



Short Communication

Effect of Graded Dietary Substitution of Soyabean Meal with Large Sour Plum (*Ximenia caffra*) Seed Meal on Erythrocyte Osmotic Fragility and the Packed Cell Volume of Growing Male Sprague Dawley Rats

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ABSTRACT

In this study, the effects on packed cell volume (PCV) and osmotic fragility were determined in rats fed *Ximenia caffra* seed meal (XCSM) as a non-conventional dietary protein source. Forty, 21-days old male Sprague Dawley rats were randomly divided into 5 groups of eight rats each and fed isocaloric and isonitrogenous diets in which XCSM substituted soyabean meal (SBM) at 0%, 25%, 50%, 75% and 100% on crude protein basis. After 38 days of feeding, the rats were anaesthetised and blood was collected by cardiac puncture, into heparinised tubes. To determine erythrocyte osmotic fragility, the blood was added to tubes containing serially diluted (0.0 to 0.85%) phosphate buffered saline (PBS) at room temperature and pH 7.5. After incubation and centrifugation, the absorbance of the supernatants was read using a spectrophotometer at 540 nm. Fragiligrams were then plotted. The packed cell volume (PCV) was determined with a micro-haematocrit centrifuge. There were no significant differences in erythrocyte osmotic fragility and PCV of the rats fed different diets. The fragiligrams showed that minimum haemolysis (> 4% haemolysis) was at 0.50% PBS, mean corpuscular fragility (50% haemolysis) was obtained at 0.45% PBS and the maximum haemolysis (> 90% haemolysis) at 0.40% PBS for blood from all of the rats. It is concluded that substituting SBM up to 100% with XCSM has no adverse effect on the osmotic fragility and PCV of growing male Sprague Dawley rats. © 2012 Friends Science Publishers

Key Words: *Ximenia caffra* seed meal; Osmotic fragility; PCV; Sprague dawley rats

INTRODUCTION

In the sub-Saharan Africa region, cost and shortage of conventional dietary protein sources such as soyabean meal (SBM) and fish meal limit livestock production (Babatunde *et al.*, 1990). There is thus a great need to search for and develop non-conventional dietary protein sources in order to increase livestock production. Recently in our laboratory we have undertaken work to characterize seeds from indigenous fruit bearing trees (IFBTs) with a view to assess their potential as food and feed ingredients (Chivandi *et al.*, 2011a & b). Our IFBTs seed characterization project is founded on the realization that the seeds, which are potential micro- and macro-nutrient sources, are largely thrown away after utilization of their fruit pulp. The Large Sour Plum, *Ximenia caffra*, family *Olacaceae*, is widely distributed in sub-Saharan Africa and bears fruit even in drought years (Mojeremane & Tshwenyane, 2004; Legwaila *et al.*, 2011). The tree seed has an oil yield of 48% (Chivandi *et al.*, 2008); the seed cake (after solvent extraction) had 43% crude protein on dry matter basis, which is comparable to 44-48% CP in SBM (McDonald *et al.*, 2002). As such,

X. caffra seed meal (XCSM) has potential to substitute SBM as a protein source in animal feeds. However, plant seeds are known to contain both heat-stable and heat-labile anti-nutritional factors including among others, lectins, terpenoids and saponins, which adversely affect the growth and health of animals (Vasconcelos & Oliveira, 2004). Haematological changes are commonly used to determine the body health status and to assess the impact of environmental, nutritional, and/or pathological stresses (Elagib & Ahmed, 2011). Certain haematological factors can be associated with particular production traits in livestock, for example, high PCV and haemoglobin contents are associated with high feed conversion efficiency (Miruka & Rawnsley, 1997), while high percentages of white blood cells, especially lymphocytes, are associated with the ability of poultry to perform well under very stressful conditions (Mmereole, 2008).

Osmotic fragility is a measure of the red blood cells resistance to haemolysis when incubated in increasingly dilute buffered saline solutions (Van der Walt & Russell, 1978). The osmotic fragility of erythrocytes is affected by extrinsic and intrinsic factors such as the size, volume and

age of erythrocytes. The temperature and pH of the test medium used to determine the osmotic fragility of the erythrocytes also has an effect on their fragility (Perk *et al.*, 1964; Schalm *et al.*, 1975). Dietary interventions such as gossypol can also affect on osmotic fragility (Matondi *et al.*, 2007). PCV is affected by a number of factors that may lead to either a decrease or increase in values such as a low in dietary iron intake, hydration state, environmental temperature, sex and age (Schalm *et al.*, 1975).

Despite the macro-nutritional potential of XCSM as a compliment and/or substitute to SBM in animal feeds, there is a dearth of information on the effects of substituting SBM with XCSM on erythrocyte osmotic fragility and PCV. As part of a larger study that is ongoing, the objective of this study was to evaluate the effects of replacing SBM with XCSM on the osmotic fragility of red blood cells and PCV in growing Sprague Dawley male rats. We chose growing rats since they have a rapid growth rate and are a sensitive model to explore dietary manipulations (Richter *et al.*, 1938).

MATERIALS AND METHODS

***Ximenia caffra* source and processing:** Ripe fruit were handpicked from trees in the Zhombe District (Latitude 14°45'S; Longitude 26°50'E) of Zimbabwe. The district is characterised by marginal soils, 550 mm rainfall per annum and mean annual temperature of 26°C. The fruit pulp was removed and then the seeds were dried under the shade. The dry seed nuts were hand-shelled to extract the inner nut. The shelled seeds were imported into South Africa for further processing under the Import Permit Number P0039683. The seeds were first crushed and then repeatedly steeped in hexane to remove the oil until the residual oil in the XCSM averaged 4%. The oil expression was done at the Centre for Scientific and Industrial Research, Mooderfontein, Johannesburg. The resultant XCSM was used in ration formulation.

Diet formulation: Diets were formulated to meet the nutrient requirements of growing Sprague Dawley rats (NRC, 1995). Briefly, the control diet was soyabean meal (protein source) and maize meal (energy source) based. SBM was substituted on a crude protein basis with XCSM at 0%, 25%, 50%, 75% and 100%, generating a control diet and four test diets as shown in Table I. The diets were both iso-caloric and iso-nitrogenous (Table I) and contained a vitamin-mineral premix from a commercial supplier.

Ethical approval and experimental animals: The project was cleared by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand (AESC Clearance Certificate Number: 2008/46/01). Forty, 21-day old male Sprague Dawley rats with an average body mass of 58.9 ± 9.9 g were divided into 5 groups of eight rats and randomly assigned to diets in which *Ximenia caffra* seed meal (XCSM) substituted soyabean meal (SBM) at 0% (control diet – D1), 25% (D2), 50% (D3), 75% (D4) and

100% (D5) on a crude protein basis. The rats were given two days to adapt to their new environment while on a commercially available rat chow (Mice Cubes, Epol, South Africa). On the third day, the rats were put on their respective treatment diet and then fed *ad libitum* for 38 days. Tap water was also provided *ad libitum* for the duration of the study. The rats were housed individually in polypropylene cages with wood shavings for bedding. A 12 h light (0600 – 1900): 12 h dark light (1900 – 0600) regime was maintained throughout the study. On day 38, the rats were fasted for 12 h but with access to clean drinking water and then they were anaesthetized with sodium pentobarbitol intraperitoneally and 4 mL blood was collected by cardiac puncture using 20 G needles into heparinised tubes (Vacutainer, BD, USA).

Erythrocyte osmotic fragility determination: Phosphate Buffered Saline (PBS) stock solutions were prepared using NaCl/Na₂HPO₄/NaH₂PO₄ according to Baker and Silverton (1980) in volumes of 500 ml for each of the samples in concentrations ranging from 0.0% to 0.85%. Throughout the execution of this experiment pH and temperature were maintained at 7.5 and 24–25°C, respectively. For each blood sample, 13 test tubes were used; 12 tubes had 5 mL each of the serially diluted phosphate buffered saline (PBS) solution and one contained 5 mL distilled water. Two hundred microlitres of blood was added into each of the thirteen test tubes and mixed by gently inverting the test tubes 5 times. The test tubes were incubated at room temperature (24–25°C) for 30 min. Thereafter, the test tubes were centrifuged (Sorvall RT 6000B, Du Pont, USA) at a controlled temperature (24°C) at 5 000 x g for 15 min. The supernatant of each test tube was decanted into plastic cuvettes (Plastibrand, Brand GMBH, Germany). The release of haemoglobin in the supernatant following haemolysis was measured using a spectrophotometer (LKB Ultrospec II, LKB Biochrom Ltd London, UK) at 540 nm with distilled water as a blank. The percent haemolysis was calculated by dividing the optical density of the supernatant from each of the 12 tubes with blood in PBS, by the optical density of supernatant of the tube with blood in distilled water which represented 100% haemolysis and subsequently multiplying the ratio by 100 (Faulkner & King, 1970).

$$\text{Percent haemolysis} = \frac{\text{Optical density of supernatant of blood in PBS}}{\text{Optical density of supernatant from blood in distilled water}} \times 100$$

The fragiligrams were obtained by plotting percent haemolysis against the PBS concentrations.

PCV determination: The packed cell volume (PCV) was determined with an IDEXX micro-haematocrit centrifuge (USA).

Test for saponins: Saponins were determined as described by Edeoga *et al.* (2005). Briefly, 2 g of the powdered sample was boiled for 5 min in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of

Table I: Ingredient and chemical composition of the control and test diets

Ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Yellow Maize Meal	12.28	12.15	12.01	11.87	11.74
Wheat Bran	0.20	0.20	0.20	0.20	0.20
Maize Gluten Feed	1.43	1.43	1.43	1.43	1.43
Soyabean Oil Cake	3.28	2.45	1.64	0.82	0.00
² <i>Ximenia caffra</i> Seed Meal	0.00	0.96	1.91	2.87	3.82
	(0.0%)	(25%)	(50%)	(75%)	(100%)
Limestone Flour Feed Grade	0.27	0.27	0.27	0.27	0.27
Salt Feed Grade	0.04	0.04	0.04	0.04	0.04
¹ Brewer's Yeast	2.05	2.05	2.05	2.05	2.05
² Tallow	0.41	0.41	0.41	0.41	0.41
³ Mineral & Vitamin Premix	0.04	0.04	0.04	0.04	0.04
Total	20.00	20.00	20.00	20.00	20.00
⁴ Chemical Composition					
Dry Matter (%)	89.87	89.90	89.99	90.05	90.24
Crude Protein (%)	18.55	19.18	18.09	18.96	18.06
Gross Energy (MJ kg ⁻¹)	15.96	15.76	15.78	15.68	16.13

¹Figures in parentheses represent substitution of SBM by XCSM on a crude protein basis

¹Brewer's Yeast obtained from Anchor Yeast, Gweru, Zimbabwe

²Tallow was obtained from Energy Oil, Wadeville, Johannesburg, RSA

³Mineral and Vitamin Premix were sourced from ADVIT Animal Nutrition, Sebenza Industrial Area, Johannesburg, RSA

⁴Chemical composition of the diets was determined at the Agricultural Research Council's Irene Animal Production Institute laboratories, RSA, a South African National Accreditation System (SANAS) accredited laboratory

Table II: Antinutritional Factors in the five diets

Anti-nutritional factor	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Saponins	Present	Present	Present	Present	Present
Terpenoids	Absent	Present	Present	Present	Present

olive oil and shaken vigorously and then observed for the formation of an emulsion.

Test for terpenoids: Terpenoids were determined as described by Edeoga *et al.* (2005) wherein 5 mL of each diet sample was mixed in 2 mL of chloroform and 3 mL of concentrated sulphuric acid (18.384 M) were carefully added to form a layer at the bottom. A reddish brown colouration of the interface indicated the presence of terpenoids.

Data analysis: All data obtained were analysed using GraphPad Prism Version 5 for Windows (GraphPad Software Inc, San Diego California, USA) and means were compared using the Student's *t*-test and values with $P < 0.05$ were considered significant.

RESULTS

The qualitative analysis of the five diets showed the presence of saponins in all five of the diets and terpenoids in only four diets excluding Diet 1, the control diet (Table II). All rats had a significant body mass gain over the study period, however, there was no significant difference over the different treatment groups (Range: 262.6-299.0 g & Mean: 286.7 g). Results for the packed cell volumes showed no significant differences across all treatments (range: 42-50% & mean 45.9%) and are shown in Fig. 1.

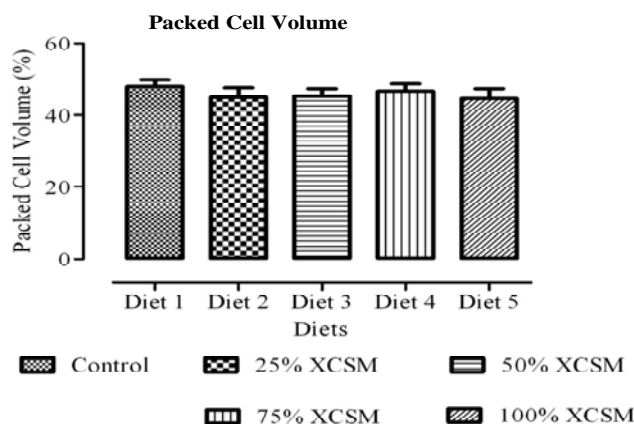
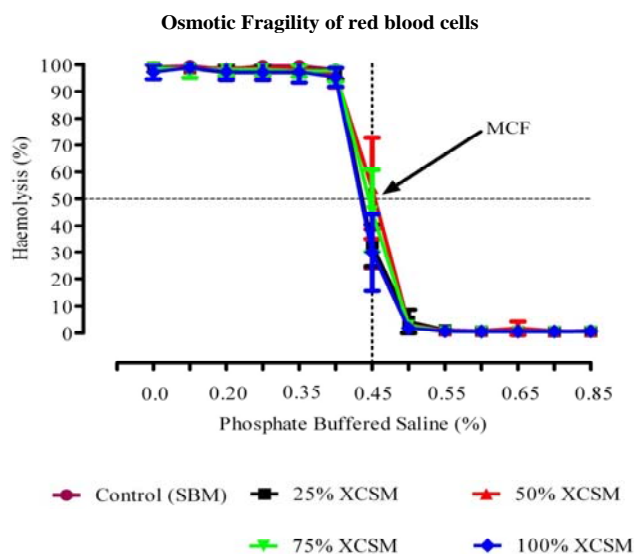
Fig. 1: Packed Cell Volume (%) of the blood of the Sprague Dawley rats after 38 days of being fed diets in which soyabean meal was substituted by graded levels of *Ximenia caffra* seed meal as a source of dietary protein**Fig. 2: The fragiligrams of the erythrocytes of the Sprague Dawley rats after 38 days of being fed diets in which soyabean meal was substituted by *Ximenia caffra* seed meal on crude protein basis. Mean corpuscular fragility (MCF) is defined as the corresponding concentration of PBS solution causing 50% lysis of red blood cells (Krogmeier *et al.*, 1993)**

Fig. 2 shows the fragiligrams of erythrocytes from the growing Sprague Dawley male rats fed graded levels of XCSM as a protein source. There were no significant differences ($P > 0.01$) in the osmotic fragility of erythrocytes from the rats fed the control and test diets. The minimum osmotic fragility ($> 4\%$ haemolysis) occurred at 0.50% PBS concentration, mean corpuscular fragility (MCF) (50% haemolysis) was obtained at 0.45% PBS concentration and the maximum osmotic fragility ($> 90\%$ haemolysis) at 0.40% PBS concentration for all samples (Suess *et al.*, 1948).

DISCUSSION

This study was conducted to determine the effects of XCSM on the erythrocyte osmotic fragility and PCV of rats in which XCSM was incorporated into maize/soyabean diets as would be done in production agriculture rather than using purified diets. This study showed that XCSM as a SBM substitute did not have an effect on the PCV and osmotic fragility of the erythrocytes of the rats. The erythrocyte osmotic fragility can indicate the presence of ANFs in the diet, which can adversely affect the indices of fragility (Matondi *et al.*, 2007). A qualitative analysis of the anti-nutritional factors on our five diets revealed the presence of saponins and terpenoids in the XCSM. The quantities present were probably minute and they did not significantly affect the erythrocyte osmotic fragility and PCV of the growing rats. It is nevertheless recommended that further analysis be done to quantify and classify the antinutritional factors present in XCSM before it can be used extensively in nutritional studies.

The PCV of the rats in this study was not affected by increasing the concentration of XCSM. Previous studies have demonstrated that dietary manipulations involving substitution of conventional protein sources with non-conventional sources such as rubber seed meal have had adverse effects on haematological profiles in pigs (Babatunde *et al.*, 1990) and broilers (Mmereole, 2008; Egbunike *et al.*, 2009). PCV alone is not useful in predicting the effects of the diets on the health status of the rats as it may be affected by environmental temperature and age (Schalm *et al.*, 1975).

The PCV values obtained for the different groups of rats in this study ranged from 42 to 50% of which the bottom end was slightly lower than bottom end of the normal range (43–50%) reported by Balkaya *et al.* (2001) for healthy Sprague Dawley male rats. The growing rats fed different diets had similar MCF values obtained at 0.45% PBS which closely agrees with the 0.44% saline reported by Viscor and Palomeque (1982) for rats.

CONCLUSION

XCSM as a protein substitute to SBM did not affect osmotic fragility, and PCV of growing Sprague Dawley male rats possibly indicating that XCSM could be used to substitute SBM without deleterious effects on the health of growing animals. To ensure adequate processing and to minimize the effects of ANFs, there is need to quantify the ANFs content in XCSM.

Acknowledgement: The Oppenheimer Memorial Trust, The University of the Witwatersrand's Faculty of Health Sciences Research Endowment Fund and the National Research Foundation (NRF) of South Africa are acknowledged for funding the study. The technical assistance of Ms Margaret Badenhorst, Ms Rachael

Dangarembizi, Mr. Trevor Nyakudya and Dr. Abdulwahid Ajibola is greatly appreciated.

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(Received 28 January 2012; Accepted 05 March 2012)