

Growth, haematology and serum biochemistry of albino rat fed diet containing raw and thermally processed Loofah Gourd (*Luffa cylindrica* Roem) seed meals

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Abstract

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The competition for food and feed arising from scarcity of conventional livestock feedstuff has resulted into search for novel feedstuff that is currently not being utilized as food by man. Thus, the present study was carried out to evaluate the nutritional potential of thermally processed loofah gourds seed in rats was evaluated in a 21-day completely randomized design feeding trials. Forty-eight Wistar strain male albino rats of 46.57 ± 0.55 g were allotted to four dietary treatments (T1–raw, T2–boiled, T3–cooked and T4–toasted) loofah gourd seed meal (LGSM) with three replicates each. Data collected included feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and carcass characteristics. Haematological and serological variables were determined using standard laboratory procedures. Results revealed that thermal processing significantly ($P < 0.05$) reduced FI by 9–13%. Rats fed toasted LGSM had significantly ($P < 0.05$) highest (7.55) weight gain and best (2.31) FCR while rats fed cooked LGSM had significantly ($P < 0.05$) lowest (4.58) WG and worst (3.96) FCR. Dressing percentage, kidney and abdominal fat and serum biochemistry indices were significantly ($P < 0.05$) affected by thermal processing. The study showed that thermal processing of LGSM enhanced growth performance and had no adverse effect on the haematology and serum biochemistry of albino rats.

Keywords: Loofah gourd seeds, thermal processing, Growth, Haematology, Serum Biochemistry

Introduction

The continued scarcity and price increase of conventional livestock feedstuff due to the competition between man and animal had resulted into search for non-conventional feedstuff as replacement for the relatively scarce and costly conventional livestock feedstuff. The prospect of using a non-conventional feedstuff for livestock feed depends on its ability to provide adequate nutrient that will meet the nutritional requirement of the animal, thus, enhancing growth and normal physiological process of the animal.

Luffa cylindrica (Roem) seed contains nutrients that can be utilized by man and animals for food and feed (Abitogun and Ashogbon, 2010; Dairo *et al.*, 2013),

however, there are dearth of information on its ability to enhance growth and normal physiological processes. Furthermore, the presence of anti-nutritional factors in loofah gourd seeds and methods of reducing/eliminating it has been reported by several workers (Akinmutimi, 2004; Muthumani *et al.*, 2010; Pallaviet *et al.*, 2011; Oyetayo and Ojo, 2012; Dairo *et al.*, 2013; Onigemo, 2015). Onigemo (2015) reported toasting, cooking and boiling as potential means of reducing the anti-nutritional factors present in loofah gourd seed.

In order to assess the potentials of thermally processed loofah gourd seed meals in sustaining growth and physiological status of man and animal, a laboratory experiment with Albino rats was conducted. Albino rat

is the first domesticated animal used in scientific research and the most commonly used laboratory mammal next to mouse probably due to their small size, high reproductive rate in short period of time, ease of handling and availability of vast bodies of knowledge about them (Festing, 1979; Jacoby *et al.*, 2002). The fact that their organs and physiological responses are similar to that of man and animals makes them the choice animal in most biological studies, *hence*, rats have been used in many disciplines, including nutrition, genetics, endocrinology, and biochemistry (Giridharan *et al.*, 2000).

This experiment was therefore designed and executed to evaluate the growth, haematology and serum biochemistry of albino rats of raw fed thermally processed LGSM on so as to establish its suitability as potential feed resource in livestock and poultry.

Materials and methods

Experimental diets

Raw Luffa cylindrica gourd seeds were sourced and processed into boiled, cooked and toasted meals as described by Onigemo et al. (2020). The test ingredients were mixed with other feedstuff in a pre-formulated manner as shown in Table 1. to produce iso-nitrogenous LGSM diets. The experimental diets were pelletized into 2mm size using cassava starch as a binder.

Experimental design

The experimental design was completely randomized design with four treatments: T1 – raw loofah gourd seed meal (RLGSM), T2 – boiled loofah gourd seed meal (BLGSM), T3 – cooked loofah gourd seed meal (CLGSM) and T4 – toasted loofah gourd seed meal (TLGSM). Each treatment was replicated three times with four rats per replicate.

Table 1: Composition of experimental diets (g/100 g)

Diets	Test ingredient diets			
	1	2	3	4
Corn Starch	44.79	45.35	45.39	44.35
Lactose	15.00	15.00	15.00	15.00
Corn Cob	8.00	8.00	8.00	8.00
Bone meal	1.50	1.50	1.50	1.50
Raw LGSM	25.21	0.00	0.00	0.00
Boiled LGSM	0.00	24.65	0.00	0.00
Cooked LGSM	0.00	0.00	24.61	0.00
Toasted LGSM	0.00	0.00	0.00	25.65
Cassava Starch	3.00	3.00	3.00	3.00
Vegetable oil	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Calculated analysis				
Crude Protein (%)	10.01	10.00	10.00	10.00
Metabolize able Energy (Kcal/Kg)	3897.05	3782.42	3703.86	3858.45

Experimental animal and management

Forty-eight, Wistar strain 24-day old male albino rats with an average weight of (46.57±0.55) sourced from Easy D Farm, Ibadan were used for the experiment. The experimental house and cages were cleaned

and disinfected with Morigad[®] veterinary Lysol solution a week before the arrival of the experimental rats. The cages were fitted with feeders and drinkers. Fresh feed and water were provided *ad libitum* at 7:00 and 17:00 hours of the day. At the end of the

twenty-one (21) days trial, one rat per replicate was sacrificed for carcass and blood samples were collected in Lithium heparin bottle and Ethylene Diamine Tetra Acetic Acid (EDTA) bottle for serological and haematological analysis respectively. Haematological variables such as haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) were all determined by the procedure of Sastry (2004). Serum chemistry variables namely total protein, albumin, and globulin were analysed according to Peters *et al.* (1982), cholesterol as described by Richmond (1973), urea using the methods outlined by Weatherburn (1967), and creatine as described by Bartels and Bohmer (1972). Serum enzymes activities of alanine aminotransferase (EC 2.6.1.1.1) and aspartic amino transferase (EC 2.6.1.1.2) were also determined as documented by Reitman and Frankel (1957).

Data collection

Data on performance indices such as feed intake (FI) and weight gain (WG) and the above mentioned haematological and serological variables were collected. The following data were generated from the above primary data:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{FI}}{\text{WG}}$$

$$\text{Mean corpuscular volume (MCV)} = \frac{\text{PCV}}{\text{RBC}}$$

$$\text{Mean corpuscular haemoglobin (MCH)} =$$

Hb

RBC

Data analysis

All data were subjected to analysis of variance (ANOVA) and differences in means were separated with Duncan Multiple Range Test using Assistat-Statistical Assistance 7.6 beta software developed by Silva and Azevedo (2009).

Results

The performance of rats fed raw and thermally processed loofah gourd seed meals is shown in Table 2. The average feed intake (AFI) of the rats fed the experimental diets did not differ significantly ($P > 0.05$). The lowest AFI of 17.45g/rat/day was observed in rats fed with TLGSM while the highest AFI of 20.0 g/rat/day was observed in the RLGSM. Generally, all the feeds were well consumed by the rats. The AFI of rats fed thermally processed LGSM were significantly ($P < 0.05$) lower than those fed the raw LGSM. The average weight gain (AWG) of rats fed the experimental diets differ significantly ($P < 0.05$) with rats fed TLGSM having the significantly ($P < 0.05$) highest AWG of 7.55g/rat/day while the lowest AWG of 4.58g/rat/day was observed in rats fed CLGSM diet. The FCR and PER of the rats was also significantly ($P < 0.05$) influenced by the experimental diets with rats fed TLGSM having the best conversion ratio (2.31) and rats fed CLGSM having the poorest (3.96).

Table 2: Growth performance of rats fed differently processed loofah gourd seeds

Diet	Average Feed Intake (g/rat/day)	Average Weight Gain (g/rat/day)	FCR
RLGSM	20.00 ^a	5.62 ^{ab}	3.56 ^a
BLGSM	18.09 ^b	4.85 ^b	3.73 ^a
CLGSM	18.14 ^b	4.58 ^b	3.96 ^a
TLGSM	17.45 ^b	7.55 ^a	2.31 ^b
SEM	0.55	0.67	0.37

Means on the same column with different superscript differ significantly ($P < 0.05$)

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Table 3 shows the carcass characteristics of rats fed raw and thermally processed LGSM. There were significant ($P < 0.05$) differences in the carcass characteristics of rats fed raw and thermally processed LGSM. The dressing percentage of the rats differed significantly ($P < 0.05$) with rats fed TLGSM having the highest (69.66%) while the lowest dressing percentage (58.23%) was observed in rats fed BLGSM. The weight of liver and spleen expressed as a percentage of the live weight differed significantly ($P < 0.05$) among rats fed differently processed LGSM with CLGSM having the highest (5.12% and 0.38%

respectively), while the lowest liver and spleen weight (4.23% and 0.30%, respectively) was observed in rats fed RLGSM. The highest kidney and abdominal fat (0.77% and 3.23%, respectively) was recorded in rats fed TLGS; the lowest kidney weight was recorded in BLGSM while the lowest abdominal fat was observed in rats fed CLGSM. The heart weight of rats fed RLGSM was significantly ($P < 0.05$) higher (0.65%) in weight while rats fed BLGSM had the significantly ($P < 0.05$) lowest heart weight expressed as percentage of live weight (0.38%).

Table 3: Carcass characteristics of rats fed differently processed loofah gourd seeds

Diets	Dressing percentage (%)	Heart (% LW)	Liver (% LW)	Kidney (% LW)	Abdominal fat (% LW)	Spleen (% LW)
RLGSM	63.23 ^{ab}	0.65 ^a	4.23 ^b	0.62 ^b	2.96 ^{ab}	0.30 ^b
BLGSM	58.23 ^b	0.38 ^c	4.41 ^b	0.60 ^c	2.25 ^{bc}	0.32 ^b
CLGSM	62.47 ^b	0.61 ^{ab}	5.12 ^a	0.74 ^{ab}	2.06 ^c	0.38 ^a
TLGSM	69.66 ^a	0.45 ^{bc}	4.25 ^b	0.77 ^a	3.23 ^a	0.31 ^b
SEM	2.36	0.06	0.21	0.04	0.28	0.02

^{abc} Means on the same column with different superscript differ significantly ($P < 0.05$)

Table 4 shows the haematological indices of raw and thermally processed LGSM fed rat. There were significant ($P < 0.05$) differences in the haematological characteristics of rats fed raw and differently processed LGSM. The highest haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white

blood cells (WBC), mean corpuscular volume and mean corpuscular haemoglobin (MCH) were observed in rats fed CLGSM while the significantly ($P < 0.05$) lowest value of the above listed haematological variable was observed in those fed BLGSM.

Table 4: Haematological indices of rats fed differently processed loofah gourd seeds

	Hb (g/dL)	PCV (%)	RBC ($10^6/\text{mm}^3$)	WBC (mm^3)	MCV (fl)	MCH (Pg)
RLGSM	5.65 ^{ab}	17.00 ^{ab}	1.97 ^{ab}	4066.67 ^{ab}	8.48 ^{ab}	28.20 ^{ab}
BLGSM	4.32 ^b	13.00 ^b	1.57 ^b	2483.33 ^b	8.12 ^b	27.01 ^b
CLGSM	7.08 ^a	21.33 ^a	2.40 ^a	5816.67 ^a	8.81 ^a	29.23 ^a
TLGSM	6.54 ^a	19.67 ^a	2.30 ^a	4183.33 ^{ab}	8.45 ^{ab}	28.12 ^{ab}
SEM	0.60	1.82	0.19	680.87	0.14	0.45

^{abc} Means on the same column with different superscript differ significantly ($P < 0.05$)

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The serum biochemistry of rats fed raw and thermally processed LGSM are presented in Table 5. It revealed significant differences ($P < 0.05$) in all the serological variables investigated. The highest serum albumin (ALB) and cholesterol (CHOL) were recorded in rats fed with BLGSM, while the lowest was recorded in rats fed raw and CLGSM. The serum protein and lactate dehydrogenase content of rats fed with diets containing raw and thermally processed LGSM were significantly ($P < 0.05$) highest (85.52 and 879.05 respectively) in rat fed TLGSM while those fed RLGSM had the lowest values of 75.92

and 240.76 respectively for both variables. The highest creatinine value (1.5) was observed in rats fed RLGSM while the significantly ($P < 0.05$) lowest creatinine value was observed in rats fed TLGSM. Rats fed CLGSM had the significantly ($P < 0.05$) highest blood urea of 47.93 while the lowest of 37.7 was observed in those fed TLGSM. The aspartate amino transferase (AST) and alanine amino transferase (ALT) of rats fed with diets containing RLGSM had significantly ($P < 0.05$) the highest (215.69 and 45.73 respectively) values while those on cooked LGSM had the lowest (145.01 and 37.28, respectively) values.

Table 5: Serum Biochemistry of Rats Fed with Differently Processed Loofah Gourd Seeds.

	ALB (g/dL)	CHOL (mg/dL)	PROT (g/L)	UREA (mg/dL)	AST (U/I)	ALT (U/I)	LDH (U/I)	CR (mg/dL)
RLGSM	5.70 ^b	98.93 ^{bc}	75.92 ^b	37.70 ^b	215.69 ^a	45.73 ^a	240.76 ^b	1.50 ^a
BLGSM	8.73 ^a	133.30 ^a	83.23 ^a	41.38 ^{ab}	150.60 ^b	41.51 ^{ab}	621.80 ^{ab}	1.33 ^a
CLGSM	7.20 ^{ab}	94.80 ^c	81.50 ^{ab}	47.93 ^a	145.01 ^b	37.28 ^b	434.71 ^b	1.17 ^a
TLGSM	8.70 ^a	127.80 ^{ab}	85.52 ^a	37.10 ^b	153.65 ^b	39.52 ^b	879.05 ^a	0.85 ^b
SEM	0.72	9.82	2.05	2.49	16.58	1.79	136.08	0.14

^{abc} Means on the same column with different alphabet differ significantly ($P < 0.05$)

Discussion

The average weight gain of rats fed with diets containing TLGSM were significantly higher than all other diets containing LGSM probably due to minimal loss of nutrient during processing as vitamins, minerals and other soluble nutrients may have leached during the cooking/boiling, hence, lost during processing (FAO, 1990). The feed conversion ratio of the rats followed a similar order with the weight gain and final weight, hence, the best FCR was observed in rats fed TLGSM, which indicated a substantial detoxification of the heat labile anti-nutritional factors. This might have made the nutrients in the diets to be more available for metabolic process. The best growth performance observed in rats fed diet containing TLGSM might be due the fact that the predicted protein efficiency ratio of TLGSM was superior to those of

raw and other thermally processed LGSM (Onigemo *et al.*, 2020). Also, the diet containing TLGSM had outstanding metabolisable energy of all the diets containing thermally treated LGSM. El-Dahhar (2000) reported that FCR or feed utilization was improved as dietary protein and energy were increased.

The weight of liver expressed as percentage of live weight of rats fed with diets containing LGSM were relatively than the values reported by Amusa *et al.* (2015) for normal rats, indicating that the liver may be undergoing overbearing activities, perhaps as a result of detoxification process. Intense metabolic activities and some metabolic syndrome have been reported to result in the inflammation of the liver (Bedogniet *al* 2005; Paschos and Paletas 2009).

The kidney weight recorded in this study was relatively higher than the values

observed by Onyeanus *et al.*, (2009) but comparable to those reported by Dunns (1967). These differences in values could be due to variations in age, breed, environmental factors and diets (Onyeanus *et al.*, 2009). The relatively high kidney weight observed in rats fed TLGSM might be attributed to the relatively high energy content of the diet. It has been observed that increased protein and energy intake rate results in the changes in kidney and liver mass (Hamad and Neifeld, 1998) and that higher protein content of diets increases kidney size (Finco, 1999). This tends to explain the significantly lowest kidney weight expressed as percentage of live weight observed in rats fed CLGSM diet.

The significantly highest spleen weight observed among rats fed CLGSM diet may probably be due to the fact that it is lower in protein content due to dissolved nutrient during processing. Intake of diets that is low in protein has been reported to result in atrophy and fibrosis in the spleen (Krustev and Popov, 1982). The abdominal fat followed a reverse order as the spleen, hence the significantly lowest abdominal fat weight was observed among rats fed toasted LGSM diet. This observation might be attributed to the relatively low energy intake of this group of rats since the amount and source of energy used in the diet after weaning influenced body growth and fat deposits (Costa *et al.*, 2010; Costa *et al.*, 2012).

Statistical analysis revealed significant ($P < 0.05$) differences in the haematological variables investigated, however, all values obtained were within the normal physiological range and do not have any serious health implications on the rats. The values of the haematological parameters observed in this study vary appreciably from those documented in literature for albino rats by other workers (Umar *et al.*, 2010; Kumar *et al.*, 2011; Bamisaye *et al.*, 2013). Haematological parameters may

vary according to breed, sex, age, reproductive status, fitness and training levels, exercise, feeding, circadian variations, handling procedure of the animals during blood withdrawal, degree of excitement, health status, season and geographical location (Jain, 1993; Rose and Hodgson, 1994; Messer, 1995; Kramer, 2000; Minka and Ayo, 2008).

The serum albumin content of rats fed with RLGSM was relatively lower than those on the thermally processed LGSM diets. The low albumin content of rats fed raw LGSM might be attributed to the loss of albumin into the intestinal tract where they are metabolized or in urine. The total blood protein content of rats fed thermally processed LGSM were significantly higher than those fed RLGSM diets. High sugar and fat has been implicated in an inflammation that increases C-reactive (pentameric) protein in the blood (Paul *et al.*, 2003; Pravin and Patel 2011; Busch 2013). The blood urea content of the rats fed boiled and CLGSM were significantly higher than those on other diets. The serum creatinine of rats used in this study did not follow any particular trend and were within the range reported as normal values for apparently healthy rats (Anderson *et al.*, 1930; Podjarny *et al.*, 1997), implying that the kidney of the rats was in healthy condition during the experimental period (Gross *et al.*, 2005). The blood cholesterol levels of rats fed BLGSM and TLGSM were similar but the value was significantly higher than other groups of rats. The relatively high cholesterol of rats fed BLGSM and TLGSM might be attributed to the higher energy intake of this group of rats. A direct relationship has been established between dietary fat and blood cholesterol (Kris-Etherton and Yu, 1997; Health Canada, 2000; Sakurai *et al.*, 2011).

The serum aspartic transaminase (EC 2.6.1.1) concentration of rats fed thermally processed LGSM diets are similar and were

within the range reported for normal rats (Anderson *et al.*, 1930; Podjarny *et al.*, 1997). However, these values were significantly less than the AST of rats fed with raw LGSM and similar trend was observed in the values of ALT concentration. The high serum aminotransferases of rats fed RLGSM might be attributed to the detoxification activities of the rat's liver due to the relatively high content of anti-nutrient in unprocessed LGSM. Serum aminotransferase activities have long been as sensitive indicators of hepatic injury (Molander *et al.*, 1955). This might also explain the high LDH concentration observed among rats fed RLGSM.

Conclusion

This study revealed that thermal processing enhanced the utilization of loofah gourd seed meal by albino rats. Rats fed toasted loofah gourd seed meal had the best growth performance and the lowest serum enzymes concentration. The adoption of thermally processed loofah gourd seed meal in the production of feed for livestock and poultry is hereby recommended.

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