

Effects of dietary fibre level on rumen pH, total microbial count and methanogenic *archaea* in Bonsmara and Nguni steers

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Abstract

A study was conducted to evaluate the effect of the level of dietary fibre on rumen pH, total microbial count and methanogenic *archaea* count of Bonsmara (225 ± 10.0 kg live weight) and Nguni (215 ± 10.0 kg live weight) steers. Nine steers per breed were used, aged 8 to 9 months. A 3 x 2 factorial experiment was executed with treatments allocated in a completely randomized design. The treatments were diets which included 64.3% neutral detergent fibre (NDF) for diet 1, 40.4% NDF for diet 2, and 29.9% NDF for diet 3. Steers were fed for 90 days and housed in individual pens. Rumen fluid was collected from steers using a stomach tube. Rumen fluid samples were taken immediately to the laboratory for microbial assay. Data were subjected to analysis of variance (ANOVA). The various levels of dietary fibre did not affect rumen pH and methanogenic *archaea* count. However, a low-fibre diet yielded high rumen total microbial count for Bonsmara steers and low rumen total microbial count for Nguni steers. Therefore, a low-fibre diet can be applied as a viable strategy to enhance rumen total microbial count in exotic beef breeds and crossbred cattle such as Bonsmara. Nguni steers fed a high NDF diet had higher rumen total microbial count than Nguni steers fed a low-fibre diet. Therefore, a high NDF diet can be used efficiently by feeding it to indigenous breeds and purebred cattle such as Nguni.

Key words: Enteric fermentation, lactic acidosis, rumen function, methanogenic *archaea*

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Introduction

The exponential growth of human population in the world compels farmers to improve livestock production to meet the increasing demand for animal protein (Delgado, 2003). Feedlot operators view cattle with a shorter production cycle, rapid growth and better feed efficiency as ideal to supply the quality protein required for the growing human population (Smil, 2002). In South Africa, most feedlot markets prefer exotic crossbred animals, such as Bonsmara, because of their outstanding feed efficiency under feedlot conditions, to indigenous and purebred animals such as Nguni (Strydom, 2008). Nguni cattle that were fed a high-fibre diet showed high feed efficiency compared with the Nguni fed a low-fibre diet (Linde *et al.*, 2016). This shows the ability of Nguni cattle to utilize high-fibre diets efficiently. When an animal consumes a high-fibre diet, fibre-digesting microbes such as *Fibrobacter spp* proliferate in its digestive system, which results in balanced microbial activity with normal (6 - 7) rumen pH (Brown *et al.*, 2006; Owens *et al.*, 1998). However, if the same animal changes to a low-fibre diet, starch-digesting microbes such as amylolytic bacteria proliferate in its digestive system, converting starch into lactic acid and ultimately reducing the rumen pH (Reddy *et al.*, 2008). Fibrous feedstuffs are commonly incorporated in diets to prevent the excessive dropping of rumen pH that may be encountered with low-fibre feedlot diets (Meissner *et al.*, 2010).

The main global concern with high-fibre diets is the production of methane, which is one of the greenhouse gases (Morgavi *et al.*, 2010; Wright *et al.*, 2004). Methanogenic *archaea* produce methane when they synthesize energy for growth by using hydrogen to reduce carbon dioxide, formed as products of the microbial feed digestion process (Hook *et al.*, 2010). Several rumen microbes are involved in enteric feed fermentation (Henderson *et al.*, 2015). However, the rumen microbial population and its diversity depend on the nutrient composition of the diet consumed by the host animal (Kamra, 2005). Methanogenic *archaea*, like

most other rumen microbes, are sensitive to low levels of ruminal pH (Popova *et al.*, 2012). Feeding beef cattle with extremely low-fibre diets might result in ruminal pH level below 6.0 for prolonged periods (Hünerberg *et al.*, 2015), which is not conducive to rumen microbial activities such as enteric fermentation (Russell & Rychlik, 2001).

The literature recommends that NDF should be maintained at 25% of dietary dry matter with at least 75% from forage for efficient rumen functioning (Banakar *et al.*, 2018; Vermaak, 2011). There is a need to determine the level of dietary fibre at which methanogenic *archaea* would be reduced without affecting the rumen pH and total microbial count (rumen functioning) negatively. Supplemental fats, starch, ionophores, concentrates, halogenated compounds, condensed tannins, probiotic yeast products and direct-fed microbials are some of nutritional interventions that have been tested and applied to reduce methane production in ruminants (Vohra *et al.*, 2016; Jeyanathan *et al.*, 2014; Beauchemin *et al.*, 2008; Guan *et al.*, 2006; Yan *et al.*, 2000; Johnson & Johnson, 1995). However, most of these nutritional interventions are costly and thus not practical for poorly resourced beef cattle farmers, especially those under communal farming systems. The purpose of this study was to evaluate the effect of the level of dietary fibre on rumen pH, total microbial count and methanogenic *archaea* count of Bonsmara and Nguni steers.

Materials and Methods

This study was conducted at Agricultural Research Council–Animal Production (ARC–AP) Institute in Irene, Pretoria. Ethical approvals were obtained from the ARC–AP Research Ethics Committee (Ref: APIEC15/047) and University of South Africa, College of Agriculture and Environmental Sciences (Ref: 2015/CAES/130). Nine Bonsmara steers and nine Nguni steers received three dietary treatments with various levels of neutral detergent fibre (NDF). These were 64.3% for diet 1, 40.4% for diet 2, and 29.9% for diet 3. A 3 x 2 factorial experiment (three dietary treatments and two cattle breeds) was used with treatments being allocated in a completely randomized design. The steers were adapted gradually to the experimental diets for 14 days to avoid ruminal acidosis and bloat (Klieve *et al.*, 2003). They were fed 2.5% of their live bodyweight on a dry matter basis each day. The orts were removed from the feed bunks and weighed weekly. Feed consumed was taken as the difference between the amount fed and the orts. The ingredients used in the formulation of treatment diets are presented in Table 1.

Table 1 Ingredients used to formulate experimental diets for Nguni and Bonsmara steers to supply various levels of neutral detergent fibre

Ingredients (kg)	Diet 1	Diet 2	Diet 3
<i>Eragrostis curvula</i>	100	75	30
Sunflower oil	-	15	5
Hominy chop	45	175	300
Wheat bran	100	75	95
Cotton oil cake	25	40	20
Molasses meal	50	50	50
<i>Medicago sativa</i>	150	50	25
Feed lime	1.0	2.5	2.5
Urea	1.5	2.0	1.75
Salt	2.5	2.5	2.5
Premix	1.25	1.25	1.25

Diet 1: 64.3% neutral detergent fibre, Diet 2: 40.4% neutral detergent fibre, Diet 3: 29.9% neutral detergent fibre

The dry matter content of the diets was determined by drying freshly mixed samples of each diet in a drying oven at 60 °C for 48 hours, following the procedure of AOAC (2010). Dried diet samples were then ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored in labelled bottles for chemical analyses. Crude protein (CP), organic matter (OM) and ether extract (EE) were analysed according to AOAC (2010). Acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and crude fibre (CF) were determined according to the technique prescribed by

Van Soest *et al.* (1991). Gross energy (GE) was assessed with a bomb calorimeter (MC-1000 modular calorimeter, energy instrumentation, 135 Knoppieslaagte, Centurion, South Africa). For the analysis of phosphorus (P) and calcium (Ca), the feed samples were compressed in a ring vessel to produce a pellet, which was then dried to a residual moisture of <3% for 3 - 4 hours at 60 °C before X-ray fluorescence analysis (West *et al.*, 2013). Table 2 shows the chemical composition of the diets.

Table 2 Chemical composition of treatment diets for Nguni and Bonsmara steers to supply various levels of neutral detergent fibre

Nutrient	Diet 1	Diet 2	Diet 3
Dry matter,%	92.5	91.9	89.9
Organic matter, %	86.1	87.3	87.9
Crude protein, %	7.7	10.1	8.9
Ether extract, %	1.5	4.2	3.4
Gross energy, MJ/kg dry matter	16.4	17.1	16.9
Crude fibre, %	41.5	18.2	10.8
Acid detergent fibre, %	46.3	16.8	12.1
Acid detergent lignin%	11.7	13.9	13.6
Neutral detergent fibre, %	64.3	40.4	29.9
Calcium, %	0.5	0.5	0.4
Phosphorus, %	0.2	0.2	0.2

A stomach tube was used to collect rumen fluid from steers fed the three diets (Geishauser, 1993). It was rinsed thoroughly with distilled water before and after every collection to avoid cross contamination of the samples from different animals. Three samples per treatment were collected. Rumen fluid was collected within two minutes per animal. The fluid was then poured into a pre-warmed collecting flask, sealed and taken to the laboratory within 20 minutes. Rumen fluid was filtered through two layers of cheesecloth and kept at 39 °C under constant flushing with CO₂. Fresh rumen fluid samples were then transferred into the anaerobic cabinet to ensure that rumen fluid samples were not exposed to oxygen to retain the anaerobic condition. A pH meter was used to measure pH level (Duffield *et al.*, 2004) from the rumen fluid samples. Monitoring rumen pH level is one of viable strategies for diagnosing acidosis (Enemark, 2009).

The method for determining microbial count from rumen fluid was adapted from Mahadevan *et al.* (1982). One ml of fresh rumen fluid was added to 9 ml volumes of anaerobic diluent. The process continued with tenfold dilution of the original sample. One ml of the dilution was placed in labelled sterile disposable petri dish. The same procedure was carried out for a control dish that contained no sample and 1 ml of uninoculated diluent. Fifteen ml per plate of MRS agar solution was added as a growth media. The contents of the dishes were then swirled to achieve thorough mixing. The agar was allowed to set. The dishes were inverted and stacked in transparent plastic canisters to protect the contents against desiccation. Incubation at 39 °C in an oxygen-free atmosphere took place for 48 hours. The control dish was also incubated and examined for possible contamination. After incubation, the colonies on each inoculated petri dish containing between 30 and 300 colonies were counted. The figures were then multiplied by the dilution factor to obtain colony-forming units (cfu) per gram/ml of sample.

The method for determining rumen methanogenic *archaea* count was adapted from Carberry *et al.* (2014). The DNA extraction from rumen fluid was done with a DNA extraction kit (Inqaba Biotec™, Africa's Genomics Company, Pretoria, RSA) according to the manufacturer's instructions. Universal primers were used to detect rumen *archaea* counts, using a real-time PCR molecular method. Quantification of methanogenic *archaea* counts was performed by quantitative real-time PCR (qRT-PCR) analysis of the 16S rRNA genes.

Shapiro-Wilk's test was performed to test for non-normality. The data were analysed using analysis of variance procedures of SAS (SAS Institute Inc., Cary, North Carolina, USA). The linear model included the main effects of breed and diet, and the interaction between them. Student's t-tests at the 5% level of probability were used to compare the treatment means.

Results and Discussion

There was no difference ($P > 0.05$) in rumen pH between diets and breeds (Figure 1). However, steers fed the low-fibre diet had rumen pH of 6.3 for Bonsmara and 6.4 for Nguni, indicating that 29.9% NDF of diet 3 was enough to maintain adequate rumen buffering capacity. This may also result from the steers being gradually adapted to experimental diets to avoid a sudden change from high- to low-fibre diets (Klieve *et al.*, 2003). An extreme drop in rumen pH usually occurs when ruminants are suddenly introduced to low-fibre diets without restriction (Reddy *et al.*, 2008). This abrupt change leads to lactic acid accumulation in the rumen because of a shift in microbial populations from gram-negative predominance to gram-positive lactic acid producers (Meissner *et al.*, 2014). These results were supported by the outcomes of Brown *et al.* (2006), who reported that a high-fibre diet resulted in balanced microbial activity and a normal (6 - 7) rumen pH level.

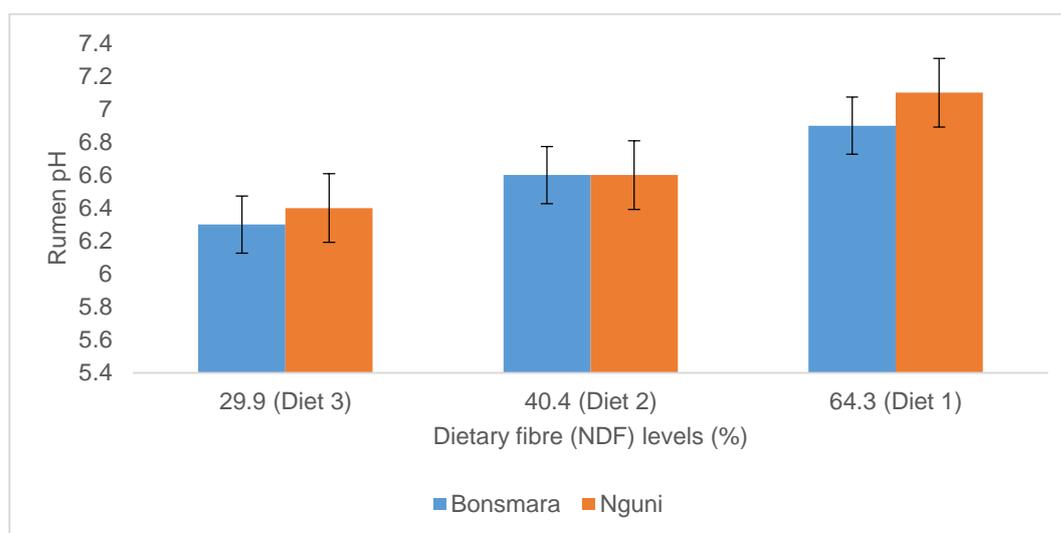


Figure 1 Effects of various levels of dietary fibre on rumen pH in Nguni and Bonsmara steers

Bonsmara steers fed the low-fibre diet had a higher ($P < 0.05$) rumen total microbial count than Nguni steers on the same diet (Table 3). This might be because Bonsmara cattle are bred to perform better under feedlot systems (Muchenje *et al.*, 2008). These results concur with those of Guan *et al.* (2008), who found that the presence of particular rumen microbes relied on the interaction between animal genetic make-up and diet composition. High rumen total microbial count is also an indication of an ideal rumen environment and good rumen function (Russell & Rychlik, 2001), which depend on the feed efficiency of the host animal and the fermentation characteristics of the feed (Hutjens, 1991). Nguni fed a high NDF diet had higher ($P < 0.05$) total microbial count than Nguni fed a low NDF diet. These results are supported by the findings that Nguni fed a high NDF diet had better feed efficiency than the Nguni fed a low NDF diet (Linde *et al.*, 2016). When fed the low-fibre diet, rumen total microbial count for the two breeds differed ($P < 0.05$) from the time that they were provided with more fibre (Table 3).

Table 3 Rumen total microbial count (cfu/ml) of Bonsmara and Nguni beef steers fed three experimental diets

Breed	Diet 1 (Mean, SD)	Diet 2 (Mean, SD)	Diet 3 (Mean, SD)	P-value
Bonsmara	1.23x10 ^{6c} , 0.1	3.85x10 ^{6c} , 0.6	9.30x10 ^{7a} , 0.1	<0.0001
Nguni	5.16x10 ^{6c} , 0.7	5.63x10 ^{6c} , 0.4	3.15x10 ^{7b} , 0.8	<0.0001

Means with a common superscript were not different with probability $P = 0.05$. SD: standard deviation
 Diet 1: 64.3% neutral detergent fibre; Diet 2: 40.4% neutral detergent fibre; Diet 3: 29.9% neutral detergent fibre; cfu: colony-forming unit

There was no difference ($P > 0.05$) in rumen methanogenic *archaea* count between diets or between breeds. However, rumen methanogenic *archaea* count in steers fed diet 3 was 0.1% for Bonsmara and 0.4% for Nguni (Figure 2). This was consequently associated with rumen pH level of 6.3 for Bonsmara and 6.4 for Nguni, which were lowest among the diets. These results are supported by the observations of H unerberg *et al.* (2015), who found that methanogens are sensitive to low rumen pH levels. On the other hand, rumen methanogenic *archaea* count in steers fed diet 1 was 0.8% for Bonsmara and 0.9% for Nguni (Figure 2). Rumen methanogenic *archaea* count for Bonsmara tended to be lower than for Nguni across all treatment diets (Figure 2). Whitford *et al.* (2001) reported that rumen *archaea* count was associated with the feed efficiency of a host animal, and Strydom *et al.* (2001) did not report substantial differences between Bonsmara and Nguni in feed conversion.

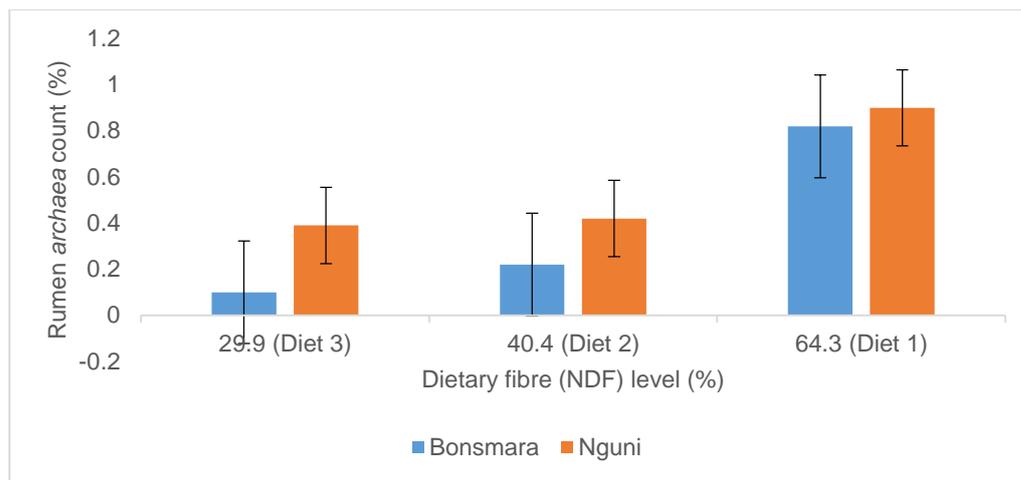


Figure 2 Effects of level of dietary fibre on rumen *archaea* as a percentage of all microbes of Bonsmara and Nguni steers fed diets containing various levels of fibre

Conclusions

Dietary fibre level did not affect the rumen pH and methanogenic *archaea* count. However, feeding a low NDF diet resulted in high rumen total microbial count for Bonsmara steers and low rumen total microbial count for Nguni steers. Consequently, NDF of 29.9% was sufficient to ensure adequate rumen buffering capacity, which is crucial to the survival of essential rumen microbes and efficient enteric fermentation. On the other hand, Nguni steers fed a high NDF diet had higher total microbial count than Nguni steers fed a low NDF diet. A low-fibre diet can be applied as a viable strategy to enhance rumen total microbial count in crossbred cattle such as Bonsmara. A high-fibre diet can be used efficiently by feeding it to indigenous breeds and purebred cattle such as Nguni, since they are able to yield high rumen total microbial count when they are fed high-fibre diets. Further research on the diversity of rumen microbial populations should be carried out to contrast the numbers of beneficial versus detrimental microbes and additional insight into growth performance and methane emission of these two beef breeds.

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Authors' Contributions

STJ, MMR, K-JL & KRM were in charge of project design and writing the manuscript. STJ, MMR & K-JL were in charge of project implementation. All co-authors participated in analysis of the data, and tabulation and interpretation of the results. This manuscript has been read and approved by all of the authors.

Conflict of Interest Declaration

The authors wish to affirm that there are no conflicts of interest associated with this work.

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