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Full Length Research Paper

Haematological and serum biochemical indices of pre-pubertal male rabbits fed with graded level of blood-wild sunflower forage meal mixture

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The effects of feeding graded levels of blood wild sunflower forage meal mixture (BWSFM) on haematological and serum biochemical parameters in rabbit were studied. Thirty-two (32) cross-bred (New Zealand-white X Chinchilla) male weaner rabbits aged between 6 and 9 weeks were randomly divided after weight balancing into four groups of eight rabbits per group and each rabbit served as replicate in a complete randomized design. Four diets were formulated in which diet 1 the control, was containing no BWSFM mixture, while diets 2, 3 and 4 had graded levels of 5, 10 and 15% BWSFM mixture respectively. The rabbits were fed with the experimental diets for 12 weeks. The results showed that though the final weight of the rabbits in the different groups were not significantly affected by the inclusion of the test ingredient, the packed cell volume (PCV), hemoglobin content (Hb), red blood cell (RBC) count, white blood cell (WBC) count, as well as the lymphocytes and serum alanine aminotransaminase (ALT) were significantly ($P < 0.05$) affected by the treatments. It was concluded that inclusion of blood/wild sunflower forage meal mixture up to 15% was well tolerated by pre-pubertal male rabbits without any adverse health condition.

Key word: Pre-pubertal, rabbits, packed cell volume, alanine aminotransaminase, blood/wild sunflower forage meal mixture.

INTRODUCTION

In most developing countries where animal protein supply to meet the animal protein need of the populace is grossly inadequate, rabbit has abundant potential in helping to meet this requirements. This is because of its obvious advantage over other livestock. Rabbits have been known to multiply quickly and have ability to utilize forage (Aduku and Olukosi, 1990). The meat is medicinal (Cheeke et al., 1986), high in protein, low in cholesterol and sodium and is therefore a cheap source of healthy meat (Beymen, 1984; Biobaku and Oguntona, 1997; Holmes et al., 1984). Furthermore, rabbit production requires low capital investment, less space per unit number and absence of cultural biases or religion hindrances to its consumption (Biobaku and Oguntona, 1997).

The wild sunflower (*Tithonia diversifolia* Helms. A. Gray) of the family, Asteraceae, the order; Asterales, was formerly identified as *Tithonia rotundifolia* or *Tithonia tageti* (Dutta et al., 1986). In Nigeria, the plant occurs on road sides and fallow land and as invader of field crops in the forest savannah transition zone (Akinola et al., 1999). Its acceptability by ruminant and non-ruminant livestock, its relative abundance and the low cost of processing the forage makes it a potential non-conventional animal feed source in the derived savannah zone.

Most emphasis by animal nutritionist is usually on nutrient digestibility, utilization and performance of the farm animals. Hardly is consideration given in extensive manner for the future health status of the animal used in feeding trials either with conventional or non-conventional feedstuffs (Uko and Ataja, 1998).

Haematological parameters refer to those factors in the blood whose levels are usually determined in order to

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Table 1. Percentage composition of experimental on diets.

Ingredient	Percentage composition diet			
	1 (0% BWSFM)	2 (5% BWSFM)	3 (10% BWSFM)	4 (15% BWSFM)
Maize	18.5	17.5	14.5	11.5
Corn bran	36.0	36.0	36.0	36.0
BWSFM mixture	-	5.0	10.0	15.0
Groundnut cake	16.0	12.0	10.0	8.0
Palm kernel cake	20.0	20.0	20.0	20.0
Fish meal	1.0	1.0	1.0	1.0
Bone meal	3.0	3.0	3.0	3.0
*Premix	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Molasses	3.0	3.0	3.0	3.0

*Premix composition per kg feed; Vitamin A, 1500 IU; Viamin D_3, 2500 IU; Vitamin E, 11 IU; Vitamin B_2, 10 mg; Vitamin B_3, 40 mg; Vitamin B_6, 20 mg; chlorine chloride 400 mg; Mn., 120 mg; Fe, 70 mg; Iodine 2.2 mg; Cu, 10 mg; Se, 0.2 mg; Zn, 45 mg; Co, 0.02 mg.

assess the degree of well being of an animal. They are important in feeding trials because it has been established that feed components affect blood constituents (Harper et al., 1979). Furthermore, for blood analysis, a readily available and fast means of assessing the health status (Anosa, 1983), clinical evaluation for survey of physiological or pathological conditions (Bush, 1991), and a diagnostic/prognostic evaluation of various diseases in animal (Nottidge et al., 1999). There is paucity of information on the effect of inclusion of blood-wild sunflower forage meal in diets on haematological and blood serum biochemistry status of rabbits. Recently, it was reported that inclusion of BWSLM mixture supported optimum caudal epididymal sperm characteristics and showed testicular morphometrics (growth) in rabbits at a 10% level of incorporation and is tolerated without adverse effects of up to 15% levels (Ajayi et al., 2009).

This study was therefore designed to investigate the effects of inclusion of graded levels of blood-wild sunflower forage meal in rabbit ration on some haematological and blood serum biochemical characteristics of pre-pubertal rabbits bucks.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Ogbomoso is located on latitude 8°15' north of the Equator and longitude 4°15' east of the Greenwich meridian. The mean annual rainfall is 1247 mm, while the mean annual temperature is about 27°C (Oguntoyinbo, 1978).

Collection and preparation of test ingredients

The wild sunflower (*T. diversifolia* Helms. A. Gray) plant materials (leaves and succulent stalks) were harvested in the uncultivated plots of the University farm when the first inflorescence had opened in 50 to 80% of plants. They were identified and authenticated by Dr. A. T. Ogunkunle, a botanist in the department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. A specimen sample of the leaf was preserved at the herbarium of the same department with the voucher no LAH 105.

The cut plants were spread on a concrete slab and air dried under shade for 10 days. The dried leaves were pruned and then grounded into the forage meal. Fresh clotted bovine blood used for the experiment was collected at the abattoir, Ogbomoso and boiled for 1 h. The lump was chopped into smaller pieces before being sun-dried to allow fast drying. The dried blood was then grounded into blood meal. The blood/wild sunflower forage meals were mixed together in the ratio of 1:2.

Experimental diets and animals

Four iso-caloric and iso-nitrogenous experimental diets were formulated as follows: diet 1 (control) contained 0% blood/wild sunflower forage meal (BWSFM) mixture. Diets 2, 3 and 4 contained 5, 10 and 15% BWSFM mixture respectively (Table 1).

Thirty-two (32) cross-bred (New Zealand White X Chinchilla) weaner male rabbits aged 6 to 9 weeks were used for the study. The rabbits were weight balanced and randomly allocated into four groups of eight (8) rabbits each, with each rabbit serving as a replicate in a complete randomized design. Groups 1, 2, 3 and 4 were placed on diet containing 0, 5, 10 and 15% BWSFM mixture, respectively. The experiment lasted for 12 weeks.

Prior to the commencement of the experiment, the rabbits were prophylactically treated against ecto- and endo-parasites by giving subcutaneous injection of 0.2 ml ivomectin per rabbit. They were equally given a broad spectrum antibiotic (Altracycline®, 0.4ml intramuscular injection per rabbit). The rabbits were housed individually in all-wire cages with provision for feed and drink *ad libitum*.

Sample collection

The rabbits were weighed weekly and the weights on the 12th week were the final weights. During the 10th week of the experiment, two sets of blood samples were collected via the ear vein of the rabbits

Table 2. Proximate composition of the experimental diets and test ingredients.

Nutrient	Blood meal	Wild flower	BWSLM	Diet 1 (0% BWSFM)	Diet 2 (5% BWSFM)	Diet 3 (10% BWSFM)	Diet 4 (15% BWSFM)
Dry matter (%)	89.76	90.16	91.80	89.21	89.18	89.25	98.31
Crude protein (%)	78.55	15.95	36.60	16.80	16.45	17.45	17.85
Crude fiber (%)	1.46	12.06	16.64	10.35	10.16	10.95	11.18
Ether extract (%)	0.72	5.00	6.21	3.15	3.29	3.32	3.46
Ash (%)	3.96	14.00	13.56	7.48	8.04	8.10	8.16
NFE (%)	15.01	52.99	26.99	62.22	62.06	60.48	59.35

BWSFM, Blood wild sunflower forage meal; NFE, nitrogen free extract; The final body weight of the rabbits is presented in Table 3. There was no significant (P > 0.05) difference between rabbits on control diet and those on experimental diets.

into EDTA-free tubes and allowed to clot for the separation of serum from the clot by decantation.

Packed cell volume (PCV) was determined by the micro-haematocrit method (Dacie and Lewis, 1991). Haemoglobin (Hb) concentration was determined by the cyanomethaemoglobin method (Kelly, 1979). Red blood cell (RBC) and white blood cell (WBC) counts were determined using improved Neubauer haemocytometer method, as described by Jain (1986). Percentage distribution of various white blood cells (differential counts) was also computed. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain (1986).

Serum albumin and globulin were analyzed colorimetrically according to the method of Douma and Briggs (1972). Activities of serum alanine aminotransaminase (ALT), serum aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) were determined colorimetrically according to the technique of Reitman and Frankel (1957).

Statistical analysis

All data collected were subjected to analysis of variance according to Steel and Torrie (1980) and where significant differences were indicated, Duncan's multiple range test (Duncan, 1955) was used to separate the means.

RESULT

The proximate composition of experimental diets and test ingredient is shown in Table 2. Haematological parameters of rabbits fed with different levels of BWSFM mixture is presented in Table 3. The PCV, Hb, RBC, WBC and lymphocytes values were significantly ($P < 0.05$) influenced by the dietary treatments. The values obtained fall within the normal range reported for rabbits by Mitruka and Rawnsley (1977). Rabbits on diet 3 (10% BWSFM) had a numerically higher PCV value (39.00%) when compared to rabbits on diet 2, (35.75%), diet 4 (33.75%) and diet 1, (30.60%). Similarly, the trend in the values obtained for Hb and RBC followed a similar pattern observed for PCV. Rabbits on diet 3 had a numerically higher Hb content (12.65 g/dl) when compared to rabbits on diet 2 (11.53 g/dl), diet 4 (11.15 g/dl) and diet 1 (9.75 g/dl). Also, diet 3 had a higher RBC value ($7.33 \times 10^6/mm^3$) when compared to rabbits on diet 2 ($6.81 \times 10^6/mm^3$) diet 4, ($6.48 \times 10^6/mm^3$) and diet 1 ($5.86 \times 10^6/mm^3$).

The white blood cells (WBC) counts for rabbits on diet 2 ($5.33 \times 10^3/mm^3$), diet 3 ($5.18 \times 10^3/mm^3$) and diet 4 ($5.05 \times 10^3/mm^3$) were comparable. However, the WBC count ($4.68 \times 10^3/mm^3$) for rabbits on diet 1 was significantly ($P < 0.05$) lower than the values obtained for diets 2, 3 and 4 separately, while the values obtained for diets 2, 3 and 4 were within the normal range, but WBC count obtained for diet 1 falls below the normal range reported by Mitruka and Rawnsley (1977).

The lymphocytes values observed for diet 1 (40.50%) and diet 2 (39.25%) were comparable and significantly ($P < 0.05$) higher than the values obtained for diet 3 (32.55%) and diet 4 (34.75%) that were similar. Other haematological parameters taken were not significantly affected.

The serum biochemical indices of the rabbits showed that albumin, globulin, total protein, ALP and ALT, were not significantly ($P > 0.05$) affected by the dietary treatments. However, the ALT value was significantly ($P < 0.05$) affected among the treatment groups. The ALT value for diet 1 (72.00 IU/L) was significantly ($P < 0.05$) higher than that of diet 2 (59.25 IU/L) and diet 4 (59.25 IU/L) which were the same but comparable with the value for diet 3 (66.50 IU/L).

DISCUSSION

The blood in an animal serves as a transport medium.

Table 3. Final weight, haematological parameters and serum biochemical indices of pre-pubertal male rabbits fed different levels of BWSLM mixture.

Parameter	Diet 1 (0% BWSFM)	Diet 2 (5% BWSFM)	Diet 3 (10% BWSFM)	Diet 4 (15% BWSFM)	SEM	**Literature value	SL (P < 0.05)
Final weight (g)	1455.60	1537.20	1543.26	1470.00	92.40	-	NS
Haematological parameter							
PCV (%)	30.60^{b}	37.75^{ab}	39.00^{a}	33.75^{ab}	2.4	31.0-48.86	*
Hb (g/dl)	9.75^{b}	11.53^{ab}	12.65^{a}	11.15^{ab}	0.86	9.8-15.8	*
RBC ($x10^{6}/mm^{3}$)	5.86^{b}	6.81^{ab}	7.33^{a}	6.48^{ab}	0.41	5.11-6.51	*
MCV (fl)	52.22	52.50	53.21	52.08	0.42	-	NS
MCH (pg)	16.64	16.93	17.26	17.21	1.17	-	NS
MCHC (g/dl)	3.19	3.23	3.24	3.30	0.44	-	NS
WBC ($x10^{3}/mm^{3}$)	4.68^{b}	5.33^{a}	5.18^{a}	5.05^{ab}	0.37	5.2-10.6	*
Neutrophils (%)	38.25	39.25	38.15	37.00	1.68	36-50	NS
Lymphocytes (%)	40.50^{a}	39.25^{a}	32.55^{b}	34.75^{b}	1.55	31-32	*
Monocytes (%)	11.75	11.25	10.95	11.05	0.65	4-13.5	NS
Eusinophils (%)	3.50	3.50	2.25	2.50	0.48	0.5-5	NS
Serum biochemical indices							
Albumin (g/dl)	3.05	2.88	3.28	2.90	0.43	2.43-4.05	NS
Globulin (g/dl)	3.08	3.00	3.12	2.98	0.26	3.58-4.25	NS
Total protein (g/dl)	6.13	5.88	6.40	5.88	0.40	6.00-8.03	NS
ALP (iU/l)	10.25	10.25	10.75	10.50	0.22	4.10-16.2	NS
ALT (iU/l)	72.00^{a}	59.5^{b}	66.50^{ab}	59.25^{b}	2.94	42.5-98.0	*
AST (iU/l)	67.25	71.25	67.75	63.50	2.58	48.5-78.9	NS

a, b: Mean along the same row with different superscript are significantly different (P < 0.05); *,significant, NS, not significant; **: Mitruka and Rawnsley, 1977; PCV, packed cell volume; Hb, haemoglobin concentration; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells; ALP, alkaline phosphatase; ALT, serum alanine aminotransaminase; AST, serum aspartate aminotransaminase; SEM, standard error of mean, SL, significant level.

It transports food materials such as glucose, fatty acids, vitamins and electrolytes from the gastrointestinal tract to body tissues where they are utilized for body building and energy. Increase or decrease in body weight from the previous weight for a specific period is the principal measure of productivity in meat animal and depends on the quality and to a lesser extent the quantity of feed given (Blood and Studdert, 1999). This means that the weight, feed and blood are related. In this study, the final weights had no significant (P < 0.05) difference. This is an indication that the test ingredients at the various inclusion levels did not have any negative effect on the final weight gain.

The following haematological parameters: PCV, Hb, RBC and lymphocytes were significantly (P < 0.05) affected. And since it has been established that feed components affect blood constituents (Harper et al., 1979), the haematological parameters can thus be used to assess the effects of the test ingredient. The percentage of the volume of whole unclotted blood occupied by the RBC is a useful prognostic indicator in dehydration or excitement when PCV is abnormally high and it aids in the diagnosis of anaemia when it is low (Blood and Studdert, 1999). Anaemia occurs when there is reduction in RBC count in circulation, haemoglobin content and PCV. Therefore, to determine the presence or absence of anaemia, these three parameters are important (Fraser et al., 1991). The result of this study indicated that the PCV, RBC count and Hb content followed the same trend with diet 3 (10% BWSFM) having the highest values numerically for the three parameters followed by diet 2 (5% BWSFM), diet 4 (15% BWSFM), while diet 1 (control) had the lowest values. Haemoglobin and PCV measurements can be used as an assay of nutritional anaemia (dietary deficiency of iron, copper, vitamins and amino acids). Since the values obtained for these parameters (PCV and Hb) fall within the normal range values, it can be interpreted that the dietary treatment did not cause any nutritional anaemia and can supply the needed nutrients to the rabbits. The RBC counts according to Swenson (1990) are influenced among other factors by nutrition, physical activities and volume and its reduction indicates anaemia. In this study, there was no clinical state of anaemic condition. This

result is an indication that the test ingredient had no negative effect on the blood parameters but instead has the ability to improve these parameters.

Ologboho et al. (1986) observed that an increase in WBC count above normal is an indication of the presence of exogenous substances and foreign bodies in the body. In this study, there was no case of such abnormal rise in values of WBC. Lymphocytes are important in forming barriers against local disease conditions and may be involved in antibody formation (Frandson, 1981). In this study, diet 1 had the highest value, while diet 3 had the least. Many authors have reported a large variation mainly in lymphocytes due to age and nutritional condition of animals (Albrilton, 1961; Schlam and Jain, 1974). This showed that the test ingredient could have affected the lymphocytes count, by producing a better health state than the control since abnormal rise in lymphocyte may be as a result of malnutrition among other factors.

Although, Blood and Studdert (1999) reported that ALT level is usually elevated when damage is done to tissues cells, especially heart and liver and also in some muscle diseases, Ademola et al. (2005) observed that an increase in ALT activity of birds on garlic and ginger treatments proved the potency of the hemolytic activities of these supplements as it affected the liver function. But in this study, all the values obtained falls within the normal range, with the control diet having the highest. The implication of this is that the utilization of protein, and therefore the blood protein levels were not negatively affected by feeding on BWSFM mixture.

Most of the values obtained for haematological as well as the serum biochemical indices in this study fall within the normal literature values for rabbits. It can therefore be concluded that up to 15% inclusion level of BWSFM mixture can be tolerated by male rabbits without a deleterious effect on their health status.

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