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Research Article
Effects of Dietary *Ximenia caffra* Meal on Nutrient Intake, Digestibility, Nitrogen Balance and Growth Performance in Sprague Dawley Rats Modelling Monogastrics

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Abstract

Background and Objective: The sub-saharan livestock feed industry depends on imported soyabean meal (SBM) as a dietary protein source in feeds thus making livestock production costly. This calls for the search and development of local dietary protein sources. Using Sprague Dawley rats to model monogastric animals, this study evaluated the potential of *Ximenia caffra* kernel meal (XCKM) to substitute SBM as a dietary protein source in feeds. **Materials and Methods:** Five diets were formulated wherein XCKM replaced SBM on a crude protein basis at 0, 25, 50, 75 and 100%. In the digestibility trial, 20 adult male SD rats were randomly assigned to the 5 diets. After a 12-day adaptation period feed and nutrient intake, faeces and urine output were determined over a 5-day collection period. Apparent Total Tract Digestibility (ATTD) of nutrients and nitrogen absorption and retention were determined. In the growth trial, 40 weanling male SD rats were randomly assigned to the five dietary treatments and fed for 38 days. The rats were weighed twice weekly. Following euthanasia, gastrointestinal viscera were harvested and their macro-morphometry determined. Linear growth was determined from tibiae and femora indices. **Results:** In adult rats dietary XCKM had no ($p>0.05$) effect on ATTD of nutrients. At 100% substitution of SBM, XCKM increased ($p<0.05$) faecal nitrogen loss while at 75% substitution level it increased ($p<0.05$) nitrogen retention. In growing SD rats, although dietary XCKM had no effect ($p>0.05$) on the terminal body and empty carcass mass and viscera macro-morphometry, at 100% SBM substitution, it significantly compromised ($p<0.05$) body mass gain and average daily gain. Femora and tibiae mass and seed or index significantly decreased ($p<0.05$) with increased dietary XCKM. **Conclusion:** The XCKM could replace SBM as a dietary protein source in adult SD rat feeds without compromising ATTD digestibility of nutrients and nitrogen utilization thus it could be speculated that XCKM can be utilized as a dietary protein source in feeds of mature monogastrics. Caution must be exercised in using XCKM in grower rat diets as its use at higher inclusion levels compromised growth performance and long bone health.

Key words: Soyabean meal, nitrogen retention, *Ximenia caffra* kernel, nutrient absorption, livestock feed industry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soyabean meal (SBM) is the major dietary protein source in feeds globally and in sub-Saharan Africa[1]. In most developing countries, including countries in sub-Saharan Africa (SSA), the local production of soya bean fails to meet the SBM requirements of the livestock feed industry[2,3]. These countries therefore rely on costly SBM imports leading to increased feed costs. The shortage militates against the intensification of animal production against a background of increased animal product demand in SSA[4]. Dependency on costly SBM imports compromises household food security. In order to facilitate intensified animal production and thus ensure household food security, there is a dire need to search and develop locally available potential non-conventional dietary protein sources for feeds.

The large Sourplum, *Ximenia caffra* (*X. caffra*), is an indigenous fruit bearing tree whose distribution spans across SSA[5] and is drought resistant[6]. *Ximenia caffra* fruit pulp has a high protein and ascorbic acid content[7]. Forty-eight percent of the *X. caffra* kernel mass is oil[8]. Research on *X. caffra* has focused on the ethnomedicinal properties of its various extracts[6], fruit pulp composition[7] and to some extent seed oil fatty acid profile[8]. Recently chemical and *in vitro* evaluations have demonstrated the potential *X. caffra* seed as an alternative dietary protein source[9-10]. However chemical analyses and *in vitro* studies do not accurately predict the nutritional value of alternative feeds *in vivo*. This two-experiment study sought to determine, *in vivo*, the potential of defatted *X. caffra* kernel meal to substitute to SBM as dietary protein source in Sprague Dawley rats modelling monogastrics.

MATERIALS AND METHODS

Feed ingredients: Ripe *X. caffra* fruit were collected from 80 trees in Zhombe District, Zimbabwe (latitude 14°45'S; longitude 26°50'E). The fruit pulp was manually removed and then the fruit "stones" were air dried under shade after which the seed was manually shelled. The kernels, packed into jute bags, were then imported into South Africa (permit number P0039683) and defatted. Yellow maize, wheat bran, gluten feed and feed grade limestone were supplied by Opti-Feeds Private Limited, Lichtenburg, Republic of South Africa (RSA). Vitamin-mineral premix and tallow were supplied by ADVIT Animal Nutrition (Johannesburg, RSA) and Energy Oil (Johannesburg, RSA), respectively. Brewers' yeast was sourced from Anchor Yeast Private Limited, Gweru, Zimbabwe.

***X. caffra* kernel meal preparation and chemical assays:** The *X. caffra* kernels were defatted at the Centre for Scientific and Industrial Research, Kempton Park, Johannesburg, RSA. Briefly, the 34 kg *X. caffra* kernels were pressed in 500 g aliquot portions at 250 bars for 12.5 min using a hydraulic press. The pressed solid paste was divided into three batches. Each pressed *X. caffra* kernel paste and 96% hexane at a ratio of 4:1 (hexane:solid paste) was then charged into a 60 L reactor and stirred for 1 h at room temperature. The mixture was then centrifuged for 30 min to separate the solid and liquid components. Recovered "solids" were subjected to an additional 5 cycles of extraction/centrifugation. The defatted *X. caffra* kernel meal (XCKM) was air dried under shade. Prior to use in experimental diet formulation the proximate, mineral and the fibre content of the XCKM were determined[11-13], respectively. The gross energy value of the XCKM was determined using an MC-1000 Modular Calorimeter (Energy Instrumentation, Centurion, RSA).

Diet formulation: The control and experimental diets were formulated such that they met the National Research Council requirements for rats[14]. The control diet (Diet 1) was SBM based with the test diets formulated such that the XCKM substituted SBM on a CP basis to generate iso-calorific and iso-nitrogenous diets. The ingredient and chemical composition of the diets are shown in Table 1.

Ethical clearance and study site: The study conducted in the Central Animal Services Unit Animal Unit at the Faculty of Health Sciences, University of the Witwatersrand, was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg, South Africa (AESC number: 2008/46/01).

Digestibility and nitrogen balance trial

Animals, housing and feeding: Twenty mature male Sprague Dawley rats, mean body mass 287.32±12.14 g were used. Each rat was individually housed in a polyethylene metabolism crate designed to separate urine and faeces in order to facilitate faeces and urine collection. The rats had *ad libitum* access to clean drinking water and feed and were on a 12 h light-dark cycle with lights on from 07:00 h. The ambient temperature was set at 22±2°C.

Study design: About 20 male adult male Sprague Dawley rats were randomly assigned to the 5 dietary treatments such that each dietary treatment was replicated 4 times. The experimental period lasted for 17 days split as 12 days of

Table 1: Ingredient and chemical composition of the control and test diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredients (g kg^{-1})					
Maize meal	614.0	607.5	600.5	593.5	587.0
Wheat bran	10.0	10.0	10.0	10.0	10.0
Gluten feed	71.5	71.5	71.5	71.5	71.5
Tallow	20.5	20.5	20.5	20.5	20.5
Soyabean meal	164.0	122.5	82.0	41.0	0.0
Ximenia caffra kernel meal	0.0 (0)	48.0 (25)	95.5 (50)	143.5 (75)	191.0 (100)
Yeast*	102.5	102.5	102.5	102.5	102.5
Limestone**	13.5	13.5	13.5	13.5	13.5
Premix***	2.0	2.0	2.0	2.0	2.0
Salt	2.0	2.0	2.0	2.0	2.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Chemical composition(g kg^{-1} DM)					
Dry natter	898.7	899.0	899.9	900.5	902.4
Organic matter	856.1	855.2	855.8	859.1	863.3
Crude protein	185.5	187.4	182.5	186.0	181.8
Ether extract	44.7	49.3	46.1	49.9	47.1
Ash	42.6	43.8	44.1	41.4	39.1
Calcium	5.9	5.8	5.5	5.2	5.7
Phosphorus	4.3	4.3	3.9	4.4	4.4
Energy (MJ kg^{-1} DM)					
Gross energy	15.78	15.62	15.67	15.61	16.13

Figures in parentheses indicate the percentage substitution of Soyabean meal CP by *X. caffra* kernel meal CP, *Brewers' yeast, **Feed grade limestone, ***Vitamin-mineral premix: Each kg contained vitamin A 30 000 000 IU, vitamin D3 166 667 IU, vitamin E 20 500 IU; vitamin K3 1.67 g, vitamin B_1 5.67 g, vitamin B_2 3.33 g, Niacin 15 g, Calcium pantothenate 6.67 g, vitamin B_{12} 0.01 g, vitamin B6 2.5 g, Choline 333.33 g, Folic acid 0.33 g, Biotin 0.04 g, Rovimix Stay C 10.83 g, $MnSO_4$ 10 g, Zn 10 g, Cu 2.33 g, KI 0.17 g, $FeSO_4$ 41.67 g, Se 0.05 g

adaptation and 5 days of faeces and urine collection and measurement of feed intake. On the last day of the adaptation period, each metabolism crate was thoroughly cleaned following which each rat was returned to its respective crate for the total collection period.

Sample collection and storage: Measurement of feed intake, refusals, faeces and urine output were done daily during the collection period. Samples of the feed and refusals for each rat were taken and bulked to generate composite samples from which chemical assays were to be done. Faeces from each rat were weighed fresh and stored in a freezer (-20°C) as described by Osuji *et al.*[15] and were kept frozen pending analysis. The volume and mass of urine from each rat was determined and stored in plastic sample bottles to which 0.5 mL of concentrated H_2SO_4 was added to prevent loss of nitrogen[15] and were stored in a freezer (-20°C) pending analysis.

Proximate analyses: A representative sample of each of the diet was ground to pass through a 1 mm screen prior to the analysis. The bulk faecal sample of each rat was freeze-dried; faecal dry matter output determined, followed by grinding through a 1 mm screen. The proximate analyses of the samples of the diets, faeces and urine were performed at the Agricultural Research Council's Irene Analytical Services Laboratories, South Africa. The diet and faeces proximate content were determined as outlined by AOAC[11]. Following thawing, homogenous 5 g urine samples were used to determine urinary N as described by AOAC[11]. The gross energy (GE) value of the diet, faeces and urine samples were determined using an MC-1000 Modular Calorimeter (Energy Instrumentation, Centurion, South Africa).

Computations: Computations of nutrient intake [dry matter (DM), organic matter (OM), crude protein (CP), nitrogen (N) and gross energy (GE)] and faecal and urinary nutrient output, Apparent Total Tract Digestibility (ATTD) coefficients for DM, OM, CP and GE and N balance were done as described by Osuji *et al.*[15].

Growth trial

Animals, housing and feeding: Forty (40) 21-day old weanling male Sprague Dawley rats of mean body mass 58.99±9.97 g were used. The rats were individually penned in Perspex cages lined with wood shavings as bedding which (bedding) was changed twice weekly. The rats had *ad libitum* access to clean drinking water and feed and

were fed on the respective diets for 37 days. Lighting was set on a 12 h light-dark cycle with lights on from 07:00 h. The ambient temperature was set at 22±2°C.

Study design: About 421 day old weanling male SD rats of mean body mass 58.99±9.97 g were randomly assigned to the five dietary treatments (D1-D5). Each dietary treatment was replicated eight times.

Body mass measurements: On commencement of the experiment, the body (induction) mass of each rat across dietary treatment groups was measured and thereafter body mass was measured twice weekly using an electronic balance (Precisa Instruments AG, Switzerland).

Terminal procedures

Determination of terminal body mass: On the 37th day of the feeding trial, the rats were fasted for 12 h overnight and then weighed on an electronic balance (Precisa Instruments AG, Switzerland) the following morning.

Euthanasia and viscera macro-morphometric measurements: Following the determination of fasting termination body mass, the rats were euthanised by intra-peritoneal administration of sodium pentobarbitone (Eutha-naze, Centaur Labs, Johannesburg, RSA) at 200 mg kg^{-1} body. Immediately thereafter each rat was carefully dissected and the GIT viscera removed for macro-morphometric determinations. Prior to determination of the mass of the GIT viscera, residual digesta in them was gently squeezed out. The length of the small and large intestines was measured by gently stretching them out on the cooled dissection board. After dissecting out all the other visceral organs, each empty carcass was then weighed on a Precisa 310 M electronic balance (Precisa Instruments AG, Switzerland).

Determination of linear growth: The femur and tibia length were used to determine linear growth of the rats. Briefly, the right femoral head was disarticulated from the acetabulum at the hip joint followed by removal of all non-calcified soft tissues from the femora and the tibiae following which the bones were dried in an oven (Salvis®, SalvisLab, Schweiz, Switzerland) at 40°C until constant mass (7 days). Bone length was measured using vernier digital calipers (KTV-150 150 mm Digital Caliper, Major Tech (PTY) LTD, Elandsfontein, RSA). Femur length was determined by measuring the distance between the distal femoral articular surface to the greater trochanter while tibia length was determined by measuring the distance between the tibia head closest to the femur and the medial malleolus. The bones were then weighed on a Precisa 310 M electronic balance (Precisa Instruments AG, Switzerland) to determine their dry mass. The femur and tibia Seedor ratio (bone density) were then determined as described by Seedor *et al.*[16] using the equation:

$$\text{Seedor ratio} = \frac{\text{Bone mass (mg)}}{\text{Bone length (mm)}}$$

Data analysis: Data is presented as Mean±SD. GraphPad Prism 5 (GraphPad Software Inc., San Diego, USA) statistical software was used to analyse data. A repeated measures ANOVA was used to analyse data on weekly body masses. A one-way ANOVA was used to analyze the rest of the data. Mean comparison was done using the Bonferroni's *post hoc* test. The level of significance was set at $p \leq 0.05$.

RESULTS

Digestibility and nitrogen balance study: Nutrient intake, ATTD of nutrients in rats across dietary treatments are shown in Table 2. Urinary N excretion, N absorption and retention were also shown in Table 2. The DM, OM, CP, N and GE intake of the rats was similar ($p>0.05$) across the dietary treatments. Despite similarities in urinary nitrogen output and nitrogen absorption by the rats across dietary treatment, at 100% substitution of SBM, dietary XCKM increased ($p<0.05$) faecal nitrogen loss while at 75% substitution it increased ($p<0.05$) nitrogen retention.

Growth study: The weekly growth profile of the rats is was shown in Fig. 1. Dietary XCKM had no effect on the termination body mass, body mass gain (%), empty carcass mass of rats (Table 3). However at 100% substitution of SBM, dietary XCKM lowered ($p<0.05$) body mass gain and average daily gain. Although, dietary XCKM had no effect on the length of the femora and tibiae from rats across dietary treatments, the bone masses and Seedor ratio decreased ($p<0.05$) with increasing dietary XCKM (Table 4). The substitution of SBM with XCKM had no effect on the absolute masses of the GIT viscera and on the length of the small and large intestines (Table 5). However, relative to body mass, complete substitution of SBM with XCKM resulted in lighter stomachs, small intestines and large intestines (Table 5).

Table 2: Effect of a graded dietary substitution of soyabean meal with defatted *X. caffra* kernel meal nutrient intake, faecal and urinary nitrogen output, apparent digestibility of nutrients and nitrogen balance in male Sprague Dawley rats

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
5 day nutrient intake						
Dry matter intake (g)	82.15±12.97^{a}	96.37±28.96^{a}	101.28±13.69^{a}	106.12±3.50^{a}	109.82±2.76^{a}	ns
Organic matter intake (g)	78.61±12.41^{a}	92.22±27.71^{a}	96.86±13.09^{a}	101.80±3.36^{a}	105.54±2.65^{a}	ns
Crude protein intake (g)	15.38±2.43^{a}	18.20±5.47^{a}	18.43±2.49^{a}	19.86±0.66^{a}	19.92±0.50^{a}	ns
Gross energy intake (MJ)	1.31±0.21^{a}	1.52±0.46^{a}	1.60±0.22^{a}	1.66±0.05^{a}	1.77±0.04^{a}	ns
Apparent nutrient digestibility						
Dry matter (g kg^{-1})	851.40±14.4^{a}	816.80±36.4^{a}	820.80±23.5^{a}	819.70±10.3^{a}	795.70±35.0^{a}	ns
Organic matter (g kg^{-1})	859.20±13.1^{a}	832.90±27.1^{a}	832.20±22.9^{a}	831.70±9.5^{a}	803.10±39.5^{a}	ns
Crude protein (g kg^{-1})	757.00±25.3^{a}	713.80±55.3^{a}	696.50±40.9^{a}	712.80±16.0^{a}	654.10±68.6^{a}	ns
Gross energy (%)	81.21±1.73^{a}	77.60±3.73^{a}	77.53±3.02^{a}	77.24±1.25^{a}	74.27±5.03^{a}	ns
Nitrogen utilization						
Nitrogen intake (g day^{-1})	0.49±0.08^{a}	0.59±0.17^{a}	0.59±0.08^{a}	0.64±0.02^{a}	0.64±0.02^{a}	ns
Nitrogen excretion						
Faecal nitrogen (g day^{-1})	0.12±0.03^{a}	0.16±0.04^{a}	0.18±0.03^{ab}	0.18±0.01^{ab}	0.22±0.04^{b}	**
Urinary nitrogen (g day^{-1})	0.31±0.06^{a}	0.21±0.06^{a}	0.27±0.05^{a}	0.22±0.02^{a}	0.22±0.05^{a}	ns
Nitrogen balance						
Absorption (g day^{-1})	0.37±0.06^{a}	0.43±0.14^{a}	0.41±0.07^{a}	0.45±0.02^{a}	0.42±0.05^{a}	ns
Retention (g day^{-1})	0.06±0.04^{a}	0.21±0.11^{ab}	0.14±0.08^{ab}	0.23±0.03^{b}	0.20±0.09^{ab}	*

ns: Not significant, p>0.05). *p≤0.05, **p≤0.01, ^{ab}Within row means with different superscripts are significantly different at p<0.05. The faecal nitrogen output of rats fed Diet 5 was significantly higher (p = 0.0159) compared to that of rats fed the control diet. Rats fed Diet 1 had significantly lower nitrogen retention compared those fed Diet 4. Diet 1: 0% substitution of Soyabean meal CP, Diet 2: 25% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 3: 50% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 4: 75% substitution of soyabean meal CP with *X. caffra* kernel meal CP, Diet 5: 100% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Data presented as Mean±SD, n = 4

Table 3: Effect of graded dietary substitution of soyabean meal with defatted *X. caffra* kernel meal on body mass, average daily gain and empty carcass mass of male Sprague Dawley rats

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Induction body mass (g)	57.75±7.73^{a}	59.88±10.07^{a}	58.00±12.35^{a}	61.12±8.24^{a}	57.20±12.61^{a}	ns
Termination body mass (g)	295.75±23.44^{a}	299.00±17.21^{a}	286.50±40.11^{a}	294.25±22.49^{a}	266.50±24.77^{a}	ns
Body mass gain (g)	237.00±18.18^{ab}	239.13±11.72^{a}	228.05±28.07^{ab}	233.13±18.61^{ab}	209.30±15.79^{b}	*
Mass gain (%)	407.18±42.07^{a}	410.61±76.83^{a}	400.92±39.80^{a}	386.37±54.18^{a}	377.23±67.34^{a}	ns
Average daily gain (g day^{-1})	6.24±0.48^{ab}	6.29±0.31^{a}	6.01±0.74^{ab}	6.14±0.49^{ab}	5.51±0.42^{b}	*
Empty carcass mass (g)	226.65±20.21^{a}	225.85±14.28^{a}	217.77±31.46^{a}	223.75±16.36^{a}	199.49±19.68^{a}	ns

ns: Not significant, p>0.05). *p≤0.05, ^{ab}Within row means with a different superscript are significantly different at p<0.05. Rats fed Diet 5 had a significantly lower body mass gain (p = 0.0282) and averagely daily gain (p = 0.0282) compared to rats fed Diet 2, Diet 1: 0% substitution of Soyabean meal CP, Diet 2: 25% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 3: 50% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 4: 75% substitution of soyabean meal CP with *X. caffra* kernel meal CP, Diet 5: 100% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Data presented as Mean±SD, n = 8

Table 4: Effect of a graded dietary substitution of soyabean meal with defatted *X. caffra* kernel meal on the length, mass and Seedor index of tibiae and femora of male Sprague Dawley rats

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Tibia length (mm)	37.06±0.56^{a}	37.50±0.89^{a}	36.94±1.32^{a}	37.13±0.69^{a}	36.13±0.88^{a}	ns
Tibia mass (mg)	385.13±15.99^{a}	383.00±21.27^{a}	358.25±40.59^{ab}	348.63±20.35^{ab}	337.50±31.14^{b}	**
Tibia density (mg mm^{-1})	10.39±0.44^{a}	10.21±0.43^{ac}	9.68±0.81^{ad}	9.39±0.46^{bcd}	9.28±0.70^{bd}	**
Femur length (mm)	32.00±0.80^{a}	32.75±0.76^{a}	32.19±1.07^{a}	32.38±0.74^{a}	31.63±0.88^{a}	ns
Femur mass (mg)	468.00±22.72^{a}	476.13±31.72^{a}	448.25±57.58^{ab}	449.13±21.12^{ab}	398.88±61.52^{b}	**
Femur density (mg mm^{-1})	14.62±0.49^{a}	14.54±0.89^{a}	13.89±1.41^{ab}	13.87±0.44^{ab}	13.00±1.18^{b}	*

ns: Not significant, p>0.05). *p≤0.05. **p≤0.01, ^{ab}Within row means with different superscripts are statistically significantly different at p<0.05. Rats fed diet 5 had significantly lower tibia and femur masses compared to rats on Diets 1 and 2, respectively. Rats fed diet 5 had the least (p<0.05) tibia density. Diet 1: 0% substitution of Soyabean meal CP, Diet 2: 25% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 3: 50% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 4: 75% substitution of soyabean meal CP with *X. caffra* kernel meal CP, Diet 5: 100% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Data presented as Mean±SD, n = 8

DISCUSSION

At any given age and physiological status animals eat to meet their energy requirements[17]. The similarity in DM, OM, CP, N and GE intake by the rats across dietary treatments suggested that SBM could be substituted with XCKM without negative impacts on energy availability to adult male SD rats. Additionally, the similarity in nutrient intake by the rats across dietary treatments suggested that the graded dietary substitution of SBM with XCKM did not negatively

Table 5: Effect of a graded dietary substitution of soyabean meal with defatted *X. caffra* kernel meal on gastrointestinal viscera macro-morphometry of male Sprague Dawley rats

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Stomach (g)	1.63±0.17^{a}	1.78±0.18^{a}	1.66±0.23^{a}	1.66±0.12^{a}	1.68±0.15^{a}	ns
Body mass (%)	0.55±0.05^{a}	0.59±0.04^{a}	0.58±0.05^{a}	0.57±0.03^{a}	0.63±0.04^{b}	**
Small intestine (g)	7.71±0.94^{a}	8.42±0.83^{a}	8.56±1.66^{a}	9.18±0.70^{a}	9.32±1.09^{a}	ns
Body mass (%)	2.61±0.23^{a}	2.81±0.19^{ac}	2.98±0.35^{ad}	3.13±0.27^{bcd}	3.50±0.32^{b}	***
Small intestine length (mm)	1326.88±113.48^{a}	1308.00±67.02^{a}	1328.13±124.55^{a}	1370.00±80.40^{a}	1340.75±186.42^{a}	ns
Large intestine (g)	1.79±0.18^{a}	1.78±0.24^{a}	1.81±0.30^{a}	1.95±0.21^{a}	2.00±0.24^{a}	ns
Body mass (%)	0.60±0.04a	0.59±0.07^{a}	0.63±0.09^{a}	0.66±0.05^{ab}	0.75±0.08^{b}	***
Large intestine length (mm)	213.75±15.06^{a}	213.75±15.98^{a}	208.13±19.99^{a}	231.25±15.53^{a}	220.00±10.69^{a}	ns
Caecum (g)	1.56±0.21^{a}	1.62±0.15^{a}	1.48±0.46^{a}	1.62±0.25^{a}	1.52±0.24^{a}	ns
Body mass (%)	0.53±0.06^{a}	0.54±0.05^{a}	0.51±0.11^{a}	0.55±0.08^{a}	0.50±0.21^{a}	ns
Visceral fat (g)	5.47±0.78^{a}	4.96±1.02^{a}	4.52±1.91^{a}	4.33±1.48^{a}	3.75±1.01	ns
Body mass (%)	1.87±0.3^{a}	1.65±0.28^{a}	1.54±0.44^{a}	1.46±0.43^{a}	1.39±0.27	ns

ns: Not significant, p>0.05. *p≤0.05. **p≤0.01, ***p≤0.001, ^{abcd}Within column means with a different superscript are significantly different at p<0.05. Rats fed diet 5 had statistically significantly larger (p = 0.0071) relative (Body mass (%)) stomach masses compared to rats on Diet 4 and Diet 1. Rats fed Diets 4 and 5, respectively had statistically significantly heavier (p = 0.0001) relative (Body mass (%)) small intestine mass compared to the relative (Body mass (%)) small intestine masses of rats on Diet 1. Rats fed Diet 2 had statistically significantly (p = 0.0001) smaller relative (Body mass (%)) small intestine mass compared to those of rats on Diet 5. Diet 1: 0% substitution of Soyabean meal CP, Diet 2: 25% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 3: 50% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Diet 4: 75% substitution of soyabean meal CP with *X. caffra* kernel meal CP, Diet 5: 100% substitution of Soyabean meal CP with *X. caffra* kernel meal CP, Data presented as Mean±SD, n = 8

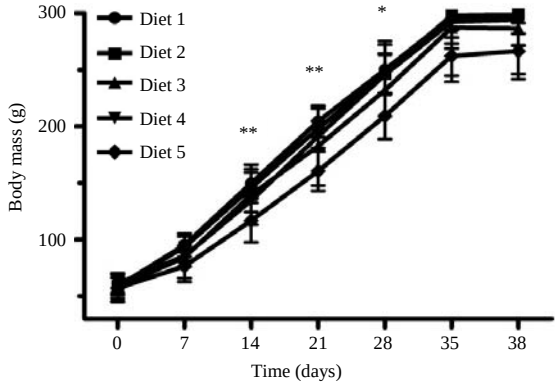

Fig. 1: Effect of a graded dietary substitution of soyabean meal (SBM) with defatted *X. caffra* kernel meal on mean weekly body masses (g) of male Sprague Dawley

From days 0-7 rats fed Diets 1 through to Diet 5 had similar (p>0.05) mean body masses, **From days 14-21 rats fed Diet 5 had statistically significantly lower mean body masses (p = 0.0054 and p = 0.0026, respectively) compared to rats on Diet 1, *On day 28 rats fed Diet 5 had a statistically significantly lower mean body mass (p = 0.0268) compared to rats fed Diet 1, From days 35-38 rats on Diets 1 through to Diet 5 had similar (p>0.05) body masses

affect the palatability of the diets. Studies on the replacement of SBM with non-conventional plant-derived protein sources have reported decreased nutrient digestibility, nitrogen absorption and retention[18,19]. The graded replacement of SBM with XCKM did not negatively impact nutrient ATTD. These results showed an ATTD of OM of over 80% for both the control and test diets which was much higher than the 74.8 and 66.3% apparent OM digestibility reported when *S. pachycarpa* and *A. digitata* seed meal, respectively, were used to replace casein in rat diets[20]. Casein is a high quality protein source with a higher protein efficiency ratio (PER) and digestibility[21]. Results from the current study showed ATTD of OM of the test diets ranged from 80.3-83.3% which approached the 91.2% OM digestibility in a casein-based diet[20]. Despite the current results showing similarity and high ATTD of nutrients across dietary treatments, the determination of true as opposed to apparent nutrient digestibilities, computation of nutrient digestibilities in the different GIT segments, determination of specific amino acid digestibilities and computation of the biological value, and net PER of XCKM could have improved results and given a clearer picture regarding the true potential of XCKM as a dietary protein source in adult rat (monogastric) feeds.

The growth profile of the rats fed the control diet and those fed various levels of SBM substitution with XCKM followed a normal sigmoid pattern in agreement with growth profiles of various rat strains[22]. Initially the weekly body mass of rats fed diet 5 (100% substitution of SBM with XCKM) lagged behind that of other groups but showed compensatory growth from the 4th week onward such that by the end of the trial their mean body mass was similar to that of counterparts on lower levels of substitution. Compensatory growth follows a period of energy restriction[23]. Since the rats were weaned onto the respective dietary treatments, the observed lag in body mass of rats fed diet 5 (100% substitution of SBM) during the first 3 weeks might have been caused by a possible reduced efficiency to extract nutrients during the early stages of the trial despite the

observed similarity in nutrient intake across dietary treatments. The observed compensatory growth can be speculated to have resulted from an improvement in nutrient extraction due to adaptation to the diet which then manifested in growth rebound.

Full body mass is influenced by gut fill and hydration status of the body, thus making it an inaccurate measure of animal growth[24]. The gastrointestinal tract (GIT) and liver protein increase independently of carcass lean mass during catch-up growth[25]. Compared to body mass, the lengths of the tibiae and femora are more accurate measures of growth in growing animals[26] since they respond to growth hormone in a dose-dependent manner[27]. At 59 days old, the termination body masses of rats across dietary treatments were similar to that reported by Lillie *et al.*[28]and Schoeffner[29] in 85 and 64 day old rats, respectively. This suggested that for their age, rats on the control and test diets grew normally and that SBM could be substituted with XCKM without adverse effects on full body and empty carcass masses. Importantly, the similarity in the empty carcass masses of the rats across diets suggested that lean tissue was accreted during the observed catch-up growth especially since similarities in visceral fat were observed despite a decrease in the mass and Seedor ratio of the long bones with increasing dietary XCKM (Table 5).

Dietary XCKM had no effect on the tibiae and femora lengths. Inadequate energy intake influences bone mass and strength by mechanisms including alteration of hormone profiles[30], reduction of bone matrix and mineral contents[31,32], respectively. While the possibility of a transient energy restriction during the first 3-4 weeks of the trial, as shown by the compensatory growth, could have influenced long bone mass and density, it could also be speculated that the XCKM might have contained chelating agents such as oxalates which negatively affect calcium absorption[33] and thus impacting on bone mass and density with an increase in dietary XCKM.

In rats (altricial mammals) the genetically programmed organogenesis that result in functional changes leading to adult GIT functions are characterized by a supremely regulated developmental pattern occurring at weaning[34]. In such mammals the programmed development of the GIT organs accelerates during lactation and early post-weaning[35].The similarity in the macro-morphometry of most GIT viscera of the rats across dietary treatments suggests that the substitution of SBM with XCKM had no deleterious effects on the programmed post-weaning growth and development of the GIT organs. The seeming trophic effects of substituting SBM with XCKM on the mass of the small intestines could have been due to the non-statistically significant relative decrease in both terminal body and empty carcass mass of the rats with an increase in dietary XCKM. The rats used in the growth study were 21 days old. Since most of the programmed development of the GIT of altricial mammals to the adult type occurs during lactation and early post-weaning, it could be inferred that by the time the study commenced, GIT organs of the rats had already developed to adult-type functions, hence the lack of differences in most of the macro-morphometry of the GIT viscera across the diets.

CONCLUSION

It is concluded that defatted *Ximenia caffra* kernel meal can be used to substitute SBM as a dietary protein source in the diets of adult male SD rat diets without compromising ATTD of nutrients and the absorption and retention of nitrogen thus it can be speculated that dietary XCKM can be used in the feeds for mature monogastric animals. However in diets of growing male SD rat diets, while dietary XCKM did not compromise terminal body mass and post weaning GIT viscera macro-morphometry development, caution needs to be taken as high levels of dietary XCKM negatively affect femora and tibiae mass and density which might be an indicator of compromised long bone health.

SIGNIFICANCE STATEMENT

This study showed that defatted *Ximenia caffra* kernel meal can replace soyabean meal as a dietary protein source in the diets of male adult Sprague Dawley rats without compromising feed intake, apparent nutrient digestibility and nitrogen utilization. Thus the meal could potentially be utilized in the diets of mature monogastric animals. However the *Ximenia caffra* kernel meal can only be utilized at low dietary inclusion levels in the diets of growing Sprague Dawley rats as at higher levels it compromised growth performance.

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