Variation in Body Weight, Organ Weight and Haematological Parameters of Rats Fed with Diets Based on Treated African kudzu (Pueraria phaseoloides, roxb. Benth) Seeds

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ABSTRACT: Food insecurity is a major problem of the developing nations. There is therefore a need to assess the nutritional potentials of some lesser-known foods particularly grains with the view of adding them to the food base. In this paper, processed kudzu seeds (cooked and autoclaved for different periods(20,40,60 minutes)) and raw kudzu seeds were made into flour and compounded into diets. The diets along with the negative and positive control diets were fed to eight groups of wistar rats for 28 days. The rats fed raw kudzu and negative control diet showed negative mean weight changes (-6.50g and -10.0g) while those fed with positive control and treated kudzu diet showed positive mean weight changes with Diets 2(cooked kudzu) and Diet 8 (positive control) having the highest values(55.10g and 90.0g). These changes were significantly different (P<0.05). The absolute and relative organ (liver, heart, kidney, pancreas, spleen and lungs) weight changes of the experimental animals did not follow any particular trend. Haematological parameters measured in the animals included, haemoglobin packed cell volume, white blood cells, neutrophils, eosinophil, monocytes, lymphocytes and basophils. Except for the values of haemoglobin and packed cell volume that were higher in positive reference diet (12.56g/100ml and 37.2%) which were not significantly different (P<0.05) from the cooked diet (12.0g/100ml and 36.33%) but significantly different from others, the remaining haematological parameters did not follow any particular trend in all the groups. The result showed that the treated flour supported growth but had no definite effect on other parameters measured. The flour can therefore support growth in higher animals. ©JASEM

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Key Words: Kudzu, cooked, autolaved, weight, organ, haematological.

Malnutrition is a major problem of the developing countries caused by several factors but mostly food insecurity. Food insecurity *per se* is a result of lack of access to food by individuals and households. Several strategies have been developed and used to reduce the menace. The strategies included investigation into the possibilities of using some wild and lesser known crops as food for man and animal. Most investigated food crops were usually legumes. Legumes are consumed universally on account of their high nutritive value. They are important sources of complex carbohydrates, unsaturated fats, minerals and vitamins. (Rajeev Bhat and Karim (2009), Yusuf et al., (2007). They also contain some non-nutrient phytochemicals which are gaining recognition due to claims of their therapeutic effects on some diseases. Recently, legumes are widely consumed because of their therapeutic value in the treatment and prevention of various non-communicable diseases like coronary diseases, some types of cancers, diabetes etc. (Rebelo et al., (2014) and Stanner et al., (2004)). However, legumes contain several antinutritional factors in the raw seeds that need to be reduced by processing so as to enhance the nutritional quality. Some processing methods including dry heat and wet heat treatment, soaking, dehulling, fermentation, spraying etc. have been used in the past. (Siddhuraju et al., (2002), Siddhuraju and Becker (2003)). These methods have been shown to reduce some of these antinutrients and to improve their overall nutritional quality. (Akande, et al., (2010), Maria et al., (2015)). Kudzu is a wild legume plant used in soil conservation, the leaf is used as forage for animals especially rabbits in some African countries while the seeds are used as feed for birds in some Asian countries ((Kaufman (1986)). In an earlier study Ife anacho et al., (2007), observed that kudzu contained substantial amount of protein, some essential some essential minerals as well as some antinutrients. However, these antinutrients were drastically reduced by some applied heat treatments (Ifeanacho et al., (2008). This paper presents the variations in body weight, organ weight as well as some haematological parameters in rats fed raw and heat-treated kudzu diets.

MATERIALS AND METHODS

Sample Collection: Kudzu seeds were obtained from International Institute for Tropical Agriculture, (IITA) Onne, Rivers State while maize (Zea mays) grains and red palm oil were purchased from the Mile 3 Market, Port Harcourt, Rivers State. Granulated pure cane sugar (sucrose) and nutrend were purchased from Sunrise Supermarket, University of Port Harcourt. Vitamins and mineral mixtures were purchased at Raf - Veterinary Store, Rumuigbo Port Harcourt, Rivers State.

Sample Preparation/Treatment: One kilogram (1kg) of the seeds was washed with clean water and boiled...
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at 100°C in a closed saucepan over a kerosene stove until it became tender in 53 minutes. Caution was exercised during the cooking such that the cooking water dried up within the cooking period and so no water was drained off at the end of the cooking. The cooked seeds were dried at 100°C, milled into fine powder, put in polythene bag and stored in the refrigerator until it was used for analysis. On the other hand, one kilogram (1kg) of raw (unprocessed) seeds of the kudzu was also milled and stored in the refrigerator until it was used for analysis. The animals were dissected; their liver, heart, kidney, spleen and pancreas were exercised, weighed and inspected for any pathological abnormalities. Haematological indices were determined using Medonic M16 Haematological Analyzer (Nelson Biochemical, Limited., UK).

**Statistical Analysis:** The results were analyzed statistically by the use of one way analysis of variance (ANOVA) to determine the differences between the mean values at P < 0.05 level (Norusis, 1986)

**RESULTS AND DISCUSSION**

| Table 1: Initial and Final Body Weight and Body Weight Change of Rats at the End of the Experimental Period (G) |
|---|---|---|
| Diet | Initial Body Weight | Final Body Weight | Weight change |
| Diet 1 | 75.25±1.20 | 68.75±2.20 | -6.50±2.20 |
| Diet 2 | 75.10±1.10 | 130.20±0.90 | 55.10±1.90 |
| Diet 3 | 72.26±2.20 | 93.50±2.20 | 21.25±2.20 |
| Diet 4 | 75.30±2.00 | 125.40±0.80 | 50.10±1.80 |
| Diet 5 | 75.20±1.40 | 123.30±0.90 | 50.00±1.00 |
| Diet 6 | 73.40±1.00 | 93.40±1.00 | -10.00±0.90 |
| Diet 7 | 74.25±2.70 | 122.35±0.80 | 48.10±1.80 |
| Diet 8 | 73.40±1.10 | 163.40±0.80 | 90.00±1.00 |

Means with different superscript letters in the same row are significantly different at 5% level (p < 0.05). The initial and final body weight changes of rats at the end of the experimental period are presented in Table 1. Rats fed Diet 1 (raw kudzu) and Diet 6 (Basal diet) had negative weight change (-6.50±12.34g and -10.0±12.25g) respectively while rats fed the remaining Diets 2, 3, 4, 5, 7and 8 recorded increases in weight. Diet 8 (reference) had the highest weight increase (90.0±13.69g) followed by Diet 2 (cooked kudzu) (51.1±8.67g), then Diet 4 (40 minutes autoclaved) (50.0±0.01g), Diet 5 (60 minutes autoclaved) (50.0±25.0g) and Diet 7 (100% cooked kudzu) (48.1±17.8g) while Diet 3 (20 minutes autoclaved) had the least weight increase (21.25±4.29g). The increase in weight by rats fed Diet 8 was significantly different (p<0.05) from the other diets while Diets 2, 4, 5 and 7 were not statistically different from each other.
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Table 2: Absolute organ weights of rats at the end of feeding study (28 days)

| Diet group | Liver (g) | Heart (g) | Kidney (g) | Pancreas (g) | Spleen (g) | Lungs (g) |
|------------|-----------|-----------|------------|--------------|------------|-----------|
| 1          | 1.91 ± 0.37 | 0.30 ± 0.09 | 0.63 ± 0.13 | 0.15 ± 0.06 | 0.19 ± 0.10 | 0.40 ± 0.12 |
| 2          | 4.11 ± 0.70 | 0.39 ± 0.11 | 0.87 ± 0.14 | 0.45 ± 0.25 | 0.32 ± 0.04 | 0.78 ± 0.06 |
| 3          | ±0.7       | ±0.02     | ±0.05      | ±0.07       | ±0.01      | ±0.06     |
| 4          | 2.50 ± 0.11 | 0.23 ± 0.04 | 0.57 ± 0.07 | 0.45 ± 0.25 | 0.25 ± 0.04 | 0.52 ± 0.02 |
| 5          | ±0.92      | ±0.07     | ±0.17      | ±0.09       | ±0.06      | ±0.02     |
| 6          | 5.07 ± 0.12 | 0.51 ± 0.04 | 0.81 ± 0.07 | 0.58 ± 0.45 | 0.92 ± 0.21 | 0.39 ± 0.19 |
| 7          | ±1.12      | ±0.01     | ±0.07      | ±0.42       | ±0.13      | ±0.19     |
| 8          | 4.02 ± 0.46 | 0.36 ± 0.04 | 0.74 ± 0.14 | 0.61 ± 0.42 | 0.23 ± 0.04 | 0.64 ± 0.18 |
| 9          | ±0.62      | ±0.11     | ±0.15      | ±0.22       | ±0.18      | ±0.10     |

Values are means ± standard deviations for 5 rats per diet group (n=5). Values in the same column with similar superscript letters are not significantly different at 5% level (p<0.05).

The values for absolute organ weights of the experimental animals are shown in Table 2. The absolute organ weights of the rats fed the various experimental diets showed that the liver weight of rats fed Diet 4 was highest (5.07 ± 1.12g) and statistically different (P < 0.05) from liver weights of rats fed Diets 6 (2.02 ± 0.87g), Diet 3 (2.50 ± 0.92g) and Diet 1 (1.91 ± 0.37g). Absolute heart weight of rats on Diet 8 (0.52 ± 0.11g) was highest but not statistically different from all other groups. This trend was observed in the kidney weights among the diet groups. Rats fed Diet 8 also had the highest absolute pancreas weight (0.81 ± 0.22g), spleen (0.61 ± 0.18g) and lungs (1.23 ± 0.10g), which were significantly higher (P < 0.05) than values in other diet groups.

Table 3: Relative organ weights of rats at the end of feeding study (28days)

| Diet group | Liver | Heart | Kidney | Pancreas | Spleen | Lungs |
|------------|-------|-------|--------|----------|--------|-------|
| 1          | 2.78 a | 0.44 a | 0.52 a | 0.22 a | 0.28 a | 0.58 bc |
| ± 0.04     | ± 0.01 | ± 0.41 | ± 0.3  | ± 0.05  | ± 0.17 |       |
| 2          | 3.16 b | 0.28 b | 0.67 b | 0.34 b | 0.24 b | 0.60 b |
| ±0.06      | ±0.21  | ±0.25  | ±0.08  | ±0.03  | ±0.09  |       |
| 3          | 4.01 c | 0.25 c | 0.60 c | 0.57 c | 0.27 c | 0.55 c |
| ±0.14      | ±0.07  | ±0.18  | ±0.01  | ±0.01  | ±0.05  |       |
| 4          | 3.26 b | 0.41 b | 0.65 b | 0.46 b | 0.36 b | 0.73 b |
| ±0.07      | ±0.11  | ±0.11  | ±0.04  | ±0.05  | ±0.09  |       |
| 5          | 4.02 c | 0.29 c | 0.60 c | 0.49 c | 0.34 c | 0.61 b |
| ±0.46      | ±0.04  | ±0.51  | ±0.08  | ±0.02  | ±0.06  |       |
| 6          | 3.18 b | 0.39 b | 0.69 b | 0.36 b | 0.21 b | 0.62 b |
| ±0.10      | ±0.02  | ±0.35  | ±0.13  | ±0.02  | ±0.01  |       |
| 7          | 3.43 b | 0.30 b | 0.56 b | 0.41 b | 0.31 b | 0.64 b |
| ±1.01      | ±0.02  | ±0.35  | ±0.20  | ±0.03  | ±0.18  |       |
| 8          | 3.05 b | 0.32 bc | 0.59 ab | 0.51 bc | 0.37 b | 0.75 b |
| ±0.13      | ±0.01  | ±0.14  | ±0.21  | ±0.03  | ±0.15  |       |

Values are means ± SD for 5 rats per diet group (n=5). Means in the same column bearing similar superscript letters were not significantly different at 5% level (p<0.05).

For the relative organ weights (Table 3), rats fed Diet 3 and Diet 5 had the highest liver weight (4.01 ± 0.14g) and (4.02 ± 0.46g) respectively which are significantly higher than other groups at (P < 0.05). The highest relative heart weight was observed in rats fed Diet 1 (0.44 ± 0.01g) which was significantly different (P < 0.05) from all other groups except Diets 4 (0.41 ± 0.11g) and Diet 6(0.39 ± 0.02g). Diet 6 produced rats with the highest relative kidney weight (0.69 ± 0.35g) which was only significantly higher than the values obtained in Diet 1 (0.52 ± 0.41). Rats on Diet 1 had the lowest relative pancreas weight (0.22 ± 0.3g) was statistically lower (p < 0.05) than values produced by all other experimental animals. Relative spleen weight was highest in rats fed Diet 8(0.37a ± 0.03g) but statistically higher than values obtained in Diets 1,2,3 and 6.

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Diet 8 produced rats with the highest lung weight (0.75 ± 0.15) which was statistically higher from values obtained in other diets except diet 4 (0.73 ± 0.09g).

Table 4: Haematological parameters of rats fed the experimental diet for 28 days

| Diet | Hb (g/100ml) | PCV (%) | WBC 10³/mm³ | N (%) | L (%) | E (%) | B (%) | M (%) |
|------|--------------|---------|-------------|--------|-------|-------|-------|-------|
| 1    | 10.75abc     | 35.5bcd | 9500f       | 55.75g | 43.25g | 0.75g | 0.00g | 0.0g  |
|      | ±0.60        | ±2.08   | ±605.5      | ±3.30  | ±2.5  | ±0.06 | ±0.00 | ±0.00 |
| 2    | 12.0bc       | 36.33bc | 9033.33bc   | 54.67g | 45.04f | 0.00g | 0.00g | 0.00g |
|      | ±0.6         | ±1.53   | ±550.76     | ±3.79  | ±3.46 | ±0.00 | ±0.00 | ±0.00 |
| 3    | 11.36b       | 33.5c   | 7850.0b     | 53.25g | 41.75g | 0.05f | 0.25g | 0.25  |
|      | ±0.36        | ±1.73   | ±369.69     | ±2.22  | ±1.71 | ±0.01 | ±0.05 | ±0.01 |
| 4    | 11.27b       | 34.0cd  | 8633.35bc   | 53.07g | 47.03g | 0.00g | 0.00g | 0.00g |
|      | ±0.64        | ±1.73   | ±1021.44    | ±4.58  | ±4.58 | ±0.00 | ±0.00 | ±0.00 |
| 5    | 11.47bc      | 32.67ab | 8733.35bc   | 57.00g | 42.08g | 0.67b | 0.23c | 0.00g |
|      | ±0.92        | ±3.06   | ±351.19     | ±6.08  | ±5.29 | ±0.08 | ±0.08 | ±0.00 |
| 6    | 9.85ac       | 29.79a  | 8300.0c     | 55.57g | 44.09g | 0.25c | 0.25g | 0.00g |
|      | ±0.76        | ±2.5    | ±1334.0     | ±2.65  | ±3.37 | ±0.5  | ±0.05 | ±0.00 |
| 7    | 11.60bc      | 35.75cd | 9225.0bc    | 56.54c | 41.25g | 0.00c | 0.00g | 0.00g |
|      | ±0.71        | ±2.36   | ±613.05     | ±3.70  | ±4.03 | ±0.00 | ±0.00 | ±0.00 |
| 8    | 12.56a       | 37.2d   | 9640c       | 58.4b  | 45.75g | 0.20b | 0.20g | 0.00g |
|      | ±0.71        | ±1.30   | ±350.71     | ±2.07  | ±2.22 | ±0.05 | ±0.05 | ±0.00 |

Values are means ± standard deviations for 5 rats per diet group (n = 5) Values in the same column with similar superscript letters are not significantly different at 5% level (p< 0.05)

LEGEND: HB - Haemoglobin, PCV – packed cell volume, WBC- white blood cells, N - Neutrophils, E- Eosinophils, M- monocytes, L - Lymphocytes, B - Basophils.

Table 4 shows the haematological parameters of the experimental animals. It was observed that the haemoglobin values for rats fed the Reference Diet (8) was highest (12.56 ± 01g/100ml) and was not significantly different from Diet 2(12.0± 0.6 g/100ml) and Diet 7 (11.60 ± 0.71 g/100ml) but significantly higher (P < 0.05) than the other groups. The same trend was observed in values for packed cell volume. The white blood cells was highest in Diet 8 (9640 ± 350.71 10⁶ min⁻¹). Neutrophils (N) were highest in diet 8(58.4±2.07%) which was significantly different from other diet groups except diet 5 which had 57.0±6.08%. Lymphocytes (L) were highest in diet 4(47.0±4.58%) which was significantly different from other diet groups. Eosinophils were highest in diet 1 (0.75±0.96) which was significantly higher than other diet groups except diet 5(0.67±0.58).Diets 5 and 6 had similar values for basophils (B) which are significantly different from the other groups. Monocytes were not detected in the blood sample of the rats fed the various diets except in diet 3(0.25±0.5) however this value did not differ significantly from the other groups.

The decreased weight observed among the rats fed Diet 6 may be due to the low protein level and this is supported by (Cousin et al., (1981)) who reported weight loss in rats fed low protein diets. For the raw diet, the negative weight change may be attributed to the presence of the ant nutritional factors prominent among which tannin and trypsin inhibitors known to interfer with protein metabolism. A similar observation was made by various authors (Ogun et al.,(1989) and Mole et al (1990)) in which the presence of trypsin inhibitor activity and tannin in uncooked feed caused diminished growth in rats, chicken and other experimental animals, however (Shaahu et al., (2014)) in their own study revealed that the final weights of the rabbits fed toasted lablab seed diets from 0-5 weeks were similar to those fed control diet, and also similar to those fed the raw seed diets. Other experimental diet showed weight increases in the rats, with the reference diet(Diet 8)having the highest value which is significantly different from the other experimental diets. This confirmed that the reference diet was superior to the other diets. However, the cooked diet supported growth more than other treatment groups. This suggested that the heat treatments reduced the levels of the inherent antinutrients to the extent that the residual quantities did not interfere with protein metabolism. This agrees with the reports of (Kaankuka et al (2000), Pathak and Ranjahan (1997) that dry heating (toasting) of legume seeds are less...
effective in the removal of ant nutritional substances and thus, performance of animals than wet heating.

The variations in the values of the absolute organ weights showed no specific trend but for the liver weights. Diets, 1, 3 and 6 had liver weights statistically different from other treatment groups. This may be attributed to the fact that the liver as the detoxifying organ is usually affected by toxins (antinutrients) and also protein deficiency. This observation disagrees with some studies including (Onyeike and Nkwuzor (2006)) which reported improvement in the organ weight of rats fed heat processed sample of cashew nut flour which they attributed to destruction of toxicants in the flour by heat.

The values for haemoglobin(Hb) and packed cell volume(PCV) for Diets 2 and 8 were higher than other groups. They were not significantly different suggesting that cooking though better than other heat treatments in destroying the antinutrient but the level of antinutrients inherent in the raw seeds and the residual in the treated seeds had little effect on the parameters. The main function of the white blood cells (WBC) is body defence and since processing did not affect the WBC levels in a particular way, it therefore means that there was neither under production or overproduction of the cells as are the cases in either protein energy malnutrition or infection and toxicity. The differences in the values for the differential counts also showed no specific trend. Altered blood parameters in animals exposed to toxicants have been reported (Amaefule and Nwagbara, (2004)). The results of this study suggest that the levels of residual antinutrients in the diets may not be high enough to elicit obvious haematological responses since most of the haematological parameters did not differ significantly in the animals on the experimental diets compared with animals on the reference diet.

Conclusion: Heat-treated kudzu formulations supported growth in the experimental animals, it can support same in higher animals including man. However, there were no specific trend in organ weight and biochemical changes, there is therefore a need to carry out further biochemical investigations and pathological examinations on the organs of these animals to rule out cellular damages. It is when this is done, that dry or wet heat treated kudzu could be recommended for either animal feed or human food.

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