

Original research Articles**SOME INDICES OF CARBOHYDRATE****METABOLISM IN RATS MAINTAINED ON HEAT-****TREATED *VIGNA UNGUICULATA* HUSK****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 ($31.11\text{g} \pm 3.32\text{g}$) 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 ($0.86\text{ mg per } 100\text{ ml blood}$) and lactate ($12.96\text{ mg per } 100\text{ ml blood}$) in rats fed 50% inclusion level of 90-minute boiled husk were not significantly different ($p = .05$) from the respective values ($0.85\text{ mg per } 100\text{ ml blood}$ and $12.53\text{ mg per } 100\text{ ml blood}$) in rats fed the control diet. Also, 50% inclusion level of 90-minute boiled husk caused no significant changes in the lipid profiles. With the exception of rats maintained on the control diet, the liver ($37.46\text{ nmol/hr/mg Protein}$) and kidney ($19.02\text{ nmol/hr/mg Protein}$) 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*

29 *unguiculata* seed pod husk remained scanty. This research was therefore designed to
30 ascertain the extent of carbohydrate metabolism in rats maintained on heat-treated *Vigna*
31 *unguiculata* pod husk.

32

33 **2.0 MATERIALS AND METHODS**

34 Fresh and mature *Vigna unguiculata* pods were bought from a farm in Katagum Local
35 Government of Bauchi State of Nigeria. Corn, soybeans, soybean oil and sucrose were
36 bought from Ilorin central market, while rice bran was collected from a rice milling factory in
37 Ilorin.

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

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

41 **Sample Preparation**

42 The pods were sorted out to remove bad ones. The pod husks were manually removed from
43 the pods, washed with water, sun-dried and then divided into 3 portions: 1, 2 and 3. Portion
44 1 was left unheated, while portions 2 and 3 were subjected to 60-minute and 90-minute
45 boiling respectively. The three portions were separately pulverised and stored in clean
46 polyethylene bags.

47 **Feed Formulation**

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

56

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

57

58 **Assigning of experimental animals**

59 The forty-six weanling albino rats were assigned into seven groups. The six rats in each
60 group were housed in standard plastic laboratory cages with stainless steel covers. The
61 groups were then maintained on their respective experimental diets and water *ad libitum* for
62 six weeks. The rats were kept in accordance with the recommendation of ARRP [5].

63

64 **Animal sacrifice, collection of blood samples and preparation of tissue homogenate**

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

75

76 **Determination of pyruvate and lactate concentration in the blood**

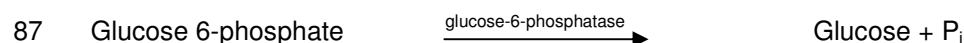
77 The method of Gloster and Harris [8] was employed in the determination of pyruvate and
78 lactate. The method is based on the reversible conversion of lactate to pyruvate catalyzed by
79 lactic dehydrogenase and is driven to completion by excess of oxidized form of nicotinamide
80 adenine dinucleotide (NAD) or reduced form of nicotinamide adenine dinucleotide (NADH).

81

82 **Determination of glucose-6-phosphatase activity**

83 The method described by Swanson [9] was used for the determination of glucose 6-
84 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.



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].

$$\text{Cardiac Risk Ratio (CRR)} = \text{TC}/\text{HDL-C}$$

$$\text{Atherogenic Coefficient (AC)} = (\text{TC} - \text{HDL-C})/\text{HDL-C}$$

$$\text{Atherogenic Index (AI)} = \log (\text{triglyceride}/\text{HDL-C}).$$

3. RESULTS & 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 ($p = .05$) 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 ($p = .05$) 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.

111 Table 2: Concentrations of lactate, pyruvate and lactate/pyruvate ratio in the serum of rats
112 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 [†]	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

113
114 * (mg per 100 ml blood)
115 Results are means of 3 determinations ± S. E. M .Values along the same row with the same
116 superscript are NOT significantly different (P = .05), and are significantly different if the
117 superscripts are different.

118 C: (control): rats fed on diet based on 100% inclusion level of corn starch
119 R: rats fed on diet based on 70% inclusion level of unheated *Vigna unguiculata* pod husk
120 B60: rats fed on diet based on 70% inclusion level of 60-minute boiled *Vigna unguiculata*
121 pod husk
122 B90: rats fed on diet based on 70% inclusion level of 90-minute boiled *Vigna unguiculata*
123 pod husk
124 CR: rats fed on diet based on 50% inclusion level of unheated *Vigna unguiculata* pod husk
125 CB60: rats fed on diet based on 50% inclusion level of 60-minute boiled *Vigna unguiculata*
126 pod husk
127 CB90: rats fed on diet based on 50% inclusion level of 90-minute boiled *Vigna unguiculata*
128 pod husk
129

130 The observed increase in the blood pyruvate and lactate levels of rats maintained on 70%
131 inclusion levels of unheated, 90-minute, and 60-minute boiled, and 50% inclusion levels of
132 unheated and 60-minute boiled *Vigna unguiculata* pod husk could have been due to low
133 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 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

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

165 C: (control): rats fed on diet based on 100% inclusion level of corn starch

166 R: rats fed on diet based on 70% inclusion level of unheated *Vigna unguiculata* pod husk

167 B60: rats fed on diet based on 70% inclusion level of 60-minute boiled *Vigna unguiculata*
 168 pod husk

169 B90: rats fed on diet based on 70% inclusion level of 90-minute boiled *Vigna unguiculata*
 170 pod husk

171 CR: rats fed on diet based on 50% inclusion level of unheated *Vigna unguiculata* pod husk

172 CB60: rats fed on diet based on 50% inclusion level of 60-minute boiled *Vigna unguiculata*
 173 pod husk

174 CB90: rats fed on diet based on 50% inclusion level of 90-minute boiled *Vigna unguiculata*
 175 pod husk

176

177 Glucose 6-phosphatase is an enzyme that hydrolyzes glucose 6-phosphate, resulting in the
 178 creation of a phosphate group and free glucose. Glucose is then exported from the cell via
 179 glucose transporter membrane proteins. This catalysis completes the final step in
 180 gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic

181 regulation of blood glucose levels [18].The increase in glucose 6-phosphatase activities
 182 could be due to increased need of gluconeogenesis. With the exception of rats on the
 183 control diet, the result also shows that rats fed 50% inclusion level of 90-minute boiled *Vigna*
 184 *unguiculata* husk had the least requirement for the endogenous supply of glucose, indicating
 185 that 50% inclusion level of 90-minute boiled *Vigna unguiculata* husk supplied more
 186 exogenous glucose. Consequently, rats fed the diet had less dependence on the activity of
 187 glucose 6- phosphatase for glucose. This is consistent with the trend in pyruvate and lactate
 188 levels. The regulation of hepatic glucose 6-phosphatase by changes in hormones and
 189 nutritional status has been reported by a number of authors [19, 20]. In particular, the
 190 expression has been shown to be regulated by dietary carbohydrates in mammals [21].

191 **3.3 Serum lipid profile of rats fed heat-treated *Vigna unguiculata* husk based** 192 **diets**

193 The serum lipid profiles of rats fed the control diet and rats fed 50% inclusion levels of 90-
 194 minute boiled *Vigna unguiculata* pod husk did not differ significantly ($p = 0.05$) from each
 195 other. Similarly, the concentrations of total cholesterol and high density lipoprotein
 196 cholesterol did not vary significantly among all the experimental groups. However,
 197 triglyceride concentrations were significantly decreased ($p = .05$) in rats fed 70% inclusion
 198 levels of unheated, 90-minute and 60-minute boiled, and 50% inclusion levels of unheated
 199 and 60-minute boiled *Vigna unguiculata* pod husk (Table 4). Although, the calculated values
 200 of Cardiac Risk Ratio (CRR) and Atherogenic Coefficient (AC) did not significantly differ from
 201 one group to another, rats maintained on 70% inclusion levels of unheated and 60-minute
 202 boiled *Vigna unguiculata* pod husk had significantly lowest values of Atherogenic Index (AI)
 203 (Table 5).

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

TC	TG	HDL-C	LDL-C
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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}

206

207 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

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

212

213 The lower level of TG in rats fed 70% inclusion levels of unheated, 90-minute and 60-minute
 214 boiled, and 50% inclusion levels of unheated and 60-minute boiled *Vigna unguiculata* pod
 215 husk is understandable; low supply of exogenous energy usually involves oxidation of fatty
 216 acids obtained from TG hydrolysis in adipose tissue. Because rats fed 50% inclusion levels

217 of 90-minute boiled *Vigna unguiculata* pod husk had adequate supply of dietary energy
 218 (carbohydrate), the TG level was unaffected when compared to the level in rats fed the
 219 control diet. The lower atherogenic index (AI) of rats fed 70% inclusion levels of unheated,
 220 90-minute and 60-minute boiled, and 50% inclusion levels of unheated *Vigna unguiculata*
 221 pod husk is understandable since rats fed on these diets had significantly lower triglyceride.
 222 The atherogenic index is calculated as $\log(\text{triglyceride}/\text{HDL-C})$. Takasaki [22] and Dobiášová
 223 [23] reported atherogenic indices to be powerful indicators of the risk of atherosclerosis with
 224 increase in their values indicating increased risk of developing cardiovascular diseases. The
 225 amount of triglycerides in blood is one important barometer of metabolic health; high levels
 226 are associated with coronary heart disease [24]. According to Woodward *et al* [25], elevated
 227 triglycerides levels are strongly associated with an increased mortality of coronary heart
 228 disease. It is evident from this finding that all the studied inclusion levels of *Vigna*
 229 *unguiculata* pod husk may not be implicated in the development of atherosclerosis and
 230 cardiovascular disease. The lower triglyceride and consequent lower AI is consistent with the
 231 low feed intake by rats fed on these diets since raised triglycerides is usually caused by
 232 regular overeating [26].

233

234 4. CONCLUSION

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

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