International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 7 (2): 86-92, 2012
ISSN 1816-4897 / DOI: 10.3923/ijar.2012.86.92

Haematology, Serum Biochemistry and Organ Histopathology of Rabbits Fed Graded Levels of Whole Kenaf (*Hibiscus canabinus*) Seed Meal

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ABSTRACT

An experiment was conducted with 36 crossbred rabbits (49 days old) averaging 650 g to assess the haematology, serum biochemistry, organ weights and organ histopathology of growing rabbits fed graded levels of Whole Kenaf Seed Meal (WKSM) of 0, 20, 40, 60, 80 and 100% as a replacement of Soyabean Meal (SBM) in 12 week feeding trial. Blood samples were collected from the animals through the ear vein for haematology and serum biochemistry while samples of visceral organs were collected from the animals after they were stunned and sacrificed after the 12 week feeding trials. Results showed that there were significant differences ($p<0.05$) in the value obtained for packed cell volume, haemoglobin concentration and red blood cell counts while there was no significant difference among the blood constants (MCV, MCH and MCHC). Among the leukocyte differential counts examined, lymphocytes, monocytes and eosinophils were not significantly different among the dietary treatments, however neutrophil was significantly ($p<0.05$) highest in animals fed 80% WKSM. The serum proteins examined were not significantly ($p>0.05$) affected by the dietary treatments except the albumin. Serum enzymes (AST and ALT) of the experimental rabbits were significantly ($p<0.05$) higher in rabbits fed 60, 80 and 100% WKSM than others. Relative organ weight of various visceral organs examined except kidney, liver and heart were significantly influenced by the dietary treatments. Pancreas weight in the control is significantly lower than in the other dietary treatments. The result of histopathological examination of selected organs (liver, kidney and spleen) revealed damage done to the organs by the dietary treatments. Severe hepatic necrosis of the liver was observed in all the treatments except the control. The results suggest that although WKSM possess good dietary protein quality for optimal growth of rabbits the inclusion levels above 20% in the diet of growing rabbits significantly altered haematological parameters and induce pancreatic hypertrophy and beyond 40% it induced damages and dysfunction of visceral organs.

Key words: Kenaf seed, rabbits, blood, organ histopathology

INTRODUCTION

The need to abridge the animal protein intake is a major problem especially in developing countries where population practically erodes increase in food production, leaving no hope of ever

increasing surplus grain sources to compound livestock feed. Hence, efforts are being made to source for alternative feed ingredients that can lead to a reduction in the cost of feed and subsequently total cost of production. Kenaf (*Hibiscus canabinus*) was formerly, primarily used as a fiber crop and secondarily as a livestock feed (Dempsey, 1991). Its leaves have an acidic flavor and are used for the production of traditional soup. Kenaf is extensively cultivated in tropical Africa, Asia, Central America and the Caribbean for the jute- like fibre. Kenaf seed contain a crude protein of 30.88%, with organic matter content of 95.15%, ether extract, 18.55% on a dry matter basis and a fatty acid profile of palmitic acid, 33.21%, stearic acid, 50.02%, oleic acid, 31.26% and linoleic acid, 30.51% (Rajashekher *et al*., 1993). Despite all the above potentials, there is no information on its suitability as a source of protein in rabbit diets. However, in the use of these non-convectional feed ingredients, assessment of the health status of the animal is paramount. A readily available and fast means of assessing clinical and nutritional status of the animal on feeding trial may be the use of blood analysis (Olabanji *et al*., 2009) and organ traits (Ewuola, 2009). Kenaf seed meal is a potential feed ingredient for use in formulating feed for livestock especially monogastric if well processed.

With the above in mind, this study was designed to evaluate the hematology, serum biochemistry and organ histopathology growing rabbits fed whole kenaf seed meal-based diets.

MATERIALS AND METHODS

Experimental plan: Kenaf seeds were collected from the Institute of Agricultural Research and Training (IAR and T), Ibadan and were cleaned to remove all the foreign materials. The seeds were then roasted for about 10-15 min until the seed coats become brownish in colour, they were frequently turned to prevent the seed from burning. The roasted kenaf seed was then milled using hammer mill. Six experimental diets comprising Whole Kenaf Seed Meal (WKSM) used as a replacement of soyabean meal at 0, 20, 40, 60, 80 and 100% which constitutes treatments 1 (control), 2, 3, 4, 5 and 6, respectively, were prepared with the addition of other ingredients as shown in Table 1.

Thirty six, 49 day old New Zealand x Chinchilla rabbits with initial average live weight of 650 g were used. The animals were treated against endo and ectoparasites. The animals were

Table 1: Gross composition of the experimental diets for rabbits

Ingredients	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)	T6 (100%)
Maize	35.00	33.80	32.40	31.40	30.20	29.00
Wheat bran	23.00	23.00	23.00	23.00	23.00	23.00
Rice bran	21.00	21.00	21.00	21.00	21.00	21.00
Soybeans meal	17.00	13.60	10.20	6.80	3.34	-
Whole kenaf seed meal	-	4.60	9.20	13.80	18.40	23.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.12	0.12	0.12	0.12	0.12	0.12
Methionine	0.12	0.12	0.12	0.12	0.12	0.12
Premix	0.32	0.32	0.32	0.32	0.32	0.32
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient						
Crude protein (%)	16.70	16.60	16.50	16.37	16.27	16.17
Metabolisable energy (kcal kg^{-1})	2327.90	2308.94	2269.20	2313.10	2290.94	2271.96

randomly divided into six main groups of the dietary treatments. Each group was then replicated thrice with 2 rabbits per replicate. The experimental feed and water were supplied *ad libitum* twice daily at 8.00 and 16.00 h and the experiment lasted for 12 weeks.

Blood collection and evaluation: At the end of the feeding trial, blood samples were collected from experimental animals through the air vein into a set of well labeled sterile bottle containing EDTA for haematological examinations. Another set of tubes without EDTA was also used to collect blood, immediately covered and centrifuged. Serum separated out, decanted, deep- frozen for serum biochemical analysis as referenced by Ewuola and Egbunike (2008).

Organ histopathology: Three animals were sacrificed per treatment at the end of the feeding trial; live weight, slaughtered weight, dressed weight and weight of liver, spleen, kidney, lung, heart, Gastro Intestinal Tract (GIT) were recorded. Selected visceral organs such as liver, kidney and spleen were removed, weighed, fixed in 10% formalin solution and later processed for histopathological examination at the department of Veterinary pathology of the University of Ibadan, Ibadan as described by Drury and Wallington (1976) and Ewuola (2009).

Data analysis: The data collected on hematology, serum biochemistry and organ weights of the experimental animals were subjected to statistical analysis using analysis of variance procedure of SAS Institute Inc (SAS, 2003). The treatment means were compared using the Duncan's procedure of the same software. Histological observations were subjected to descriptive statistics (percentage).

RESULTS

The results of haematological parameters examined are presented in Table 2. There was significant difference ($p<0.05$) in the values obtained for PCV, HB and RBC. There was no significant difference in the values obtained for the all the blood constants (MCV, MCH and MCHC) examined. Apart from neutrophils, there was no significant difference among the leukocyte differentials examined such as lymphocytes, monocytes and eosinophils. The serum biochemical response of rabbits to the experimental diets is shown in Table 3. Serum proteins examined were not significantly ($p<0.05$) affected by the dietary treatments except Albumin. Serum total protein,

Table 2: Haematological parameters of growing rabbits fed graded levels of WKSM

Parameters	T1(0%)	T2(20%)	T3(40%)	T4(60%)	T5(80%)	T6(100%)	SEM*
Packed cell volume (%)	25.00^{ab}	18.25^{b}	27.25^{ab}	29.25^{a}	25.50^{ab}	31.00^{a}	1.37
Haemoglobin (g dL^{-1})	8.48^{ab}	6.08^{b}	9.08^{ab}	9.75^{a}	8.52^{ab}	10.33^{a}	0.46
Red blood cell (x10^{6} L^{-1})	2.47^{b}	2.46^{b}	3.79^{a}	3.03^{ab}	2.52^{b}	2.72^{ab}	0.17
Mean cell volume (μ^{3})	102.78	74.06	73.07	101.12	111.28	117.89	6.11
Mean cell hemoglobin ($\mu\mu g$)	35.04	24.68	24.34	33.70	35.74	39.31	2.09
MCHC**(%)	33.78	32.09	33.29	33.34	33.36	33.31	0.22
White blood cell (x10^{3})	7.11	5.53	6.94	5.29	5.95	6.04	0.28
Neutrophils (%)	26.26^{ab}	21.50^{ab}	19.25^{b}	23.25^{ab}	30.75^{a}	26.25^{ab}	1.33
Lymphocytes (%)	65.25	73.50	73.00	68.50	64.25	65.25	1.47
Monocytes (%)	2.25	2.00	2.25	2.75	2.75	2.25	0.17
Eosinophil (%)	6.25	3.00	5.50	5.50	4.00	6.50	0.52

ab: Mean in the same row with diff. superscript are significantly ($p<0.05$) different, *SEM: Standard error of mean, ** MCHC: Mean cell haemoglobin concentration

Table 3: Serum biochemistry of rabbits fed graded levels of whole kenaf seed meal

Parameters (g)	T1	T2	T3	T4	T5	T6	*SEM
Total protein (g dL^{-1})	6.10	5.96	5.77	5.82	5.96	6.19	0.17
Globulin (g dL^{-1})	3.17	3.06	2.85	2.62	2.16	3.07	0.16
Albumin (g dL^{-1})	2.83^{b}	2.90^{b}	2.92^{b}	3.20^{a}	3.80^{a}	3.12^{a}	0.29
Albumin/Globulin	1.01	1.13	1.12	0.98	1.06	1.14	0.07
*AST (IU L^{-1})	8.54^{b}	8.62^{b}	8.64^{b}	10.10^{a}	10.33^{a}	10.37^{a}	0.11
**ALT (IU L^{-1})	7.46^{b}	7.71^{b}	7.77^{b}	9.94^{a}	10.98^{a}	11.04^{a}	0.13

ab: Mean in the same row with diff. superscript are significantly ($p<0.05$) different, *SEM: Standard error of mean, *Aspartate Amino transferase, **Alanine Amino transferase

Table 4: Organ weight of growing rabbits fed graded level of WKSM

Parameters	T1	T2	T3	T4	T5	T6	SEM*
Kidney	5.98	6.87	6.76	5.49	5.12	5.98	0.24
Liver	39.35^{a}	34.22^{ab}	34.80^{ab}	31.61^{bc}	30.69^{c}	32.45^{b}	1.87
Lung	7.65^{a}	5.53^{ab}	4.95^{b}	3.82^{b}	5.06^{b}	4.73^{b}	0.37
Heart	2.67	2.67	2.56	2.44	2.16	2.28	0.10
Spleen	0.57^{a}	0.33^{ab}	0.48^{ab}	0.44^{ab}	0.30^{b}	0.25^{b}	0.04
Pancreas	0.17^{b}	0.49^{a}	0.37^{ab}	0.41^{ab}	0.51^{a}	0.36^{ab}	0.04
Adrenal gland	0.16^{b}	0.12^{bc}	0.07^{c}	0.21^{a}	0.22^{a}	0.25^{a}	0.03
GIT**	261.42^{ab}	303.19^{a}	257.33^{ab}	260.26^{ab}	216.44^{a}	244.92^{ab}	8.17

abc: Mean in the same row with diff. superscript are significantly ($p<0.05$) different, *SEM: Standard error of mean, **GIT: Gastro-intestinal tract

Table 5: Organ histopathology of rabbits fed varied levels of whole kenaf seed meal {% (number)}

Organs	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)	T6 (100%)
Liver	0(0/3)	67(2/3)	100(3/3)	100(3/3)	100(3/3)	100(3/3)
Kidney	0(0/3)	0(0/3)	33.33(1/3)	33.33(1/3)	33.33(1/3)	33.33(1/3)
Spleen	0(0/3)	0(0/3)	0(0/3)	0(0/3)	0(0/3)	33.33(1/3)

Globulin and Globulin/Albumin ratio of rabbits fed graded level of WKSM were not significantly different from those fed the control diet. However, Albumin concentration of rabbits fed treatments 4, 5 and 6 were significantly ($p<0.05$) higher than those fed the control diet, diets 2 and 3. Serum enzymes of experimental rabbits were significantly ($p<0.05$) influenced by the dietary treatments (Table 3). Aspartate amino transferase and Alanine amino transferase of rabbits fed treatments 4, 5 and 6 containing 60, 80 and 100% WKSM were significantly ($p<0.05$) elevated than those fed diets 3, 2 and the control which is an indication of organ toxicity in the animals.

Relative organ weights of rabbits fed graded levels of WKSM are as shown in Table 4. The values obtained for the weight of visceral organs, apart from kidney and heart, were significantly ($p<0.05$) different among the dietary treatments. The values obtained for the pancrease showed that pancreatic weight significantly ($p<0.05$) increased as the level of WKSM increases in the diet while liver weight decreases significantly with increased dietary level of WKSM. The result of histopathologcal examination of selected organs (liver, kidney and spleen) of rabbits fed varied levels of WKSM (Table 5), revealed damage done to the organs by the dietary treatments. It was observed that 67% of rabbits fed 20% WKSM showed liver hepatic degeneration and necrosis while 100% of rabbits fed 40, 60, 80 and 100% WKSM suffered same effect as compared to no visible lesion seen in the control rabbits. No visible lesion was also observed in the kidney of rabbits fed

control diet and diet 2 that contain 20% WKSM, however, 33.33% of animals that fed 40% WKSM and above showed tubular necrosis with interstitial cellular infiltration by mononuclear cells. 33.33% of animals fed 100% WKSM showed lesion in the spleen while no visible lesion was observed for those that fed diets 5, 4, 3, 2 and the control.

DISCUSSION

Blood is an important index of physiological, pathological and nutritional status in the organism (Olorode *et al.*, 2007; Ewuola *et al.*, 2004). Reports by Aletor and Egberongbe (1992) and Aletor (1989) indicated that the blood variables most consistently affected by dietary influence includes RBC, PCV and plasma protein. Although, PCV, HB and RBC were significantly influenced by dietary treatments they were still within the reported normal physiological range of rabbit as reported by Mitruka and Rawnsley (1977). The higher values obtain for lymphocytes and eosnophils than the standard value reported by Mitruka and Rawnsley (1977) is an indication that the rabbits immune system may have been challenged by toxic substance probably the antinutritional factors in the test ingredient used in formulating the diets. The results of the serum proteins imply that animals on the test ingredient utilized and synthesized the dietary protein from WKSM adequately with the control group fed convectional SBM. Albumin utilization and synthesis was enhanced in rabbits fed 60, 80 and 100% WKSM probably because of the increase in the level of crude protein available from the test ingredient at higher inclusion level in the diet, since albumin synthesis has been reported to be related to the amount of available protein (Iyayi and Tewe, 1998) in the diet. Besides Rajashekher *et al.* (1993) also observed that WKSM contain high level of crude protein as high as 30.88% with organic matter content of 95.15%. Serum protein variables of animals fed test ingredients compared favourably with the control indicating that treatment with test ingredient possessed the same dietary qualities with the control diet. Significance increase in the activities of ALT and AST in rabbits fed 60, 80 and 100% WKSM may be probably attributed to the dietary treatments effect at higher WKSM inclusion levels. It could be that as the inclusion level increases, the tendency of increase in the anti-nutritional factor in the test ingredient increases, this may probably be responsible for the cellular destruction within the organs that is responsible for the significant increase in the enzyme concentration observed in this study. Harper *et al.* (1997) and Ewuola and Egbunike (2008) observed in their separate studies that increase in the Alanine amino transferase and Aspartate amino transferase are clinical indication of diagnosing state of damage done to the visceral organs by toxic substance or infections.

The liver and spleen weight that were significantly reduced in rabbits fed 60% WKSM and above could probably be due to destruction of hepatocytes as well as spleen cells and consequently shrinkage of the organ, induced by toxic substance (anti-nutritional factors in this case) as reported by Ewuola (2009). The heavier values reported for pancrease and adrenal gland probably represent hypertrophy condition as a result of protease inhibitors in the WKSM (Koong *et al.*, 1985; Bond and Smith, 1989; Makkah, 1991). Also, since WKSM is high in fibre, its consumption may have led to increase in pancreatic weight.

The histopathologic examination of the liver, kidney and spleen of experimental rabbits adjudged the increase in serum enzymes observed in the same animals. Animals on test ingredient suffered from liver hepatic degeneration and necrosis which were diffused and severe most especially at 40% WKSM and above. There were cases of tubular necrosis with interstitial cellular infiltration by mononuclear cells which was also accompanied with periportal cellular infiltration

(multifocal). The lesion/damage observed in the visceral organ examined could probably be attributed to the presence of toxic substances in the kenaf seed in form of anti-nutritional factors alongside with other nutrients as reported by Rajashekher *et al.* (1993). It is obvious that, when diet contain toxic substances such as antinutrient or toxin contaminants the effect is always result in histopathological damage to the body organs most especially liver, spleen and kidney (Tolleson *et al.*, 1996; Ewuola *et al.*, 2003; Ewuola, 2009).

CONCLUSION

This study has demonstrated that whole kenaf seed meal possess good dietary protein quality for optimal growth of rabbits. However, the results revealed that feeding WKSM above 20% to rabbits will significantly alter the haematological and serum biochemical variables and probably induce anaemic conditions, damage and dysfunction of visceral organs which can lead to their poor performance.

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