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Growth Performance of Rats Maintained On *Citrullus colocynthis* Seed Coat-based Diet

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Abstract: *Growth performance of rats maintained on Citrullus colocynthis seed coat based diet was evaluated. The experiment was carried out to ascertain whether replacing corn starch in animal feed with raw or heat-treated Citrullus colocynthis seed coat can support proper growth of the animals. Forty-nine weanling albino rats, with mean weight of 36.76g ± 2.56g were divided into seven (7) groups of equal average weight. The groups were then randomly assigned to seven (7) experimental diets. Diet C (Control) contained 100% inclusion level of corn starch. Diets R, A and B contained 100% inclusion levels of raw, autoclaved and boiled Citrullus colocynthis seed coat respectively, while diets CR, CA and CB contained 50% inclusion levels of raw, autoclaved and boiled Citrullus colocynthis seed coat respectively. The rats were fed on their respective experimental diets and water ad libitum for six weeks after one week of acclimatization period to the laboratory environment. Feed intake, growth response, carbohydrate metabolism and organ to body weight ratio were determined. The results obtained revealed that 50% inclusion levels of autoclaved or boiled Citrullus colocynthis seed coat diets did not significantly affect (p>0.05) the average weekly feed intake, weight gain, feed conversion ratio and carbohydrate digestibility but significantly reduced (p < 0.05) metabolizable energy. Organ to body weight ratio and concentrations of blood pyruvate, lactate and lactate-to- pyruvate ratio were also not significantly (p>0.05) affected by these diets. 100% inclusion levels of raw, boiled, autoclaved Citrullus colocynthis seed coat diets and 50% inclusion level of raw Citrullus colocynthis seed coat diet significantly (p < 0.05) reduced the average weekly feed intake, weight gain, carbohydrate digestibility and metabolizable energy, but significantly increased feed conversion ratio, lactate, pyruvate and lactate/pyruvate ratio. Present findings showed that 50% replacement of corn starch by boiled or autoclaved Citrullus colocynthis seed coat in animal feed supports proper growth of the animals. However, further researches to ascertain the safety of the seed coat is necessary.*

Key words: *Citrullus, colocynthis, growth, coat, seed, digestibility*

I. Introduction

Hunger, which afflicts one in five of the developing world's people, is a profound impediment to the advancement of individuals and societies. As population increase particularly in developing nations continue to outstrip food production, FAO[1] believes that consumers still face at least until 2018 more expensive food, and with the spikes hitting all major foods in most countries at once. In an effort to avert the menace of hunger, World Food Summit convened in 2009 adopted unanimously a declaration committing all the nations of the world to eradicate hunger at the earliest possible date. It pledged to substantially increase aid to agriculture in developing countries, so that the world's 1 billion hungry can become more self-sufficient. Unfortunately, this seems unachievable due in large part to freak weather, dramatic change in global economy, lower food reserves and growing consumer demand across the globe. This has remarkably led world's population into food problems that has particularly made the provision of qualitatively and quantitatively adequate protein difficult. Animal protein is essential in human nutrition, due to its balanced amino acid profile and ease of utilization.[2]

The demand for protein of animal origin in Nigeria is greater than the supply[3]which makes increasing livestock production imperative. However, a limiting factor in achieving this is the provision of adequate and affordable food as there is competition between man and livestock for the conventional feed stuffs like maize. It is therefore necessary to search for inexpensive, but nutritionally adequate feeds for livestock. Consequently, nutritional researches are being directed to alternative sources of feed ingredients such as agricultural by-products. One such by-products of potential importance are *Citrullus colocynthis* seed coats.

Citrullus colocynthis, commonly known as the as the bitter apple, bitter gourd, and locally called egusi bara, belongs to the species of the genus Citrullus of cucurbitaceae family, which usually consists of a large number of varieties[4]. Cucurbitaceae plant family is a crawling crop and is known for its great genetic diversity and widespread adaptation which includes tropical and subtropical regions, arid deserts and temperate locations[5] and occupies the vast area extending from the west coast of northern Africa. The nutritional value of Citrullus colocynthis seed is well documented[6,7].

Citrullus colocynthis seeds are covered by seed coats which are shelled in the process of processing. These seed coats are thrown away as waste. Earlier reports indicate that 100g *Citrullus colocynthis* seed coats typically contain 15.5- 19.14% proteins, 1.17-2.05% fats (oils), 61-69.59 % carbohydrate, 6.07- 8.12% fibre and 6.79 - 7.13% ash content[8,9]. *Citrullus colocynthis* seed coat is available in abundance in Nigeria and presently constitutes potential environmental pollutant. If raw or heat- treated *Citrullus colocynthis* seed coat could replace maize in animal feed, it will go a long way in bringing the cost of livestock production to affordable level. However, prior to the utilization of such novel sources, either in human food or feed formulation for animals, it is absolutely necessary to investigate the possible effect of their ingestion on some tissues and organs of the body. This is because many plants are endowed with substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects[10]. Although, some researchers have indicated the nutritional potential of *Citrullus colocynthis* seed coat[11, 12], information on the growth response of rats maintained on raw or heat-treated *Citrullus colocynthis* seed coat remained scanty. This research was therefore conducted to evaluate the growth response of rats maintained on raw or heat-treated *Citrullus colocynthis* seed coat.

II. Materials And Methods

2.1 Sample Preparation

Citrullus colocynthis seeds, as identified at University of Ilorin herbarium were bought from a farmer in En kwura, Kano, Nigeria and screened to remove bad ones. The seed coats were manually removed from the seeds, washed with tap water to remove dirt, sun-dried and then divided into portions 1, 2 and 3. Portion 1 was left raw, while portions 2 and 3 were subjected to heat treatments. Portion 2 was boiled by immersing the seed coats in boiling water and allowing boiling for 90 minutes and then sun-dried. Portion 3 was autoclaved for 30 minutes at 15 Ib pressure and 120°C. The three portions were separately ground to flour and separately stored in clean polyethylene bags[8].

2.2 Feed Formulation

The raw, autoclaved and boiled *Citrullus colocynthis* seed coats were separately mixed with other ingredients to formulate seven experimental diets to replace dietary corn starch at 0%, 50% and 100% (Table 1).

Table 1: Components of the formulated diets (gkg^{-1})

	A	B	R	C	CA	CB	CR
A	516	-	-	-			
B	-	516	-	-			
R	-	-	516	-			
C	-	-	-	516			
CA	-	-	-	-	258+258	-	-
CB	-	-	-	-	-	258+258	-
CR	-	-	-	-	-	-	258+258
Soy meal	250	250	250	250	250	250	250
Soybean oil	40	40	40	40	40	40	40
Rice bran	40	40	40	40	40	40	40
D-L methionine	04	04	04	04	04	04	04
sucrose	100	100	100	100	100	100	100
Vit/min mix	50	50	50	50	50	50	50

Diet A: Diet based on 100% inclusion level of autoclaved *Citrullus colocynthis* seed coat
Diet B: Diet based on 100% inclusion level of boiled *Citrullus colocynthis* seed coat
Diet R: Diet based on100% inclusion level of raw *Citrullus colocynthis* seed coat
Diet C (control): Diet based on 100% inclusion level of corn starch
Diet CA: Diet based on 50% inclusion level of autoclaved *Citrullus colocynthis* seed coat
Diet CB: Diet based on 50% inclusion level of boiled *Citrullus colocynthis* seed coat
Diet CR: Diet based on 50% inclusion level of raw *Citrullus colocynthis* seed coat

The food items were thoroughly mixed together and manually made into pellets to feed albino rats (Table 1).

2.3 Proximate analysis

The carbohydrate, ash, crude fat, crude fiber and crude protein contents of the formulated diets were determined using the recommended methods of the Association of Official Analytical Chemists (AOAC)[13].

2.4 Management of Experimental Animals

Forty-nine weanling albino rats **with mean weight of 36.76g ± 2.56g** were obtained from the small animal holding unit of the department of Biochemistry, University of Ilorin. The rats were randomly assigned into the seven (7) dietary treatment groups of 7 animals each which were equalized for body weight. The rats in each group were housed together in standard plastic laboratory cages with stainless steel covers and were offered their respective experimental diets and water ad libitum after one week of acclimatization period to the laboratory environment. The feeding trial lasted for six weeks.

2.5 Determination of Feed Intake and Growth Response

The feed intake was determined as the differential between the quantity of feed served and the quantity of feed left over. The change in weight was determined by weighing the rats at the commencement of the feeding trial and thereafter on a weekly basis until termination of the experiment. The weekly feed conversion ratio was calculated by dividing the total feed consumed by total weight of the rats. Energy values, digestibility and metabolizable energy were determined using the methods of CAES[14].

2.6 Animal sacrifice, Collection and Treatment of Blood Samples

After 42 days, the animals were weighed and sacrificed by anaesthetizing them in a jar containing cotton wool soaked in diethyl ether. Blood was obtained through the jugular veins in tubes containing EDTA as anticoagulant (Akanji and Ngaha, 1989). The method of Gloster and Harris (1962) was employed in the determination of pyruvate and lactate.

2.7 Statistical analysis

Data were expressed as mean ± SEM of the numbers of determinants.

III. Results

The components of the formulated feeds were adjusted to have proximate compositions not significantly different (p>0.05) from the proximate composition of the control feed (Table 2). The average weekly feed intake, weight gain and feed conversion ratio in rats fed on the control diet did not differ significantly (p>0.05) from the values obtained in rats fed on 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat. Compared with the control, rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat had significantly (p< 0.05) higher feed conversion ratio but lower feed intake, metabolizable energy and weight gain (Table 3). The percentage organ (liver, kidney and heart) to body weight ratio obtained from rats fed on control diet did not differ significantly (P>0.05) from the values obtained from rats fed on 50% inclusion levels of autoclaved or boiled *Citrullus colocynthis* seed coat. The percentage organ (liver, kidney and heart) to body weight ratios were significantly (P< 0.05) decreased in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat when compared to the control (Table 4). The concentration of blood pyruvate, lactate and lactate-to- pyruvate ratio shown in Table 5 were significantly (P< 0.05) higher in the blood of rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat in comparison with the control group. 50% inclusion levels of autoclaved or boiled *Citrullus colocynthis* seed coat diets had no significant effect (P>0.05) on these intermediates.

Table 2: Proximate composition of the formulated diet

	C	R	A	B	CR	CA	CB
DM (%)	88.16 ±1.65^{a}	92.27 ±2.63^{a}	91.56 ±2.96^{a}	89.75±1.70^{a}	90.56±1.72^{a}	90.14±1.14^{a}	88.81±0.72^{a}
Protein (%)	21.03 ±1.60^{b}	24.85 ±1.13^{a}	21.53 ±2.09^{ab}	22.24 ±1.99^{ab}	20.91 ±0.18^{ab}	20.88 ±0.65^{ab}	20.50 ±0.32^{ab}
Fat (%)	6.28 ±0.97^{a}	5.3 8±0.59^{a}	5.92±0.28^{a}	6.11±0.17^{a}	6.06±0.24^{a}	5.94±0.14^{a}	5.88±0.85^{a}
Ash (%)	6.00 ±0.19^{a}	6.01 ±0.20^{a}	6.00±0.30^{a}	6.21±0.03^{a}	6.04±0.27^{a}	6.06±0.14^{a}	5.89±0.15^{a}
Fibre (%)	6.07 ±0.69^{b}	9.27 ±0.41^{a}	7.82 ±0.13^{ab}	7.66 ±0.12^{ab}	8.51 ±0.62^{ab}	7.24 ±0.04^{ab}	7.33 ±0.31^{ab}
CHO (%)	60.62 ±1.78^{b}	54.49 ±1.14^{a}	58.72 ±1.76^{ab}	57.77 ±2.00^{ab}	58.48 ±0.49^{ab}	59.88 ±0.67^{b}	60.30 ±1.28^{b}
Energy (Cal/100g)	381.51±8.25^{b}	365.75± 3.68^{a}	374.31 ±0.35^{ab}	375.08 ±1.38^{ab}	372.10 ±3.22^{ab}	376.52 ±1.41^{ab}	376.15 ±3.92^{ab}

Results are means of 3 determinations ± S. E. M

Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different.

DM: dry matter

CHO: carbohydrate

Table 3: Growth performance of experimental rats fed on raw or heat-treated Citrullus colocynthis seed coat based diets for six (6) weeks

	C	R	A	B	CR	CA	CB
WG(g)	7.17±0.06[a]	0.98 ±0.01[d]	1.83 ±0.02[c]	1.94 ±0.01[c]	2.01 ±0.07[c]	5.50 ±0.03[b]	6.06 ±0.03[ab]
FI(g)	75.83 ±1.37[a]	39.81 ±3.65[c]	47.91 ±3.03[b]	48.35 ±3.14[b]	50.48 ±3.23[b]	71.14 ±2.22[a]	71.67 ±2.65[a]
FCR	10.84 ±0.70[a]	43.06 ±1.89[c]	26.35 ±1.91[b]	24.55 ±1.82[b]	26.88 ±1.68[b]	13.37±1.14[a]	12.47±1.29[a]
CHO. D	89.38±0.21[a]	82.12±0.78[b]	86.56±0.50[c]	86.74±0.53[c]	86.54±0.46[c]	88.65±0.28[a]	88.56±0.48[a]
ME	1553.44±2.16[a]	827.79±3.32[c]	1005.01±1.7[d]	1016.09±2.37[d]	1043.93±2.22[d]	1456.27±2.56[b]	1468.98±2.01[b]

Results are means of 6 determinations ± S. E. M

Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different.

FI: feed intake

WG: weight gain

FCR: feed conversion ratio

CHO.D: carbohydrate digestibility

ME: metabolizable energy

Table 4: Percentage organ to body ratio of experimental rats fed heat-treated Citrullus colocynthis seed coat based diets for six (6) weeks

Organ	C	R	A	B	CR	CA	CB
Liver	6.21±0.29[c]	3.58±0.21[a]	3.85±0.19[ab]	4.15±0.14[ab]	4.15±0.34[b]	5.59±0.40[c]	6.29±0.21[c]
Kidney	0.72±0.1[bc]	0.50±0.02[ab]	0.67±0.03[b]	0.63±0.06[b]	0.41±0.02[a]	0.75±0.05[bc]	0.87±0.07[cd]
Heart	0.76±0.09[b]	0.44±0.02[a]	0.43±0.02[a]	0.45±0.02[a]	0.42±0.02[a]	0.65±0.05[b]	0.70±0.04[b]

Results are means of 3 determinations ± S. E. M

Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different.

Table 5: Concentrations of lactate ((mg per 100 ml blood)), pyruvate ((mg per 100 ml blood)) and lactate/pyruvate ratio in the blood of rats fed on raw or heat treated Citrullus colocynthis seed coat.

	C	R	A	B	CR	CA	CB
Pyruvate	0.76±0.02[a]	1.53±0.07[d]	1.25±0.02[c]	1.24±0.02[c]	1.08±0.05[b]	0.83±0.01[a]	0.80±0.02[a]
Lactate	9.78±0.27[a]	39.38±0.21[d]	28.81±0.28[c]	28.51±0.32[c]	20.70±0.35[b]	11.91±0.29[a]	11.23±0.05[a]
Lac:Pyr	12.87±0.08[a]	25.77±0.22[d]	22.98±0.26[c]	22.87±0.16[c]	19.28±0.14[b]	14.30±0.03[a]	13.99±0.07[a]

Results are means of 3 determinations ± S. E. M

Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different.

Lac:Pyr: lactate - to -pyruvate ratio

IV. Discussion

The lower carbohydrate digestibility, metabolizable energy and higher feed conversion ratio observed in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat (Table 3) may be due to the presence of some antinutrients, particularly amylase inhibitor, oxalate, tripsin inhibitor and lectin. Unlike other antinutrients detected in the *Citrullus colocynthis* seed coat, their levels in *Citrullus colocynthis* seed coat remained significant even after treatments[8]. Tripsin inhibitor and amylase inhibitor have been reported to decrease the activity of digestive enzymes, which may decelerate the digestion of the nutrients, thus resulting in poor growth. Amylase inhibitors prevent the action of enzymes that break the glycosidic bonds of starches and other complex carbohydrates, preventing the release of simple sugars and consequently absorption by the body[17], while tripsin inhibitor prevent proper protein digestion[18]. Like amylase inhibitor, lectins negatively interfere with carbohydrate utilization. Dietary lectins act as protein antigens which bind to surface glycoproteins (or glycolipids) on erythrocytes or lymphocytes[19] leading to poor nutrient utilization, while high levels of oxalate may interfere with carbohydrate metabolism, particularly by succinic dehydrogenase inhibition[20]. Also, phytate was reported to interact with, and reduce the bioavailability and digestion of carbohydrate (starch)[21]

Also known as feed conversion rate or feed conversion efficiency (FCE), feed conversion ratio (FCR) is a measure of an animal's efficiency in converting feed mass into increases of mass gained by the animal. A low FCR is a good indication of a high quality feed[22]. The combined effects of low feed intake by rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat, and the presence of antinutritional factors could have resulted in the rats not converting the feed consumed into useful nutrients required by the body, thus accounting for the remarkable low weekly weight gain by rats fed on these diets as depicted by the growth chart (Table 3). The low feed intake could be due to the taste and/or texture of these diets. The finding is in line with the report of Farran *et al*[23] that taste and texture of finished feeds influence intake in animals. The improvement observed in rats fed on 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat both in feed intake and growth performance gives credence to the earlier suggestion that low feed intake and presence of antinutritional factors are responsible for the low growth performance observed in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat. In 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat, the concentrations of these antinutrients were lowered and the palatability increased, hence the improved feed intake, metabolism and growth. The finding is consistent with the reports of Martinus van *et al* [24] that heat treatment improved digestibility and bioavailability of nutrients, improved palatability, taste, texture and flavour, and Bamikole *et al* [25]that dry matter intake and weight gain declined with increasing levels of inclusion of heat processed jack bean in diets of rabbit.

The percentage organ (liver, kidney and heart) to body ratio obtained from rats fed on control diet did not differ significantly from that obtained from rats fed on 50% inclusion levels of autoclaved and boiled Citrullus colocynthis seed coat. This indicates that 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat may supply necessary nutrients for proper organ development and may have no adverse effect on the development of the organs. On the other hand, 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat (Table 4) may not supply enough nutrients necessary for proper organ development. Nutrients provide structural material (amino acids from which proteins are built, and lipids from which cell membranes and some signaling molecules are built) and energy[26].

The non significant difference between the levels of gluconeogenic substrates (lactate and pyruvate) in blood of rats fed on 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat and in rats fed on the control diet (Table 5) is indicative of normal carbohydrate metabolism in rats fed on 50% inclusion levels of autoclaved and boiled *Citrullus colocynthis* seed coat. Adequate nutrient intake by rats fed on these diets prevented muscle breakdown and overproduction of acetyl CoA (an inhibitor of pyruvate dehydrogenase complex) by β-oxidation of fatty acids by muscle. Under this condition, the pyruvates generated from carbohydrate metabolism flow towards TCA cycle, which makes the substrate unavailable for conversion to lactate.

On the other hand, the elevated circulating levels of gluconeogenic substrates (lactate and pyruvate) in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat confirm altered carbohydrate metabolism in these rats and could be due to low feed intake by rats on these diets (Table 5) and/or due to presence of antinutritional factors in these diets. This is in harmony with metabolic processes. During low food intake or food deprivation, glucose is scarce and glucose utilization becomes decreased[27]. In a process called catabolysis, the body breaks down its own muscles and other tissues in order to keep vital systems such as the nervous system and heart muscle functioning. The β-oxidation of fatty acids by muscle halts the conversion of pyruvate into acetyl CoA, because acetyl CoA stimulates the phosphorylation of the pyruvate dehydrogenase complex, which renders it inactive. Hence, pyruvate, lactate, and alanine are exported to the liver for conversion into glucose[26]. The inactivation of pyruvate dehydrogenase (PDH) in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat as indicated by the elevated level of lactate and pyruvate in these rats indicate unaltered glycolytic pathway leading to pyruvate formation and reduced flow of pyruvate towards TCA cycle, which makes the substrates available for gluconeogenesis. Elevated lactate-to-pyruvate ratio was also observed in rats fed on 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat which may be due to increased production of lactate. Inhibition of pyruvate dehydrogenase (PDH) complex causes rapid accumulation of pyruvate, and pyruvate metabolism is subsequently shifted almost in entirety toward lactate formation. Subsequently, intracellular lactate concentration rapidly increases, leading to excretion into the bloodstream. The high elevation of lactate may also be due to decreased lactate clearance which occurs principally in the liver with important contributions from the kidney and to a lesser extent other organs (heart and skeletal muscle)[28]. This elevation therefore indicates possible liver and kidney diseases in rats fed on these diets. Mizock[29] reported that during liver disease, lactate clearance is diminished, thus also contributing to elevated blood levels.

V. Conclusion

From the foregoing, it is our conclusion that replacement of cornstarch up to 50% inclusion levels by autoclaved or boiled melon seed coat in the diet of rat would produce no adverse effect on the growth performance and may therefore be incorporated into feedstuff. However, investigation into the possible toxic effect of such feedstuff is highly recommended.

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