



EFFECTS OF THE INTEGRATION OF *TRIDAX PROCUMBENS* INTO THE DIET OF MALE RABBITS ON GROWTH, HEMATOLOGY, SERUM CHEMISTRY AND TESTICULAR PARAMETERS

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ABSTRACT

Fifteen weaned crossbred male rabbits were used for the study of the effects of different diets of *Tridax procumbens* on the growth, haematology, serum chemistry and testicular development, over a period of about eight months. The animals were grouped into three of five animals each, and housed in a standard hutch. The first was fed *Tridax procumbens*, second group received a mixture of *Tridax procumbens* and concentrate, while the third group was placed on concentrate only, and were tagged groups A, B and C respectively. Body weights of all the animals were taken on weekly basis, and the differences (i.e. weight changes) were recorded. Some of the weight changes of note are those taken after acclimatization (i.e. commencement of experiment), at testicular descent, just before castration, and eight weeks post castration. Blood sample was collected from all the animals on the day of castration (i.e. six months of receiving the diet) and used for haematology and serum chemistry evaluations. The testes were processed for histology. Animals placed on a diet of *Tridax procumbens* only, performed poorly with an initial drop in body weight (-140g), when compared with those on other diets (B: +557g and C: +546.4g). The same trend was recorded in the age at which testicular descent was observed in the animals; those with exclusive diet of *T. procumbens* (25weeks) were the last to have their testicles descended, while the concentrate only group experienced testicular descent earlier than the other two (18 weeks). The group with a mixture of *T. procumbens* and concentrate came between the other two (22 weeks). The pattern was not different with the weights taken at testicular descent and pre-castration, as well as erythrogram values like the Rbc and Hb. The values recorded across the three groups for serum chemistry were not statistically significant ($P>0.05$).

INTRODUCTION

For several decades, Africa has been plagued by high poverty levels that have portrayed an increasingly worrisome trend over time. According to a World Bank report (World Bank, 2008), between 1993 and 2002, poverty levels in Sub-Saharan Africa increased from 200 million to 220 million in rural areas and from 80 to 100 million in urban areas. This report noted that the number of rural poor has continued to rise and will likely exceed the number of urban poor by 2040.

Small livestock species, including rabbits, have been promoted as tools in poverty alleviation programmes (Owen *et al.*, 2005). Rabbits are particularly favoured for poverty reduction programmes on account of their low investment and early benefits, and subsistence on renewable resources for feeding, housing and general management.

Animal protein intake has been on steady decline among Nigerians owing to high cost of meat and other animal products. This shortage of protein particularly of animal origin in human diets in all parts of Africa and most

developing countries of the world has been well documented (Bawa *et al.*, 2009). The production of non-ruminant species represents the fastest means of correcting the shortage of animal protein in tropical Africa (Apata and Ojo, 2000).

Rabbits (*Oryctolagus cuniculus*) are prolific animals with short gestation period and have enormous potentials in alleviating the problem of animal protein supply in developing economies (Biobaku and Dosumu, 2003). Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria (Ajala and Balogun, 2004). The rabbit has immense potentials and good attributes which include unique attributes such as the ability to convert forage crop residues and agro-industrial by-products more efficiently into meat than most other livestock (Sese *et al.*, 2013), their small sizes, the ease of management, their high reproductive potential, high growth rate (Odimba, 2006), short gestation period, and high prolificacy, relatively low cost of production, high nutritional quality of rabbit meat which includes low levels of fat, sodium, and cholesterol. It also has a high protein level of about 20.8% and its consumption is

bereft of cultural and religious biases (Biobaku and Oguntona, 1997).

Nutrition, drugs and toxic substances, stress, temperature, humidity and photoperiod have been reported as some of the important external or environmental factors affecting sperm production and development (Farombi *et al.* 2007; Oyeyemi *et al.* 2006).

Some studies on the potentials of leaf meals in the diet of livestock (Amata and Bratte, 2008; Amata *et al.*, 2009) have shown significant growth responses by animals fed such meals.

Tridax procumbens, It has crude protein values ranging from 15-22% depending on time of harvest (Kalu *et al.*, 1986), high in mineral depending on stage of maturity (Aduku, 1993) and high in essential amino acid content (Anthony *et al.*, 2007) and because of its nutrient profile, *Tridax procumbens* can be used to supplement nutrient deficiencies found in other diets (Ojobe, 1998).

Tridax procumbens (coat buttons), is one of the most common alternative feed, fed to rabbits in Nigeria. *Tridax procumbens* has been used by farmers to reduce cost of production and maximize their profit margins. It is a tropical forage of the Compositae family, and has great potential for use as livestock feed ingredient and has the potential to improve reproduction efficiency in rabbits and thus increase availability of rabbit meat and economic power of rabbit farmers and general ease of sourcing rabbits for research and other uses.

Literature is however scanty on how the consumption of *Tridax procumbens* affects growth, haematology, serum chemistry and testicular development in rabbits. This study therefore intended to determine the performance of growing rabbits when they are fed different levels of *Tridax procumbens*, with the view to decide the diet that supports optimum growth with respect to weight gain, blood picture and testicular parameters.

MATERIALS AND METHODS

Study Design

The case groups were rabbits that were fed *Tridax procumbens* only diet, mixture of *Tridax procumbens* and concentrate diet. The control group was fed concentrates only diet. The three groups matched each other in initial body weight, water supplementation, and all other environmental conditions. The concentrate i.e. commercial rabbit feed (Vital Feed[®]) has the following nutritional components; crude protein (13%), crude fibre (15%), fat (8%), calcium (0.9%), phosphorus (0.35%), metabolizable energy (2,600 Kcal/kg).

Experimental Animals

Fifteen weaned crossbred male rabbits aged 7-9 weeks were used for this study. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water.

Acclimatization of the animals to laboratory conditions was done over a period of four weeks before the commencement of the experiments. All the procedures were carried out in compliance with the recommendations on the standard use of laboratory animals (World Medical Association and American Physiological Society, 2002).

The rabbits were divided into three groups as follow:

Table 1: Study Groups.

Group	Diet
A	<i>Tridax procumbens</i> only
B	Concentrate and <i>Tridax procumbens</i>
C	Concentrate only

Group A was on a diet of *Tridax procumbens* in the morning and evening; Group B was fed a diet of only *Tridax procumbens* in the morning and concentrate in the evening; while Group C entirely on concentrate until castration was done.

Following castration, all the animals (irrespective of their groups) were placed on a diet of *Tridax procumbens* in the morning and concentrate in the evening.

Blood Sampling

Blood samples were collected from each rabbit in duplicate after 24 weeks of experimental feeding. This was achieved by puncturing the orbital sinus and allowing free flow of blood into labeled sterile universal bottles through a capillary tube. The initial 5ml was collected over labelled sterile universal bottles containing 1.0 mg/ml ethyldiamine tetracetic acid (EDTA). This was used to determine the haematological component according to the method of Ajagbonna *et al.* (1999) and Uko *et al.* (2000). The other 5ml was collected into labelled sterile sample bottles without coagulant and used to determine the biochemical components (Ajagbonna *et al.*, 1999; Uko *et al.*, 2000).

Reproductive Organ Evaluation

The age at which the testicles descended was recorded for individual animal. Testicles were removed for evaluation 24 weeks after the commencement of the experiment. Prior to the procedure, the rabbits were starved overnight to clear the guts and their weights were recorded. The animals were restrained chemically using Xylazine, and lignocaine was used as a local anaesthetic. An incision was made carefully through the scrotal tunics, the spermatic cord was ligated with chromic catgut, the gubernaculum was transected and the testicles were then picked out with forceps. The animals were closed up and the incision sites were sprayed with Oxytetracycline spray to prevent contamination. The testes were weighed and put into the Bouins' solution.

Parameters Determined

Weight – Some specific weights were taken, and the weight changes determined there from. They include: Initial weight: taken at the commencement of the experiment (W_0),

Weight at testicular descent: recorded at the point of testicular descent (W_1),

Weight at castration: recorded just before castrating the animals (W_2)

Weight Post- Castration: taken eight (8) weeks after the castration was done (W_3),

Weight Gain at Descent (WA) = $W_1 - W_0$,

Weight Gain after Testicular Descent, Pre-Castration (WB) = $W_2 - W_1$

Weight Gain Pre-Castration (WC) = $W_2 - W_0$

Weight Gain Post Castration (WD) = $W_3 - W_2$

Age

The age at testicular descent was recorded for each animal as **A1**.

Haematological parameters

This included haemoglobin, red blood cell, white blood cell, packed cell volume, lymphocyte, eosinophil and neutrophil.

Serum Biochemistry

This included urea, serum creatinine, bilirubin (total and conjugated), total protein, globulin, AST and ALT.

Data Analysis

Data collected were recorded as Mean \pm SEM, and were analyzed using the Statistical Package for Social Sciences (SPSS 19). Descriptive statistics such as mean, analysis of variance were used and a Duncan post hoc test. Level of significance was set at 5%.

RESULTS

Table 2: Differences between Pre- and Post-Castration Weight.

Parameters	Group A	Group B	Group C
Gained pre castration (g)	325	1272	1410
Gained post castration (g)	500	270	130
Difference(g)	175	-1002	-1280

Table 3: Weight changes across the different groups, at different stages.

	Weights (g)	
WA (g)	Group A	-140.0 \pm 37.58 ^{ab}
	Group B	557.0 \pm 88.07 ^b
	Group C	546.4 \pm 101.76 ^a
WB (g)	Group A	479.0 \pm 86.62 ^a
	Group B	710.0 \pm 76.08
	Group C	863.6 \pm 145.18 ^a
WC (g)	Group A	339.0 \pm 62.42 ^{ab}
	Group B	1267.0 \pm 21.42 ^a
	Group C	1410.0 \pm 133.22 ^b
WD (g)	Group A	496.0 \pm 72.22 ^{ab}
	Group B	250.0 \pm 44.72 ^a
	Group C	130.0 \pm 83.73 ^b

Values with similar superscripts are statistically significant at $p < 0.05$

Table 4: Erythrogram.

Parameters	Group	Mean \pm SEM
Pcv	A	42.00 \pm 0.70 ^{ab}
	B	47.80 \pm 1.28 ^a
	C	48.20 \pm 2.35 ^b
Hb	A	13.80 \pm 0.07 ^{ab}
	B	15.66 \pm 0.37 ^b
	C	15.64 \pm 0.79 ^a
Rbc	A	7.130 \pm 0.01
	B	7.866 \pm 0.21
	C	7.860 \pm 0.36
MCV	A	58.9023 \pm 0.93
	B	60.7808 \pm 0.69
	C	61.3143 \pm 0.75
MCH	A	19.3548 \pm 0.10
	B	19.9175 \pm 0.15
	C	19.8824 \pm 0.15
MCHC	A	32.8913 \pm 0.54
	B	32.7777 \pm 0.25
	C	32.4365 \pm 0.24
Platelets (10^3)	A	176.000 \pm 1224.74 ^{ab}
	B	244.400 \pm 17825.82 ^a
	C	248.600 \pm 19951.44 ^b

Values with similar superscripts are statistically significant at $p < 0.05$

Table 5: Leucogram.

Parameters	Group	Mean \pm SEM
Total Wbc 10^3 (g/dl)	A	4.600 \pm 70.71 ^{ab}
	B	6.640 \pm 201.49 ^a
	C	6.690 \pm 412.43 ^b
Lym 10^2	A	30.388 \pm 97.70
	B	47.404 \pm 153.96
	C	47.980 \pm 295.95
Neut 10^2	A	13.368 \pm 70.99
	B	16.036 \pm 51.41
	C	16.165 \pm 182.30
Mon 10^2	A	1.004 \pm 16.03
	B	1.474 \pm 27.75
	C	1.226 \pm 28.08
Eos 10^2	A	1.378 \pm 14.68
	B	1.468 \pm 35.25
	C	1.405 \pm 26.76

Values with similar superscripts are statistically significant at $p < 0.05$

Table 6: Serum Chemistry.

Parameters	Group	Mean ±SEM
PROTEIN	A	7.68 ±0.13
	B	7.86 ±0.21
	C	7.90 ±0.21
ALB	A	2.6 ±0.11
	B	2.6 ±0.13
	C	2.7 ±0.15
GLOB	A	5.04 ±0.10
	B	5.26 ±0.07
	C	5.20 ±0.06
AST	A	15.00 ±1.41
	B	17.00 ±0.95
	C	16.20 ±1.56
ALT	A	65.00 ±1.64
	B	104.80 ±25.90
	C	106.60 ±26.01
ALP	A	62.00 ±1.92
	B	36.20 ±3.34
	C	61.00 ±3.96
BUN	A	16.06 ±0.20
	B	16.24 ±0.39
	C	16.00 ±0.27
CREAT	A	0.84 ±0.05
	B	1.06 ±0.11
	C	1.02 ±0.13

Values with similar superscripts are statistically significant at p<0.05

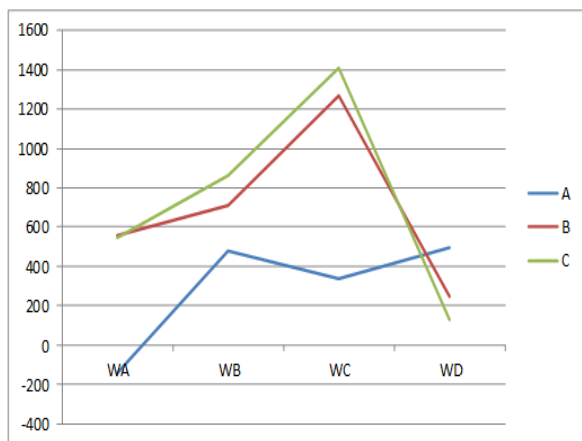


Fig1: Weight gain (in grams) at different stages of the experiment.

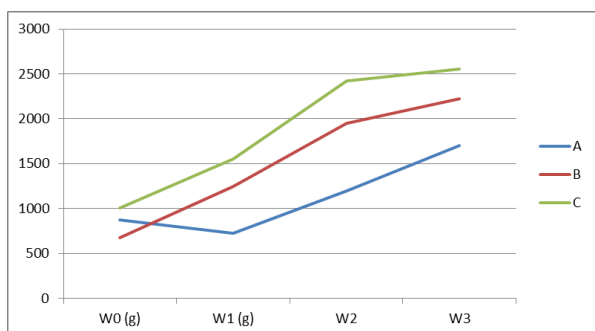


Fig 2: Weight (in grams) at different stages of the experiment.

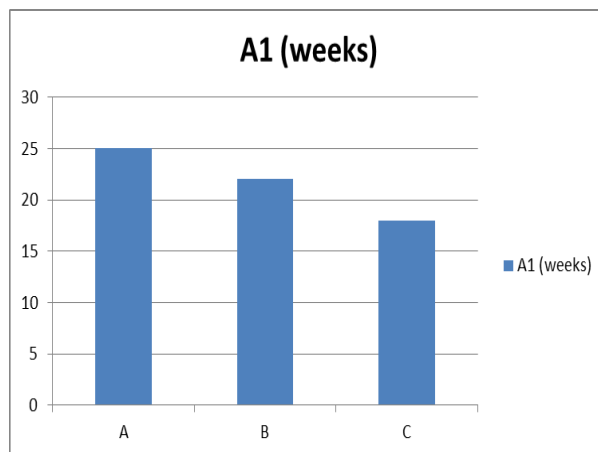


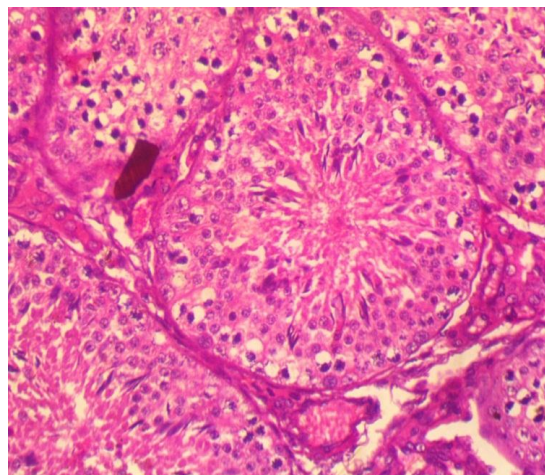
Fig3: Age at which Testicular descent was observed across the groups.

Testicular histology



X400

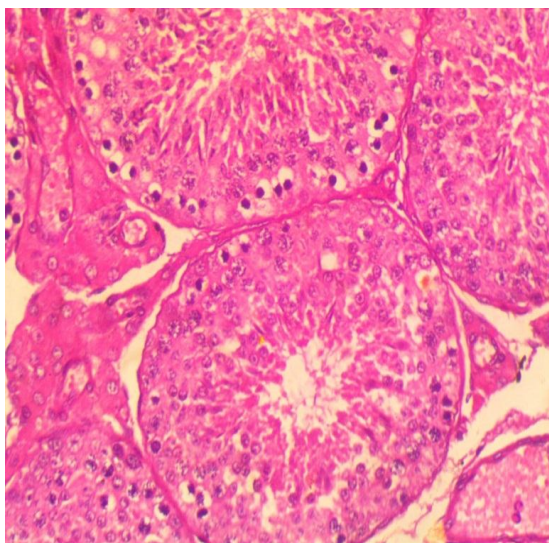
Plate 1 (from Group A): There are numerous, closely-packed, uniformly-sized seminiferous tubules (STs) with regular outlines. The STs are packed full with spermatogenic cells. Spermatozoa predominate. There is moderate congestion of interstitial testicular blood vessels. Interstitial cells appear normal.



X400

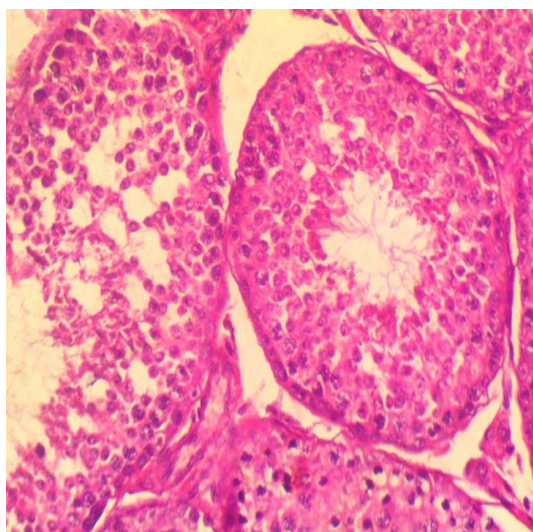
Plate 2: (From Group B): There are numerous, closely-packed, uniformly-sized seminiferous tubules with regular outlines. The STs are packed full with spermatogenic cells. Spermatozoa and elongate

spermatids predominate. There is mild vacuolar change of spermatogenic cells in the basal compartment. There is moderate congestion of interstitial testicular blood vessels. Interstitial cells appear normal.



X400

Plate 3: (from Group C): There are numerous, closely-packed, uniformly-sized seminiferous tubules with regular outlines. The STs are packed full with spermatogenic cells. Spermatocytes and elongate spermatids predominate. There is mild vacuolar change of spermatogenic cells in the basal compartment. There is moderate congestion of interstitial testicular blood vessels. Interstitial cells appear more numerous than expected.



X400

Plates 4: (from Group C): There are numerous, closely-packed, uniformly-sized seminiferous tubules with regular outlines. There are a few STs which are mildly depleted of spermatogenic cells. Affected tubules show reduced height of the germinal epithelium, as well as loss of cohesion between cells of the germinal epithelium. Spermatocytes predominate. Vascular changes are unremarkable.

DISCUSSION

In the course of this study, three different groups of rabbit were fed *Tridax procumbens* only, *Tridax procumbens* with supplemented concentrate and concentrate only.

The animals fed only concentrates showed the highest growth rate, while the animals fed with only *Tridax procumbens* had the least values for growth rate. The growth rate for rabbits placed on a diet of both concentrates and *Tridax procumbens* was however comparable to those of rabbits on concentrate alone. These values are statistically significant ($P < 0.05$).

The result of this experiment thus differs slightly from those of Okonkwo *et al.*, 2010 and Taiwo *et al.*, 2004. They reported that marked improvement in weight changes were obtained with higher crude protein content of concentrate feed supplemented with *Tridax procumbens*, and that the difference was due to the ability of the rabbits to gain more weight on diet formulated to contain forage than diet based solely on concentrate.

Also, there appears to be a relationship between the age of testicular descent and weight of the animals; rabbits fed concentrate only showed the earliest signs of descent at about four and a half month, while those fed concentrate and *Tridax procumbens* at about 5 and a half month, and those on *Tridax procumbens* did not show signs of testicular descent till about 6 months. The rabbits placed on concentrate only were noted with the highest values of body weight at the time of testicular descent, followed by those on mixture of concentrate and forage whereas the *Tridax procumbens* only group had the least weight. This is an indication of the role of nutrition in the attainment of sexual maturity and puberty in bucks.

Eight weeks after castration, the rabbits on *Tridax procumbens* showed a statistically significant ($P < 0.05$) increased growth rate when compared with the other groups. This can be adduced to the fact that these animals which had hitherto been on forage only tend to be responding positively to the introduction of concentrate into their diet.

It goes further to show that the initial slow growth rate experienced by the rabbits fed with *Tridax procumbens* only can be reversed, once the nutrition is improved upon by the incorporation of concentrate into their diet.

All the values obtained for haematological parameters in this study are within the normal ranges for rabbits as reported by Medirabbit (2011).

The result also showed that the rabbits on concentrate alone, and *Tridax procumbens* supplemented with concentrate had higher hematological values (PCV & Hb concentrations) compared with rabbits on *Tridax*

procumbens alone ($P < 0.05$). This may be an indication of the physiological state of the animals (Aro *et al.*, 2013), since none of the values is indicative of anaemia, and that the diets are not necessarily toxic to the animals (Isaac *et al.*, 2013). In like manner, this is suggestive of the fact that animals in groups B and C may be healthier than those in group A (Aro and Akinmoyegun, 2012).

Some of the white blood cells values (i.e. lymphocytes and monocytes) showed the same pattern with the haematological parameters. However, reverse was the case for the neutrophils and eosinophils in which the group fed *T. procumbens* only had higher values than the first two groups. Notwithstanding, in both instances, the differences were not statistically significant ($P > 0.05$), except for the lymphocytes as well as the platelets count ($P < 0.05$).

With regards to the serum chemistry, all the values obtained in this study are within the normal ranges for rabbits reported by Medirabbit (2011). However, there are variations in the values recorded for the different groups, with the group fed with *T. procumbens* only having the least values for most of the parameters measured, with the exception of Alkaline Phosphatase. The observed differences were however not statistically significant ($P > 0.05$).

On the whole, there were no significant aberrations in the testicular histology of the rabbits across the three groups under consideration. The architectures are considered normal for a matured rabbit buck. The few abnormalities observed could be occasional findings as may be the case for different species of animals.

It is therefore concluded that *Tridax procumbens* and concentrate diet at that inclusion level in this experiment may not adversely affect the hematology, serum chemistry and reproductive parameters of rabbit bucks. And, this may be sufficient for proper growth in these animals.

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