Differential gene expression in the Longissimus dorsi of Bonsmara and Nguni cattle associated with diet in South Africa

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Summary

Nguni cattle are farmed mostly under extensive production systems with production of weaners for the feedlot. Due to the relative smaller frame and slower growth compared to other composites such as the SA Bonsmara, South African feedlots tend to discriminate against the Nguni weaners. In this study the effect of a lower energy diet compared to a traditional high energy feedlot diet has been investigated using a transcriptome approach. Twenty Nguni and twenty Bonsmara bulls were divided into two groups (10 Bonsmara and 10 Nguni per groups and fed a low or a high energy diet, respectively for 120 days. At slaughter, *L. dorsi* muscle samples were collected for transcriptome analysis. Performance results show a higher, carcass weight and marbling score for all bulls fed the high energy diet compared to the bulls on the low energy diet, irrespective of breed. A total of 73 differentially expressed genes were observed across breeds. PITX2 and PRKAG3 were expressed in the bulls that received the high energy diet. Genes that are involved in intramuscular fat deposition were expressed on a higher level in the bulls that received the low energy diet compared to the bulls that received the high energy diet. Further studies are required for a better understanding of the effect of the diet on gene expression associated with muscle and fat deposition in Nguni cattle.

Keywords: low and high energy diet, transcriptome, myogenesis, marbling

Introduction

The Nguni is a tropically adapted *Bos taurus* Sanga breed, indigenous to Southern Africa and is known for its adaptability to harsh conditions (Makina *et al.*, 2014). Nguni cattle are mostly used in extensive commercial production. Nguni cattle are furthermore kept in communal systems where they contribute to the livelihoods of resource poor farmers in South Africa (Mapiye *et al.*, 2009). These systems do not have the carrying capacity to finish the cattle off from the veldt and therefore the producers have a need to market their cattle to feedlots. In South Africa 75% of beef are produced in feedlots. One of the breeds most commonly found in the feedlot is the SA Bonsmara, a medium frame breed, well adapted composite breed (Strydom, 2008) that is also the most numerous beef breed in South Africa (Bosman *et al.*, 2016). The Nguni being a small frame breed is disadvantaged, despite an acceptable adaptability to the feedlot conditions, due to their tendency for early fat deposition in the growth stage and lower carcass weights (Strydom *et al.*, 2008).

It has been reported that environmental factors, such as nutrition, can modify gene expression, where relatively small changes in gene expression may result in can significant changes in the performance of animals (MacNamara, 2015). An improved understanding of the association between the environment and the underlying genes holds potential for more effective livestock management (Scholtz *et al.*, 2014). The aim of this paper was to determine differences in the transcriptomes (*Longissimus dorsi*) of Nguni and Bonsmara bulls fed a high or a low energy diet.

Materials and Methods

The animals and sample collection.

Ethical approval was received from the Animal Ethical Committee of the University of Pretoria (eco90-15). Twenty Nguni bulls and twenty Bonsmara (control) bulls were given an adaptation period of 28 days where after they were fed for 90 days. The bulls (10-14 months old) were divided into two groups per breed; one group received a low energy diet (10.9 MJ ME/kg) and the other a high energy diet (12.5 MJ ME/kg). No growth hormones or implants were added to the diets or injected in the animals. They were penned 10 animals per pen and individual feed intake was measured via Calan gates (American Calan, USA). The animals were weighed weekly and real time ultrasonic scanned at the commencement and completion of the study. The bulls were slaughtered after 120 days in the feedlot. At slaughter, *Longissimus dorsi* muscle samples were taken for RNA extraction from at the 13th rib, using an 8mm biopsy punch. The samples were taken 15 minutes after death, immediately frozen in liquid Nitrogen and stored in a -80 °C fridge until RNA extraction could be performed.

RNA extraction and RNA sequencing

RNA was extracted from the frozen *L. dorsi* muscle samples. Each sample had three replicates (each replicate was 120 mg). TRIzol® Reagent (Ambion, USA) was used to extract the RNA with chloroform, isopropanol and ethanol. Each sample was treated with the RNase-free DNase set (Qiagen, Hilden, Germany) and purified with the RNeasy mini kit according to manufacturer's guidelines (Qiagen, Hilden, Germany). Extracted RNA from the samples were immediately stored in a -80 °C fridge. The extracted RNA was quantified using Qubit Fluorescent meter (Invitrogen, USA) according to the manufacturer's protocols. Three samples per treatment group (n=12) was sequenced with three replicates of each sample (36). Sample preparation was done with TruSeq stranded mRNA protocol (Illumina, USA). Sequencing was performed using a HiSeq 2500 (Illumina, USA) and ran pair-ended 125 x 125. Four GB data per sample was received for downstream analyses.

Statistical Analysis

The weight, feed intake and RTU measurements of the bulls were recorded and analysed using SAS v 9.4 Proc MIXED (SAS, 2004). The transcriptomic data was analysed using various software programs. FastQC was used to determine the quality of the data received for downstream analysis. Trimmomatic (Bolger *et al.*, 2014) trimmed the data and removed the adapters. Quality control was performed again before further analysis. Salmon (Patro *et al.*, 2016) was used to build the index and to align to the reference genome (UDM3.1.1), R Statistical package for visual conformation of the differential expressed genes and the Panther Classification System (Mi *et al.*, 2016) for annotation of the genes. The data from the breeds were pooled together to analyse the influence of the diets for the purpose of this paper.

Results

The performance results are shown in Table 1. The following traits differed ($p<0.05$) between the breeds: live weight (LW), average daily gain (ADG), feed efficiency (FCR), rib fat, marbling and carcass weight (CW). Traits that showed a significant difference between the high and the low energy diet were the marbling score and the carcass weight.

Table 1. The performance traits of the Bonsmara and the Nguni fed a high or a low energy diet.

Traits	Bonsmara			Nguni		
	Average	High Energy diet	Low energy diet	Average	High energy diet	Low energy diet
LW (kg)	337.9[a]	354.9[a]	330.6[a]	303.9[b]	301.1[b]	306.6[b]
ADG	1.56[a]	1.65[a]	1.50[a]	1.32[b]	1.34[b]	1.30[b]
FCR	7.14[a]	6.88[a]	7.32[a]	8.33[b]	8.36[b]	8.30[b]
Rump fat	5.5	5.3	5.6	5.3	5.3	5.2
Rib fat	3.19[a]	3.25[a]	3.29[a]	2.88[b]	2.87[b]	2.89[b]
EMA	57.0	57.0	57.5	54.5	54.3	54.7
Marbling	3.31	3.43[a]	3.34[b]	2.98	3.40[c]	2.56[d]
CW	176.76	187.70[a]	168.39[b]	156.23	159.11[c]	153.35[d]

[abcde] – different superscripts across columns indicate significance at $p<0.05$

Between the bulls that were fed the low energy diet and those fed the high energy diet, 73 genes were differentially expressed (adjusted p-value < 0.10), irrespective of breed. Of these genes, 62 genes were annotated. Fold change indicated that 43 genes were up-regulated (fc > 0) (SPARC, CRHR2, Kreuppel-like factor 15) and 30 genes were down-regulated (fc < 0) (PAX8, PITX2, PRKAG3) in the low energy diet. These differentially expressed genes are predominantly involved in the metabolic pathways as well as cell proliferation across both breeds.

Discussion

South African farmers experience a number of environmental challenges in extensive production systems and grazing is not always available to finish cattle from the veldt. Marketing to feedlots provides an alternative. Feedlots, however, are reluctant to purchase Nguni weaners compared to fast growing and larger framed breeds (Strydom, 2008). Feedlot diets have been formulated at relative high energy content for faster growing breeds and it is unknown if Nguni cattle might perform better when fed a lower energy diet. Transcriptome analysis may be useful to gain insight in gene pathways related to muscle and fat deposition.

In this study, the Nguni fed the low energy diet had a slightly higher live weight (306.6 kg) compared to the Nguni fed the high energy diet (301.1 kg), however this difference between the diets were not significant ($p > 0.05$). The breed average for live weight is 338 kg (Scholtz, 2010), which is higher than what was found in this project.

Nutrition has an influence on the gene expression of the animal. PAX8 and PITX2 genes involved in myogenesis were upregulated in the bulls that received the high energy diet. Myogenesis requires specific transcription factors, such as PAX3 (Goulding *et al.*, 1999) and these genes are precursors of the muscle regulatory factors that lead to muscle growth. PAX3 is correlated with PITX2 (Shih *et al.*, 2007) and expressed in all cells where these genes are present. Despite playing a crucial role in myogenesis in the embryo, PITX2 can be found in most muscles of the adult animal where it seems to play a role in the maturation or maintenance of muscle cells. As these genes were upregulated in the bulls that received the high energy diet, it can be suggested that the high energy diet being fed to the animals result in a heavier live weight. This is strengthened by the significantly higher carcass weight of the bulls fed the higher energy diet compared to the bulls fed the low energy diet.

The bulls fed the high energy diet had a higher rump fat (P8) and rib fat measurement compared to the bulls that received the low energy diet. Schoonmaker *et al.* (2004) found that feeding a high energy diet caused an appreciable amount of energy to be partitioned towards subcutaneous fat deposition. Marbling is the amount of intramuscular fat between bundles of muscle fibres and an indicator of meat quality (Hocquette *et al.*, 2010). Wang *et al.* (2009) suggests that a prerequisite for intramuscular fat development might be the expansion of the extracellular matrix. The SPARC gene influences the synthesis and interaction with the extracellular matrix. In this study, the SPARC gene was upregulated in the bulls of both breeds that received the low energy diet compared to the bulls that received the high energy diet. However, the bulls that received the high energy diet had a higher marbling score as diets high in energy increase intramuscular fat deposition. It has been reported that Sanga cattle have a capacity to deposit more fat intramuscularly compared to *Bos taurus* cattle and crosses, that tend to deposit fat subcutaneously (Shaffer *et al.*, 1981). Differences in fat deposition can have implications for fatty acid mobilisation in terms of thermoregulation and energy reserves (Nonoka *et al.*, 2008) and could therefore be seen as adaptive mechanisms. The higher expression of CRHR2 as well as Kreuppel-like factor 15 in the bulls that were fed the low energy diet strengthens this observation, as both are associated with higher marbling score and adipogenesis (Wibowo *et al.*, 2007). However, in this study the bulls that received the high energy diet had a higher marbling score. Further studies are required to study the breed diet interaction in terms of marbling and carcass weight.

Conclusion

Gene expression profiling provides an effective tool to discover changes in the expression of genes linked to production traits. In this study, genes that are involved in myogenesis pathways were expressed higher in the bulls that received the high energy diet compared to the bulls that received the low energy diet. This concludes that the high energy diet is advantageous for higher muscle development. However, the genes involved in intramuscular fat development exhibited higher expression in the bulls fed a low energy diet compared to the bulls fed the high energy diet. In contrast, the high marbling scores were observed in the bulls that received the high energy diet. Further studies are required for a better understanding of the effect of the diet on gene expression associated with muscle and fat deposition in Nguni cattle.

List of References

Bolger, A.M., M. Lohse & B. Usadel, 2014. Trimmomatic: a flexible trimmer for Illumina

sequence data. Bioinformatics. 30: 2114-2120
Bosman, L.M., R.R van der Westhuizen, C.D. Visser & E. van Marle-Köster, 2016. Determination of genome-wide linkage disequilibrium in the South African Bonsmara reference population. J. Anim. Sci. 94 Suppl 4: 36
Goulding, M., A. Lumsden & A.J. Pacquette, 1999. Regulation of PAX3 expression in the dermomyotome and its role in muscle development. Development 120: 957-971.
Hocquette, J.F., F. Gondret, E. Baéza, F. Médale, C. Jurie & D.W. Pethick, 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4: 303-319
Makina, S.O., F.C. Muchadeyi, E. van Marle-Köster, M.D. MacNeil, & A. Maiwashe, 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. Front. Genet. 5: 333
Mapiye, C., M. Chimonyo, K. Dzama, J.G. Raats, & M. Mapekula, 2009. Opportunities for improving Nguni cattle production in the smallholder farming systems of South Africa. Livest. Sci. 124: 196-204.
McNamara, J.P., 2015. Triennial lactation symposium: systems biology of regulatory mechanisms of nutrient metabolism in lactation. J. Anim. Sci. 93: 5575 – 5585.
Mi, H., X. Huang, A. Muruganujan, H. Tang, C. Mills, D. Kang & P.D. Thomas, 2017. Panther version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. 45: D183 – D189.
Nonaka, I., N. Takusari, K. Tajima, T. Suzuki, K. Higuchi & M. Kurihara, 2008. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. Liv. Sci. 113 (1), 14-23
Patro, R., G. Duggal, M.I. Love, R.A. Irizarry & C. Kingsford, 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods. 14 (4): 417-419.
SAS, 2004. Statistical analysis systems user's guide (V 9.4). SAS Institute, Inc. Cary, North Carolina, USA.
Scholtz, M.M., Ed, 2010. Beef breeding in South Africa. 2nd Edition. Agricultural Research Council, South Africa. pp211-213.
Scholtz, M.M., A. Maiwashe, F.W.C. Neser, A. Theunissen, W. J. Olivier, M.C. Mokolobate, & J. Hendriks, 2014. Livestock breeding for sustainability to mitigate global warming, with the emphasis on developing countries. S. Afr. J. Anim. Sci. 43: 269-281.
Schoonmaker, J.P., M.J. Cecava, F.L. Fluharty, H.N. Zerby & S.C. Loerch, 2004. Effect of source and amount of energy and rate of growth in the growing phase on performance and carcass characteristics of early- and normal-weaned steer. J. Anim. Sci. 82: 273-282
Shaffer, L., J.D. Roussel & K.L. Koonce, 1981. Effect of age, temperature, season and breed on blood characteristics of dairy cattle. J. Diary Sci. 64: 62-70
Shih, H.P., M.K. Gross & C. Kioussi, 2007. Expression pattern of the homeodomain transcription factor PITX2 during muscle development. Gene Expr. Patterns 7: 441-451.
Strydom, P.E., 2008. Do indigenous Southern African cattle breeds have the right genetics for commercial production of quality meat? Meat Sci. 80: 86-93
Strydom, P.E., L. Frylinck, J. van der Westhuizen & H.M. Burrow, 2008. Growth performance, feed efficiency and carcass and meat quality of tropically adapted breed types from different farming systems in South Africa. Aust. J. Exper. Agri. 48: 599-601
Wang, Y.H., N.I. Bower, A. Reverter, S.H. Tan, N. De Jager, R. Wang, S.M. Williams, L.M. Cafe, P.L. Greenwood & S.A. Lenhert, 2009. Gene expression patterns during intramuscular fat development. J. Anim. Sci. 87: 119-130.
Wibowo, T.A., J.J. Michal & Z. Jiang, 2007. Corticotropin releasing hormone is a promising

candidate gene for marbling and subcutaneous fat depth in beef cattle. Genome. 50: 939-945.