



Some Indices of Carbohydrate Metabolism in Rats Maintained on Heat-treated *Vigna unguiculata* Husk

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASI designed the experiment, performed the statistical analysis and interpreted the results. Author NL was in charge of the literature search and writing of the first draft. Author AB administered the diets and typed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The high cost of feed ingredients such as corn is one of the most important factors in livestock production. If agricultural by-products such as *Vigna unguiculata* husk could replace corn starch in animal feed, the cost of raising livestock would be cheaper. Some indices of carbohydrate metabolism in rats maintained on heat-treated *Vigna unguiculata* husk were investigated to ascertain the extent of carbohydrate metabolism in rats maintained on the husk. Forty-six weanling albino rats (31.11 g \pm 3.32 g) were divided into seven groups of equal average weight. The groups were then assigned to seven experimental diets. The rats were fed on their respective experimental diets and water *ad libitum*. Blood pyruvate and lactate concentrations, glucose 6-phosphatase activities in selected tissues, and serum lipid profiles were determined. The results revealed that the concentrations of pyruvate (0.86 mg per 100 ml blood) and lactate (12.96 mg per 100 ml blood) in rats fed 50% inclusion level of 90-minute boiled husk were not significantly different ($p = .05$) from the respective values (0.85 mg per 100 ml blood and 12.53 mg per 100 ml blood) in rats fed the control diet. Also, 50% inclusion level of 90-minute boiled husk caused no significant changes in

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the lipid profiles. With the exception of rats maintained on the control diet, the liver (37.46 nmol/hr/mg Protein) and kidney (19.02 nmol/hr/mg Protein) concentrations of glucose 6-phosphatase activities in rats fed 50% inclusion level of 90-minute boiled husk were significantly lower ($p = 0.05$) than the concentrations in the liver and kidney of rats maintained on other diets. The findings show that diets based on 50% inclusion level of 90-minute boiled husk are well metabolized. Therefore, 50% replacement of corn starch by 90-minute boiled *Vigna unguiculata* pod husk may be considered as a way of reducing the cost of livestock production.

Keywords: Heat-treated; *Vigna unguiculata*; pod husk; carbohydrate; pyruvate; lactate.

1. INTRODUCTION

The need for increased production of live stock had long been established. This in part is due to the rapidly growing demand for animal products. Animal products such as meat, milk and eggs provide much required nutrition, but developing country consumption levels are still low due to the high cost of these products. Apart from the importance of animal production to national economies in contributing to national income, improved human nutrition and foreign exchange, livestock play an important role in contributing to rural livelihoods, employment and poverty relief [1]. Since high cost of feed ingredients such as corn is one of the most important factors in livestock production, looking closely at locally available feed resources becomes necessary. If agricultural by-products such as *Vigna unguiculata* pod husk could replace corn starch in animal feed, the cost of raising livestock would be much cheaper. Most *Vigna unguiculata* are grown on the African continent, particularly in Nigeria and Niger which account for 72% of world *Vigna unguiculata* production [2]. Commonly called cowpea beans, *Vigna unguiculata* is an annual herbaceous legume cultivated for its edible seeds or for fodder. It is one of several species of the widely cultivated genus *Vigna* [3]. *Vigna unguiculata* seeds are contained in cylindrical pods, 6 to 2 cm long and 3-12 mm broad and each pod may contain 8 to 20 seeds. The seeds are usually separated from the pods by threshing. The pod husks are used as they are in many places to feed livestock [4]. However, information on the metabolizability of carbohydrate in rats maintained on *Vigna unguiculata* seed pod husk remained scanty. This research was therefore designed to ascertain the extent of carbohydrate metabolism in rats maintained on heat-treated *Vigna unguiculata* pod husk.

2. MATERIALS AND METHODS

Fresh and mature *Vigna unguiculata* pods were bought from a farm in Katagum Local

Government of Bauchi State of Nigeria. Corn, soybeans, soybean oil and sucrose were bought from Ilorin central market, while rice bran was collected from a rice milling factory in Ilorin.

Forty-six (46) weanling albino rats (31.11 g \pm 3.32 g) were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

All the assay kits used in this study were from Randox Laboratories Ltd. (United Kingdom).

2.1 Sample Preparation

The pods were sorted out to remove bad ones. The pod husks were manually removed from the pods, washed with water, sun-dried and then divided into 3 portions: 1, 2 and 3. Portion 1 was left unheated, while portions 2 and 3 were subjected to 60-minute and 90-minute boiling respectively. The three portions were separately pulverised and stored in clean polyethylene bags.

2.2 Feed Formulation

The pulverised unheated, 60-minute boiled and 90-minute boiled *Vigna unguiculata* husk were separately mixed with other ingredients to formulate seven experimental diets to replace dietary corn starch at 0%, 50% and 70%. Diet C (Control) contained 100% inclusion level of corn starch. Diets R, B60 and B90 contained 70% inclusion levels of unheated, 60-minute boiled and 90-minute boiled *Vigna unguiculata* husk respectively, while diets CR, CB60 and CB90 contained 50% inclusion levels of unheated, 60-minute boiled and 90-minute boiled *Vigna unguiculata* husk. The food items were thoroughly mixed together and manually made into pellets to feed albino rats.

2.3 Assigning of Experimental Animals

The forty-six weanling albino rats were assigned into seven groups. The six rats in each group were housed in standard plastic laboratory cages

Table 1. Components of the formulated diets (gkg⁻¹)

	C	R	B60	B90	CR	CB60	CB90
B60	-	-	361.2	-	-	-	-
B90	-	-	-	361.2	-	-	-
R	-	361.2	-	-	-	-	-
C	516	154.8	154.8	154.8	258	258	258
CA	-	-	-	-	258	-	-
CB	-	-	-	-	-	258	-
CR	-	-	-	-	-	-	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

with stainless steel covers. The groups were then maintained on their respective experimental diets and water *ad libitum* for six weeks. The rats were kept in accordance with the recommendation of ARRPP [5].

2.4 Animal Sacrifice, Collection of Blood Samples and Preparation of Tissue Homogenate

After 42 days of feeding trial, the animals were weighed and sacrificed by anaesthetizing them in a jar containing cotton wool soaked in diethylether. Blood was obtained through the jugular veins in tubes containing EDTA as anticoagulant and in plain tubes. Serum was separated from the blood in plain tubes by allowing the blood to clot for 3 hours. The clotted blood was spun in a bench top centrifuge at 1500 rpm for 15 minutes to obtain serum. The serum samples were thereafter separated into another set of plain sample tubes and kept in the refrigerator [6]. The sacrificed rats were dissected to remove the liver and kidney. The isolated tissues were weighed and a portion cut out, cut into very small pieces and then homogenized in ice-cold 0.25 M sucrose solution to make a 1 in 6 dilution. The tissue homogenates were kept frozen while being used [7].

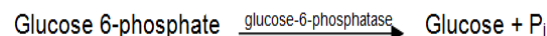
2.5 Determination of Pyruvate and Lactate Concentration in the Blood

The method of Gloster and Harris [8] was employed in the determination of pyruvate and lactate. The method is based on the reversible conversion of lactate to pyruvate catalyzed by lactic dehydrogenase and is driven to completion by excess of oxidized form of nicotinamide

adenine dinucleotide (NAD) or reduced form of nicotinamide adenine dinucleotide (NADH).

2.6 Determination of Glucose-6-Phosphatase Activity

The method described by Swanson [9] was used for the determination of glucose 6-phosphatase activity in the liver and kidney. This method is based on the incubation of the specific substrate with the enzyme source and the determination of liberated inorganic orthophosphate.



2.7 Determination of Serum Lipid Profile and Atherogenic Indices

The total cholesterol (TC) was estimated according to the methods of Abell et al. [10] while triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were estimated according to the methods of Tietz [11]. Low density lipoprotein cholesterol (LDL-C) was computed from the expression: LDL-C (mmol/l) = TC-TG/ (2.2-HDL-C) [12]. Both the Cardiac Risk Ratio (CRR) and Atherogenic Coefficient (AC) were determined using the method of Ikewuchi and Ikewuchi [13], while the atherogenic index was by the method of Tan et al. [14].

Cardiac Risk Ratio (CRR) = TC/HDL-C.

Atherogenic Coefficient (AC) = (TC- HDL-C)/HDL-C

Atherogenic Index (AI) = log (triglyceride/HDL-C).

2.8 Statistical Analysis

Results were expressed as means \pm SEM. Using statistical package program (SPSS 16.0 version),

one-way ANOVA was used to assess statistical significance where p value less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Concentrations of Lactate, Pyruvate and Lactate/Pyruvate Ratio in the Serum of Rats Fed *Vigna unguiculata* Husk Based Diets

The concentrations of pyruvate and lactate were not significantly affected ($p > 0.05$) by 50% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk (Table 2). On the other hand, 70% inclusion levels of unheated, 90-minute boiled and 60-minute boiled, and 50% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod husk caused significant increase ($p < 0.05$) in both the levels of pyruvate and lactate. The rats fed on 70% inclusion level of unheated *Vigna unguiculata* husk had significantly highest concentrations of pyruvate and lactate. However, lactate:pyruvate ratio did not significantly vary among the groups.

The observed increase in the blood pyruvate and lactate levels of rats maintained on 70% inclusion levels of unheated, 90-minute, and 60-minute boiled, and 50% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod husk could have been due to low supply of exogenous energy by these diets. This could have resulted from the high feed conversion ratio coupled with the low intake of the diets (Idoko and Ilouno, Federal University Dutsinma, Katsina State, Nigeria). Low supply of exogenous energy

necessitates the mobilization of triacylglycerols in adipose tissue and gluconeogenesis by the liver. The oxidation of triacylglycerols causes the concentrations of acetyl CoA and citrate to increase [15]. Acetyl CoA is an allosteric activator of pyruvate carboxylase and an allosteric inhibitor of pyruvate dehydrogenase, and thus pushes pyruvate to gluconeogenesis [16]. As the pyruvate accumulates, its metabolism shifts towards lactate formation, the excess of which is excreted into the bloodstream. The result also shows that 50% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk has the potential to supply adequate energy. Under such metabolic condition, pyruvates are channelled towards acetyl CoA formation and triacylglycerols are synthesized rather than broken down. This finding is consistent with the pattern of observed weight gains by the rats on these diets (Idoko and Ilouno, Federal University Dutsinma, Katsina State, Nigeria). Idoko et al. [17] reported similar increase in circulating levels of lactate and pyruvate in rats fed 100% inclusion levels of raw, boiled, autoclaved *Citrullus colocynthis* seed coat and 50% inclusion level of raw *Citrullus colocynthis* seed coat, which they attributed to altered carbohydrate metabolism.

3.2 Concentrations of Glucose 6-Phosphatase Activities in Selected Tissues of Rats Fed *Vigna unguiculata* Husk Based Diets

The concentrations of glucose 6-phosphatase activities in the liver and kidney of rats maintained on the control diet were significantly lower ($p < 0.05$) than the concentrations in the

Table 2. Concentrations of lactate, pyruvate and lactate/pyruvate ratio in the serum of rats fed *Vigna unguiculata* pod husk based diets

	Pyruvate (*)	Lactate (*)	Lac:Pyr
C	0.85±0.01 ^a	12.53±0.23 ^a	14.68±0.27 ^b
R	2.08±0.03 ^f	29.09±0.46 ^f	13.99±0.25 ^a
B60	1.91±0.01 ^e	25.18±0.02 ^e	13.16±0.08 ^{ab}
B90	1.43±0.03 ^c	21.29±0.15 ^c	14.93±0.21 ^b
CR	1.50±0.04 ^d	22.95±0.64 ^d	15.33±0.79 ^b
CB60	1.16±0.01 ^b	17.43±0.21 ^b	15.03±0.26 ^b
CB90	0.86±0.00 ^a	12.96±0.02 ^a	15.02±0.08 ^b

* (mg per 100 ml blood), Results are means of 3 determinations ± S. E. M. Values along the same row with the same superscript are NOT significantly different ($P = .05$), and are significantly different if the superscripts are different, C: (control): rats fed on diet based on 100% inclusion level of corn starch, R: rats fed on diet based on 70% inclusion level of unheated *Vigna unguiculata* pod husk, B60: rats fed on diet based on 70% inclusion level of 60-minute boiled *Vigna unguiculata* pod husk, B90: rats fed on diet based on 70% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk, CR: rats fed on diet based on 50% inclusion level of unheated *Vigna unguiculata* pod husk, CB60: rats fed on diet based on 50% inclusion level of 60-minute boiled *Vigna unguiculata* pod husk, CB90: rats fed on diet based on 50% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk

liver and kidney of rats fed other diets. With the exception of rats maintained on the control diet, the liver and kidney concentrations of glucose 6-phosphatase activities in rats fed 50% inclusion level of 90-minute boiled *Vigna unguiculata* husk were significantly lower ($p < 0.05$) than the concentrations in the liver and kidney of rats maintained on other diets (Table 3).

Table 3. Concentrations of glucose 6-phosphatase activities (nmol/hr/mg protein) in selected tissues of experimental rats fed *Vigna unguiculata* pod husk based diets

	Liver	Kidney
C	30.11±0.30 ^a	18.13±0.27 ^a
R	59.67±0.73 ^e	28.67±0.04 ^e
B60	51.16±0.51 ^d	23.12±0.04 ^d
B90	50.47±0.30 ^d	22.82±0.24 ^d
CR	50.04±0.02 ^d	22.18±0.02 ^c
CB60	40.78±0.46 ^c	21.99±0.03 ^c
CB90	37.46±0.36 ^b	19.02±0.00 ^b

Results are means of 3 determinations ± S. E. M. Values along the same row with the same superscript are NOT significantly different ($P = .05$), and are significantly different if the superscripts are different. C: (control): rats fed on diet based on 100% inclusion level of corn starch, R: rats fed on diet based on 70% inclusion level of unheated *Vigna unguiculata* pod husk, B60: rats fed on diet based on 70% inclusion level of 60-minute boiled *Vigna unguiculata* pod husk B90: rats fed on diet based on 70% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk, CR: rats fed on diet based on 50% inclusion level of unheated *Vigna unguiculata* pod husk, CB60: rats fed on diet based on 50% inclusion level of 60-minute boiled *Vigna unguiculata* pod husk, CB90: rats fed on diet based on 50% inclusion level of 90-minute boiled *Vigna unguiculata* pod husk

Glucose 6-phosphatase is an enzyme that hydrolyzes glucose 6-phosphate, resulting in the creation of a phosphate group and free glucose. Glucose is then exported from the cell via glucose transporter membrane proteins. This catalysis completes the final step in gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic regulation of blood glucose levels [18]. The increase in glucose 6-phosphatase activities could be due to increased need of gluconeogenesis. With the exception of rats on the control diet, the result also shows that rats fed 50% inclusion level of 90-minute boiled *Vigna unguiculata* husk had the least requirement for the endogenous supply of glucose, indicating that 50% inclusion level of 90-minute boiled *Vigna unguiculata* husk supplied more exogenous glucose. Consequently, rats fed the

diet had less dependence on the activity of glucose 6-phosphatase for glucose. This is consistent with the trend in pyruvate and lactate levels. The regulation of hepatic glucose 6-phosphatase by changes in hormones and nutritional status has been reported by a number of authors [19,20]. In particular, the expression has been shown to be regulated by dietary carbohydrates in mammals [21].

3.3 Serum Lipid Profile of Rats Fed Heat-treated *Vigna unguiculata* Husk Based Diets

The serum lipid profiles of rats fed the control diet and rats fed 50% inclusion levels of 90-minute boiled *Vigna unguiculata* pod husk did not differ significantly ($p > 0.05$) from each other. Similarly, the concentrations of total cholesterol and high density lipoprotein cholesterol did not vary significantly among all the experimental groups. However, triglyceride concentrations were significantly decreased ($p < 0.05$) in rats fed 70% inclusion levels of unheated, 90-minute and 60-minute boiled, and 50% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod husk (Table 4). Although, the calculated values of Cardiac Risk Ratio (CRR) and Atherogenic Coefficient (AC) did not significantly differ from one group to another, rats maintained on 70% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod husk had significantly ($p < 0.05$) lowest values of Atherogenic Index (AI) (Table 5).

The lower level of TG in rats fed 70% inclusion levels of unheated, 90-minute and 60-minute boiled, and 50% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod husk is understandable; low supply of exogenous energy usually involves oxidation of fatty acids obtained from TG hydrolysis in adipose tissue. Because rats fed 50% inclusion levels of 90-minute boiled *Vigna unguiculata* pod husk had adequate supply of dietary energy (carbohydrate), the TG level was unaffected when compared to the level in rats fed the control diet. The lower atherogenic index (AI) of rats fed 70% inclusion levels of unheated, 90-minute and 60-minute boiled, and 50% inclusion levels of unheated *Vigna unguiculata* pod husk is understandable since rats fed on these diets had significantly lower triglyceride. The atherogenic index is calculated as $\log(\text{triglyceride}/\text{HDL-C})$. Takasaki [22] and Dobiášová [23] reported atherogenic indices to be powerful indicators of the risk of atherosclerosis with increase in their values

Table 4. Serum lipid profile of experimental rats fed heat-treated *Vigna unguiculata* husk based diets

	TC	TG	HDL-C	LDL-C
C	1.90±0.09 ^a	0.72±0.02 ^a	0.84±0.02 ^a	0.87±0.07 ^a
R	2.07±0.07 ^a	0.43±0.01 ^c	0.83±0.01 ^a	1.20±0.06 ^{dbc}
B60	1.99±0.01 ^a	0.44±0.01 ^c	0.84±0.00 ^a	1.14±0.01 ^c
B90	2.00±0.05 ^a	0.54±0.01 ^b	0.84±0.01 ^a	1.07±0.07 ^{bc}
CR	2.06±0.03 ^a	0.56±0.03 ^b	0.84±0.02 ^a	1.10±0.04 ^c
CB60	1.99±0.02 ^a	0.60±0.01 ^b	0.81±0.01 ^a	1.00±0.01 ^{abc}
CB90	1.98±0.06 ^a	0.70±0.01 ^a	0.82±0.01 ^a	0.93±0.05 ^{ab}

indicating increased risk of developing cardiovascular diseases. The amount of triglycerides in blood is one important barometer of metabolic health; high levels are associated with coronary heart disease [24]. According to Woodward et al. [25], elevated triglycerides levels are strongly associated with an increased mortality of coronary heart disease. It is evident from this finding that all the studied inclusion levels of *Vigna unguiculata* pod husk may not be implicated in the development of atherosclerosis and cardiovascular disease. The lower triglyceride and consequent lower AI is consistent with the low feed intake by rats fed on these diets since raised triglycerides is usually caused by regular overeating [26].

Table 5. Atherogenic indices of rats fed heat-treated *Vigna unguiculata* husk based diets

	CRR	AC	AI
C	2.27 ^a	1.27 ^a	-0.07 ^d
R	2.49 ^b	1.49 ^b	-0.28 ^a
B60	2.37 ^{ab}	1.37 ^{ab}	-0.28 ^a
B90	2.39 ^{ab}	1.39 ^{ab}	-0.19 ^b
CR	2.44 ^{ab}	1.44 ^{ab}	-0.18 ^{bc}
CB60	2.47 ^{ab}	1.47 ^{ab}	-0.13 ^{cd}
CB90	2.43 ^{ab}	1.43 ^{ab}	-0.07 ^d

Results are means of 3 determinations ± S. E. M. Values along the same row with the same superscript are NOT significantly different ($P = .05$), and are significantly different if the superscripts are different

4. CONCLUSION

Having investigated some indices of carbohydrate metabolism in rats maintained on *Vigna unguiculata* pod husk, it is our conclusion that 50% inclusion level of 90-minute boiled pod husk are well metabolized in rats. Therefore, 50% replacement of corn starch by 90-minute boiled *Vigna unguiculata* pod husk may be considered as a way of reducing the cost of livestock production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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