Growth performance and meat quality of weaner steers adapted to starter diets containing potassium humate in the feedlot

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DECLARATION

I, **Nthabiseng Precilla Mokotedi** declare that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof U Marume and Mr Klaas-Jan Leeuw. All assistance towards the production of this work and all the references contained herein have been duly acknowledged

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DEDICATION

This work is dedicated to my daughter, Oratile Letlotlo Mokotedi. I pray to God to continue showering His blessings on your life. You have been my strength through it all.

"My great hope is to laugh as much as I cry; to get my work done and try to love somebody and have the courage to accept the love in return." Maya Angelou

ABSTRACT

Humates can be described as raw material used in animal husbandry and agriculture in the form of a humate drink or dry feed as a source of mineral and organic substances for growth stimulation. Most commercially available humic products are extracted from deposits of soft brown coal with an alkali solution. In this study, the effects of inclusion of potassium humate on growth performance, meat and carcass quality parameters were examined in weaner steers. To achieve this, 22 steers of age 6-7 months were randomly divided into control and treatment groups each containing 11 steers. The control group was fed basal diet composed of a total mixed ration for growing steers, whereas treatment group was fed basal diet containing potassium humate at a rate of 1.5g/kg feed. The experimental period lasted for 4 months. Growth performance parameters, including average daily feed intake (ADFI), average daily gain (ADG), food conversion efficiency (FCE). At the end of the trial, all animlas were slaughtered humanely for determination of meat quality parameters. All data were subjected to appropriate statistical analysis. Overall, steers fed diet with potassium humate had greater average daily than the steers in the control group during adaptation period. However, there was no significant difference in the ADG, FI and FCR of steers among the two treatment groups. At the end of the trial there were no significant effects of potassium humate inclusion on growth performance, meat quality parameters and carcass weights compared with control. Inclusion of PH in starter diets of weaner steers significantly ($P < 0.05$) improved meat tenderness and caused a greater in meat pH, two of the most important parameters affecting meat quality. From the study, although the inclusion of Potassium humate in diets did not affect total fat content, individual fatty acids and nutritional indices, it was evident that composition of fatty acids in meat is not fixed and can be changed by differences in dietary components. Overall, although the results fom the study appeared to be inconclusive, it appears that inclusion of PH in diets can positively influence adaptability of weaners to feedlots diets with ultimate desirable effects on meat quality characteristics. PH inclusion in steer diets can therefore provide an alternative in the production of safe and healthier meat in the feedlot.

Key word: Potassium humate, growth performance, carcass characteristics, meat quality, fatty acids

TABLE OF CONTENTS

ABBREVIATIONS

A-Ampere

ADG-Average Daily Gain

AOAC- Association of Official Analytical chemists

BCS-Body conditioning score

D-day

DM-Dry matter

FCR-Feed Conversion Ratio

IBR-Infectious Bovine Rhinotracheitis

K-Potassium

Hz-Hertz

HS-Humic substance

MIC-Meat Industry Centre

NSPCA-National Society for the Prevention of Cruelty to Animals

T-Treatment

Vs-versus

V –volt

WHC-Water holding capacity

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background

In the feedlot industry the feeding management of weaner calves which are not adapted to concentrate diets remains a challenge. The possibility of deficiencies of nutrients and unwanted changes to rumen microbial populations does exist for stressed cattle, adapting to high concentrate rations in feedlots. This has provoked a need to derive strategies to reduced stress of weaner calves during adaptation to feedlot conditions. One of the major strategies that has gained a lot of interest is the inclusion of humate in weaner diets. Humates are natural performance enhancer that is increasingly gaining interest (Peña-Méndez *et al.*, 2005; Cusack, 2008 & McMurphy *et al.*, 2009). Studies on humates and other similar compounds have brought awareness to their physicochemical properties, which led to the idea of the use of these natural compounds in animal nutrition (Kocabagli *et al.*, 2002; Peña-Méndez *et al.*, 2005; McMurphy *et al.*, 2009). Information on the use of humates (humic acid and fulvic acids) indicate growth promoting effects in farm animals when added to their diets (Kocabagli *et al.*, 2002; Wang *et al.*, 2008).

Research has shown that humates can help relieve stress effects in calves entering feedlots for first time (Islam *et al.,* 2005). Weaned calves are susceptible to stress that may reduce performance and increase morbidity (Loerch & Fluharty, 1999). In addition, fulvic acids contained in the humates have been shown to have beneficial effects on animal health by boosting immune system as a result of the antipyretic effect, antiviral effect and detoxifying effects of toxic substances (Agazzi *et al.*, 2007; Islam *et al.*, 2005). Moreover, humates have been observed to reduce the volatile ammonia in animal waste (Islam *et al.*, 2005). Also Humates have been shown to enhance NDF digestion, reduce NH_3 concentrations and increased pH in the rumen as well (Bell et al., 1997). In some studies, some researchers have also suggested that humates can help relieve stress effects in calves entering feedlots for first time through inhibition of mycotoxins absorption in the gastrointestinal tract (Islam *et al.*, 2005; Ramos *et al.*, 1996a). The use of humates as feed additives has been of great promise to modern animal husbandry, but this practice is not without criticism as it's still new. Despite the high awareness of humate potential in horticulture and poultry nutrition, there is still very little understanding of the potential in animal husbandry.

1.2 Problem statement and Justification

Although studies have been conducted on the use of potassium humate as a growth promotant in animal feedings systems, there are still a lot of questions that needs to be answered. Most of the studies conducted on the use of potassium humate have focused on pigs and poultry were observation were made that inclusion of humic acid can influence digestion dynamics, general health and immune development (Ragaa and Korany, 2016). Moreover, organic acids, like humic acid have inhibition properties against acid intolerant bacteria including *E. coli*, Salmonella spp and *Clostridium perfringens* and hence can be used as alternatives to antibiotics (Fascina et al., 2012; Naseri et al., 2012).There appears to be no conclusive information on the beneficial effects of potassium humates on feedlot steers. Therefore, there is a need to investigate the efficacy of use of Potassium humate as a stress reliever and growth promotant in feedlot diets of steers.

1.3 Objectives

The broad objective of the study was to establish efficacy of Potassium humate as a growth promotant in feedlot diets, by assessing its effect on growth performance, carcass characteristics and meat quality attributes of feedlot weaner steers.

The specific objectives for this study are:

- ✓ To evaluate the effects of Potassium humate on feed intake, average daily gain and feed conversion efficiency of feedlot steers;
- ✓ To evaluate the effect potassium humate on carcass characteristics and meat quality of feedlot steers
- ✓ To evaluate the effect potassium humate on, fatty acid profiles, nutritional indices and oxidative stability of the meat

1.4 Hypotheses

- ✓ Potassium humate has no effect on feed intake, average daily gain and feed conversion efficiency of feedlot steers
- ✓ Potassium humate has no effect on carcass characteristics and meat quality of feedlot steers
- ✓ Potassium humate has no effect on fatty acid profiles, nutritional indices and oxidative stability of the meat

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The animal feed industry has put a lot of emphasis on improving livestock productivity. Currently, there are a number of publications from Scientific and Medical journals reporting on the effect the use of growth promoters in animal production has on humans and animal health. (Gazette,No. 31005). According to a report compiled by AFMA Technical Committee (AFMA: Viewpoint on Growth-Promoting Hormones (GPHs), Compiled by AFMA Technical Committee, May 2013) there are strict regulations governing the use of GPHs intended for use in food-producing animals in South Africa. These must be approved by the Registrar under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947, (Act No. 36 of 1947), and are further controlled under both the Meat Safety Act, 2000 (Act No. 40 of 2000) and the Medicines and Related Substances Control Act, 1965 (Act No. 101 of 1965) to ensure that food produced from hormone-treated animals is safe for human consumption. Furthermore, the use of medicated feed additives is controlled by the Food and Drug Administration (FDA).

2.2 Use of growth promotants in cattle

McMurphy (2007) states that implantation of growth promotants into finishing cattle has been used in beef industry for decades in order to improve efficiency. The use of growth-promoters as feed additives has been widely accepted by the cattle production industry as a stratergy to improve feed utilisation and productivity, ultimately reducing the cost of production and enhancing affordability of beef by consumers. Esterhuizen *et al.* (2008), reported an increase in demand for meat produced naturally over the conventionally produced meat animals globally in recent years which has been complemented by significant research supporting the positive benefits of use natural growth promotants in livestock production.

Currently consumers are increasingly becoming anxious about meat produced using chemicals and hormones due to risks of persistent residues in the meat (Van Ryssen, 2003a; Walshe *et al.*, 2006). The ban on the use of antibiotics as growth promoters in the European Union and the potential for a ban in most parts of the world has provoked the search for alternative feed supplements in animal production (Ozturk *et al.*, 2009). Thus the use of humates has stimulated increasing interest in animal nutrition as they have been proved to play a major role in soil and plant nutrition. Some of the benefits include improved functioning of soil bacteria that make nutrients available to plants at higher levels and also more of the limiting nutrients.

Today use of natural organic substances, such as humates, is becoming increasingly apparent in various sectors of the agriculture industry. The use of humic substances is promising. Presently, the benefits of use of natural humic substances are common knowledge and many commercial companies have begun commercial production of a different humic products, particularly the organic fertilizers, plant growth stimulators, reducing agents for disturbed soils, and sorbents for toxic pollutants which are commonly used in the crop and horticulture production sectors (Gosteva *et al.*, 2012).

2.3 Effect of humates on growth performance

Humates can be included as feed additives in animal diets in the form of a humate drink or dry feed providing minerals and organic acids that act as growth stimulants (Galip *et al.*, 2010). In Europe, humates have been used as growth-promoters in ruminants, following the ban of antibiotic use in feeds (Karaoglu *et al.*, 2004). Although humic acids have not been approved as feed additives but rather as veterinary drugs at Europe Union, several reports have highlighted its therapeutic and growth promoting effect. EMEA (1999) report indicated that diarrhoea, dyspepsia and acute intoxications in horses, ruminants, pigs and chickens are treated with humic acids at an oral dose of 200-500mg/kg of body weight. Lyons *et al.*, 2016 reports that humic substances have been the topic of numerous studies due to their mitigating effects on biota and abiotic stress conditions such as low/high temperatures.

According to Islam *et al.*, (2005) organic acids with antimicrobial properties such as humates are used all over the world to improve the animal gastrointestinal ecology and ultimately promoting efficiency of feed utilisation, increasing growth rate and diminishing the risk of diseases. With increased production and demand of livestock products, the South African feed additives market has been steadily growing. The demand for animal products has been largely due to an increase in the population, urbanization and increase in income levels. Over the years, the feed prices have been inconsistent due to many factors and this has driven the market to look for better feeds.

2.4 Effects of humates on carcass characteristics, meat quality and fatty acid profiles

Various parameters are used in the measurement of nutritional value of meat including carcass characteristics, instrument-based quality measurements and assessment of fatty acid profiles. All these measure are affected by factors such as diet, age, breed and sex. Dietary influences on nutritional value of meat have been extensively explored (Sabow *et al.*, 2015). Nevertheless, gaps still exist on the influence of natural feed additives such as humic acid on nutritional value of beef. The available information on the effects of humic acid on meat quality is largely inconsistent. Ozturk *et al.* (2012) reported that humic acid inclusion in animal diets can improve digestion dynamics and nutrient absorption ultimately regulating growth rates and altering the metabolic processes that enhance meat quality traits. Kocabagli *et al.* (2002) and Ozturk *et al.* (2012) also demonstrated a linear increase in body and carcass weights with inclusion of humic acids in the diets. Although the underlying mode of action is still not well understood, humic acid salts have been associated with some meat quality parameters (Berg *et al.*, 2001; Wang *et al.*, 2008; Ozuturk *et al.*, 2012). In chicken and pork, humic acid salt was observed to desirably modify meat colour mainly due to accelerated myoglobin synthesis (Ozuturk *et al.*, 2012).

2.5 Use of potassium humate in livestock production

According to Kocabagli *et al.*, (2002) humates are substances formed from decayed plant matter with the help of the bacteria living in the soil and are composed of humus, humic acid, fulvic acid, ulmic acid and trace elements (McMurphy *et al.*, 2011). They exist as humic acid salts mainly either of Potassium or Sodium (Kocabagli *et al.*, 2002). They can be added in dry feeds and can be dissolved in water forming the humate drink providing minerals and organic substance for stimulation of growth (Galip *et al.*, 2010; Mayhew, 2004). Nevertheless, information on the use of humate in animal diets remains largely scarce (Islam *et al.*, 2005). McMurphy *et al.* (2009, 2011) evaluated supplementation of humates on Holstein steers and on beef cattle respectively.

Kocabagli *et al.* (2002) observed an improved gain in body weights of broilers by feeding humate from day 22-42. In some studies, humates have been reported to stabilise the animal's intestinal ecology consequently improving feed utilisation efficiency resulting in 5%- 15% weight gains in pigs, cattle and poultry (Shermer *et al.* 1998; Humitech, 2004; Anon, 2009). The biological activity of humates is not well understood, their hormone–like properties may be responsible for the observed effects in animals (Canellas *et al.* 2008; Nardi *et al.* 2002).

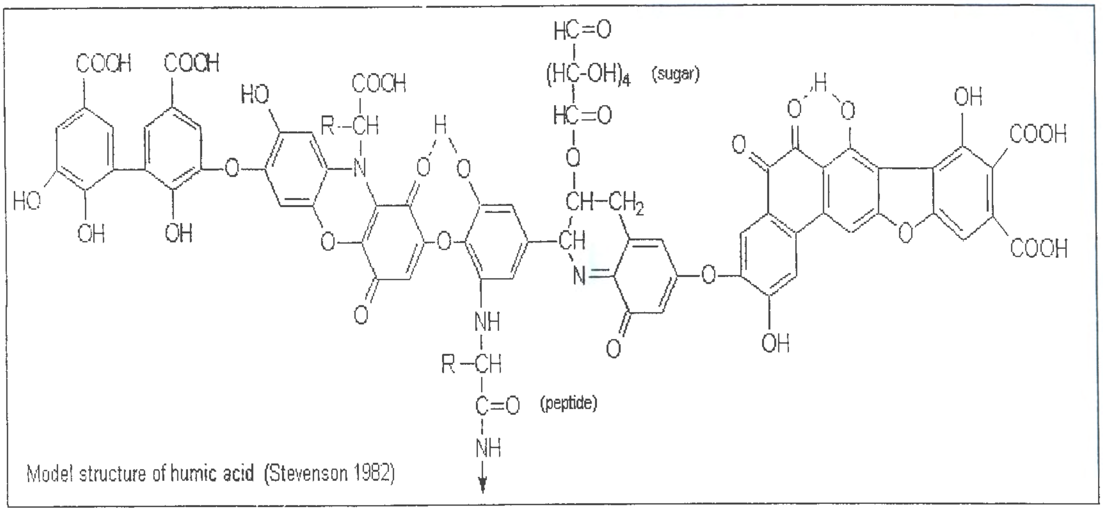

Figure 2. 1: Model structure of humic acid. R can be alkyl, aryl or aralkyl (Stevenson, 1982; Peňa-Méndez et al., 2005).

The uniqueness of a humate substance is accredited to its specific properties, method of preparation, as well as its source. More importantly, the ability of humic substance is to effectively increase metabolic processes in vegetable cells has been recognised. A number of scientific researchers have shown that this is also applies to animal organisms, more specifically, in broiler poultry (Anon, 2009).

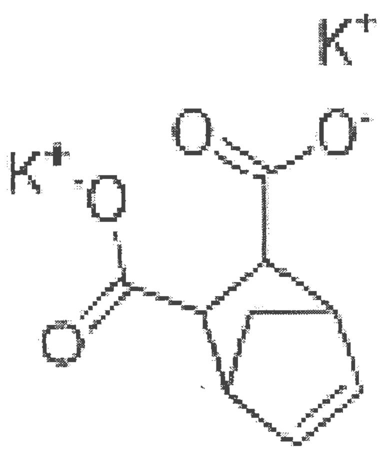

Figure 2. 2: Potassium Humate with Molecular formula-$C_9H_8K_2O_4$

Liquid humate extracts, primarily humic acid, have been evaluated in numerous trials in poultry, USA beef cattle and dairy trials. Through these well-conducted trials, there has been enhanced weight gain and decrease in feed dry matter conversion. Trial with monogastrics fed different levels of humates and humic acids have shown significant improvement in growth and in animal performance. In addition, utilization of nutrients in animal feed can be improved by feeding humic acids (Kucukeersan *et al.*, 2005). Moreover, the benefits of inclusion of humic acids in diets can be realised through its use in stress management in animals (Enviromate, 2002). The anti-inflammatory activity and antiviral properties enables the animals to withstand the effects of certain intestinal diseases, mainly diarrhea in animals. The limited numbers of articles that are currently published depict a consistent agreement that shows that humates promote growth by altering the partitioning of nutrient metabolism and improving feed conversion efficiency (Karr, 2001).

2.6 Effect of Potassium Humate on Mycotoxins in animal feeds

According to Van Rensburg (2005), mycotoxins can be described as a structurally diverse group of secondary metabolites produced by different genera of fungi. These toxins are involved in several animal and human toxicoses causing suppression on immune responses and immunomodulation in domestic animals. Ramos & Hernández (1996) states that mycotoxins can furthermore cause serious health problems which leads to production losses in livestock. Traditionally, the South African beef industry uses different tactics such as implantations of growth promotants into finishing cattle however efforts to protect livestock from the effects of mycotoxicosis have created an entrance point into the market for many companies to develop different products for combating mycotoxins.

A research has shown that humates are composed of different functional groups (Steveson, 1992), which has raised assumption of their adsorption capacity leading to a considerable believe that they can bind with several compounds. Due to the colloidal properties and the ability of humates to form chelates, humic acids together with their salts can change the toxic effects of numerous mycotoxins and unwanted substances that enter digestive tract when livestock consume feed (Livens, 1991; Jansen van Resnburg *et al.*, 2006). Many humic substances are modified chemically forming humates with improved functional properties. The carboxyl and phenolic OH groups enables the humates to have greater capacities to form complexes with metal ions and hence protecting them from being assimilated in the digestive tract (Dogan *et al.*, 2015). Findings from *in vitro* binding studies showed that humates have high mycotoxin adsorption capacity (Jansen van Rensburg *et al.*, 2006). The protective effect of humates appears to involve forming complexes with aflatoxins, reducing their bioavailability in the gastrointestinal tract (Ghahri *et al.,* 2010; Lin and Lee, 1992).

2.7 Factors affecting effectiveness of Potassium Humate in animal diets

2.7.1 The raw material used

Process for the manufacturing of humic acids and salts follows sequential steps of fermentation of raw material under selected controlled temperature, time, and aeration conditions. Raw material such as peat, lignite, coal or leornadite must go through an alkaline extraction process. Several literatures suggested that humic acid composition varies due to different preparations processes adopted by different companies and also due to the different extraction sources. (Islam *et al.*, 2005; Trckova *et al.*, 2005). Humates are commercially available in a number of different formulations including liquids, powders and granules of several sizes. According to a report by Astute Communications (The Use of Humic Substances in Agriculture: Origins, Science and Applications, 2012), high quality powders, granules and dry soluble products may contain up to 80% or more organic matter (humin, humic acid and fulvic acids combined). Humates derived from coal are common (Avena *et al.*, 1998; Hertkorn *et al.*, 2002; Mikkelsen, 2005). They are dark in colour and are readily soluble in water. The humates derived from coal may differ according to the grade of coalification and conditions under which they were formed (Mackowiak *et al.*, 2001; Li *et al.*, 2003; Karaca *et al.*, 2006; Imbufe *et al.*, 2004; Skhonde *et al.*, 2006).

2.7.2 Rate of dosage

Different workers have conducted various research using different inclusiom levels of humates in animal diets. McMurphy *et al.* (2009, 2011) reported that including dietary humate at 5.0 g/kg slightly altered dry matter intake while reducing available rumen ammonia nitrogen. In addition, Levinsky (1996) reported a case where animals were fed sodium humate at 10 mg per 10 kg of active (live) weight, in addition to the fodder for 21-30 day. Finding from study showed that within a four months period, calves born from cows fed humates had a 13.4% increase in weight (Levinsky, 1996).

2.7.3 Animal species

Humates have been evaluated as a dietary supplement in pigs and poultry industries. Shortened time to market, higher carcass weight, better gain to feed ratios and reduced ammonia emissions have all been shown to result from adding humic substances to livestock feed McMurphy *et al.* (2009, 2011). Kocabagli *et al.* (2002) observed an improved gain in body weights of broilers by feeding humate from day 22-42. However, Hassan (2014) found out that adding humate negatively affected the production performance of broiler chickens.

2.8 Summary

The ban on the use of antibiotics as feed additives in animal diets by the European Union and worldwide has provoked the search for alternative feed additives in animal diets. An incareasde interest has been shown on exploration of the use of humates as feed additives in animal diets. Nevertheless it appears the information available of the use of humic acids in animal diets is inconsistent. Moreover, the mechanism of action of humates on digestion dynamics and general health of animals is not well understood. The beneficial effect of humic acid on growth in different species of animals has been considered based on its capacity of changing gut physiology and interference in immunity. Nevertheless, to be sustainable and accepted by the industry, humates additives require further long term studies in the live ruminant to determine how effective they are in commercial systems.

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CHAPTER 3

GROWTH PERFORMANCE OF WEANER STEERS FED STARTER DIETS CONTAINING POTASSIUM HUMATE

Abstract

This study was done to evaluate the influence of humates in diets on growth performance of weaner calves in a feedlot. Twenty-two bull-calves, housed in single stanchions (1m by 2m), fed a starter diet regime (first two weeks) and a grower diet (second two weeks) during the adaptation period (first 4 weeks at feedlot) with no added humate source (control or CT, n=11) or with added humate source (potassium humate, or KH, n=11). During the adaptation period, calves in the CT and KH gained 1.28 and 1.54 kg/ day respectively, no difference between the treatments occurred for feed intake. Feed conversion ratio (FCR) tended strongly to differ between treatments with CT at 5.12 kg/kg and for KH at 4.25 kg/kg. Potassium humate intake levels were for the first two weeks 24.2 gram/animal/day and 15.3 gram/animal/day for week 3 and 4. The 17% reduction in FCR for KH is of significant economic value to the feedlot industry. This study indicates the positive value of KH as a feed additive in rations fed during the adaptation period to feedlot calves.

3.1 Introduction

Growth performance is one of the most important factors for the efficiency of beef production thus growth promoters such as implants are available for use in cattle to optimize production efficiency (Mantiziba, 2014). According to Radunz *et al.* (2011), implants are described as products containing natural and synthetic hormones that are implanted in the ear and affect the hormone status of the animal to optimize growth whereas humates are natural bioactive growth-promoting agent primarily decomposed from organic matter by living bacteria in the soil (Senn and Kingman, 1973; Shermer *et al.*, 1998; MacCarthy, 2001). These growth promoters primarily change division of energy from feed and take more to muscle instead of fat deposition, thereby increasing feed utilisation and weight gain (Mantiziba, 2014). However the use of these growth promotants has raised a lot of concerns from the health conscious consumer's promoting a need to find alternative and safe growth promoting agents (Wang *et al.*, 2008; Avcı *et al.* 2007; Galip, 2006).

An alternative natural growth promoting agents that has raised a lot of interest among researchers and animal producers in Potassium humate. Humates are natural performance enhancers that are increasingly gaining a lot of interest (Peña-Méndez et al., 2005; Cusack, 2008 & McMurphy et al., 2009). Studies with humates and other similar compounds have brought awareness to their physicochemical properties, which led to the idea of the use of these natural compounds in animal nutrition (Kocabagli et al., 2002; Peña-Méndez et al., 2005; McMurphy et al., 2009). Potassium humate is defined as a humic acid salts, normally called humate. Information on the use of humates (humic acid and fulvic acids) indicate growth promoting effects in farm animals when added to their diets (Kocabagli et al., 2002; Wang et al., 2008). In addition, fulvic acids contained in the humates have been shown to have beneficial effects on animal health by boosting immune system as a result of the antipyretic effect, antiviral effect and detoxifying effects of toxic substances (Agazzi et al., 2007; Islam et al., 2005). Moreover, humates have been observed to reduce the volatile ammonia in animal waste (Islam et al., 2005).

Humates have been shown to enhance nitrogen detergent fibre (NDF) digestion, reduce ammonia (NH_3) concentrations and increased pH in the rumen as well (Bell et al., 1997). Research has also shown that humates can help relieve stress effects in calves entering feedlots for first time (Islam et al., 2005). Weaned calves are susceptible to stress that may reduce performance and increase morbidity (Loerch & Fluharty, 1999). Despite the potential of potassium humate in improving performance in feedlot animals, it appears the results obtained are inconclusive. This trial was therefore done to investigate the effect of adding a humate source to the diets on performance (ADG and FCR) of weaner bulls during their feedlot adaptation period.

3.2 Materials and Methods

3.2.1 Study site

The study was carried out at cattle feedlot situated at the Animal Production Institute of the Agricultural Research Council (Irene, SA longitude 28°13 S: latitude 25°55 E, altitude 1526m). The area is characterized by an ambient temperature of 18 to 29°C during summer and between 5 and 20° C during winter.

3.2.2 Diets and experimental design

Twenty-two yearling male steers (average weight: 249.4 ± 5.62), weaned at 6-7 months were randomly allocated to two treatments: T1 (Control, n = 11) fed a standard mixed feedlot diet and Treatment 2 (Potassium Humate, PH, n = 11) fed a standard diet mixed with added potassium humate (5.8g/kg feed). The animals were housed individually with each animal as the experimental unit. PH inclusion was aimed to ensure an intake of 15 grams/animal per day. The steers were allowed to adapt to their environment for 6 days where the single stanchions were left open for the animals to access water and hay. They were then allowed an adaptation period of 14 days to experimental diets before the start of growth performance measurements. All steers had had free access to water. Daily feed allocations were changed according to amount of orts in the feed troughs. Orts were removed weekly or as dictated by feed trough conditions and noted. Daily feed allocation was initially based on an estimated intake of 4kg/day for the first batch of feed. The second batch was mixed according to feed intake by the treatment group. Feed analysis for chemical composition was done according to the Official methods for analytical chemistry (AOAC, 2005). The ingredients and nutritional composition of the starter and grower feeds are shown in Table 3.1.

3.2.3 Potassium humate source

The humate product (Potassium Humate-S100) used for this study was black in colour and was supplied by Omnia Nutriology. K-Humate is produced by alkaline extraction of Leonardite or peat during which potassium hydroxide is used. Heat is used to increase its solubility (product properties obtain from Omnia). The properties of the product are specified on Table 3.2.

3.2.4 Animal management

The animals were raised and kept according to feedlot standard practices. Before the beginning of the experiment, the animals were ear-tagged for identification and vaccinated against internal parasites using Gardal 10% and external parasite using Delete All and treated against any possible bacterial and viral infectious diseases (Clostridium ssp., anthrax, botulism, IBR) using Ivotan,Covexin, Botu-thrax and Bovitech III. The steers were then placed in individual pens and fed adaptation ration for 14 days. The pens (2m2 per animal) were developed to meet the welfare standards as guided by National Society for the Prevention of Cruelty to Animal (NSPCA, South Africa). The trial was run over a period of 112 days. Ethical clearance for the study was obtained from the ARC-API Ethics committee (Ethics clearance no. APIEC15/013). The animals were raised and kept according to feedlot standard practices.

Table 3. 1: Feed ingredients (kg) and estimated nutrient composition (% / kg DM, unless stated otherwise) of the starter and finishing diet

Item Ingredient	Starter 1 (kg)	Finisher (kg)	Nutrient composition	Starter 1	Finisher
Hominy chop	615	615	ME (MJ/kg DM)	11.83	12.05
Wheat bran	150	150	Fat (%)	5.69	5.95
Molasses meal	100	100	CP (%)	15.21	16.15
Cotton OCM[1]	50	50	CF (%)	13.4	8.4
Grass hay	50	50	NDF (%)	31.2	24.1
Feed-lime	15	15	Ca (%)	0.68	0.77
Urea	14	14	P (%)	0.59	0.63
Salt	5	5	K (%)	1.21	1.12
Premix	1	1			
Potassium Humate	5.8g/kg	1.7g/kg			

[1] Oil cake meal, extracted; CP = crude protein, CF = crude fibre, NDF = neutral detergent fibre, Ca = calcium, P = phosphorus, K = potassium.

Table 3. 2: Product Properties of Potassium Humate-S100

	SI Unit	Value
K-Humate	g/kg	920 (min)
Humic and Fulvic Acids	g/kg	780 (min)
Organic Carbon	g/kg	675 (min)
Potassium	g/kg	130 (min)
pH (in water)	pH	9.5-12 typical
Density	g/cm^3	0.94 typical
Solubility (in water)	g/L	Complete Solubility
Arsenic	mg/kg	2 (max)
Cadmium	mg/kg	5 (max)
Lead	mg/kg	10 (max)
Mercury	mg/kg	0.1 (max)

3.2.5 Measurements

3.2.5.1 Body weight and Average Daily Gain

The animals were weighed on the day of arrival (initial weight), and fortnightly after the beginning of experiment using Livestock weigh scale (Model LS4, Libra Measuring Instruments Pty Ltd). Scales used were calibrated prior to use on a weekly basis prior to cattle weighing. The average amount of weight an animal has gained each day for the experimental period of time the animal has been on feed will be calculated as follows:

$$ADWG\,(t_0,T) = \frac{W(T) - W(t_0)}{T - t_0}$$

Where: t_0 = initial time, T = final time (90^{th} day), W (T) = final body weight, and W (t_0) = initial body weight

3.2.5.2 Feed intake and feed conversion ratio

Feed intake (FI) was determined weekly, as the difference between the amount of feed offered and refusals. The refusals were removed, weighed, and discarded on a weekly basis. Feed Conversion Ratio (kg feed/kg gain) was calculated by dividing Total FI with BW gain.

FI = Feed offered – Feed refusals (taken the following morning)

3.2.6 Statistical analysis

The effect of diet on growth performance of feedlot steers was analysed using the Proc Mixed procedure (PROC MIXED) of SAS (2008) for repeated measures.The model used was:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where Y_{ij} = response variable (body weight, average daily gain, feed conversion ratio), the i^{th} observation form j^{th} treatment group, μ = is the overall mean (general mean), D_j = is the j^{th} Diet effect (Control and potassium humate), and E_{ij} = is the random experimental errors distributed independently and normally with mean zero and common variance, $\sigma 2$. Where differences were significant, mean separation was done using the *t-test* for comparison of mean.

3.3 Results

The results of body weight changes, FI, ADWG and feed conversion ratio (FCR) during the adaptation period are presented in Table 3.3. There were no statistical differences between all analysed growth performance parameters. However, during the adaptation period, calves in the KH gained more weight than those in the CT group (1.54 kg/day vs 1.28 kg/day). No difference between the treatments was observed with regards to feed intake. Feed conversion ratio (FCR) was higher in the CT group (5.12) than the KH group (4.25). Expressed as a percentage change, ADG was 20% higher for KH and FCR was 17% lower for KH.

Overall, body weight gains of steers fed diet supplemented with potassium humate tended to decrease as compared to the control group. During this period, the animals in the KH group were eating less feed with a higher body weight gain (Figure 3.1). Feed conversion ratio (FCR) tended (P=0.06) to differ between the treatments, with CT at 5.47kg/kg and for KH at 4.38kg/kg. For first two weeks potassium humate intake levels were 24.2 gram/animal/day and 15.3 gram/animal/day for week 3 and 4 (Figure 3.2). As the trial progressed, the control group started eating more feed compared to the K-humate group, thereafter by the end of the trial the animals had more body weight. Overally, steers fed diet with potassium humate had greater average daily than the steers in the control group during adaptation period. However, there was no significant difference in the ADG, FI and FCR of steers among the two treatment groups.

Table 3. 3: Performance of the weaner calves in the feedlot during the adaptatation period (first 4 weeks).

	Treatments			
Growth parameters	Control	Potassium humate	SEM	Sig
n	11	11		
Starting weight (kg)	246.9	246.3	18.3	0.98
End weight (kg)	282.7	289.8	17.6	0.41
ADG[1] (kg/day)	1.28	1.54	0.31	0.09
FI[2] (kg/day)	6.55	6.55	0.81	0.99
FCR[3]	5.12	4.25	1.16	0.06

[1] average daily gain; [2] Feed intake; [3] Feed conversion ratio ; n = number of animals

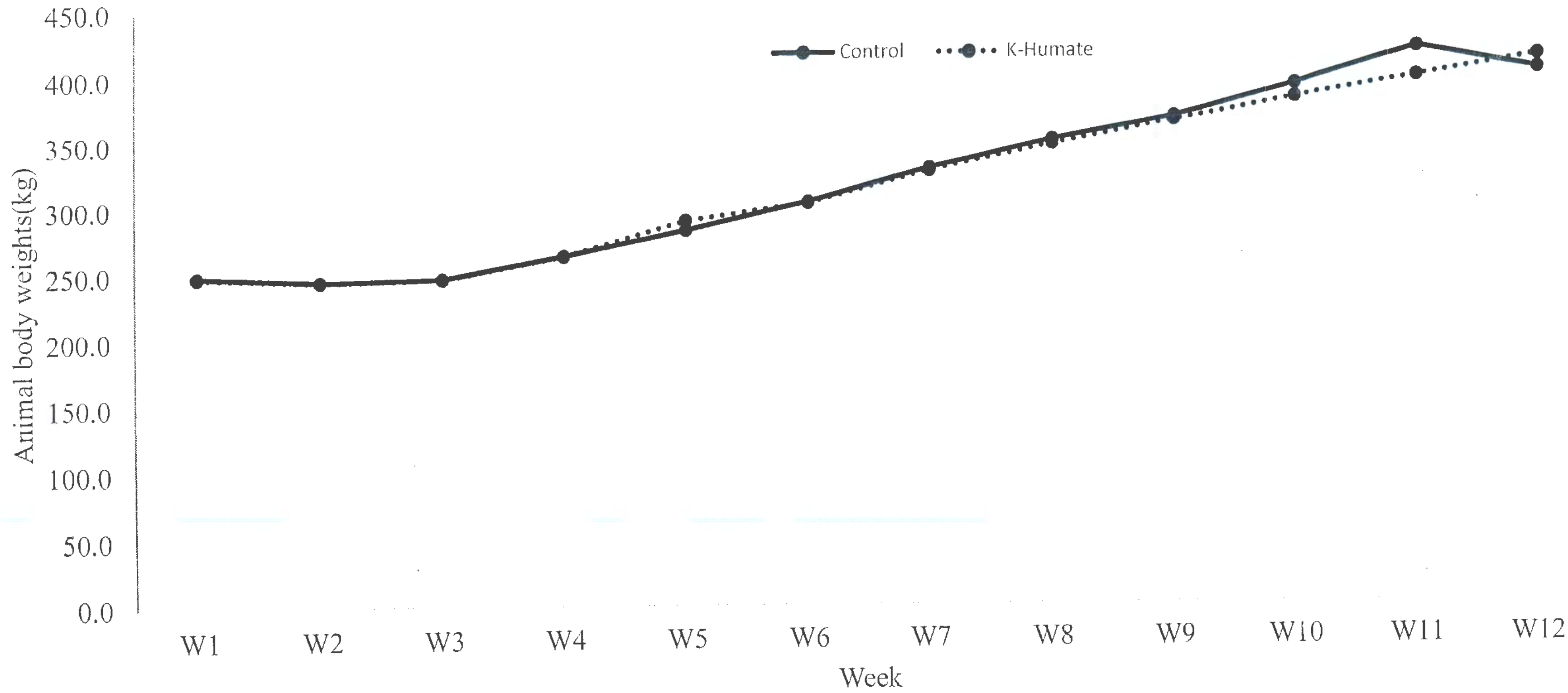

Figure 3. 1: Weekly animal body weights of both treatments

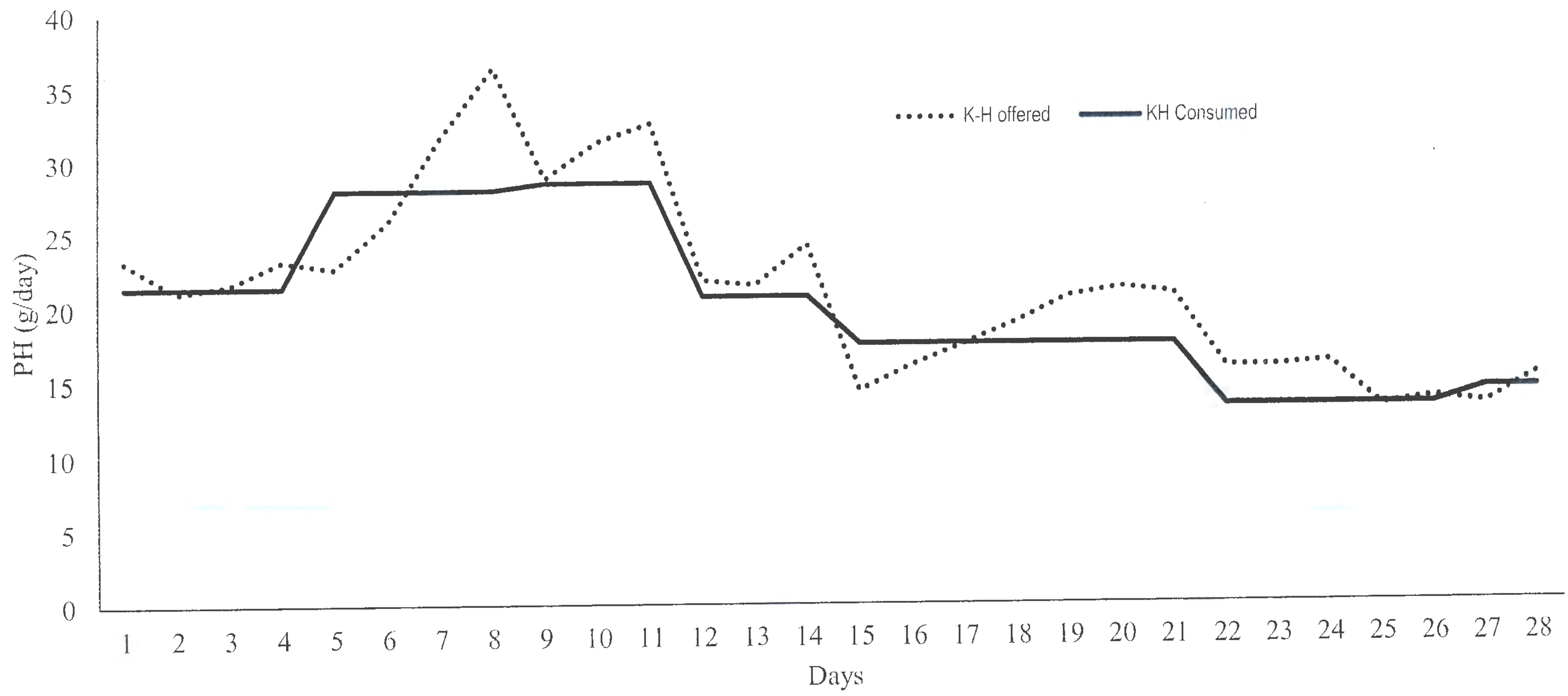

Figure 3. 2: Daily potassium humate intake by cattle for treated group

3.4 Discussion

The study intended to assess the influence of KH in diets on the growth performance of weaner steers in the feedlot. The small quantities of feed mixed and unpredictability of feed intake contributed to the higher level of KH consumed initially than intended. The variation between KH offered and consumed is due to the correction by subtracting the orts. The combination of higher fibre levels in the starter diets and KH have been reported to be beneficial to the digestion dynamics in animals (Váradyová *et al.*, 2009). Feed intake did not vary and is in accordance with Brown et al. (2007), however, results of McMurphy *et al.* (2009, 2011) and Chirase *et al* (2000, as reported by McMurphy *et al.*, 2011), showed a numerical or significant decrease in feed intake. The variation in ADG did dampen the results but was still numerically higher for KH (P=0.09) this seem opposite when compared with McMurphy *et al.* (2009). However, it is in line with the results of Cusack (2008) on the whole feeding period with feedlot cattle and in line with results from pigs (Wang *et al.*, 2008). A strong tendency (P=0.06) for improved FCR for KH was observed, where Brown *et al.* (2007) with steers and Covington *et al.* (1997) with lambs, found no difference for FCR.

There was no statistical difference between the control and potassium humate group in the case of growth performance parameters, at the end of the trial the result showed insignificant result in which growth performance of steers were not affected by humate added to the diet. These results were consistent with those of Chirase *et al.,* (2000) who also observed that inclusion of humates in feedlot diets had no impact dry matter intake (DMI), average daily gain (ADG) or feed to gain (F:G) ratio. Moreover, many studies (Ceylan *et al.*,2003; Yalcin *et al.*,2003;Ozcelik and Yalcin.,2004) also showed that humates or humic substances had no effect on live weight gained as reflected in this present study. However, in 2000, Eren *et al* observed that addition of 2.5g/kg of humates significantly increase live weight and live weight gains. This results differs from the current study done and can possibly be explained by the difference of the origin of the humate and its treating procedure.

According to a study conducted by Degirmencioglu (2012), different levels of humic acids did not affect feed intake in Saanen goats. These results were consistent with a previous reports by Vucskits *et al.* (2010), who reported that low or high doses of humate did not affect the feed intake and body weight in rats. Chirase *et al.* (2000) demonstrated a similar decrease in intake during first 28 days for cattle fed a lower humic substance concentration (0.78%) vs. control and increased concentrations (1.56% and 3.12% humic subsatnce). Brown *et al.* (2007) also reported no changes in performance or feed efficiency with the inclusion of humates in the diet. However, in a study with pigs by Wang *et al.* (2008) showed a beneficial increase in the ratio of body weight gain to feed intake.

The use of humates in livestock production still on an infant stage. More studies need to be conducted to ascertain the benefits of humic acid inclusion in diets on performance in ruminant animals (Galip, 2009). Differences in performance of livestock due to humate supplementation observed in the literature and in the study might be due to the compositional differences humate products used in different studies. Despite the fact that not enough evidence is available to argue for the use of humates in ruminant diets, Shermer *et al.* (1998) suggested that humates might influence animal performance by altering the microflora in the gastrointestinal system.

3.5 Conclusion

The variability in results from the different studies (published and un-published) highlights the need to quantify the various trace minerals and other compounds present in a specific source of humate. Also it was difficult to draw comparisons of the findings obtained in the present study with those from literature due to the different composition of humate products used in different studies and a generally lack of understanding on how the mechanism of actions between the various components work together. Nevertheless this research strongly indicates the positive value of KH as a feed additive in diets fed during the adaptation period to feedlot steers. It is therefore interesting also to evaluate the effect of potassium humate inclusion on meat quality of the steers

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CHAPTER 4

EFECT OF POTASSIUM HUMATE INCLUSION IN DIETS ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF WEANER STEERS

Abstract

The objective of the study was to determine the meat quality responses of weaner steers fed starter diets containing Potassium humate in a feedlot. Twenty-two yearling male steers were randomly allocated to two Treatments: Control (n = 11) fed a standard feedlot diet and Potassium Humate (PH, n = 11) fed a standard diet mixed with added potassium humate (5.8g/kg feed). The steers were house in individual stanchion with each steer as the experimental unit. After being fed over a period of 112 days, the steers were slaughtered and the *m. longissimus thoracic et lumborum* was sampled for carcass and meat quality measurements. From the results, PH in the diet improved meat tenderness and increased shear force values and meat pH (Figure 4.1). Shear force values were significantly higher ($P < 0.05$) in the Control group than the PH group at both Day 1 and Day 7 of measurement respectively. The post-mortem decline in pH was steeper ($P < 0.05$) in the Control group although ultimately, the PH group had a lower ($P < 0.05$) ultimate pH compared to the Control group.

4.1 Introduction

In the meat industry the greatest objective is to attend to the consumer demands for fresh and wholesome meat and meat products. Recently not only the quantity but also the quality of produced meat has become increasingly important. Beef producers face many challenges as the result of growing public concerns regarding the use of chemicals and antibiotics in animals. In South Africa beef animals are primarily finished in feedlots, with the administration of feed additives such as revalor and beta-antagonists a common practice. According to Nilzén *et al.*, 2001; Walshe *et al.*, 2006 and Shongwe *et al.*, 2007, the demand for meat produced naturally and organically with the exclusion of commercial feed additives has increased worldwide in recent years. The use of organic substance and acids such as humate as a feed additive, have recently been touted as potential alternative replacements for the commercial feed additives.

Meat quality is affected by both intrinsic and extrinsic factors. Among the extrinsic factors, diet, including addition of feed additives and other growth promotants has been observed to affect meat quality (Frylinck *et al.*, 2013), owing to their effects on growth rates, carcass fat and fatty acid content, meat instrumental measurements. Diet has also been observed to affect muscle energy status which in turn affects the conversion of muscle to meat and subsequently meat quality (Webb & Erasmus, 2013). Intrinsic meat quality aspects of animal products that are affected include carcass composition and conformation, carcass fat content and colour, meat composition, colour, tenderness and flavor. These parameters determines desirability and acceptability of meat products by consumers and hence their purchasing decisions (Lawrie, 2006).

Although studies have been cdone on the use of potassium humate as a feed additive in animal feedings systems, there are still a lot of questions that needs to be answered. Most of the studies conducted on the use of potassium humate have focused on monogastric animals. There appears to be no conclusive information on the beneficial effects of potassium humates on feedlot steers. Moreover, its effects meat on quality have not been elucidated. In Chapter 3, it was observed that inclusion of potassium humate in diets can have a positive influence on adaptation and growth performance of steers. I can therefore be expected that inclusion of potassium humates in diets can also have an influence on meat quality. Therefore, the objective of this study was to establish efficacy of Potassium humate as a feed additive in feedlot diets, by assessing its effect on carcass characteristics and meat quality attributes of weaner calves in a feedlot.

4.2 Materials and Methods

4.2.1 Study site

At the end of the feeding trial (Chapter 3), animals were transported from the feedlot to the Meat Industry Centre situated in the farm for slaughter and carcass and meat quality measurements.

4.2.2 Transportation and slaughter

The animals were transported using light duty vehicle to the ARC- Meat Industry Centre (MIC) abattoir and subsequently slaughtered according to the standard slaughter procedures (SAMIC, 2006; Leeuw *et al.,* 2009). The steers were subjected to a twelve-hour fasting period to empty the stomach, and where weighed before slaughter to determine the live weight at slaughter (LWS). The steers were electrically stunned using a captive bolt. Slaughtering was done following the normal procedures of the abattoir, whereby they were first stunned with a captive volt. Carcasses was electrically stimulated, using a voltage of 300V,a frequency of 50Hz, a current of 5A in 30 seconds at a pulse of 12/s (Mapiye *et al.*,2010).

4.2.3 Carcass characteristics

4.2.3.1 Carcass weights and dressing percentage

After slaughter the head, skin, limbs, viscera, as well as lungs and trachea were removed to obtain hot carcass weight (HCW). Carcasses were then split into two halves according to dissection methods described by Fisher and de Boer (1994). Then carcasses were chilled in refrigerator for 24 hours at -4 °C, then after they were weighed to obtain the cold carcass weight

(CCW). The eye muscle (*Longissimus thoracis*) cross-sectional area was measured using a plastic grid between the 10th and 11th ribs. Dressing percentage was calculated as warm carcass weight expressed as a proportion of final live-weight.

$$\textit{Dressing out \%} = \textit{Hot carcass wt. /slaughter wt.} \times \textit{100 \%}$$

The *m. longissimus thoracis et lumborum (LTL)* of the left side was sampled, a day after slaughter, from the 10^{th} rib in the direction of the rump for meat quality analysis.

4.2.3.2 Fat code

The carcass fatness was graded on a scale from 0 to 6 (0=no visual fat cover, 1=very lean, 3=medium, 4=fat, 5=over fat, 6=excessively over fat) based on the South African meat industry conformation scale (SAMIC, 2006).

4.2.3.3 Carcass conformation

Conformation of carcass from each individual steer was visually appraised using a 5-point scale. The South African meat industry (SAMIC, 2006) conformation scale of 1–5 (with 1=a very flat carcass, 2=a flat carcass, 3=medium carcass, 4=a round carcass, 5=very round carcass) was used.

4.2.4 Meat quality responses

4.2.4.1 pH and drip loss measurement

The pH and temperature values of the LTL were measured with a digital hand-held meat pH meter at 1 h, 2 h, 3 h and 24 h after slaughter. For drip loss measurement, two blocks of meat measuring 15 × 15 × 30 mm were cut from the LTL steak with the fibres running longitudinal to the axis of the sample. The meat samples were hooked on to the bottle caps using metal hooks and suspended in plastic sample bottles. The samples were suspended is such a way that they would not touch the side of the bottle. Subsequently, the bottles stored at 2 °C for 72 % in a cold room. Drip loss was calculated as the difference between the weight before storage and weight after storage, expressed as a percentage.

% Drip loss = [(Initial weight of meat - Final weight of meat)/initial weight] × *100%*

4.2.4.2 Determination of meat colour

A Minolta meter (Model CR200, Minolta, Japan) was used to measure meat colour on fresh samples (2 days post-mortem). A white calibration tile was used to calibrate the Minolta meter on three locations on the cut surface of individual steaks, the following CIE (1976) colour coordinates were measured: lightness (L*), redness (a*) and yellowness (b*) from. The measurements were taken on areas that had no connective tissue and intramuscular fat on them.

4.2.4.3 Cooking loss and Warner - Bratzler shear force (WBSF).

An Instron (3344, Universal Testing cross head speed at 400mm/min, one shear in the centre of each core) was used to measure meat tenderness (shear force). After cooking, sub samples of meat were cored parallel to the fibre of the meat. A Warner Bratzler (WB) shear blade mounted on an Instron 3344 (Universal Testing) was used to shear the meat samples perpendicular to the direction of fibres and mean maximum load (N) were recorded for each sample. Shear force values and cooking loss were determined on Day 1 after slaughter and day 7 of aging. Cooking loss was computed as:

$$Cookingloss\ \% = \frac{wt\ of\ raw\ steak\ after\ thawing - wt\ of cooked\ meat}{wt\ of\ raw\ steak\ after\ thawing} \times 100\%$$

4.2.5 Statistical analysis

The general linear models procedure (PROC GLM) of SAS (2008) was used to analyse the effect of potassium humate inclusion in diet on carcass characteristics and meat quality of feedlot steers was analysed using. The model used was:

$$Y_{ij}= \mu + D_i + E_{ij}$$

Where Y_{ij} = response variable (carcass characteristics and meat quality measures), the i^{th} observation form j^{th} treatment group μ = is the overall mean (general mean), D_j= is the j^{th} Diet effect (Control and potassium humate), and E_{ij} = is the random experimental errors distributed independently and normally with mean zero and common variance, σ2. Where differences were significant, mean separation was done using the *t-test* for comparison of mean.

4.3 Results

4.3.1 Carcass characteristics

Diet had no effect on all carcass measurement. Although there were no differences, generally the Control group had higher carcass measurements compared with the PH Group. Means values (±standard errors) obtained for slaughter weights, carcass traits and pH at 24 h post-mortem for all the animals used in this study are presented in Table 4.1. Slaughter weights were slightly greater for the steers in the control group than for the steers in the potassium group; however, this difference was not significant. All the steers had a conformation of 3 and a carcass fat score of 2 (lean) which is desirable for most consumers in South Africa. Eye muscle area was not significantly different ($P > 0.05$) between the two treatment groups. Carcasses in the PH group steers were lighter than those of the control steers (133 kg vs. 128 kg), and with

Table 4. 1: Effect of potassium humate dietary inclusion on carcass quality characteristics of weaner steers

	Treatments		
Parameter	Control	Potassium humate	Sig
Carcass Measurements			
Slaughter weight (kg)	477.9 ± 9.45	428.4 ± 9.01	NS
HCW (right side) kg	133.3 ± 2.99	128.1 ± 2.86	NS
HCW (left side) kg	132.5 ± 3.33	126.2 ± 3.17	NS
CCW (right side) (kg)	130.2 ± 3.05	125.1 ± 2.91	NS
CCW (left side) (kg)	129.4 ± 3.2	123.9 ± 3.05	NS
Dressing out %	57.9 ± 0.53	58.1 ± 0.51	NS
Eye Muscle Area (mm^2)	7849.3 ± 276.9	7747 ± 263.9	NS
Fat score (corrected)	3.70 ± 0.65	3.18 ± 0.63	NS
Carcass conformation	3.30 ± 0.16	3.09 ± 0.15	NS

*Significant at $P < 0.05$; NS = not significant; HCW = hot carcass weight; CCW =cold carcass weight a lower yield. In this present study, there were no significant differences ($P > 0.05$) between the two groups.

4.3.1 Meat quality

Diet had no effect on all meat quality measurements (Table 4.2) except for the shear force values and meat pH (Figure 4.1). Shear force values were significantly higher ($P < 0.05$) in the Control group than the PH group at both Day 1 and Day 7 of measurement respectively. The post-mortem decline in pH was steeper ($P < 0.05$) in the Control group although ultimately, the PH group had a lower ($P < 0.05$) ultimate pH compared to the Control group. No differences were observed in terms of post-mortem meat temperature decline (Figure 4.2).

4.4 Discussion

The objective of this study was to establish the effects of PH inclusion in starter diets of weaner steers on carcass characteristics and meat quality attributes. This study is one of the few attempts that has been made with regards to the use of non-conventional growth promotants in beef with the objective of producing meat with reduced residues that is acceptable to the consumers. From the results, diet had no effect on all carcass measurements although the Control group had marginally higher carcass values than the PH fed steers. This indicates that inclusion of PH in the diets of steers can probably result in similar effects of carcass characteristics as with the conventional growth promotants. Moreover, no differences were observed with regards to the objective meat quality parameters measured in the study except for the shear force values and meat pH. The comparable values of carcass and meat quality parameters may be a reflection of similar treatment of animals prior to slaughter, as observed in other studies (Coleman et al., 2016, Mapiye et al., 2010).

Table 4. 2: Effect of potassium humate dietary inclusion on meat quality characteristics of weaner steers

	Treatments		
Parameter	Control	Potassium humate	Sig
Cooking loss (Day 1) (%)	27.7 ± 4.44	26.9 ± 3.34	NS
Cooking loss (Day 7) (%)	26.7 ± 2.77	26.6 ± 3.46	NS
Drip loss (Day 1) (%)	5.4 ± 1.9	5.18 ± 1.06	NS
Drip loss (Day 7) (%)	5.24 ± 1.25	4.78 ± 1.25	NS
Shear Force (Day 1) (kg)	6.58 ± 1.23	5.12 ± 0.86	*
Shear Force (Day 7) (kg)	4.91 ± 1.04	4.14 ± 0.74	*
Meat colour			NS
L*1h	34.4 ± 0.75	36.6 ± 0.72	NS
a*1h	17.5 ± 1.08	17.1 ± 1.02	NS
b*1h	15.2 ± 0.53	14.7 ± 0.51	NS
C*1h	23.4 ± 1.04	22.7 ± 0.99	NS
H 1h	41.6 ± 1.57	41.7 ± 1.50	NS
L*24	42.3 ± 0.62	41.2 ± 0.62	NS
a*24	15.4 ± 0.93	15.2 ±0.86	NS
b*24	6.42 ± 0.55	5.73 ± 0.51	NS
C*24	16.8 ± 0.99	16.4 ± 0.94	NS
H24	22.2 ± 1.27	20.1 ± 1.22	NS

Significant at $P < 0.05$; NS = not significant; L = lightness; a* = redness; b* = yellowness; C* = chroma; H = hue

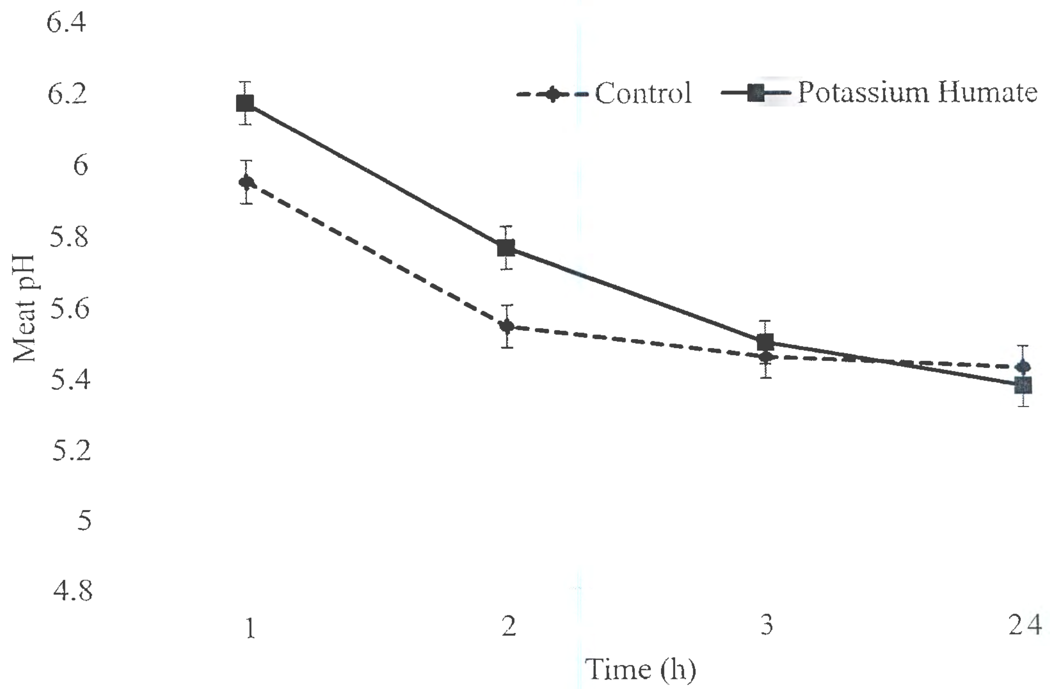

Figure 4. 1: The effect of potassium humate inclusion on post-mortem decline in meat pH of weaner steers

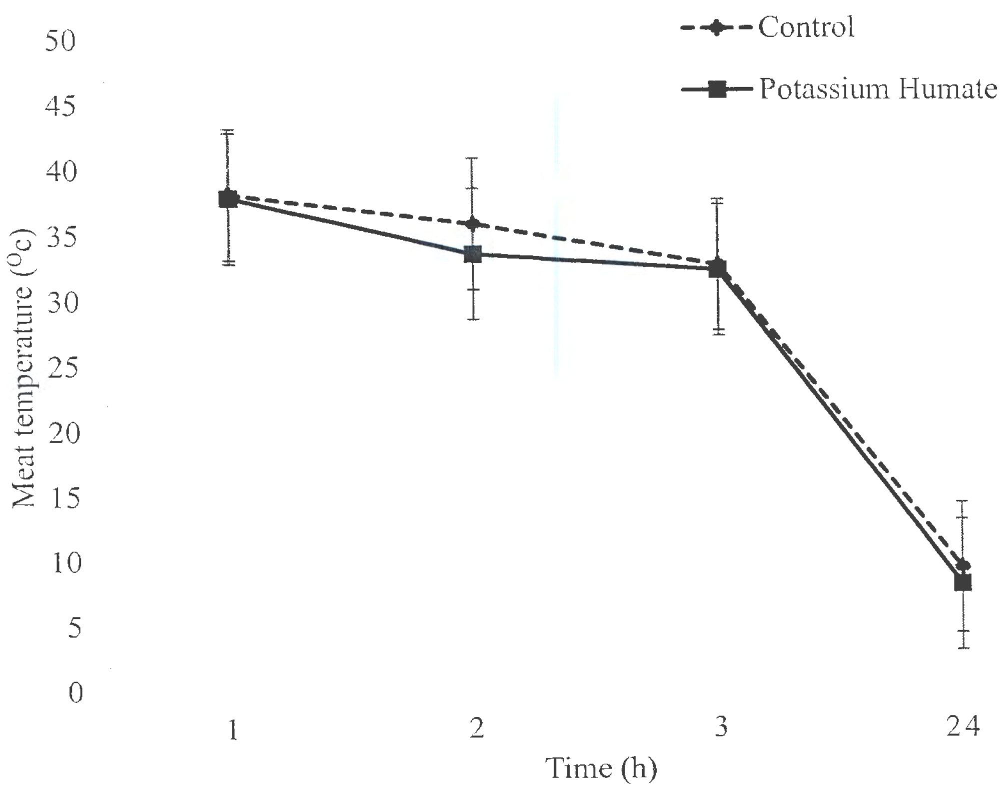

Figure 4. 2: The effect of potassium humate inclusion on post-mortem decline in meat temperature of weaner steers

Shear force is one of the critical indicators of meat quality that have a significant effect on consumer purchasing decisions. Factors such as ultimate pH, intramuscular fat and extent of proteolytic activities in meat have a significant on the level of tenderness of meat (Coleman *et al.*, 2016; Muchenje et al., 2009). In the current study, the observed significant effect on diet on shear force on day 1 and day 7 of aging is contrary to the findings from previous reports (Mapiye et al., 2010; French et al., 2001). The lower shear force values in the PH fed steers could be ascribed to the effects of PH in modulating energy and protein digestion dynamics during the growth phase (Wang et al., 2008; Kocabagli et al., 2002) and proteolytic activities during the rigor phase and the 7-day aging period (Jung et al., 2010). Nevertheless, this need further verifications.

The pH of meat is indicative of the level of acidity and normally the ultimate pH of meat is determined by the extent of the pH decline 24 hours after slaughter (Muchenje et al., 2009). In the current study, the post-mortem decline in meat pH was more pronounced in the control group compared with PH group although ultimately, the PH group has lower pH than the Control group. The pH_u in the two treatment groups were, however, on the lower end of the acceptable pH of a good quality meat, which is usually from 5.4 to 5.7 (Kerry and Ledward, 2002, 2009). Although the was lack of apparent variation in in temperature decline between the two treatments, it has been alluded that, generally, the rates of muscle pH and temperature decline and the pH-temperature interaction during the immediate post-mortem period may have a significant effect on meat tenderness (Xazela *et al.*, 2012; Kannan *et al.*, 2006).

The amount of fat and cholesterol food products contain, as well as the long term effect thereof are of an increasing concern. Although high quantities of visible fat are undesirable, they contributes to meat quality and are important to the nutritional value of meat (Nieto & Ros. 2012). The differences in fatty acid compositions generally effects the quality of meat, due to the fact that fatty acid composition affects both firmness of adipose tissue and the oxidative stability of meat, ultimately affecting flavour and muscle colour. High *PUFA* levels may alter meat flavour due increased susceptibility to rapid oxidation and the release of unpleasant volatile components during cooking (Wood *et al.*, 1999). Nevertheless, high PUFA levels are desirable and there has been increased attempts to modify the fatty acid composition in meat, especially reducing the concentration of *SFA* and increasing *PUFAs* through research. It is therefore imperative to assess the effects of Potassium humates inclusion in diets on the modification of fatty acid profiles meat and their oxidative stableness.

4.5 Conclusion

Inclusion of PH in starter diets of weaner steers significantly improved meat tenderness and caused a greater in meat pH, two of the most important parameters affecting meat quality. In addition, PH inclusion resulted in comparable values for carcass characteristics and objective meat quality measurements. Therefore, PH inclusion in steer diets can provide an alternative in the production of safe and healthier meat in the feedlots although more verification needs to be done to provide conclusive evidence. Since meat quality measures are closely associated with fatty acid composition of meat, it is also necessary to evaluate the effect of potassium humate inclusion in diets of fatty acid composition and oxidative stability of the meat.

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CHAPTER 5

THE EFFECT OF POTASSIUM HUMATE INCLUSION IN FFEDLOT DIETS FATTY ACID PROFILILES, ATHEROGENICITY AND LIPID STABILITY OF WEARNER STEERS

Abstract

The objective of the study was to determine the fatty acids profiles and lipid stability responses of weaner steers fed starter diets containing Potassium humate in a feedlot. Twenty-two yearling male steers were randomly allocated to two groups: Control (n = 11) fed a standard feedlot diet and Potassium Humate (PH, n = 11) fed a standard diet mixed with added potassium humate (5.8g/kg feed). After being fed over a period of 112 days, the steers were slaughtered and the *m. longissimus thoracic et lumborum* was sampled from the 10^{th} rib in the direction of the rump for fatty acid and oxidative stability analysis. From the results, PH in the diet improved increased the total amount IMF ($P < 0.05$) and some SFAs but there was a decrease in the level of some n-6 PUFAs ($P < 0.05$). The PH group had comparable values for all other nutritional indices as the Control group.

5.1 Introduction

Fatty acids composition and concentration of several nutrients depend largely on the diet fed of the animal. Thus, there has been an increased interest in the manipulation the fatty acid (FA) composition of meat for the benefit of human health. Meat is considered to be a major source of dietary fat for humans. Altering the lipid content and FA composition of foods can therefore be an effective way of providing desirable fats that benefits consumers' health (Qi, 2010). Intramuscular fat, whether in adipose tissue or muscle, have an important contribution towards the various aspects of meat quality and are central to the nutritional value of meat.

The important fat acid indices include among other, total Polyunsaturated fatty acids (PUFA), total omega 6 fats (n-6), total omega 3 fats (n-3), the ratios of PUFAs over saturated fats (SFA) and finally the ratio of n-6 to n-3 fatty acids (Wood *et al.*, 2004). These indices are normally used to evaluate the nutritional value of meat. A low proportion of polyunsaturated fatty acid and high proportion of saturated fatty acids in meat may increase the risk of cardiovascular diseases (Mapiye, et al., 2011; Wood *et al.*, 2004). Ruminants conserve PUFA in muscle and the ratio of 18:0/18:2n _ 6 in adipose tissue declines as fattening proceeds. Diet therefore can significantly alter the amounts and proportions of different fatty acids (Wood *et al.*, 2004). This can be achieved by the effects of different nutrients and feed additives in diets that can modulate beneficial fatty acids synthesis. Although the underlying mechanism is still not well understood, in pork, organic acids such as humic acid were observed to affect marbling values and to reduce back fat thickness probably due their influence on protein and lipid distribution (Wang *et al.*, 2008).

In Chapter 4, it was observed that potassium humate inclusion in diets affected some carcass characteristics and meat quality parameters. Fat score and body conformation may be indicative of the intramuscular fat and the amount of fat in the carcass and muscle influences the fatty acid composition. This study therefore intents to evaluate the effect of inclusion of potassium humate in diets on fatty acid composition, nutritional indices, athrogenicity and oxidative stability of meat from weaner steers.

5.2 Materials and Methods

5.2.1 Study site

The study site was described in Chapter 3 (Section 3.2.1)

5.2.2 Meat samples

A day after slaughter, the *m. longissimus thoracis et lumborum (LTL)* of the left side was sampled, a day after slaughter, from the 10^{th} rib in the direction of the rump for fatty acid and oxidative stability analysis. The samples were vacuum packed and send for analysis of fatty acids at the Microbial, Biochemical and Food Biotechnology Centre, University of Free State (SA).

5.2.3 Proximate analysis and fatty acids profiles

Quantitative extraction of total fat from meat samples was done according to the method of Folch *et al.* (1957) using chloroform and methanol in a ratio of 2:1 with the addition of butylated hydroxytoluene, an antioxidant, at a concentration of 0.001 % to the chloroform: methanol mixture. Drying of the fat extracts was done overnight in a vacuum oven at 50°C using a rotary evaporator, with phosphorus pentoxide as the moisture adsorbent. A gravimetric method was used to determine the total extractable fat expressed as percent fat (w/w) per 100 g tissue. The extracted fat from the meat samples was stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and froze then it was stored at–20°C pending fatty acid analyses.

Quantification of Fatty Acid Methyl Esters (FAMEs) of the meat samples was done using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Identification of the FAME samples was done by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Merck Chemicals (Pty Ltd, Halfway House, and Johannesburg, South Africa) provide all solvents and reagents. Individual fatty acids were expressed as a proportion of total fatty acids present in the sample. The following fatty acid indices were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio,

5.2.4 *Athrogenicity and Desaturase activity*

Atherogenicity indices were computed as the ratio of SFA/ unsaturated FA using the following formula proposed by Ulbricht and Southgate (1991):

$$Atherogenicity\ index\ (AI) = [C12{:}0 + 4\ (C14{:}0) + (C16{:}0)] / \sum(MUFA + PUFA) \quad (1)$$

The Δ^9 desaturase indices were computed using the following models proposed by Lock and Garnsworthy (2003):

$$Desaturase\ index\ (DI) = C14{:}1/C14:0 \quad (2)$$

$$Desaturase\ activity\ (DA) = (\sum \Delta 9 \text{ desaturase products})/$$
$$(\sum \Delta 9 \text{ desaturase substrates} + \text{products}) \quad (3)$$

The desaturase indices were used as an indicator of the Δ^9 desaturase activity using fatty acids that are substrates and products for Δ^9 desaturase

5.2.5 *Lipid peroxidation*

Lipid peroxidation was determined by the method described by Nur Alarm, (2013) that involved thiobarbituric acid reactive species (TBARS) with Malondialdehyde (MDA) as standard. A volume of 0.1 mL meat supernatant was treated with 2 mL of (1:1:1 ratio) TBA–TCA–HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and allowed to cool. The amount formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm using a spectrophotometer (Hewlett Packard, UV/visible light) against a reagent blank. Percentage inhibition was calculated using the equation:

$$\%\ of\ lipid\ oxidation\ Inhibition = \{Ao- A1\}/Ao \times 100$$

Where; Ao = the absorbance of the control and A1 = the absorbance of the sample extract.

5.2.6 *Statistical analysis*

Data on fatty acid profiles, athrogenicity and lipid stability of the meat was analysed using the general linear models procedure (PROC GLM) of SAS (2008). The model used was:

$$Y_{ij}= \mu + D_i + E_{ij}$$

Where Y_{ij} = response variable (fatty acid profiles, athrogenicity and lipid stability), the i^{th} observation form j^{th} treatment group μ = is the overall mean (general mean), D_j= is the j^{th} Diet effect (Control and potassium humate), and E_{ij} = is the random experimental errors distributed independently and normally with mean zero and common variance, σ2. Where differences were significant, mean separation was done using the *t-test* for comparison of mean.

5.3 Results

5.3.1 *Fatty acid composition*

The effects of Potassium humate inclusion in diets on marbling and muscle fatty acids composition are presented in Table 5.1, 5.2 and 5.3. The total IMF was higher (by 16%, $P < 0.05$) in the potassium fed weaner steers compared with the control group. With regards to the SFA, higher ($P < 0.05$) amounts of Myristic acid (C14:0) and Heneicosanoic acid (C21:0) were

Table 5. 1: Effect of potassium humate dietary inclusion on proximate fat composition (%) of L. dorsi muscle from weaner steers

	Treatments			
[1]Parameter	Control	Potassium humate	SE	Sig
IMF	1.66	1.97	0.09	*
FFDM	22.07	22.04	0.24	NS
Moisture	76.27	76	0.27	NS

[1]Parameter: IMF = Intramuscular fat; FFDM = Fat free dry matter; *Significant at $P < 0.05$; NS = not significant;

Table 5. 2: Effect of potassium humate dietary inclusion on individual fatty acid composition (%) of L. dorsi muscle from weaner steers

	Treatments			
Fatty Acids	Control	Potassium humate	SEM	Sig
Saturated fatty acids (SFA)				
C12:0	0.004	0.02	0.01	NS
C14:0	2.47	3.03	0.17	*
C15:0	0.32	0.34	0.02	NS
C16:0	26.11	27.01	0.6	NS
C17:0	1.76	1.6	0.07	NS
C18:0	16.58	16.94	0.49	NS
C20:0	0.06	0.07	0.01	NS
C21:0	0.29	0.23	0.01	*
C23:0	3	2.35	0.27	NS
Monounsaturated fatty acids (MUFA)				
C14:1c9	0.34	0.45	0.05	NS
C16:1c9	2.07	2.32	0.14	NS
C17:1c10	0.33	0.45	0.06	NS
C18:1t9	0.25	0.18	0.08	NS
C18:1c9	31.38	31.93	0.87	NS
C18:1c7	3.43	3.52	0.3	NS
Polyunsaturated fatty acids(PUFA)				
C18:2c9,t11 (n-6)	0.2	0.21	0.02	NS
C18:2c9,12 (n-6)	9.26	7.60	0.74	NS
C18:3c6,9,12 (n-3)	0.01	0.02	0.01	NS
C18:3c9,12, 15 (n-3)	0.42	0.4	0.02	NS
C20:2c11,14 (n-6)	0.02	0.02	0.01	NS
C20:3c8,11,14 (n-6)	0.06	0.04	0.01	*
C20:4c5,8,11,14 (n-6)	0.5	0.37	0.04	*
C20:5c5,8,11,14,17 (n-3)	0.01	0.01	0.02	NS
C22:5c7,10,13,16,19 (n-3)	0.42	0.35	0.04	NS

*Significant at $P < 0.05$; NS = not significant; SEM = standard error of the mean; TBARS+ = Thiobarbituric acid reactive substances; n-6 = omega 6; n-3 = omega 3.

Table 5. 3: Effect of potassium humate dietary inclusion on total fatty acids and ratios of L. dorsi muscle from weaner steers

	Treatments			
Fatty Acids	Control	Potassium humate	SEM	Sig
Total SFA	47.6	49.3	0.82	NS
Total MUFA	38.3	39.2	1.15	NS
Total PUFA	14.1	11.5	1.11	NS
Total n-6	12.5	10.2	1.01	NS
Total n-3	1.56	1.29	0.11	NS
PUFA:SFA	0.26	0.21	0.02	NS
PUFA:MUFA	0.3	0.23	0.02	*
n-6: n-3	8.05	7.86	0.3	NS
Atherogenicity index	0.55	0.6	0.02	NS
Desaturase index	1.92	1.89	0.09	NS
TBARS	0.04	0.04	0.01	NS

*Significant at $P < 0.05$; NS = not significant; SEM = standard error of the mean; TBARS+ = Thiobarbituric acid reactive substance

observed in the potassium humate fed steers compared to the control group. However, diet had no effect on all other SFAs. Similarly, diet had no effect on all MUFAs. On the contrary, diet affected some PUFAs with the Control group having more (P < 0.05) Eicosatrienoic acid [(C20:3c8, 11, 14 (n-6)] and Eicosatetraenoic acid [C20:4c5, 8, 11, 14 (n-6)]. With regards to nutritional indices, only the PUFA: MUFA ratio was affected with the Control group having a higher (P < 0.05) ratio compared to the PH group. All other indices were not affected by diet.

5.3.2 Athrogenicity, desaturase indices and oxidative stability

Diet had no effect on atherogenicity and desaturase indices (Table 5.3). The ratios of C14:0/C14:1 did not differ significantly between the muscles of steers receiving potassium humate or control. No statistically significant correlation was found between the Δ9 desaturase index and total lipid content. The use of humate in diets had no effect on the lipid oxidation of meat cuts between the two treatments groups, results are shown in (Table 5.3). TBARS values were similar between the two treatments. There is no information about the effect of dietary humate on the lipid oxidation in muscle tissue of feedlot steers for discussion of the results. However, the effect of humate on lipid oxidation in the present study may have been arisen from various fatty acid profiles which humate caused.

5.4 Discussion

The fatty acid composition of meat is one of the critical determinants of the nutritional value of meat (Coleman *et al.,* 2016; Vessby, Gustafsson, Tengblad, & Berglund, 2013). A low proportion of polyunsaturated fatty acid and high proportion of saturated fatty acids in meat may increase the risk of cardiovascular diseases (Mapiye, Chimonyo, Dzama, Hugo, Strydom, & Muchenje, 2011; Wood *et al.*, 2004). In the current study, subtle differences were observed in some individual SFAs and PUFAs between the two treatment groups although their ratios were not significantly different. Generally the PUFA/SFA ratio, together with the omega-6 to omega-3 ratio, are indicators of the indicators of nutritional value of meat and the consumption higher proportions of omega-3 fatty acids is associated with reduced cardiovascular diseases and cancer (Aldai, Nájera, Dugan, Celaya, & Osoro, 2007; Simopoulos, 2004). In our study, although there were no significant differences in the PUFA/SFA ratio and the n6/n3 ratio, the PUFA/SFA ratios for both treatments were well below the desired ratio of 0.45. Nevertheless, the ratios obtained in the current study were higher than those obtained by Coleman et al. (2016). The observed low total omega-3 fatty acids obtained in the current study could be due to the low amounts of C18:3 n-3 and C20:5 n-3 that normally characterizes the total mixed rations that were given to the steers. This is consistent with observation from other studies (Vasta *et al.*, 2009; Warren *et al.,* 2008). Contrary to the findings by Warren et al. (2008), the n6:n3 ratio were well above the recommended values of < 5:1, pointing the unbalanced levels of PUFAs in meat from both treatments. However, the ratios were lower than those reported for yearling steers of different genotypes by Aldai *et al.* (2007).

The nutritional value of meat is not only determined by the proportions of fatty acids therein but also by the endogenous metabolism of the fatty acids, particularly the Δ9 desaturase activity, atherogenicity and oxidative stability of the meat (Vessby *et al.*, 2013, Vasta *et al.*, 2009). Δ9 Desaturase (Stearoyl-coenzyme-A desaturase 1 (SCD-1) activity plays a critical role in modulating the intracellular effects of SFA as well as the production of CLA and MUFA (Nantapo, Muchenje, & Hugo, 2014; Vessby et al., 2013; Souyert et al., 2006). In the current study, the lack of differences with regards to DI and atherogenicity index between the two treatment groups concurs with findings from other studies (Vasta et al., 2009). High values for DI and Atherogenicity index of meat may be associated with increased risks of cardiovascular diseases (Vessby *et al.*, 2013). The carcass fat, as fat thickness, or meat fat, as intramuscular fat, contents have a significant effect on the fatty acid profile of the meat. In general, there is an increase in the SFA and a decrease of MUFA with the increase in the fat content (Orellana *et al.*, 2009).

Interestingly, no differences in the TBARS was observed between the two treatments in our study. Due to the presence of phenolic compounds that have been observed to have antioxidave properties, PH was expected to increase the oxidative stability of the meat by significantly reducing the TBARS levels in the meat (Rababah *et al.*, 2006). A possible explanation for this observation could be that the PH levels used in the diets were probably low to cause any significant reduction in lipid oxidation, hence the similarities observed between the two treatments.

5.5 Conclusion

From the study, although the inclusion of Potassium humate in diets did not affect total fat content, individual fatty acids and nutritional indices, it was evident that composition of fatty acids in meat is not fixed and can be changed by differences in dietary components. The nutritional value of beef including the amounts and composition of fatty acids may influence consumers´ perception of meat that may influence their purchasing decisions. Therefore, inclusion natural feed additives cattle diets can provide an alternative in the production of desirable meat in the feedlots although more verification needs to be done to provide conclusive evidence.

5.6 References

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CHAPTER 6

GENERAL DISCUSSION AND RECOMMENDATION

6.1 General discussion

In the present study humic acid was used in the form of a commercial K-Humate S100 feed additive preparation. However, there was little information in the literature concerning the use of humic salts as a feed additive for promoting growth in ruminants. Limited studies on the effects of humate or humic acid on feedlot performance, health and production in ruminants showed the positive effect on the utilisation of carbohydrates and protein. When analysing the data, there are indications that breed might play a role in the results for some of the parameters. Average daily gain showed a tendency to have a breed*treatment interaction (P0.09) and time could also play role (P=0.10). However, this was not pursued further as these breeds and crosses are regarded as fairly similar. The results are analysed on the two treatments only for the purpose of this report, but that breed could play a role should be taken into account.

The proposed inclusion level was to achieve a 15 gram intake of humate per animal per day. Around this some confusion occurred and this level is lower than proposed by McMurphy *et al.* (2011), who proposed 5 g/kg humic acid (Bovigro®) in a cannulated dairy cow trial. Tomassen and Faust (2000) used in their dairy cow trial 2, 3 or 4 g HA/day, they used Lithicin™ which has 74% humic acids. The report on tests done at West Virginia University, by Miller Webster and Hoover, where they used 5 g, 10 g or 15 g humate per day equivalent to a continuous batch culture. From Table 6.1 it is clear that administration levels vary and that there is in some instances no report on humic acid levels, or mineral composition. Further there is also no information on the bioavailability of the humic acid or its minerals.

Table 6. 1: Humate trials and their inclusion levels

Article	Administration levels	Species	Humic acid %	% P
Tomassen & Faust (2000)	0, 2, 3, 4 g/day Lithicin™	Dairy cows	74	
Kocabağli et al. (2002)	0, 2.5 g/kg Farmagültör	Poultry	90	0.3
McMurphy at al. (2009)	DRY™	Beef cattle		
Kaya & Tuncer (2009)	0, 5, 10, 15 g/kg Bovigrow	Poultry		
Písaříková et al. (2010)	0, 2.5 g/kg	Pigs		
McMurphy et al. (2011)	30 g/kg	Dairy steers	90	0.3
Degirmencioglu & Ozbilgin	0, 5, 10, 15 g/kg Bovigro®	Dairy goats	68	
(2013)	0, 1, 3 g/kg Bovifarm	Sheep	70	13
Omnia – unpublished (2013)	0, 10 g/day	Beef Cattle	70	13
This study	0, 15 g/day (±2.2 g/kg)			

Humates have also been given to newborn goat kids (Agazzi *et al.*, 2007) in liquid form for the first 8 weeks and show an improved growth performance. Most research seems to use the g/kg indication or g/day, at a level of 2 g/kg as the inclusion level most are recommending. Tomassen and Faust (2000) seemed very low whilst McMurphy *et al.* (2011) is very high. Again the level of bioavailable humate and its mineral composition is unknown and adding to the variability of the results obtained by these authors and in this study. However, further research is warranted, developing a negative control and a larger population which is more equivalent to beef industry setup.

6.2 Recommendations

There is a range of commercial humates available for use in agriculture however there is a lack of solid evidence which highlight the positive outcome of adding potassium humate on ruminant's diets. Many of the manufacture of commercial humates products make unsubstantiated and claims which portray poor image of these products when compared to few research trials. It is clear that there is a need for further research trials, more specifically, field trials are need to explain the effect of commercial humates on ruminants. Inconsistent results, exaggerated claims and correct inclusion rates are some of the issues that clearly highlight the need for such research. In conclusion, in order to determine if commercial humate play a major role in animal nutrition or agricultural industry, it is of recommendation that more scientific trial are conducted at sufficient inclusion rates that would have an effect, targeted to the growth, health, carcass and meat quality of animals.

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CHAPTER 7

ETHICS, GENERAL HUSBANDRY AND CARE OF EXPERIMENTAL ANIMALS

- This experimental trial was conducted in accordance with standard ethical norms of ARC-API and North West University. Ethical clearance for the study was obtained from the ARC-API Ethics committee (Ethics clearance no. APIEC15/013).
- The trial was conducted at ARC-API feedlot situated in Irene. General veterinary care was monitored by a qualified personnel.
- Animals were correctly ear-tagged and pens were labelled accordingly
- The steers were housed on single pens with free movement and provided with water ad-libitum.
- A Data sheet of daily observations on the health status of each animal and its morbidity was taken and recorded. Mortality was recorded as they occurred.
- Cattle slaughter and weighing was done humanely, following the usual commercial procedure at the MIC.
- Feed-bunkers and water troughs were cleaned daily