetin *et al.*, 2009). Numerous researchers (Chulayo and Muchenje, 2013; Mpakama *et al.*, 2014; Katiyatiya *et al.*, 2015) have studied the concentrations of different HP in various seasons. Many of them reported that HP differs with seasons, however, high concentrations are mostly found during extreme temperatures (Chulayo and Muchenje, 2013; Katiyatiya *et al.*, 2015).

2.8 Common tick-borne diseases and their effect on production

Several studies have reported that tick-borne diseases (TBDs) pose a greater limitation on livestock production and they have been rated high in relation to their impact on the livelihood of resource-limited farming communities in many countries (Makala *et al.*, 2003; Minjauw and McLeod, 2003; Jongejan and Uilenberg, 2004). Tick-borne diseases also have been reported to have a significant economic influence on people in the communal areas, affecting both their food supply and their daily income (Minjauw and McLeod, 2003). In addition, TBDs are associated with great economic losses which include death and morbidity of livestock (Jongejan and Uilenberg, 2004). Tick borne diseases are the limiting agents of genetic improvement of indigenous livestock breeds, because they inhibit the introduction of more high performing foreign breeds (Simuunza *et al.*, 2011). They also have a significant impact on meat and milk production (Ndlovu *et al.*, 2009), since they reduce growth rate and lead to fertility problems (Minjauw and McLeod, 2003). The most prevailing TBDs of livestock in communal areas of South Africa are anaplasmosis, babesiosis, ehrlichiosis or cowdriosis and theileriosis (Dreyer *et al.*, 1998; Mbatia *et al.*, 2002; Ndhlovu *et al.*, 2009).

2.8.1 Anaplasmosis

Anaplasmosis is classified as a rickettsial disease (Parola, 2006). It is caused by infection with the rickettsial pathogen *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) (Dumler *et al.*, 2001; Kocan *et al.*, 2004; Kocan *et al.*, 2010). According to Dreher *et al.* (2005), *A. marginale* is biologically conveyed by certain tick species and mechanically by other parasitic arthropods and fomites. *A. marginale* is broadly distributed around the world, and it results to illnesses characterized by progressive anemia, weakness, fever, decreased milk yield jaundice, abortion, anorexia, and sometimes death (Dumler *et al.*, 2001; Kocan *et al.*, 2010). Similar clinical signs were observed by Hofmann-Lehamann *et al.* (2004) in a cattle farm in Switzerland, where there was an outbreak of bovine anaplasmosis in August 2002. Anaplasmosis has been reported to be endemic in numerous Mediterranean countries (Cringoli *et al.*, 2002; Shkap *et al.*, 2002; De La Fuente *et al.*, 2004). It has also been reported in France, Austria and in several eastern other European countries (OIE, 2004). De Waals (2000) reported that a large number of cattle production farmers in South Africa arise in the endemic areas of anaplasmosis. In the case of developing preventive measures, serology tests have been developed for the evaluation of anaplasmosis (Barros *et al.*, 2005).

2.8.2 Babesiosis

Babesiosis is one of the most common tick-borne diseases (TBD) in South Africa, which has been reported to have a substantial economic impact on livestock and companion animals particularly in arid and semi-arid lands (de Waal and Cambrink, 2006). Babesiosis is caused by an infection from two bovine protozoa parasites namely; *Babesia bovis* and *Babesia bigemina* (Makala *et al.*, 2003; Iseki *et al.*, 2007). These two parasites are known to induce similar clinical signs, characterized by fever, anemia and icterus in the diseased cattle (Makala *et al.*, 2003).

Several studies used polymerase chain reaction method for diagnosis of this disease (Smeenk *et al.*, 2000; Almeria *et al.*, 2001; Oliveira-Sequeira *et al.*, 2005). However, this strategy has not generally been adjusted for research centre conclusions for economic and functional reasons (Oliveira-Sequeira *et al.*, 2005).

2.8.3 Ehrlichiosis/ Cowdriosis

Ehrlichiosis is a tick-borne disease caused by intracellular bacteria known as *Ehrlichia ruminantium* (Makala *et al.*, 2003). The disease is conveyed by hard ticks (Loftis *et al.*, 2008), particularly of the genus *Amblyomma*. In the United States, Ehrlichiosis is known to only affect dogs and people (Buller *et al.*, 1999). However, in African countries and the Caribbean, *Ehrlichia ruminantium* causes infection in ruminants, fluctuating from mellow febrile illness to lethal heart-water disease (Allsopp *et al.*, 2007). This disease becomes more serious when predisposed animals are relocated from *Ehrlichiosis*-free to *Ehrlichiosis*-infected regions (Allsopp *et al.*, 2007). About 150 000 000 animals have been reported to be at risk of *Ehrlichiosis* in Africa. Of these animals, approximately 82% were adult cattle (Allsopp, 2010). A proficient vaccine is only cost-effective technique by which control may be attained. Therefore, future research should be done to provide efficient vaccines that are not expensive.

2.8.4 Theileriosis/ East Coast fever

Theileriosis (East Coast Fever) is a tick-borne disease caused by infection with a protozoan parasite called *theileria pava* (Muraguri *et al.*, 1999; Dobbelaere and Mckeever, 2002). It is transmitted by ixodid ticks (Perera *et al.*, 2013). This protozoan parasite is known to affect a range of domestic and wild animals, particularly ruminants (Dobbelaere and Mckeever, 2002). The clinical symptoms of theileriosis may include haemolytic anaemia, depression, lymph node swelling, tachypnea, dyspnea, pneumonia jaundice, abortions, still births and metritis, and

sometimes death (Dobbelaere and Mckeever, 2002; Izzo *et al.*, 2010; McFadden *et al.*, 2011). Theileriosis is one of the significant tick-borne diseases that pose a serious challenge to commercial cattle producers in Africa and it is endemic in regions like Eastern Congo, Tanzania, Sudan and South Africa (Editorial, 2007). Effective molecular diagnostic and analytical tools have been used to explore the incidence, distribution and possible control of this disease (Islam *et al.*, 2011; Eamens *et al.*, 2013). Infection and treatment method was first introduced in early 1960, in South Africa, and it is widely used by the United Nations and other countries (Editorial, 2007).

2.9 Control methods of ticks

Ticks and their related diseases are a main constraint to livestock production all over the world (Moyo and Masika, 2009), particularly in Africa where financial variables oversee the degree to which livestock farmers can control ticks (Mugabi *et al.*, 2009). Willadsen (2006) reported that the strategic way to fight TBDs is through the control of ticks. Various tick control schemes have been proposed (Pegram *et al.*, 2000; Jonsson, 2004; Peter *et al.*, 2005) and these include the use of acaricides (Willadsen, 2006), biological control, pasture management (Mandal *et al.*, 2013) and the use of resistant breeds (Marufu *et al.*, 2011; Manjunathachar *et al.*, 2014).

2.9.1 Acaricides

The direct application of acaricide to the animal host has been widely used throughout the world (Mandal *et al.*, 2013). Direct application of acaricides can be achieved through dipping, spraying, spoton, pour-on, horn bands, hand dressing and injections (Manjuathachar *et al.*, 2014). However, if these are incorrectly used, they lead to drawbacks, such as development resistance, environmental pollution, and residues in meat, milk, hides, skin and natural toxicity (Willadson, 2006). In addition, acaricides are expensive and oblige consumption in foreign currencies, thus

constituting noteworthy economic strain on the advancement of the cattle industry, especially in emerging countries (Jongejan and Uilenberg, 2004). Therefore, all the afore-mentioned factors permit different tick control methods (Sudhakar *et al.*, 2013).

2.9.2 Biological control

This is the method whereby predators (e.g beetle spiders and ants) or parasites (e. g insects, mites, and nematodes) are introduced in a habitat of others to manage the control of target parasites, by decreasing the population of growth of the latter below the threshold (Parola and Raoult, 2001; Manjuathachar *et al.*, 2014). Most African countries use chickens (*Buphagus africanus*) as the natural predators of ticks (Soulsby, 1982; Duffy *et al.*, 1992). Osegboka (2012) reported that guinea fowl expends mass amounts of ticks, as shown in Figure 2.1.



Figure 2.1: Natural predators of ticks

(Source: Osegboka, 2012)

2.9.3 Pasture management

Fluctuations in management practices helps in reducing tick loads, however, few farmers are practicing this method due to the expenses of hiring more labour (Ikpeze, 2004). Rotational grazing and burning of the grazed pastures are mostly used in African countries to reduce the environmental infestation by ticks (Mandal *et al.*, 2013). Moreover, burning of pastures encourages reduction in tick populace, since some tick species use the blades of grass and vegetation as their habitat (Muhammad *et al.*, 2008).

2.9.4 Use of resistance cattle breeds

Rearing of adaptive and tick resistance breeds has been credited as one of the effective control of tick-borne diseases (Marufu *et al.*, 2011). Mirkena *et al.* (2010) characterized adaptability of an animal as the capacity to survive and reproduce in a defined environment. Zebu cattle are more impervious to ticks than European cattle because of their thick flexible hides secured with short straight, non-regulated hair, and high thickness of sweat glands (Kiss *et al.*, 2012). Additionally, bos indicus breeds are reported to be more resistant to *B. microplus* than B Taurus breeds in tropical regions (Mattioli *et al.*, 2000; Shyma *et al.*, 2013). Muchenje *et al.* (2008a, b) reported that Nguni cattle are environmentally friendly animals, having tick resistance abilities. Hence, the Nguni cattle Society of South Africa dishearten the dipping of the Nguni cattle (Hobbs, 2005).

2.10 Summary

The interest in the ecological adaptation of cattle and versatile qualities in various environments is the consequence of several factors that incorporate climatic changes, the need to enhance animal production, and elevated consumer's attention to utilization of chemicals in cattle, which in turn affect the meat products. The resistance of cattle to ticks is of specific importance as it is

connected with both welfare and production issues. Ticks and tick-borne diseases are the central constraints to cattle farming, especially to emerging communal farmers. Moreover, there are still unresolved contradictions amongst farmers as into which breed is most resistant to ticks and TBD's. Therefore, information with respect to tick species, tick loads, seasonal change in haematological parameters of Boran cattle is limited. Hence, the current study aims at focusing on the seasonal variations in tick loads, coat characteristics, temperature-humidity index and blood metabolite profiles of pasture based Boran caows.

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Chapter 3: Effects of physical and production traits on tick loads at different anatomical positions of Boran cows reared in a semi-arid rangeland of South Africa

Abstract

A study was conducted to determine the effects of physical and production traits on tick loads at different anatomical positions of Boran cows reared in a semi-arid rangeland of South Africa. A total of 20 Boran cows from Edendale farm were used in this study. The experimental cows of different age groups were randomly selected from the Boran herd. The cows' physical traits (body length and body condition score), production traits (body weight) and coat characteristics (coat score, and hair length) were recorded at the beginning and end of the post-rainy, cold-dry, hot-dry and hot-wet seasons. Ticks were observed and counted from different anatomical sites, namely anterior (neck, head, ears, and around eyes), ventral (belly, udder, and limbs) and posterior (back and under the tail). The tick species observed from the Boran cows were *Rhipicephalus evertsi evertsi* (34.58%), *Amblyomma hebraeum* (43.35%), *Rhipicephalus simus* (4.48%) and *Rhipicephalus (Boophilus) decoloratus* (17.59%). Significant differences were observed in tick burdens at different anatomical sites, with posterior position having the highest tick loads during the post-rainy (15.05 ± 1.177), cold-dry (4.55 ± 1.177) and hot-dry (12.40 ± 1.177) seasons. The ventral position recorded the highest tick loads during the hot-wet season. It was also observed that the cattle had long hairs ($P < 0.05$) during the cold-dry season (16.33 ± 0.842 mm). Furthermore, coat score ($r = -0.188$) and hair length ($r = -0.080$) were observed to be negatively correlated ($P < 0.05$) with the tick counts, while coat scores were positively correlated ($P < 0.05$) with hair length. It was concluded that physical and production traits had significant effects on tick loads at different anatomical positions of Boran cows.

Key words: *Amblyomma*, anatomical positions, physical traits, production traits, *Rhipicephalus*, tick loads

3.1 Introduction

Approximately 80% of South African land is regarded as rangeland which is classified under arid or semi-arid land (ASAL) (Van den Berg and Kellner, 2005). These lands are characterized by high temperatures, disease, and parasite challenges (Mpfu, 2002). These make land un-suitable for crop production since crops require relatively more resource inputs in order to grow (Okeyo *et al.*, 1998). On the other hand, livestock production can be extensively done on the ASAL. Consequently, people residing on ASALs depend more on livestock for their livelihood and food security (Thornton, 2010) than on crop production. Cattle production in ASAL is of high potential because of the moderate to low rainfall availability. However, cattle in these lands are usually exposed to parasites and tick borne diseases which negatively affect their performance and production (Foster *et al.*, 2008; Katiyatiya *et al.*, 2015).

Ticks are one of the major problems that limit livestock production in most arid countries (Jonsson, 2006; Rony *et al.*, 2010) and have been reported to affect about 1.4 billion cattle worldwide (FAO, 2011). Ticks transmit disease such as redwater, heartwater, anaplasmosis, theileriosis and gallsickness which pose a health problem to animals and humans. In cattle, ticks have been reported to reduce live weight gains, milk and meat production, fertility and as well cause deaths (de la Fluente *et al.*, 2007; Muchenje *et al.*, 2008a). Currently, the most commonly used method to control ticks, is the application of acaricides (Moyo and Masika, 2009; Katiyatiya *et al.*, 2014). However, improper and extended use of these acaricides results to an improvement of tick resistance. Thus, the selection of adaptive breeds which exhibit increased overall resistance, high reproductive potential and maximum performance is recommended.

The Nguni breed is widely reared by farmers in South Africa (Marufu *et al.*, 2010; Katiyatiya *et al.*, 2014). This is mainly because of its pliability to tick-borne diseases (Muchenje *et al.*, 2008a), high reproductive performance (Ndlovu *et al.*, 2007) and its ability to adapt in poor grazing areas (Muchenje *et al.*, 2008a, b; Musemwa *et al.*, 2010). However, its small body size has resulted in low returns on the market. The Boran is another indigenous breed of interest; it is well adapted to arid and semi-arid environments characterized by high temperatures, disease, and parasite challenges as well as poor feed quality (Mpofu, 2002). It is also known to be a tick resistant breed, because of its short hairs and excessively smooth coat characteristics amongst other breeds (Ojango *et al.*, 2006).

Despite these characteristics, there is a dearth of information on the influence of physical traits on tick loads in Boran cattle. Maiwashe *et al.* (2002) reported that animals with longer body length have more tick burdens than the ones with shorter body length. Information on tick infestation of cattle can be utilized to assess and contrast the level of resistance of breeds to ticks (Mirkena *et al.*, 2010). To estimate the level of resistance in Boran cattle breed, a study was done on the effects of physical and production traits on tick loads of Boran cows. It was hypothesized that physical and production traits have no effect on tick loads of Boran cows reared in a semi-arid rangeland of South Africa.

3.2 Materials and Methods

3.2.1 Ethical Consideration

The experiment was approved by the Ethical Clearance Committee (Ethical clearance reference number: MUC311SNTI01) of the University of Fort Hare and all trial procedures were as per the moral standards of experimentation built up by the committee of ethics on Animal Use of the Society for the Prevention of Cruelty to Animals (SPCA).

3.2.2 Study Site

The experiment was conducted from late cold-dry (August 2015) to early cold-dry (June 2016) seasons at the Edendale farm which is situated at Fort Beaufort, in the Bisho thornveld vegetation of the Eastern Cape (Rutherford *et al.*, 2012). The farm is located 429 metres above sea level, and 32°54'18.72"S and 26 °37'41.77"E in the Amathole District Municipality. It receives an average rainfall and temperature of 498 mm and 18.3 °C, respectively. The veld type is classified as a sweetveld, with a nutritive value that is partially good (Acocks, 1988). The vegetation is composed of numerous tree, shrub and grass species. *Acacia karoo* is the most abundant tree species followed by other woody species such as *Ziziphus mucronata* and *Scutia indica*. *Themeda triandra* is the most dominant grass species with very few shrubs such as *Grewia* spp. The region is generally flat with delicate inclines (Acocks, 1988).

3.2.3 Study Animals

A total number of 20 Boran cows between the ages of 2 and 8 years were utilized in this experiment. Experimental cows were selected according to their age as shown in Table 3.1 below.

Table 3.1: Age of Boran cows used in the experiment

Age (years)	Number of cows
8	1
6	1
5	4
4	5
3	7
2	2

The experimental animals solely depended on the natural veld, with no supplementary feed. Existing management practices within the farm were used throughout the experimental period. Veld condition was partially good at the beginning of the trial (August 2015) and it improved rapidly during the summer rains. At the end of the trial (June 2016) the veld condition was slowly declining. The experimental animals were not dipped throughout the study period. However, heavily infested animals were treated.

3.2.4 Body weight, body condition score (BCS) and body length

Body weights were measured twice in a season (beginning and at the end) for four seasons using a cattle scale (LS4, Taltec, South Africa). Body conditions were visually appraised twice a season, by the same independent assessor throughout the experimental period. A 5-point scale was used to score the Boran cows with score 1 being thin and a score 5 being very fat or obese (Osoro and Wright, 1992). Body lengths were measured using a 5m flexible measuring tape.

3.2.5 Tick counts, types and location

Experimental animals were individually restrained in a crush pen before inspected for tick infestation. Tick counts were done from different anatomical sites. These include the posterior (back and under the tail), ventral (belly, udder, and limbs) and anterior (includes neck, head, ears and around eyes) positions of each animal. Two well-trained enumerators were used for tick counts. Adult ticks were collected and transferred separately in bottles containing (70%) ethyl alcohol for further identification.

3.2.6 Coat score and hair length

Coat scores were assessed visually by the same independent assessor throughout the experimental period. The coat of each animal was scored using a 1-5 scale based on the level of smoothness of the coat, with; 1 being excessively smooth coat and 5 being excessively wooly coat (Taylor *et al.*, 1995). A shaving stick was used to gather hair samples from the upper part of the tail. Hair samples were then stored in plastic bags and the measurement of hair length (mm) was done using a 30 cm ruler that was set on a spotless white A4 paper. A total of 15 hairs were randomly selected and measured for each animal, and then the average was calculated and recorded.

3.2.7 Statistical analysis

The data for body condition score (BCS), coat score (CS), hair length (HL), body length (BL) and tick count (TC) were normally distributed. The effect of body length, body weight, body condition score, coat score, hair length, season and anatomical sites on tick counts were analysed using PROC MIXED model procedures for repeated measurements (SAS, 2003). Pearson correlation between x-variables (body length, body weight, body condition score, coat score and hair length) and y-variable (number of ticks) were determined using PROC CORR in SAS

(2003). The frequencies were determined using PROC FREQ (SAS, 2003). The output were regarded significant at $P < 0.05$.

3.3 Results

3.3.1 Effect of season on tick loads and different anatomical sites

A total of 1631 ticks from four species, namely *Rhipicephalus evertsi evertsi*, *Amblyomma hebraeum*, *Rhipicephalus simus* and *Rhipicephalus (Boophilus) decoloratus* were counted from the 20 examined Boran cows in this study. Of these, *Amblyomma hebraeum* was the most common tick species as summarized in Table 3.2. The result of seasonal prevalence of ticks is shown in Figure 3.1. The highest tick counts were observed during the hot-wet season followed by post-rainy, hot-dry and least in cold-dry season. Significant differences were observed in tick burdens at different anatomical sites, with the posterior position having the highest tick loads during post-rainy (15.05 ± 1.177), cold-dry (4.55 ± 1.177) and hot-dry (12.40 ± 1.177) (Table 3.3). However, during the hot-wet season, the ventral position had the highest tick load (11.25 ± 1.177) compared to posterior (10.05 ± 1.177) and anterior (3.25 ± 1.177) positions.

Table 3.2: Abundance of different tick species counted in Boran cows ($n=20$)

Tick type	Total number	Percentage (%)
<i>Rhipicephalus evertsi evertsi</i>	564	34.58
<i>Amblyomma hebraeum</i>	707	43.35
<i>Rhipicephalus simus</i>	73	4.48
<i>Rhipicephalus (Boophilus) decoloratus</i>	287	17.59

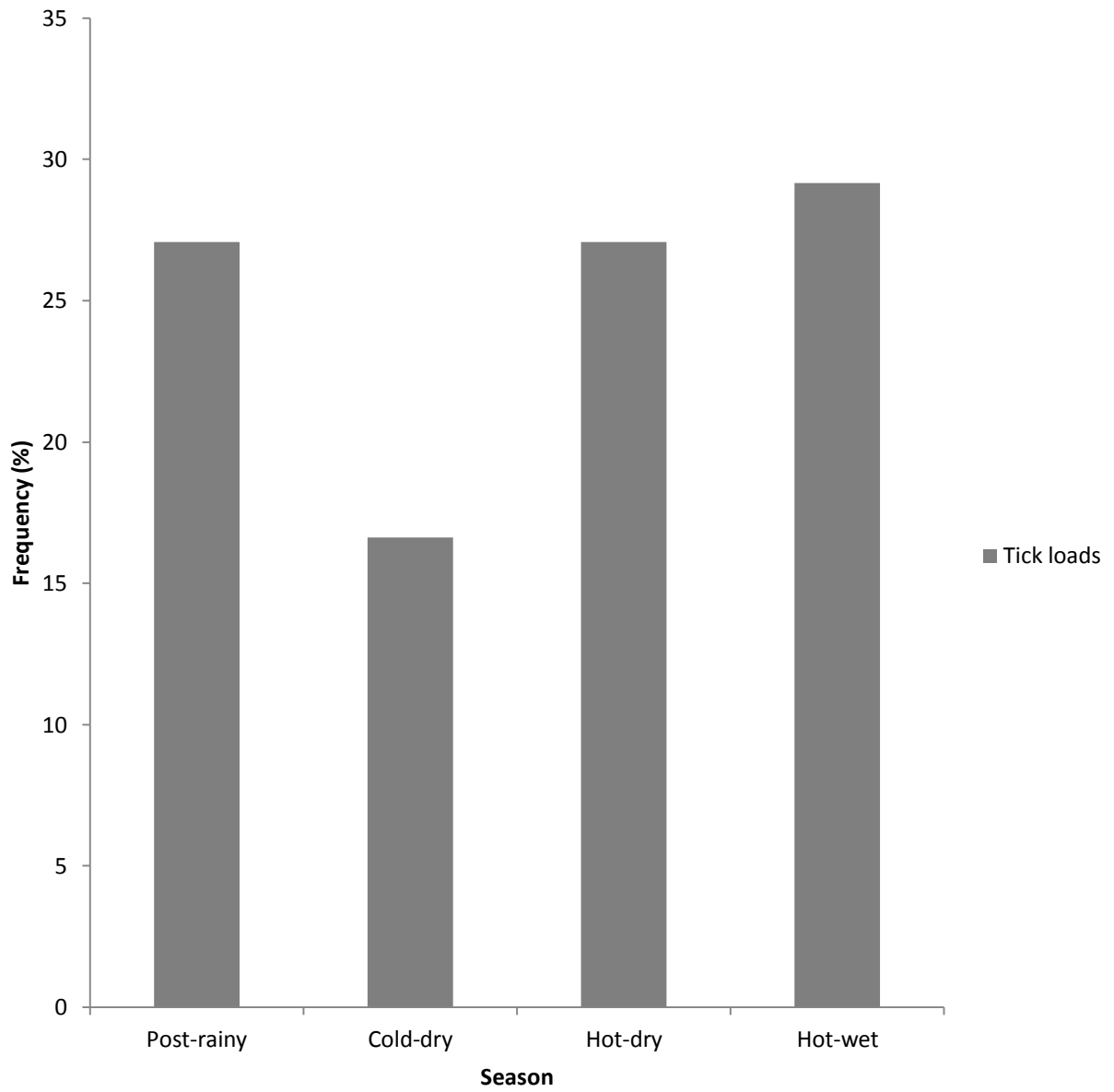


Figure 3.1: The tick counts in Boran cows ($n=20$) across different seasons

Table 3.3: Seasonal least square mean (\pm Standard error) tick loads in anterior, ventral and posterior positions of Boran cows (n=20) raised on natural pastures

Season	Position	Tick loads
Post-rainy	Anterior	2.25 ^{de} \pm 1.177
	Ventral	8.05 ^c \pm 1.177
	Posterior	15.05 ^a \pm 1.177
Cold-dry	Anterior	0.80 ^e \pm 1.177
	Ventral	3.20 ^{de} \pm 1.177
	Posterior	4.55 ^d \pm 1.177
Hot-dry	Anterior	2.15 ^{de} \pm 1.177
	Ventral	8.55 ^c \pm 1.177
	Posterior	12.40 ^{ab} \pm 1.177
Hot-wet	Anterior	3.25 ^{de} \pm 1.177
	Ventral	11.25 ^{bc} \pm 1.177
	Posterior	10.05 ^{bc} \pm 1.177

^{abcde} Values with different superscripts in the same column differ significantly ($P < 0.05$); Anterior (neck, head, ears and around eyes); Ventral (belly, udder and limbs); Posterior (back and under the tail)

3.3.2 Effect of season on body weight, body condition score, coat score and hair length

The results revealed a lower body weights during the hot-wet (440.84 ± 5.695) than cold-dry (452.69 ± 6.889) season. Seasonal change in body condition scores of Boran cows is shown in Figure 3.2. The body condition score of 3.5 dominated throughout the four experimental seasons (Figure 3.2). There was a significant difference in hair lengths of Boran cows across the seasons. Longer hair lengths were observed during the cold-dry season (16.33 ± 0.842 mm), followed by hot-dry (14.54 ± 0.691 mm), post-rainy (13.27 ± 0.691 mm) and least in hot-wet (10.22 ± 0.696 mm) seasons. As shown in Figure 3.5 and Table 3.5, the average hair lengths of Boran cows were (13.27 ± 0.691 mm) post-rainy, (16.33 ± 0.842 mm) cold-dry, (14.54 ± 0.691 mm) hot-dry and (10.22 ± 0.696 mm) in hot-wet season.

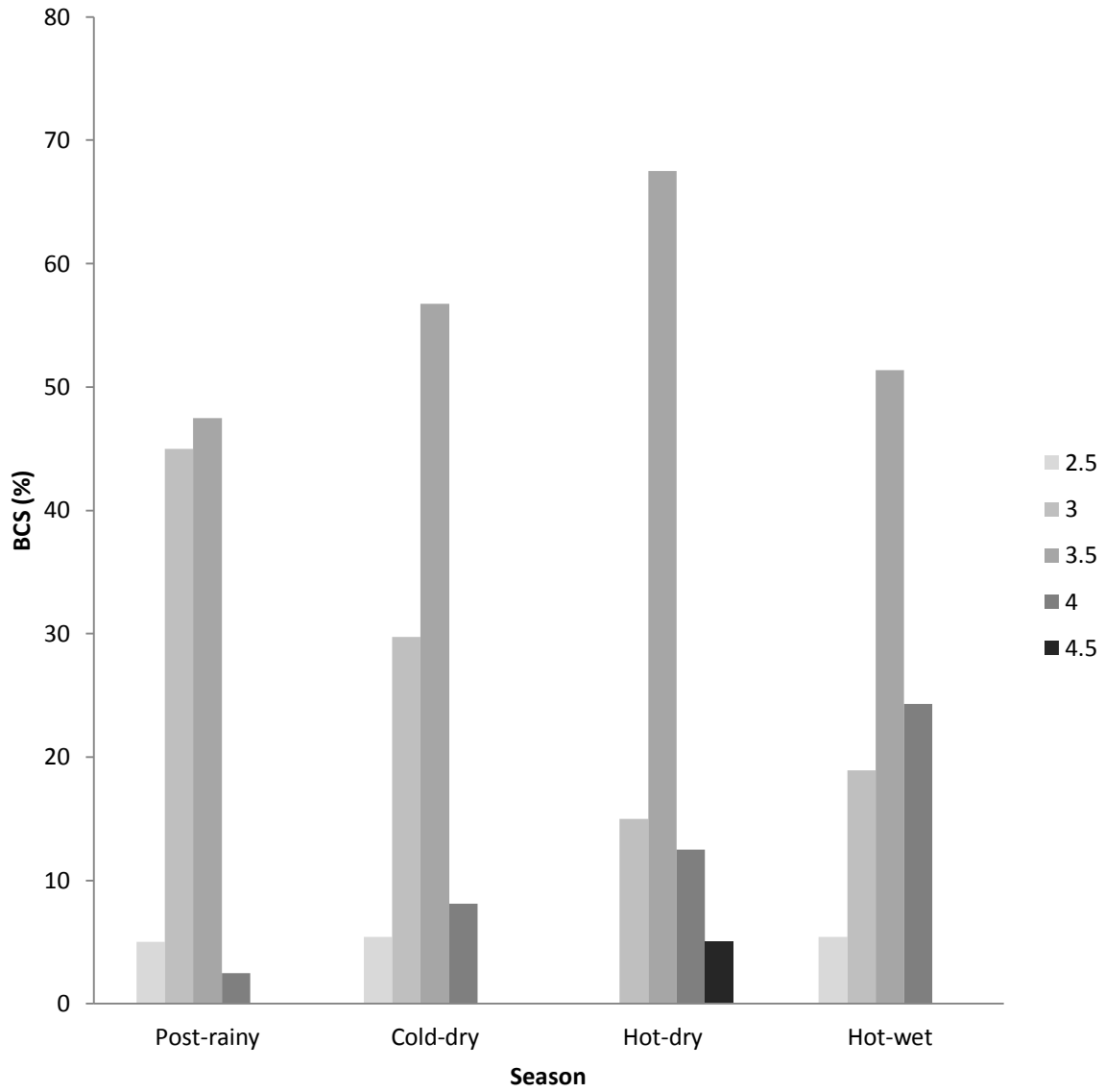


Figure 3.2: Seasonal changes in body condition score (BCS) of Boran cows ($n=20$) raised on natural pastures

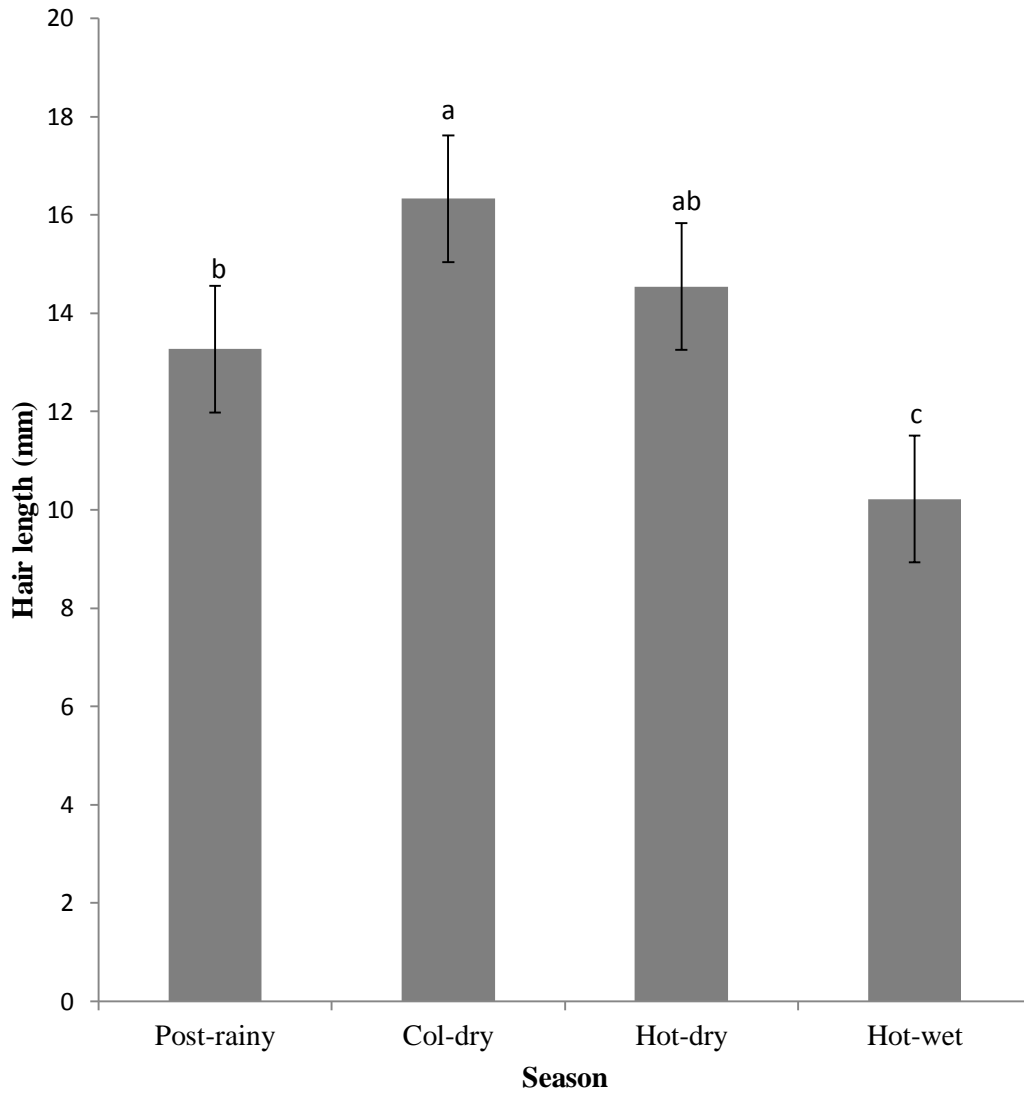


Figure 3.3: Least square mean hair length variations of Boran cows ($n=20$) across different seasons

^{a,b,c} Least square means in the same season with different superscripts differ significantly ($P<0.05$)

Table 3.4: Seasonal least square mean (\pm Standard error) of the body length (BL), body weight (BW), body condition score (BCS), coat score (CS) and hair length (HL) in Boran cows ($n=20$) raised on natural pastures

Variable	Post-rainy	Col-dry	Hot-dry	Hot-wet
BL	3.28 ^a \pm 0.098	3.28 ^a \pm 0.120	3.28 ^a \pm 0.098	3.26 ^a \pm 0.099
BW (kg)	423.22 ^c \pm 5.65	452.69 ^b \pm 6.889	506.53 ^a \pm 5.653	440.84 ^b \pm 5.695
BCS	3.36 ^b \pm 0.032	3.31 ^b \pm 0.039	3.55 ^a \pm 0.032	3.53 ^a \pm 0.032
CS	2.84 ^b \pm 0.033	3.04 ^a \pm 0.041	2.81 ^b \pm 0.033	2.63 ^c \pm 0.034
HL(mm)	13.27 ^b \pm 0.691	16.33 ^a \pm 0.842	14.54 ^{ab} \pm 0.691	10.22 ^c \pm 0.696

^{abc} Values with different superscripts in the same row differ significantly ($P<0.05$)

3.3.3 Correlations among tick counts and coat characteristics

Correlations between tick counts and coat characteristics are shown in Table 3.5. Coat score ($r=-0.188$) and hair length ($r=-0.080$) were negatively correlated ($P<0.05$) with the tick counts. Body length, body condition score and body weight were not significantly correlated with tick counts. The results show that the coat scores were positively correlated ($P<0.05$) with hair length.

Table 3.5: Correlations between tick counts, physical and production traits

Variable	TC	BL	BW	BCS	CS
BL	0.042 ^{ns}				
BW(kg)	-0.022 ^{ns}	0.384 ^{***}			
BCS	0.004 ^{ns}	0.134 ^{***}	0.513 ^{***}		
CS	-0.188 ^{***}	-0.289 ^{***}	0.020 ^{ns}	-0.116 ^{***}	
HL(mm)	-0.080 ^{***}	-0.014 ^{ns}	0.173 ^{***}	-0.008 ^{ns}	0.429 ^{***}

***Significant at (P<0.05), ns not significant

3.4 Discussion

The tick species that were found in the present study were *Rhipicephalus evertsi evertsi*, *Ambloomma hebraeum*, *Rhipicephalus simus* and *Rhipicephalus (Boophilus) decoloratus*. These findings are in line with the reports of Muchenje *et al.* (2008a), Marufu *et al.* (2011b) and Katiyatiya *et al.* (2015) who observed analogous species in cattle. However, Muchenje *et al.* (2008a) and Marufu *et al.* (2011b) found *Hyalomma*, which were absent in the current study. The presence of *A. hebraeum* in this study can be attributed to the influence of extreme drought and dry periods which have been reported to increase their infestation (Estrada-Pena *et al.*, 2008). The Eastern Cape Province apparently provides a perfect habitat for *A. hebraeum*; hence it has migrated and expanded to the inland semi-dry areas of South Africa (Nyangiwe *et al.*, 2011). Therefore, the variation of tick species depends on different factors, and the presence of certain species vary with favorable conditions.

In the present study, high tick infestations were observed during hot-dry and post-rainy seasons. These findings are in agreement with Muchenje *et al.* (2008a) and Marufu *et al.* (2011b) who noticed more tick prevalence during the summer and autumn seasons. Likewise, Pukuma *et al.* (2011) and Lorusso *et al.* (2013) stated that tick infestation increases after the major rains of the year. In addition, Katiyatiya *et al.* (2014) found that 93% of farmers faced tick problems during the summer season. This could be attributed to the fact that these seasons are more favourable for tick multiplication (Moyo and Masika, 2009), since they are characterised by high ambient temperatures and relative humidity. Ticks breed during the warm seasons, when the ambient temperatures are high. However, Andrade *et al.* (1998) reported contrasting results whereby high tick numbers were observed in the dry season. These distinctive findings might be clarified by the impact of environmental factors in the tick's production cycle.

The tick species were observed from all anatomical sites in the current study, however, tick counts varied among the different sites ($P < 0.05$) on each cow's body. Observations from this study showed that ticks preferred the posterior position than other experimental anatomical sites. These findings agree with Muchenje *et al.* (2008b), Marufu *et al.* (2010; 2011b) and Katiyatiya *et al.* (2015) who noted that the perineum position provides a more conducive environment for tick survival. Similar results were reported by Ndlovu *et al.* (2009) who also found the majority of ticks under the tail. These findings contrast with those of Web and David (2002) who found higher tick prevalence on the belly and sternum, which normally has longer hairs. In addition, these authors validated the findings by Verissimo *et al.* (2002) who reported that tick resistance in cattle is related to coat characteristics.

The distinctions in the attachment sites among the observed tick species in this study suggest privileged feeding behaviour within the species. The selection of attachment site of ticks may also be impacted by attractive smells from different predilection sites (Wanzala *et al.*, 2004). Moreover, seasonal variations have been reported to cause alterations in tick abundance (Olwoch *et al.*, 2008). Further observations from this study showed that during the hot-wet season, higher tick abundance was observed from the ventral position as compared to the anterior and posterior sites. This could be attributed to the fact that the ventral position is exposed to the external environment, which can assist in cooling during the extremely hot temperatures. Furthermore, the region under the tail is mainly considered to be warm and moist, therefore, ticks could not withstand such conditions under hot-wet temperatures, hence they were evenly dispersed all over the belly, udder and limb regions of Boran cows. To our knowledge, seasonal change may also influence the host-seeking tick activity and can prompt expanding or diminishing tick abundance. Estrada-Peña and de la Fuente (2014) reported that long phases of extreme

temperatures during the hot-wet season may advance an ascent of the mortality rates in ticks. Hence, the present study demonstrated that ticks scattered all over the ventral region, where there is a high power of air for cooling. Furthermore, a rise in temperature contributes to desiccation through water losses (Silva *et al.*, 2007).

Further observations from this study showed that the measured hairs were longer and curly during the cold-dry season than the other seasons. This was ideal for high tick loads, as it would provide suitable habitat for ticks to hide from predators. However, the tick loads were low during this season. Comparable discoveries with respect to hair length were accounted by Katiyatiya *et al.* (2015) who observed longer hairs during winter which was a preference for keeping the cows warm and allegedly offer a conducive microclimate for ticks. On the other hand, Marufu *et al.* (2011a) reported that short hairs debilitate tick attachment; hence the findings from this study showed high tick loads in the hot-wet season when the hair length was commonly short. The results on hair length are in contrast to those of Taylor *et al.* (2006) and Ibelli *et al.* (2012), who found lower tick counts from animals with short hairs as compared to others with longer hairs. Therefore, to our knowledge, these results confirm that seasonal alterations have greater influence to hair length; which in turn encourages tick infestation in animals.

The differences in coat scores ($P < 0.05$) across different seasons in this study validates that tick loads may be related to coat characteristics (Verissimo *et al.*, 2002). This study ascertained the presence of smoother and shorter coats during the hot-dry season, when the tick counts were also high. These results, however, are in contrast with Bonsma (1981) who reported that smoother coats have a tendency to demoralize tick attachment. The observed positive correlation between coat score and hair length ($r = 0.429$) is in agreement with Marufu *et al.* (2011a) who reported a positive correlation of $r = 0.48$ in the Bonsmara breed. As the coat score increased the hair length

increased as well. On the other hand, hair length and coat scores were negatively related to tick loads ($r = -0.080$ and -0.188 , respectively) in the present study. These results are in contrast to those of Veríssimo *et al.* (2002) who found a positive correlation between these factors. Likewise, Martinez *et al.* (2006) and Machado *et al.* (2010) reported a significant positive correlation between tick loads and coat score. However, the results from the current study discredit the earlier affirmation by Foster *et al.* (2008) that animals with plane smooth coat tend to have lower tick counts than those with wooly coats.

We could not detect any significant relationship between body condition score and tick loads. This can be attributed to the fact that the majority of the experimental animals were rated body condition 3.5 throughout the four different seasons. In addition, the consistent body condition score throughout the four seasons was mainly because of the pasture condition, which remained palatable with adequate nutrition to maintain animals throughout the year.

3.5 Conclusion

The selected physical and productive traits had an effect on the tick loads of Boran cows. Seasonal changes influenced tick species distribution in various anatomical sites of the cows. Smoother coated cows experienced high tick infestations, which were mostly found in the posterior position, except for the hot-wet season where they were evenly distributed in the ventral region of the cows. There were negative correlations between coat score, hair length and tick counts. The Boran cows sustained good body condition score even during the hostile conditions in the cold-dry season. It can subsequently be concluded that Boran breed can contend favourably under South African conditions, however, further research should be done to compare this breed with other indigenous breeds such as the Nguni cattle. Additionally, cardinal use of acaricides for tick control during hot-wet and post-rainy seasons is advocated.

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Chapter 4: Seasonal variations in creatine kinase activity, temperature-humidity index and haematological parameters of Boran cows reared in a semi-arid rangeland of South Africa

Abstract

The objective of this study was to determine the influence of seasonal variations in creatine kinase activity (CK), temperature-humidity index (THI) and haematological parameters (HP) of Boran cows reared in a semi-arid rangeland of South Africa. A total of 15 Boran cows were randomly selected and grouped according to their age as follows: 2-3 years ($n=5$), 4 years ($n=5$), 5-8 years ($n=5$). A palm-sized temperature-humidity meter was used to determine the average temperature and relative humidity values during data collection. Blood samples were taken seasonally (post-rainy, cold-dry, hot-dry and hot-wet) for the analysis of CK activity and HP. Results showed differences ($P<0.05$) in CK activity across various seasons, with higher levels of CK recorded during the hot-dry (246.54 ± 36.324 U/L) and hot-wet (207.98 ± 35.406 U/L) season compared to post-rainy (81.29 ± 37.512 U/L) and cold-dry (157.42 ± 42.452 U/L) season. The highest THI value was observed during the hot-dry (74.12 ± 0.680), followed by hot-wet season (71.59 ± 0.680), post-rainy (69.99 ± 0.680) and least during cold-dry (65.64 ± 0.680) seasons. Seasonal variation had an effect ($P<0.05$) on some HP (HB, HCT, MCV, MCH, EOS, and BAS). Neutrophils (-0.267) and basophils (0.268) were significantly correlated with THI. It was concluded that seasonal variations had an effect on creatine kinase activity, temperature-humidity index and haematological parameters of Boran cows.

Key words: Haematological parameters, seasonal variation, temperature-humidity index

4.1 Introduction

Blood is a unique form of connective tissue composed of elements that consist of different essential elements including red blood cells, white blood cells and platelets (Bacha and Bacha, 2000). An animal's physiological equilibrium (normal state) is known to be maintained by the blood; however, there are many physiological conditions that may alter this equilibrium. Seasonal variation (Feldman *et al.*, 2002) and environmental factors such as ambient temperature and humidity may deviate an animal's equilibrium. On the other hand, stressors such as dehorning, castration (Mellor *et al.*, 2002) and tick infestation (Mans and Neitz, 2004) may possibly alter an animal's normal physiology. Mazzullo *et al.* (2014) reported thermal environment as a major factor that can adversely affect cattle performance, particularly animals of good hereditary makeup.

Temperature-humidity index (THI) is used as an indicator of thermal climatic exposure and stress gauge on cows (Kadzere *et al.*, 2002; Gashaw, 2005; Dikmen and Hansen, 2009). Exposure of cows to extremely hot environment and stressful conditions can stimulates thermoregulatory mechanisms and results in reduced metabolic rates (Abdelatif and Alameen, 2012). Studies have shown that cattle react to stressful settings with increased concentrations of catecholamines and creatine kinase (Vojtic, 2000; Muchenje *et al.*, 2009). Creatine kinase (CK) is one of the physiological stress indicators in animals, which is seen to increase in the blood mainly due to tissue damage and a lack of reperfusion in muscular tissues (Vojtic, 2000) after an animal is exposed to a stressful condition.

Many authors have also examined haematological parameters (HP) and blood cell morphology to determine the health status of an animal and to determine their response to stresses caused by environmental and pathological factors (Afolabi *et al.*, 2010; Wood and Quiroz-Rocha, 2010;

Grünwaldth *et al.*, 2005). The levels of HP such as erythrocytes, leukocytes (Koubkova *et al.*, 2002), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and hemoglobin (HB) (Kumar and Pachaura, 2000) may also indicate the adaptability of a breed to adverse environmental conditions (Wood and Quiroz-Rocha, 2010). Various researchers (Chulayo and Muchenje, 2013a, b; Mpakama *et al.*, 2013; Katiyatiya *et al.*, 2015) have documented welfare indexes such as haematological and biochemical parameters in different cattle breeds in South Africa. Hence, some species evolved endogenous annual rhythmicity as an adaptive mechanism to react in advance of regulating environmental changes associated with the seasons (Piccione *et al.*, 2009). However, there is still dearth of information on haematological parameters of Boran cattle under semi-arid conditions. Therefore, the current study aims to determine the seasonal variation on some haematological parameters and creatine kinase activities of Boran cows reared in semi-arid rangelands of South Africa.

4.2 Materials and Methods

4.2.1 Study Site

Study site is described in Section 3.2.1

4.2.2 Study animals

A total of 15 Boran cows between the age of 2 and 8 years were used in this experiment. Experimental cows were further grouped according to their age as shown in Table 4.1 below.

Table 4.1: Age of experimental animals

Age group (years)	Animal number
2-3	5
4	5
5-8	5

The experimental animals solely depended on the natural veld, with no supplementary feed. Existing management practices within the farm were used throughout the experimental period. Veld condition, in terms of nutrient content, was fairly good at the beginning of the trial (August 2015) and it improved rapidly during the summer rains. At the end of the trial (June 2016) the veld condition was slowly declining. The experimental animals were dipped twice in a season throughout the study period. However, heavily infested animals were treated.

4.2.3 Data collection

4.2.3.1 Tick counts

Animals were properly restrained in a crush pen before inspected for tick infestation. Tick counts were done from different anatomical sites. These include the posterior (back and under the tail), ventral (belly, udder, and limbs) and anterior (neck, head, ears and around eyes) positions of each animal. Two well-trained enumerators were used for tick counts. A total number of ticks in each animal were recorded.

4.2.3.2 Blood sampling

Blood sampling was done twice (beginning and end) in every season for a period of 1 year (from August 2015- June 2016). Two test tubes (one for full blood count and the other for creatine

kinase) with different colors were used to collect blood samples from each animal. These samples were drawn from the jugular vein using an 18 gauge needle attached to a syringe into labeled empty vacutainer tubes. To reduce the variation, blood samples were taken from cattle at the same hours (09-11:30h). The yellow topped tubes containing thrombin solution were used to collect blood for Creatine Kinase (CK) analysis. All blood samples collected from the farm were kept in ice during the transportation for approximately 20 minutes until they were centrifuged at on arrival at the laboratory. At arrival, the blood samples were then centrifuged for 10 minutes at 3500 rpm (Model 5403 Centrifuge, Geratebay Eppendorf GmbH, Engelsdorf, Germany) and were later stored at -21°C. Tubes with lavender/purple top (containing K₃EDTA) were used to collect blood for full blood count (FBC).. Blood analysis for CK levels, Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (HB), Haematocrit (HCT), Mean Cell Volume (MCV), Neutrophils (NEUT), Lymphocytes (LYMP), Monocytes (MON), Eosinophils (EOS) and Basophils (BAS) was done using Model DXC 600 machine (Beckman Coulter, Ireland).

4.2.3.3 Temperature humidity index (THI)

A Palm-Sized Temperature/ Humidity meter was used to measure temperature (T_{min} and T_{max}) and humidity (H_{min} and H_{max}) during data collection. THI was calculated using the readings.

The following formula was used:

$$THI = (1.8 \times T_{max} + 32) - (0.55 - 0.0055 \times RH_{min}) \times (1.8 \times T_{max} - 26) \quad (\text{Bohlouli } et al., 2013)$$

In the formula, T_{max} and RH_{min} are represented in °C and %, respectively.

4.2.4 Statistical analysis

The data was subjected to a one-way repeated measure analysis of covariance (ANCOVA) using the General Linear Model Procedure of SAS (2003) to determine the effect of age and season on

creatine kinase activities and some haematological parameters. Tick loads were used as covariance. Differences in least square means were determined using the Fisher's least significant differences (LSD) method. Least square means were considered statistically different at $P < 0.05$. Correlations among Temperature humidity index and haematological parameters were done using PROC CORR of SAS (2003).

4.3 Results

4.3.1 Effect of season on creatine kinase activities

Results in Figure 4.1 show the seasonal variation in creatine kinase (CK) activities of Boran cows. CK levels during the post-rainy (81.29 ± 37.512) were, however, different ($P < 0.05$) from those of hot-dry (246.54 ± 36.324 U/L) and hot-wet (207.98 ± 35.406 U/L) seasons. In descending order, the CK activities observed in this study across different seasons were (246.54 ± 36.324 U/L) hot-dry, (207.98 ± 35.406 U/L) hot-wet, (157.42 ± 42.452 U/L) cold dry and (81.29 ± 37.512 U/L) post-rainy.

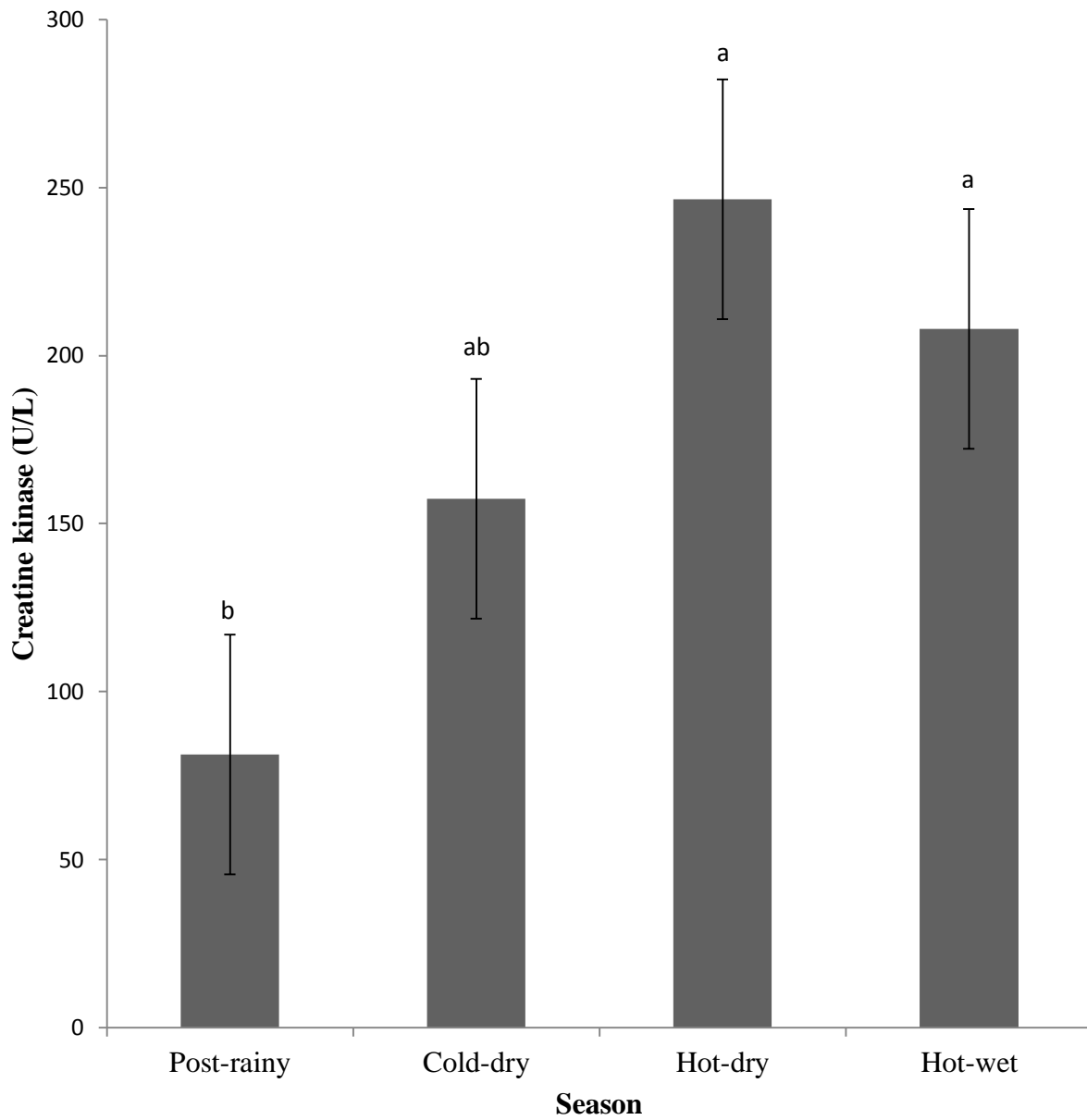


Figure 4.1: Seasonal variations in least square mean (\pm standard error) of creatine kinase activity in pasture based Boran cows ($n=15$)

4.3.2 Seasonal variation on haematological parameters

Season variations had a significant effect on some of the haematological parameters (HP) of Boran cows as represented in Table 4.2 and 4.3. Table 4.2 showed that, the Red cell count (RCC) and Mean corpuscular haemoglobin concentration (MCHC) of Boran cows were not affected ($P>0.05$) by seasonal variations (post-rainy, cold-dry, hot-dry and hot-wet) considered in this study. The Haemoglobin (HB) and Haematocrit (HCT) of Boran cows were significantly higher during the cold-dry season but similar to HB and HCT of Boran cows recorded during Hot-dry and Hot-wet seasons. The least values of HB and HCT of Boran cows were documented during the Post-rainy season. During Hot-dry and Hot-wet seasons, the Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) of Boran cows were higher ($P<0.05$) but they are similar to MCV and MCH of Boran cows that was recorded during cold-dry season. However, the MCV and MCH values of Boran cows recorded the least values during the Post-rainy season.

White cell count (WCC), Neutrophils (NEU), Lymphocytes (LYMP), Monocytes (MON), Eosinophils (EOS) and Basophils (BAS) were presented in table 4.3. Seasonal variations had no effect ($P>0.05$) on WCC, NEU, LYMP, and MON of Boran cows. EOS and BAS of Boran cows were significantly ($P<0.05$) influenced by seasonal variations. Boran cows had a significantly higher EOS count during Hot-dry season with a similar value recorded during Cold-dry and Hot-wet seasons. The Post-rainy season had the least effect on the EOS count of Boran cows. The Hot-wet season had a higher ($P<0.05$) influence of the BAS count of Boran cows even though they are similar to BAS count of Boran cow during Hot-dry. Hence, among the four seasonal variations (post-rainy, cold-dry, hot-dry and hot-wet) considered in this study, Post-rainy and Cold-dry seasons recorded the least effect on BAS count of Boran cow.

Table 4.2: Seasonal least square mean (\pm Standard error) concentrations for RCC, HB, HCT, MCV, MCH and MCHC

Season	RCC ($10^{12}/L$)	HB (g/dL)	HCT($10^9/L$)	MCV(FL)	MCH (pg)	MCHC(g/dL)
Post-rainy	7.04 ^a \pm 0.235	11.61 ^b \pm 0.340	0.33 ^b \pm 0.009	47.41 ^b \pm 0.696	16.54 ^b \pm 0.289	34.88 ^a \pm 0.307
Cold-dry	7.44 ^a \pm 0.249	12.65 ^a \pm 0.360	0.36 ^a \pm 0.009	48.70 ^{ab} \pm 0.737	16.99 ^{ab} \pm 0.305	34.87 ^a \pm 0.324
Hot-dry	6.96 ^a \pm 0.234	12.04 ^{ab} \pm 0.339	0.35 ^{ab} \pm 0.009	49.99 ^a \pm 0.694	17.39 ^a \pm 0.288	34.77 ^a \pm 0.306
Hot-wet	7.10 ^a \pm 0.245	12.30 ^{ab} \pm 0.355	0.35 ^{ab} \pm 0.010	50.11 ^a \pm 0.727	17.47 ^a \pm 0.301	34.84 ^a \pm 0.320

^{abc} Values with different superscripts in the same column differ significantly ($P < 0.05$). RCC= red cell count; HB= haemoglobin, HCT= haematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration.

Table 4.3: Seasonal least square mean (\pm Standard error) concentrations for WCC, NEU, LYMP, MON, EOS and BAS

Season	WCC ($10^9/L$)	NEU ($10^9/L$)	LYMP ($10^9/L$)	MON ($10^9/L$)	EOS ($10^9/L$)	BAS ($10^9/L$)
Post-rainy	9.17 ^a \pm 0.708	1.30 ^a \pm 0.097	4.55 ^a \pm 0.216	2.83 ^a \pm 0.143	0.25 ^b \pm 0.105	0.05 ^b \pm 0.006
Cold-dry	8.53 ^a \pm 0.749	1.33 ^a \pm 0.102	4.13 ^a \pm 0.228	2.72 ^a \pm 0.152	0.42 ^{ab} \pm 0.111	0.05 ^b \pm 0.006
Hot-dry	8.94 ^a \pm 0.705	1.16 ^a \pm 0.096	4.55 ^a \pm 0.215	2.65 ^a \pm 0.143	0.66 ^a \pm 0.105	0.07 ^{ab} \pm 0.006
Hot-wet	9.75 ^a \pm 0.739	1.12 ^a \pm 0.101	4.54 ^a \pm 0.225	2.87 ^a \pm 0.150	0.42 ^{ab} \pm 0.111	0.08 ^a \pm 0.006

^{abc} Values with different superscripts in the same column differ significantly ($P < 0.05$). WCC= white cell count; NEU=neutrophils;

LYMP= lymphocytes; MON= monocytes; EOS= eosinophils; BAS= basophils.

4.3.2 Effect of age and season on haematological parameters

Age showed changes ($P < 0.05$) within season for the parameters red cell count (RCC), haemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes (LYMP), eosinophils (EOS) and basophils (BAS). Moreover, in all seasons, age showed no difference ($P > 0.05$) for haematocrit (HCT), white cell count (WCC), neutrophils (NEU) and monocytes (MON). The concentrations for RCC and HB were higher during the cold-dry season within different age groups, except for HCT concentration which was higher during the hot-wet season for animals at the range of 5-8 years. Animals at the age of 4 years showed significantly high mean cell volume (51.58 ± 1.066) concentrations in the hot-wet season. On the other hand, a significantly lower MCV (47.65 ± 1.095) concentration for animals in 5-8 year range was observed during the post-rainy season. Table 4.4 show that the animals in 2-3 year range had different ($P < 0.05$) MCH concentrations during the cold-dry season. LYMP concentration differed significantly for animals in 2-3 (5.21 ± 0.359) years and 4 (3.67 ± 0.359) years range in the hot-wet season, as demonstrated in Table 4.5.

Table 4.4: Seasonal least square mean (\pm Standard error) concentrations for RCC, HB, HCT, MCV, MCH and MCHC from Boran cows of different age groups (2-3, 4 and 5-8 years)

Age(years)	Season	RCC ($10^{12}/L$)	HB (g/dL)	HCT($10^9/L$)	MCV(FL)	MCH (pg)	MCHC(g/dL)
2-3	Post-rainy	7.47 ^{abc} \pm 0.390	12.05 ^{ab} \pm 0.583	0.34 ^{ab} \pm 0.016	45.60 ^c \pm 1.066	16.16 ^{bc} \pm 0.497	35.42 ^a \pm 0.495
	Cold-dry	8.01 ^a \pm 0.421	12.88 ^a \pm 0.630	0.36 ^{ab} \pm 0.016	45.81 ^c \pm 1.151	16.01 ^c \pm 0.536	34.90 ^{ab} \pm 0.495
	Hot-dry	7.47 ^{abc} \pm 0.400	12.80 ^a \pm 0.599	0.33 ^{ab} \pm 0.016	48.55 ^{bc} \pm 1.095	17.15 ^{abc} \pm 0.510	35.31 ^a \pm 0.508
	Hot-wet	7.90 ^{ab} \pm 0.390	13.27 ^a \pm 0.583	0.35 ^{ab} \pm 0.016	48.16 ^{bc} \pm 1.095	16.90 ^{abc} \pm 0.497	35.08 ^{ab} \pm 0.508
4	Post-rainy	6.71 ^c \pm 0.421	11.06 ^b \pm 0.630	0.32 ^b \pm 0.016	48.45 ^{bc} \pm 1.151	16.50 ^{abc} \pm 0.536	34.01 ^{ab} \pm 0.534
	Cold-dry	7.02 ^{abc} \pm 0.421	12.28 ^{ab} \pm 0.630	0.35 ^{ab} \pm 0.016	50.67 ^{ab} \pm 1.151	17.42 ^{ab} \pm 0.536	34.39 ^{ab} \pm 0.534
	Hot-dry	6.82 ^{bc} \pm 0.390	11.63 ^{ab} \pm 0.583	0.34 ^{ab} \pm 0.016	50.48 ^{ab} \pm 1.066	17.08 ^{abc} \pm 0.497	33.80 ^b \pm 0.495
	Hot-wet	6.67 ^c \pm 0.390	11.61 ^{ab} \pm 0.583	0.34 ^{ab} \pm 0.016	51.58 ^a \pm 1.066	17.46 ^{ab} \pm 0.497	33.76 ^b \pm 0.495
5-8	Post-rainy	7.16 ^{abc} \pm 0.400	11.88 ^{ab} \pm 0.599	0.34 ^{ab} \pm 0.016	47.65 ^c \pm 1.095	16.63 ^{abc} \pm 0.510	34.91 ^{ab} \pm 0.508
	Cold-dry	7.25 ^{abc} \pm 0.400	12.26 ^{ab} \pm 0.630	0.37 ^{ab} \pm 0.016	49.69 ^{ab} \pm 1.151	17.58 ^{ab} \pm 0.536	35.37 ^a \pm 0.534
	Hot-dry	6.65 ^c \pm 0.400	11.63 ^{ab} \pm 0.583	0.36 ^{ab} \pm 0.016	50.79 ^{ab} \pm 1.095	17.83 ^a \pm 0.510	35.09 ^{ab} \pm 0.508
	Hot-wet	7.06 ^{abc} \pm 0.421	11.61 ^{ab} \pm 0.583	0.38 ^a \pm 0.016	49.83 ^{ab} \pm 1.151	17.58 ^{ab} \pm 0.536	35.25 ^a \pm 0.534

^{abc} Values with different superscripts in the same column differ significantly ($P<0.05$). RCC= red cell count; HB= haemoglobin, HCT= haematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration.

Table 4.5: Seasonal least square mean (\pm Standard error) concentration for WCC, NEU, LYMP, MON, EOS and BAS from Boran cows of different age groups (2-3, 4 and 5-8 years)

Age(years)	Season	WCC ($10^9/L$)	NEU ($10^9/L$)	LYMP ($10^9/L$)	MON ($10^9/L$)	EOS ($10^9/L$)	BAS ($10^9/L$)
2-3	Post-rainy	10.36 ^a \pm 1.263	1.30 ^{abc} \pm 0.167	4.86 ^{ab} \pm 0.359	3.02 ^a \pm 0.246	0.35 ^b \pm 0.18	0.05 ^b \pm 0.011
	Cold-dry	8.78 ^a \pm 1.363	1.19 ^{abc} \pm 0.181	4.16 ^{abc} \pm 0.387	2.81 ^{abc} \pm 0.265	0.21 ^b \pm 1.95	0.05 ^b \pm 0.011
	Hot-dry	9.48 ^a \pm 1.297	1.04 ^{ab} \pm 0.172	4.92 ^{ab} \pm 0.369	2.87 ^{abc} \pm 0.252	0.43 ^b \pm 0.181	0.07 ^{ab} \pm 0.011
	Hot-wet	9.97 ^a \pm 1.263	1.10 ^{abc} \pm 0.167	5.21 ^a \pm 0.359	2.96 ^{bc} \pm 0.246	0.44 ^b \pm 0.181	0.09 ^a \pm 0.010
4	Post-rainy	8.91 ^a \pm 1.363	1.07 ^{bc} \pm 0.181	4.30 ^{abc} \pm 0.387	2.64 ^{abc} \pm 0.265	0.35 ^b \pm 0.195	0.04 ^b \pm 0.011
	Cold-dry	7.73 ^a \pm 1.363	1.19 ^{abc} \pm 0.181	3.98 ^{bc} \pm 0.387	2.68 ^{abc} \pm 0.265	0.40 ^b \pm 0.195	0.06 ^b \pm 0.011
	Hot-dry	7.77 ^a \pm 1.263	1.08 ^{bc} \pm 0.167	4.30 ^{abc} \pm 0.387	2.72 ^{abc} \pm 0.246	1.05 ^a \pm 0.181	0.07 ^{ab} \pm 0.011
	Hot-wet	10.01 ^a \pm 1.263	1.22 ^{abc} \pm 0.167	3.67 ^c \pm 0.359	2.86 ^{abc} \pm 0.246	0.54 ^b \pm 0.181	0.06 ^b \pm 0.010
5-8	Post-rainy	7.54 ^a \pm 1.297	1.41 ^{ab} \pm 0.172	4.43 ^{abc} \pm 0.369	2.65 ^{abc} \pm 0.252	0.20 ^b \pm 0.186	0.04 ^b \pm 0.011
	Cold-dry	7.45 ^a \pm 1.363	1.57 ^a \pm 0.181	4.32 ^{abc} \pm 0.387	2.29 ^{bc} \pm 0.265	0.33 ^b \pm 0.195	0.04 ^b \pm 0.011
	Hot-dry	8.91 ^a \pm 1.297	1.31 ^{abc} \pm 0.172	4.44 ^{abc} \pm 0.369	2.19 ^c \pm 0.252	0.39 ^b \pm 0.186	0.05 ^b \pm 0.011
	Hot-wet	9.09 ^a \pm 1.363	0.92 ^c \pm 0.181	4.61 ^{abc} \pm 0.387	2.68 ^{abc} \pm 0.265	0.30 ^b \pm 0.195	0.06 ^b \pm 0.011

^{abc} Values with different superscripts in the same column differ significantly ($P < 0.05$). WCC= white cell count; NEU=neutrophils; LYMP= lymphocytes; MON= monocytes; EOS= eosinophils; BAS= basophils.

4.3.3 Effect of season on temperature humidity index

Seasonal variations in the temperature-humidity index (THI) values are reported in Figure 4.2. Comparing the seasonal average THI, highest ($P<0.05$) least square mean value was observed in the hot-wet (74.12) season, while the least values were observed in the cold-dry (65.64) season. Animals were in thermoneutral condition (no stress phase) during post-rainy and cold-dry season. However, the hot-dry and hot-wet season showed an increase ($P<0.05$) to a mild stress phase. In ascending order, the THI values were cold-dry (65.64), post-rainy (69.99), hot-dry (71.59) and hot-wet (74.12).

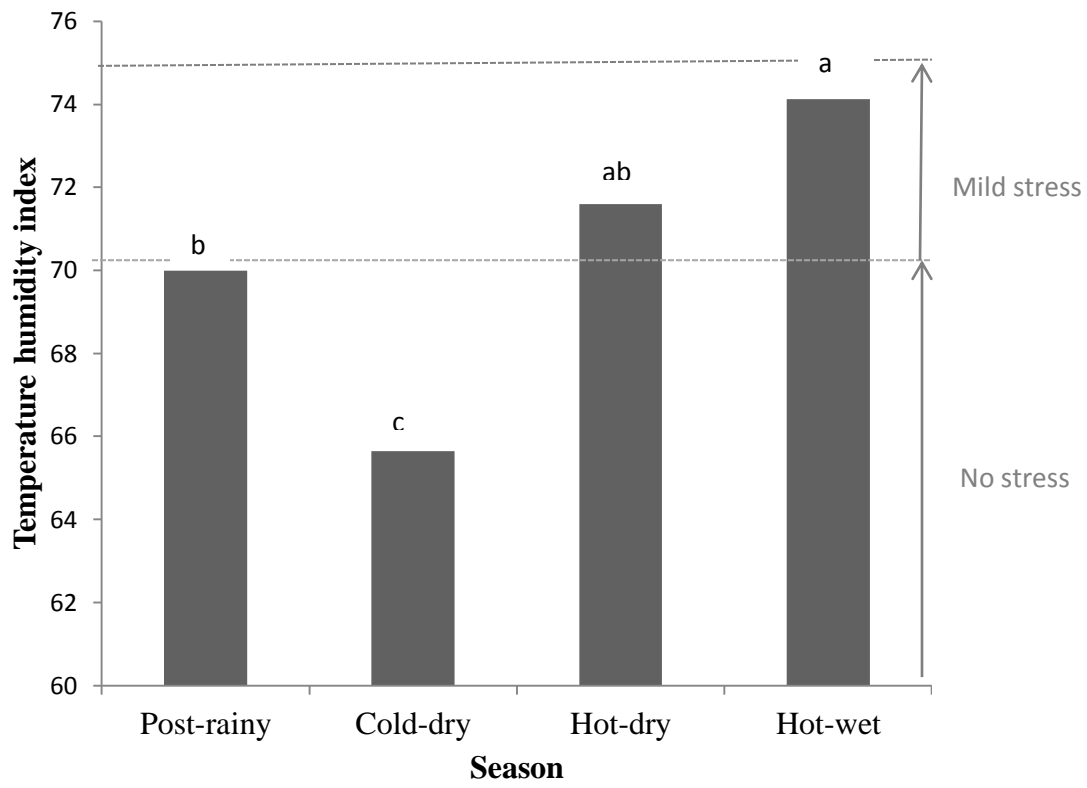


Figure 4.2: Seasonal least square mean (\pm standard error) values for temperature-humidity index (THI)

4.3.4 Correlations among temperature-humidity index and haematological parameters

Correlations between temperature-humidity index (THI) and different haematological parameters (HP) are shown in Table 4.6. Neutrophils (NEU) (-0.267) and basophils (BAS) (0.268) were significantly correlated with THI. On the other hand, parameters such as red cell count (RCC), white cell count (WCC), haemoglobin (HB), haematocrit (HCT), mean corpuscular volume (MCV), lymphocytes (LYMP), monocytes (MON), eosinophils (EOS), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not correlated ($P>0.05$) with THI. Red cell count showed a significant correlation with HB, MCV, LYMP and MCH. Moreover, a significant positive correlation was observed between white cell count and parameters like LYMP, MON, EOS and BAS.

Table 4.6: Correlation between temperature-humidity index and hematological parameters

Variable	THI	RCC	WCC	HB	HCT	MCV	NEU	LYMP	MON	EOS	BAS	MCH
RCC	-0.096 ^{ns}											
WCC	0.174 ^{ns}	-0.025 ^{ns}										
HB	-0.075 ^{ns}	0.855 ^{**}	0.023 ^{ns}									
HCT	0.053 ^{ns}	-0.085 ^{ns}	-0.291 ^{***}	-0.114 ^{ns}								
MCV	0.165 ^{ns}	-0.561 ^{**}	0.128 ^{ns}	-0.125 ^{ns}	0.058 ^{ns}							
NEU	-0.267 ^{***}	-0.011 ^{ns}	0.105 ^{ns}	-0.002 ^{ns}	0.063 ^{ns}	-0.024 ^{ns}						
LYMP	0.161 ^{ns}	0.368 ^{***}	0.371 ^{***}	0.344 ^{***}	-0.069 ^{ns}	-0.238 ^{ns}	0.041 ^{ns}					
MON	0.077 ^{ns}	0.038 ^{ns}	0.393 ^{***}	0.117 ^{ns}	0.018 ^{ns}	0.093 ^{ns}	-0.132 ^{ns}	0.231 ^{ns}				
EOS	0.125 ^{ns}	-0.230 ^{ns}	0.261 ^{***}	-0.130 ^{ns}	0.001 ^{ns}	0.309 ^{***}	-0.143 ^{ns}	-0.060 ^{ns}	0.063 ^{ns}			
BAS	0.268 ^{***}	0.111 ^{ns}	0.406 ^{***}	0.153 ^{ns}	0.038 ^{ns}	0.109 ^{ns}	-0.088 ^{ns}	0.096 ^{ns}	0.181 ^{ns}	0.305 ^{ns}		
MCH	0.102 ^{ns}	-0.475 ^{***}	0.123 ^{ns}	0.047 ^{ns}	-0.034 ^{ns}	0.864 ^{***}	0.008 ^{ns}	-0.101 ^{ns}	0.128 ^{ns}	0.240 ^{ns}	0.079 ^{ns}	
MCHC	-0.084 ^{ns}	0.025 ^{ns}	0.007 ^{ns}	0.310 ^{***}	-0.152 ^{ns}	-0.008 ^{ns}	0.057 ^{ns}	0.201 ^{ns}	0.090 ^{ns}	-0.046 ^{ns}	-0.039 ^{ns}	0.0494 ^{**}

***Significant at ($P < 0.05$), ** Significant at ($P < 0.001$), *ns* not significant

4.4 Discussion

From the current study, the levels of creatine kinase (CK) activity during the hot-dry (246.54 ± 37.512) and hot-wet (207.98 ± 35.406) season than post-rainy (81.29 ± 37.512) and cold-dry (157.42 ± 42.452) season are in line with reports by Kannan *et al.* (2000); Tadich *et al.* (2005) and Miranda-de la Lama *et al.* (2010, 2012), who found high levels of CK during the hot seasons. This may be attributed to the physical stress at extreme temperatures during hot seasons that results in tissue damage and muscular injury (Liu *et al.*, 2008). During the hot seasons, high amounts of adenosine triphosphate (ATP) are needed by animals for respiratory and metabolism processes, however, high ATP levels in the blood results in increased CK activity (Chulayo and Muchenje *et al.*, 2013a). Similar results were reported by Chulayo and Muchenje (2013b) who found high CK activity during the summer season than the winter season. Thus, according to Partida *et al.* (2007) and Melesse *et al.* (2011), high levels of CK are associated with high temperatures. On the contrary, Zimmerman *et al.* (2011) and Mpakama *et al.* (2014) found higher levels of CK during the winter season than the summer season, which were caused by poor conditions prior slaughter. However, in the present study the high levels may be due to high tick infestation, which was influenced by high temperatures during the hot-dry and hot-wet seasons.

Furthermore, according to Wood and Quiroz-Rocha (2010), haematological parameters (HP) are used to indicate a breed's adaptability to hostile environmental conditions. In this study, the haemoglobin (HB) and haematocrit (HCT) concentration levels showed a significant increase during the cold-dry season than other seasons. While significantly lower levels of HB and HCT were observed during the post-rainy season than other seasons. These findings are in agreement with those of Casella *et al.* (2013), who observed higher concentrations of HB and HCT during winter than summer season. According to Shehab-el-deen *et al.* (2010), these high concentration

levels of HB and HCT could be attributed to the decreased water consumption during the cold-dry season in which temperatures are low. Temperature variations within seasons result in severe changes in water balance. Lower temperatures during the cold-dry season decreases the chances of haemodilution effect, which decreases the concentration of HP in blood (Kumar and Pachauri, 2000), hence the HB and HCT concentrations were high. However, several studies (Kumar and Pachauri, 2000; Fadare *et al.*, 2012; Katiyatiya *et al.*, 2015; Farooq *et al.*, 2015) have reported contrasting results whereby high concentrations of HB and HCT were observed during the hot-dry and hot-humid seasons.

Further observations from this study showed a significantly higher mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and basophile (BAS) concentrations during the hot-dry season compared to other seasons. High trends of MCV during the hot-dry season could have been caused by the increased temperatures which lead to heat stress. In addition, these results suggest adaptation in the investigated Boran cows, which is related to an increase in oxygen levels due to high environmental temperatures during the hot-dry season. These results are in line with the reports by Mazzullo *et al.* (2014) and Ribeiro *et al.* (2015) who observed higher MCV concentration during the hot season. Similar findings by Zakari *et al.* (2015) observed an increased MCV in donkeys when they were exposed to heat stress during the hot seasons. Furthermore, the lower MCH during the post-rainy season could be attributed to the reduction in environmental temperatures. The observed significant ($P < 0.05$) interaction between age and season on HP revealed high concentrations of RBC and HB during the cold-dry season within different age groups. In addition, the higher concentration of HCT for older cows (5-8 years) during hot-wet season is in contrast with the report by Vengust *et al.* (2002), who observed highest values in younger fallow deer's. High trends of MCH in animals of 2-3 year

range during the cold-dry season could be attributed to the presence of high immunity in younger animals, which benefits them to cope and survive under cold stress conditions.

The probability for incidence of heat stress in cows can be viewed through calculated THI values. The presence of heat stress as depicted by the THI values during hot-dry and hot-wet season could be attributed to high environmental temperatures. According to Abdelatif and Alameen (2012), exposure of cows to extreme temperatures causes heat stress and reduces the ability to sustain thermoregulatory mechanism. The current results showed that the THI values were above 70 in hot-wet and hot-dry season, indicating that the cows experienced mild stress conditions. Similar results were reported by Teke and Akdag (2012) who also found high values of THI which were above 70, thus emphasizing the presence of mild to elevated heat stress levels in their study. However, our findings are in contrast with the report by Katiyatiya *et al.* (2015) who recorded lower values of THI when they monitored Nguni cows from different farming systems. These results revealed that THI variations could be breed dependent, therefore, further research should be done to compare THI variations among different cattle breeds.

The observed relationship between NEU and BAS with THI could be attributed to the capability of indigenous breeds to cope and survive under arid conditions, where temperatures are high. However, the negative correlation between NEU and THI revealed that as the temperatures rise within different seasons the NEU levels decreases. The positive correlation between WBC with LYMP, MON, EOS and BAS indicates high immune stimulation in the examined Boran cows, which enables them to withstand the seasonal variation in arid environments. A negative association between RBC and its indices (MCV and MCH) could be ascribed to alterations in oxygen carrying potential from the blood, which can be affected by an animal's physiological state (Farooq *et al.*, 2011).

4.5 Conclusion

Seasonal variations had an influence in creatine kinase activity, temperature-humidity index and haematological parameters of Boran cows. Creatine kinase activity varied across various seasons, with high levels in the hot-dry and hot-wet season. Temperature-humidity index levels also differed across seasons, with higher levels showing a mild stress condition during the hot-dry and hot-wet seasons. The results indicated that the investigated Boran cows showed adaptation to various environmental conditions. This was showed by the less variation in haematological parameters during various seasons. However, further research should be designed to consider the interaction between age and sex on haematological parameters of the Boran breed. On the other hand, farmers should not only pay attention to heat stress during the summer season, but be conscious throughout the year. Therefore, farmers are advised to make routine blood tests to ensure heat tolerance and good performance in their animals.

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Chapter 5: General Discussion, Conclusion and Recommendations

5.1 General Discussion

The main objective of this study was to investigate the seasonal variations in tick loads, coat characteristics, temperature-humidity index, creatine kinase activity and haematological parameters of pasture based Boran cows. Boran cows have a superior ability to adapt and survive under harsh environmental conditions, characterized by high ambient temperature and humidity (Herlocker, 1999; Wasike *et al.*, 2009). This breed has been documented by numerous authors (Mpofu, 2002; Wasike *et al.*, 2009; Kahi, 2015) in various countries, however, very few publications have been done in South Africa. In chapter 3, the effect of physical and production traits on tick loads of Boran cows reared in a semi-arid rangeland of South Africa was investigated. The results revealed that the experimental cows were naturally infested by four different tick types (*Rhipicephalus evertsi evertsi*, *Amblyomma hebraeum*, *Rhipicephalus simus* and *Rhipicephalus (Boophilus) decoloratus*), which were observed in different anatomical sites within various experimental seasons. The highest tick counts were observed from the posterior region during the post-rainy, cold-dry and hot-dry season as also noted by various authors (Muchenje *et al.*, 2008; Marufu *et al.*, 2011; Katiyatiya *et al.*, 2015). While interesting findings during the hot-wet season revealed that the highest counts were observed from the ventral position. This was attributed to the fact that the ventral position is exposed to the external environment, providing the cooler environment for ticks during hot temperatures in the hot-wet season.

Therefore the results from chapter 3 agreed with the earlier findings by Wasike *et al.* (2009) and KBCBS (2010) who reported the Boran breed as the highly adaptive breed in tropical environments. This was further validated by the results observed in chapter 4. The Boran cows

have shown a potential to sustain the majority of their haematological parameters pretty much at the similar levels without indicating much variation during different seasons. On the other hand, the enzymes are generally located in various tissues and once they are present in the blood they serve as a sign of muscle damage. In our study, the muscle damage may be brought about by tick infestation and hot temperatures within various seasons. Thus, the highest creatine kinase activity was observed during the hot-dry season when the temperatures and tick infestation were high.

Cows in this study experienced a mild stress condition during the hot-dry and hot-wet season when the temperature-humidity index values were above 70. These high values were in agreement with Gatner *et al.* (2011) who observed high values of THI during hot-dry and hot-wet season. Heat stress encourages the activation of heat stress mechanism (Hansen, 2004) including an escalation in sweating rate and respiratory volume (AI-Haidary *et al.*, 2001). According to the results from this study, Boran cows adapted quite well under arid rangelands of South Africa because of their thermotolerance traits. Hence Hansen (2004) also reported that Boran cattle have high density of sweat glands which helps them to adapt and survive under stressful environmental conditions.

5.2 Conclusion

It was concluded that seasonal variations have an influence in tick loads, coat characteristics, creatine kinase activity and haematological profiles of pasture based Boran cows. These cows showed high adaptation under semi-arid conditions. This was ascertained when experimental trials showed low tick infestations, temperature-humidity index and pretty less variation in levels of haematological parameters across different seasons. Experimental cows revealed good adaptation performance in semi-arid conditions; therefore this breed can be reared by communal farmers who are exposed to harsh climatic conditions.

5.3 Recommendations

- ❖ Farmers are advised not to only pay attention to heat stress during the summer season, but be conscious throughout the year.
- ❖ Therefore, there is a need to further investigate the heat tolerance abilities of the Boran cattle.
- ❖ Further research should be geared towards comparing different genders within the breed and also comparing the breed with other indigenous cattle breeds.

5.4 References

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Appendices

Appendix 1: Ethical clearance certificate



University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: MUC311SNTI01

Project title: **Tick loads, coat characteristics and blood metabolite profiles of pure and crossbred Boran cattle.**

Nature of Project: Masters

Principal Researcher: Wonga Ntilini

Supervisor: Prof V Muchenje

Co-supervisor: N/A

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

Appendix 2: Plagiasim report