The development and application of approaches in protein profiling for Nguni cattle

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INTRODUCTION

As a consequence of natural selection in stressful environmental conditions, the Nguni cattle have been reported to be metabolically superior under unfavourable conditions, thus indicating an adaptive measure to survive times of poor feed quality. Compared to European breeds such as the Hereford, this indigenous Nguni breed is less susceptible to drought, parasites, diseases and insects. It is also widely acknowledged to be the outstanding beef breed for optimal production under harsh African conditions. One of the features that enable the Nguni to survive under these adverse conditions is their nitrogen cycle pathway which is believed to result in the animals being less dependant on dietary protein than other breeds. It is speculated that this could be partly due to the maintenance of high blood urea levels when the nitrogen content of the pasture drops.

OBJECTIVE

To use proteomics to characterise genetic-borne polymorphisms that may contribute to this phenomenon in the Nguni, and to conserve and enhance the trait to be able to select for it in other breeds. Data are presented on techniques such as 2D-electrophoresis that have been developed and optimised to separate the Nguni kidney and liver proteome, decreasing its complexity, allowing mining of low abundance proteins. Mass spectrometry (LC-MS/MS) will be used to compare selected tissues of interest in the Nguni and Hereford breeds in terms of protein identification and expression, to identify proteins potentially associated with increased tolerance to drought.

PROJECT SUMMARY

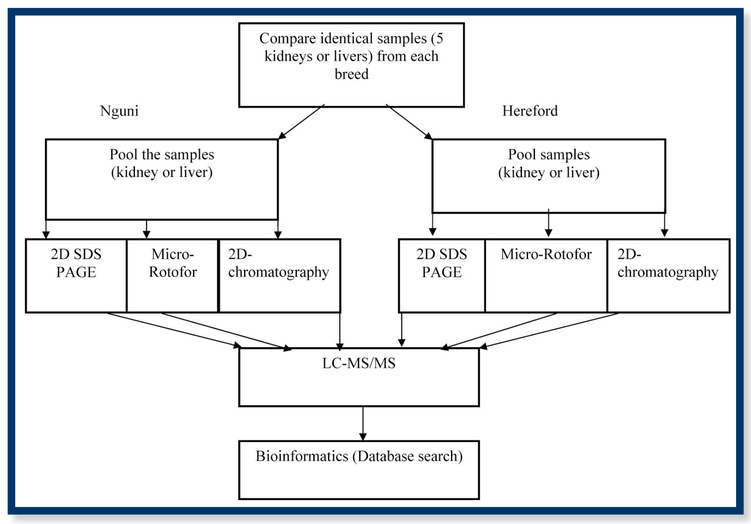


Figure 1: Study design to analyse kidney and liver samples from the Hereford and the Nguni cattle. The samples from both species will be pooled, analysed and compared. The cell lysates will be separated by 2DE, MicroRotofor and 2D Chromatography then analysed by LC MS/MS

MATERIALS AND METHODS

Nguni and Hereford cattle tissues are obtained from the Agricultural Research Council (ARC) Animal Production Institute in Irene, Gauteng. Different methods were explored to identify the best method for liver and kidney extraction.

- **TCA Method:** Frozen tissue was crushed and 0.2 g of the tissue was weighed out and resuspended in ice-cold 1 ml 10% (w/v) TCA-in-acetone solution with DTT. Samples were vortexed and incubated at -20 °C for 14 hours.
- **Protein extraction according to Xu et al., (2005):** Frozen tissue was crushed and mixed with a solution containing 8 M urea, 4% CHAPS (w/v), 65 mM DTT, 50 mM Tris-HCl. The samples were centrifuged; supernatant was retained for protein concentration determination.
- **Protein extraction using the Isopropanol method:** Frozen tissue was crushed and mixed in a solution containing known concentrations (10%, 20%, 30%, 40%, 50%, 60% and 70%) of isopropanol. The protein extract was centrifuged, supernatant was then removed and cold acetone was added to it. It was then incubated at -20 °C overnight (12-16 hours). The samples were centrifuged and the dried pellet was resuspended in lysis buffer.

RESULTS

- **TCA Method:** This method resulted in the tissue samples freezing. In the end, this method was not followed because protein could not be precipitated from the tissues.
- **Protein extraction using the Isopropanol method: Tables 1A** and **B** show the amounts of proteins extracted from different isopropanol concentrations this procedure was repeated three times. The protein sample that was extracted with 20% isopropanol was observed to yield the highest protein concentration, and this sample was subsequently selected for profiling on a 2DE gel (the gel was run twice) (**Figures 2A** and **B**). Few protein spots were identified and both gels were observed to be of poor quality due to the horizontal streaks.
- **Protein extraction according to Xu *et al.*, (2005):** This method was repeated three times and it led to the highest amount of protein spots identified compared to the others and it will be adopted for downstream analysis. After method selection gels were ran and stained with the more sensitive SYPRO Ruby stain (**Figures 3A** and **B**).

Table 1A: Amounts of liver proteins extracted from different isopropanol concentrations

Isopropanol concentration (%)	Protein concentration (µg/ml)
10	4 470
20	5 000
30	4 950
40	4 710
50	4 530
60	4 730
70	4 340

Table 1B: Amounts of kidney proteins extracted from different isopropanol concentrations

Isopropanol concentration (%)	Protein concentration (µg/ml)
10	4 590
20	4 850
30	4 800
40	4 800
50	4 610
60	4 390
70	4 320

A

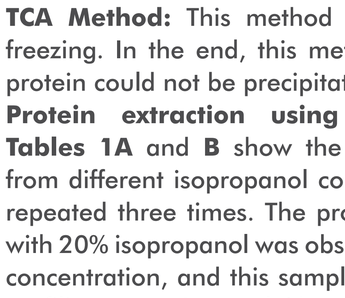


Figure 2A: Silver stained 2D-E gel of 100µg kidney proteins extracted with 20% isopropanol

B

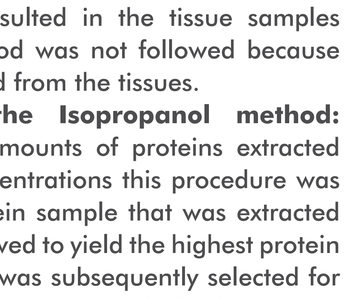


Figure 2B: Silver stained 2D-E gel of 100µg liver proteins extracted with 20% isopropanol

A

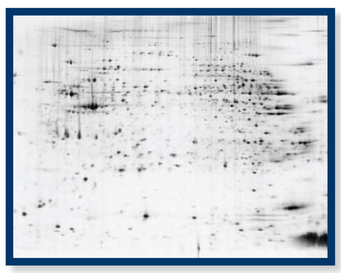


Figure 3A: SYPRO Ruby stained 2D-E gel of 100µg kidney proteins extracted according to Xu *et al.*, 2005

B

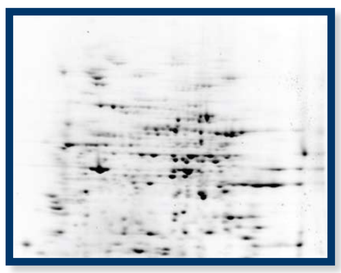
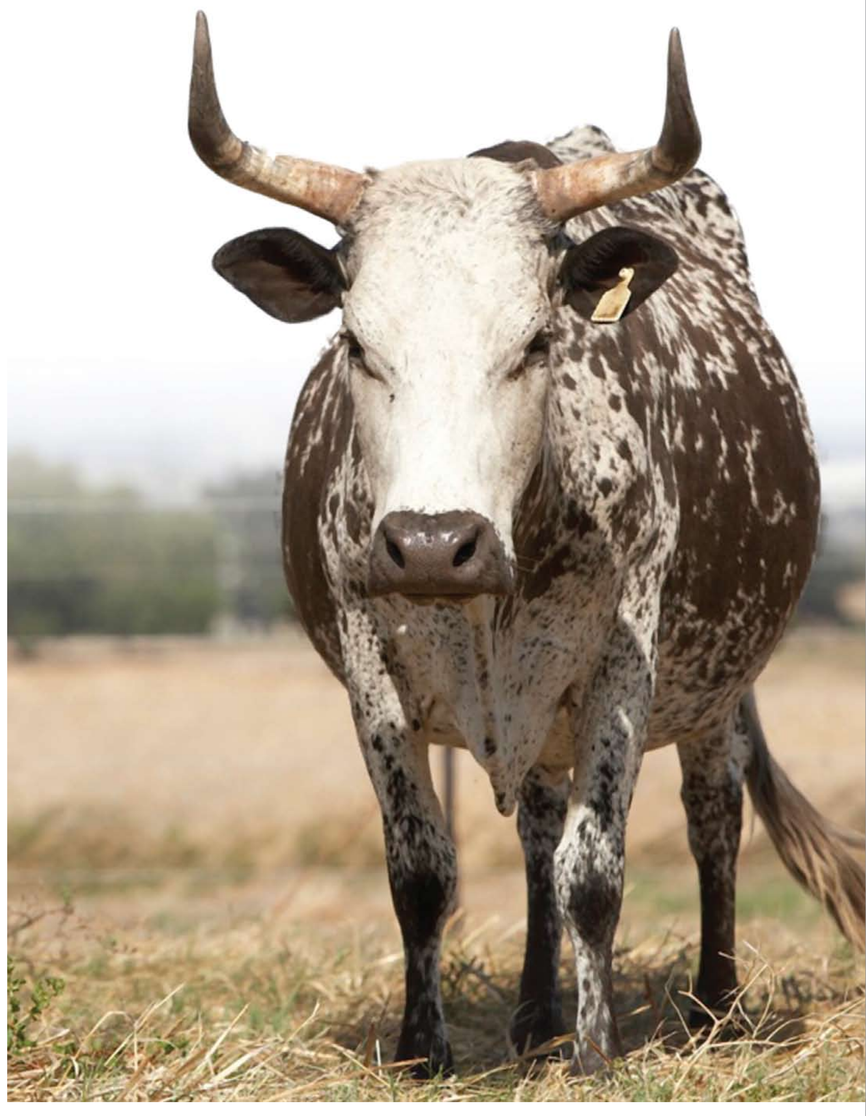


Figure 3B: SYPRO Ruby stained 2D-E gel of 100µg liver proteins extracted according to Xu *et al.*, 2005.

DISCUSSION AND CONTINUING WORK

The protein extraction method by Xu *et al.*, 2005 will be used for the profile comparisons of liver and kidney tissues isolated from Nguni and other cattle breeds. Bio-Rad's PDQuest 2D analysis software will be used to analyse the 2D-E gels. In addition, this software will enable statistical analysis of gels to be performed, to determine which protein differences are significant between tissues and animals of interest. To ensure that the maximum level of proteins are characterised in tissues obtained from Nguni and Hereford cattle, 2D chromatography-based fractionation protocols will also be employed. Ultimately, this work will result in the identification of bovine proteins that confer increased tolerance to drought.

The African breed, Nguni, is metabolically superior over the European breed, Hereford, under unfavourable conditions. CSIR is using proteomics as a tool to determine biological traits that could assist in establishing the extent to which the two breeds differ in order to conserve and enhance these traits, and to be able to select them in other breeds.



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