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THE EFFECT OF FEEDING DIFFERENTLY PREPARED BREADFRUIT (*ARTOCARPUS ALTILIS*) ON THE HEMATOLOGY, SERUM BIOCHEMISTRY, LIVE AND RELATIVE ORGAN WEIGHTS IN ALBINO RATS

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SUMMARY

A total of seventy five male albino rats divided into five groups of fifteen rats each were used to investigate the effect of feeding a 50 % feed supplementation with breadfruit (*Artocarpus altilis*), prepared differently as raw unpeeled sun dried (T_1), parboiled (T_2), unpeeled roasted (T_3) and unpeeled cooked (T_4) breadfruit meals on hematology, serum biochemistry, live and relative organ weights. These treatments were offered to four groups, while the fifth group receive a 100 % commercial basic diet (poultry growers mash, vital® feed) served as the control. The experiment lasted for six weeks. The result of the study showed a significantly ($p<0.05$) reduced total leucocyt count (TLC), erythrocyte count (EC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and live weight (LW) in T_1 and T_2. Total leucocyte was normal in T_3 and T_4, but significant increase ($p<0.05$) in Hb, PCV and MCV was observed in T_4. Treatment 4 had a significant increase ($p<0.05$) in LW. Also was significant ($p<0.0$) increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB) in T_1 and T_2, but not for T_3 in which they increased slightly and T4 which remained unchanged. Total plasma proteins were significantly ($p<0.05$) reduced in T_1 and T_2, but significantly increased ($p<0.05$) in T_4. Relative organ weights differed between treatments with significant increase of liver and spleen in T_1 and T_2 and of kidney in T_1 only. The heart was significantly reduced ($p<0.05$) in T_1, T_2 and T_3.There was no treatment effect on testicular weight. These findings show that the nutritional merits of the breadfruit (*Artocarpus altilis*) as a feed resource for livestock can be fully exploited only when it has been properly heat-treated otherwise it could produced some toxic effects which probably result from antinutritional factor(s) n its raw or poorly heat treated forms.

KEY WORDS: *Artocarpus altilis*, Hematology, Biochemistry, Weights, Rats

INTRODUCTION

Current researches in animal nutrition are focused on the use of alternative energy feed resources that could replace cereals and supply the required nutrients in adequate amounts. This quest had over time placed much demand on researchers, especially those involved in developing feed formulae for meeting the nutrient requirements of poultry and other livestock. In developing countries, especially those with increasing human population, there is high competition for food commonly grown cereals between humans and livestock, a situation that leads to shortage in their supply, and consequently, makes them expensive. It becomes imperative that, in other to sustain the feed industry in such countries, the dependence on commonly available cereals should be reduced. In other to achieve this, cereals that are not popularly consumed and hence not well grown in such

countries, should be exploited as feed sources for livestock.

Breadfruit (*Artocarpus altilis*) is one of the cereals that lack popular attention and use. Though not in large quantity due to neglect, it is readily available in Africa and in the tropical West Africa in particular. This cereal could largely serve as a good replacement for some of the commonly available cereals, if popularly used in livestock feed industry. Breadfruit (*Artocarpus altilis*) is a tropical tree crop that has been successfully introduced to most part of South- Western Nigeria, (Oladunjoye *et al.*, 2005). Though present in the South- Eastern Nigeria, less attention is paid to its cultivation, nurturing and expansion as an edible fruit. In most parts of Nigeria, its use for food is not very common; hence it is not popularly and routinely consumed. In most cases, it serves as food for humans at odd times and in times of food scarcity, especially when other cereals become scarce or very expensive (Oladunjoye *et al.*, 2004).

According to Morton (1987), breadfruit (*Artocarpus altilis)* is capable of producing 16-32 ton/ha of edible fruit, which makes it very considerable for use in the feed industry. Breadfruit has a short shelf live as it requires temperature as low as 12°C for its storage (Gohl, 1981). This means that it cannot be stored raw for too long, at normal ambient temperature of 27°C once it is harvested from the pulp. Therefore, in other to preserve it for longer period, it follows that some form of treatment (heat or chemical) are required. This therefore shows that treatment, for adequate preservation, may be one of the limiting factors in the utilization of breadfruit as a profitable feed source for livestock nutrition and production. In other to highlight the importance and effects of heating on the nutritional qualities or merits of breadfruit and possibly arrive at the best form for storage, this study was conducted to investigate the effect of dietary intake of a 50 % feed supplementation with different heat-treated breadfruit on the hematology, serum biochemistry, live weight and relative organ weights in albino rats.

MATERIALS AND METHODS

Animals (feeding and management) / experimental design

Seventy five male albino rats breed at the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and weighing an average of 186 ±4.11 g body weight (BW) were used for this study. They were kept in metallic cages for an adjustment period of 14 days. During which they were fed *ad libitum* with growers mash (vital® poultry feed). Clean tap water was provided daily in nipple drinkers. After the 14 days adjustment period, they were randomly separated into five (5) groups of fifteen rats each. The various groups and their experimental treatments were as follows:

T_1 (group A) = fed with unpeeled raw sun dried breadfruit meal.

T_2 (group B) = fed with unpeeled parboiled breadfruit meal.

T_3 (group C) = fed with unpeeled roasted breadfruit meal.

T_4 (group D) = fed with unpeeled cooked breadfruit meal.

T_5 (group E) = fed with 100 % growers mash (control).

N.B: In all cases a 50 % supplementation with treatments (different modes of heat treated breadfruit, T_1-T_4) was performed before feeding them to the rats.

Preparation of breadfruit

Unpeeled raw breadfruit (*Artocarpus altilis)* seed freshly removed from the pulp were treated as described for each experimental group. Four kilograms of the unpeeled raw breadfruit was divided into four parts of 1kg each and treated differently. Sun drying was by exposure to ultraviolet radiation for 21 days during which it was kept away from moisture. Parboiling was by soaking in water for 5 minutes before heating to boiling point which was maintained for 5 minutes. The set up was sieved and allowed to air

dry at room temperature and kept in a refrigerator. Roasting was by soaking in water for 5mins, sieved and air dried at room temperature, continuous stirring on fire until they were roasted to brown coloration. Roasting was for 15 minutes. Cooking was by soaking in water for 5 minutes and then heating to boiling point which was maintained for 1 hour. The set up was then sieved after 1 hour and allowed to air dry at room temperature and kept in a refrigerator.

Sample collection

Blood samples were colleted after a six weeks feeding (experimental period) from eight randomly selected rats per group. Through the ophthalmic venous plexus around the retro-orbital sinus using a non-heparinized micro-haematocrit tube. Blood was collected into separate Bijou bottles) one with ethylenediaminetetraacetic acid (EDTA) and the other without EDTA.

Body weight (live weight)

Each rat per group was weighed weekly (1-6) using a Methler balance (Devender Instrument, England) and the mean weight/week for each group was calculated.

Relative organ weight

At the end of the experiment, the eight rats per group from which blood was collected were eventually sacrificed by cervical decapitation. Hearts, spleen, liver, kidneys, lungs and testes of each rat was removed and weighed. The mean weight of each organ and its relative weight (in relation to mean carcass weight of each group) were calculated. Relative organ weight was calculated as percentages (Adejumo, 2004).

Hematological analysis

Red blood cell count (RBCC), white blood cell count (WBCC) and packed cell volume (PCV) were determined as described by Schalm *et al.*, (1975). Hemoglobin concentration Drakins reagent (ICSH, 1965) adopted as the International reference standard for hemoglobinometry by the International Council for Standardization in Hematology was used to assay for hemoglobin. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were determined as described by Jain (1986) and values expressed in femtolitres (fl) and picograms (pg), respectively. Mean corpuscular hemoglobin concentration (MCHC) was determined as described by (Melvin, 2004) and value expressed in percentage.

Serum biochemical analysis

Total serum protein was determined using the biurette methods of Coles (1986). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) kits (Quimica Clinica Applicada S.A 473671 Amposta, Spain) were used for liver enzyme activity analysis as described by Reitman and Frankel (1957. Total bilirubin was determined by the method of Kelly (1967). Total bilirubin was determined by the method of Kelly (1967).

Statistical analysis

Data collected were analyszed statiscally by one way analysis of variance (ANOVA). Differrences in means were separated statistically by the Duncans multiple range test of mean comparism. Level of significance was tested at probability of 0.05 (Steel and Torrie, 1980).

RESULTS

The results of this experiment are as presented in Tables I, II, III, IV and V. Table I shows the chemical composition of the different rations on percentage dry matter basis. The crude protein content was highest in T_1 and T_2 compared to other treatments Crude fiber, ether extract and ash contents of the breadfruit was reduced in the treatment groups compared to the control.

TABLE I: Chemical composition (% dry matter) of differently prepared bread fruit (*Artocarpus altilis)*

Constituents	*Treatments*				
	T1	T2	T3	T4	control
Crude protein	15.39	12.12	11.08	10.02	18.03
Crude fibre	5.36	5.18	5.15	4.87	9.97
Ether extract	3.39	2.38	3.17	3.57	5.87
Ash	13.56	12.01	12.65	9.72	14.53

Table II shows the mean hematological values of the experimental groups and the control after a six week feeding period with the different heat treated breadfruit There was a significant reduction ($p<0.05$) in all the hematological indices in T1. Treatment 2 had a significant reduction ($p<0.05$) in RBCC, WBCC, Hb and PCV. Treatment 3 and T4 had a significantly increased ($p<0.05$) MCV. Treatment 4 only had a significant increase ($p<0.05$) in Hb compared to the control group and other treatment groups.

TABLE II: Mean hematological of inbreed albino rats fed differently prepared unpeeled breadfruit (*Arocarpus altilis*)

Hematological	**Treatments**				
parameters	**T1**	**T2**	**T3**	**T4**	**Control**
Rbc (x $10^6\mu l$)	4.38 ± 0.27^a	4.47 ± 0.38^a	5.3 ± 1.19^b	7.36 ± 0.63^c	6.83 ± 0.63^c
Wbc (x $10^3\mu l$)	4.69 ± 1.17^a	4.86 ± 1.24^a	6.17 ± 1.83^b	6.84 ± 1.91^b	$7.22sss\pm0.71^b$
Hb (g/dl)	6.41 ± 1.11^a	8.36 ± 1.63^a	10.82 ± 1.07^b	16.27 ± 1.57^c	12.64 ± 2.64^b
Pcv (%)	20.19 ± 2.01^a	23.11 ± 1.34^a	28.33 ± 0.24^b	36.17 ± 1.23^c	34.00 ± 1.24^c
Mcv (fl)	46.09 ± 7.44^a	51.70 ± 3.54^b	53.45 ± 1.26^c	55.94 ± 2.12^c	49.78 ± 1.31^b
Mch (pg)	$14.6s3\pm4.11^a$	18.70 ± 4.29^b	20.42 ± 0.90^b	22.11 ± 2.46^b	19.94 ± 2.41^b
Mchc (%)	31.75 ± 0.60^a	36.17 ± 1.22^b	38.19 ± 4.46^b	39.52 ± 1.24^b	40.12 ± 2.12^b

a, b, c = means with different superscript along row are significantly different ($P<0.05$)

Table III shows the mean serum biochemical parameters. There was significant ($p<0.05$) increase in serum total proteins in T_4 and significant reduction ($p<0.05$) in T1 and T_2. Serum ALT,AST and total bilirubin significantly ($p<0.05$) increased in T_1 and T_2.

Table IV shows the mean weekly body weight of rats fed with different treatments. There was significant ($p<0.05$) decrease in weight after six weeks in T_1 and T_2. The decrease in T_3 was not significant ($p>0.05$) between week one and the end of the experiment (week 6). Conversely, T_4 showed a significant ($p<0.05$) increase in weight.

Table V shows the mean relative organ weights after the experiment. There was significant ($p<0.05$) increase in liver and spleen weights in T_1 and T_2. Kidney was significantly ($p<0.05$) increased only in T_1. The heart was significantly ($p<0.05$) reduced in T_1, T_2 and T_3.

TABLE III: Mean serum biochemical parameters of inbreed rats fed differently prepared breadfruit (*Artocarpus altilis*)

Biochemical parameters	Treatments				
	T1	T2	T3	T4	Control
Serum Total Protein (g/dl)	4.12±1.93[a]	4.16±2.71[a]	6.3±1.83[b]	10.23±1.46[c]	7.28±1.14[b]
ALT (μL)	126.4±1.21[a]	112.8±3.11[a]	93.5±2.19[b]	90.81±2.78[b]	88.43±2.64[b]
AST ((μL)	166.8±1.26[a]	161.3±2.66[a]	141.9±1.36[b]	136.1±1.47[c]	134.62±2.88[c]
Serum Total dilirubin (mg/dl)	1.6±0.01[a]	0.8±0.02[a]	0.4±0.02[b]	0.3±0.11[b]	0.3±0.01[a]

a,b,c = means with different superscript are significantly different at $p<0.05$

TABLE IV: Mean weekly body weight (g) of inbreed rats fed differently prepared unpeeled breadfruit (Artocarpus altilis)

Exp. Period (Wks)	Treaments				
	T1	T2	T3	T4	Control
0	188.64 ± 3.11[a]	191.61±2.61[a]	190.66±1.86[a]	189.64±2.46[a]	188.43±2.19[a]
1	183.73±3.61	185.[b]2±2.41	190.11±1.22	191.22±2.61	184.86±1.92
2	178.16±3.44	181.18±1.25	198.13±2.16	196.14±2.38	190.37±2.43
3	170.11±2.17[a]	176.28±1.19[a]	186.28±1.42[a]	211.96±1.63[b]	190.73±1.34[b]
4	160.37±2.61[a]	172.82±2.13[a]	191.14±1.83[b]	222.16±1.93[b]	196.21±3.61[b]
5	121.91±2.43[a]	166.13±2.42[b]	191.26±1.41[b]	226.83±2.[11ab]	189.67±1.56[b]
6	121.91±2.43[a]	143.19±2.14[a]	192.83±2.56[B]	229.96±1.14[ab]	198.76±2.46[b]
% Change in BW	-33.37	-29.97	+1.14	+19.49	+5.48

a, b, ab = means with different superscript along row are significantly different ($p<0.05$)
-,+ = - indicates % weight loss, + indicates % weight gain

TABLE V: Mean relative organ weight (% of carcass weight) of inbreed albino rats fed differently prepared breadfruit (*Artocarpus altilis*)

Organs	Treatments				
	T1	T2	T3	T4	Control
Heart	2..31±0.02^{a}	2.33±0.02^{a}	3.36±0.01^{a}	2.89±0.16^{a}	3.17±0.03^{a}
Liver	5.86±0.19^{a}	4.88±0.17^{a}	3.16±0.67^{b}	1.68±0.21^{ab}	2.09±0.06^{ab}
Spleen	2.93±0.46^{a}	2.06±0.06^{a}	1..94±0.02^{a}	1.02±0.001^{b}	0.83±0.00^{b}
Kidney	1.93±0.01^{a}	1.89±0.11^{a}	1.96±0.01^{a}	1.27±0.12^{b}	1.79±0.04^{a}
Lungs	3.03±0.02^{a}	2.66±0.01^{a}	2.02±0.02^{b}	1.92±0.01^{b}	1.06±0.002^{ab}
Testes	1.98±0.12^{a}	1.86±0.04^{a}	1.85±0.02^{a}	1.64±0.06^{a}	2.01±0.05^{a}

a, b, = means with different superscript along row are significantly different ($p<0.05$)

DISCUSSION

The results of this study showed that various degrees of heat treatment alter mainly the crude protein constituent of the breadfruit. As shown in Table I, raw unpeeled sun dried breadfruit had a crude protein value of 8.39 % before experimentation compared to parboiled, roasted and cooked forms which had values of 5.6 %, 5.08 and 4.02 %, respectively. It is interesting to note that as the degree of heating increased, the crude protein level decreased correspondingly. It could be said that prolonged heating denatured most of the protein fraction of the feed. The value of 8.39 % crude protein of raw sun dried breadfruit as found in this experiment varies from a value of 5.54 % reported by Oladunjoye *et al.* (2004), but agrees with the report of Ravindrin and Sivakanesan (1995). These differences probably resulted from length of exposure to heat or differences in method of processing. Oladunjoye *et al.* (2004), used raw breadfruit that was oven dried, while in this study sun drying was applied. Since heat treatment, as observed, reduces crude protein level, the intense heat obtainable in oven drying process could have accounted for the more reduced protein recorded by Oladunjoye *et al.* (2004).

It was observed that all the hematological parameters were reduced in rats that received treatments 1 and 2, except for MCV, MCH, and MCHC in T_2 which did not vary significantly from other treatments and control. There was no significant difference in the red blood cell count (RBCC) white blood cell count (WBCC), MCHC, MCH and MCV in T_3, T_4 and control.However, there was a significant increase in PCV and Hb in T_4. The decrease in all the hematological parameters observed in T_1 as well as the significant decrease in RBCC, WBCC, Hb and PCV in T_2 suggest that feeding raw or poorly heat treated unpeeled breadfruit likely interferes with the hemopoietic system, leading to reduced cellular elements of the blood, resulting in anaemia and leucopenia as observed in this study. These could be due to metabolic inhibition of the bone marrow or primary autoimmune hemolitic anaemia by some chemicals and/or antinutritional factors probably present in the raw forms of the breadfruit.

Certain chemical constituents of food, such as copper and lead are known to produce hemolytic anaemina (Coles, 1986). Excessive intake of copper containing foods lead to high liver concentration of copper and under stress condition are released into the blood stream, resulting in rapid hemolysis of erythrocytes

(Guyton and Hall, 2000). It could therefore, raw breadfruit (*Artocarpus altilis*) contain such elements in high concentration and therefore capable of eliciting hemolytic conditions. Since parboiling and other forms of heat treatment did not produce a significant change, from the control, in MCV, MCH and MCHC, it goes therefore, to suggest that the anemic condition produced by raw breadfruit (T_1) was more etiological than morphological. On the basis of etiology, this form of anaemia can be described as hemolytic. Hemolytic form of anaemia is known to be produced by excessive destruction or shortened erythrocyte life span (Shutt and Macdonald, 1965) Again, since leucopenia accompanied the anaemic condition in treatments T_1 and T_2; the probability that bone marrow activity was depressed becomes suggestive in this study. As such, the activity of a chemical agent in the feed ($T_1$and T_2) may be involved. In chemically induced anaemia, the primary alteration is a marked reduction of thrombocytes followed by a rapid reduction in the number of leukocytes and erythrocytes (Akinmutimi, 2001). This probably explains the mechanism responsible for the reduction in the cellular elements of blood (RBC and WBC) following treatments 1 and 2. In other to understand this better, there is the need to study the mineral and phytochemical profile of raw and heated unpeeled breadfruit (*Artocarpus altilis*).

Significantly decreased serum total protein with a concomitnat significantly increased ALT, AST occurred in T_1 and T_2 (Table III). The decrease in serum proteins probably resulted from hepatic dysfunction especially as it was accompanied by a corresponding increase in ALT, AST and total bilirubin. Increase in serum ALT is specific for hepatic disorder (*Obidike et al., 2005*), especially in dogs and cats (Coles, 1986) and severe hepatitis produces very high levels of bilirubin (Chessbruogh, 2002). Since bilirubin is essentially an excretory product, the assessment of serum activity of AST and ALT provided better clues to the hepatic condition following treatments. Increase in serun activity of ALT is directly related to the amount of damage that has occurred to the hepatocytes. On the other hand, increase in serum AST is associated with cell necrosis of many different tissues (William, 2004).

Pathologies involving either skeletal or cardiac muscles or hepatic cell may allow for the escape of large quantities of AST enzyme into the blood (Coles, 1986). Even though increase in AST cannot be considered specific for liver necrosis, hepatic necrosis was strongly suspected in this experiment. This is buttressed by the fact that there were increased AST and total bilirubin, decreased total protein and increased liver weight. Copper poisoning has been shown to cause increased serum AST level (Guyton and Hall, 2000).Therefore, since copper has been shown to produce the classical features seen in this study, it is probable that copper content of raw unpeeled breadfruit (*Artocarpus altilis*) may be high and could be responsible for the observation such as increase in liver enzymes and weight, made in this study.

Tables IV and V, indicate a significant ($p<0.05$) increase in body weight in T_4 and significant decrease in T_1 and T_2. There was no significant change in T_3 and the control. Olandunjoye *et al.* (2005) had earlier reported a significant increase in body weight in broilers that recieved a 55 % feed supplementation with raw unpeeled breadfruit (*Artocarpus altilis*) meal. In this experiment, it was found that unpeeled raw breadfruit reduced body weight as well as other hematological parameters at 50 % supplementation. The differences in these reports could be attributed to variations in specie tolerance of the probable chemical and/or anti-nutritional factors constituents of unpeeled raw breadfruit. Thus, poultry appears to tolerate raw unpeeled breadfruit better than mammalian specie (e.g rats). Increase in liver, spleen, and kidney weights which were significant in T_1 and T_2 (Table IV), probably was more of a pathological than physiological response, especially in response to hemolytic and hepatic disturbances as earlier suggested. The increase in heart weight in T_4 probably was due to increased protein deposition and

consequently an increase in cardiac muscle mass since there was no gross pathologies at post mortem.

CONCLUSION

In conclusion, the findings of this study suggest that breadfruit (*Artocarpus altilis)* meal could be used as a good feed resource at 50 % supplementation for livestock but should not be used in the raw state, especially in mammalian species as it could lead to hemolytic anaemia, spleenic and hepatic disorder and other pathologies which could be averted by adopting suitable method(s) of heat treatment such as roasting and cooking or even prolonged sun-drying. It is recommended that such heat treatment could provide better ways of exploiting the potentials of breadfruit (*Artocarpus altilis)* as a valuable feed resource for the livestock feed industry.

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