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Effect of graded levels of ginger rhizome (*Zingiber officinale*) meal on serum biochemistry of pubertal New Zealand White rabbits

Ilo, S.U.[1] and Egu, U. N.[2*]

[1]Department of Animal Science, University of Nigeria Nssuka, Enugu State, Nigeria.
[2]Department of Animal Science and fFsheries Abia State University PMB 7010 Umuahia, Nigeria.

*Corresponding author. Email: ucheegu1@gmail.com

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ABSTRACT: Forty-eight sexually matured (7 to 8 months old) healthy male and female New Zealand White rabbits were used to determine the effect of graded levels of ginger rhizome meal on serum biochemical indices. The rabbits were divided into 4 treatment groups, namely T_1, T_2, T_3 and T_4 and assigned to four treatment diets in a Complete Randomized Block Design (CRBD). Each of the treatment groups consisted of 12 rabbits (6 does and 6 bucks) replicated 3 times with 2 rabbits per replicate. The levels of inclusion of the ginger rhizome meal were 0.00, 5.00, 10.00 and 15.00% for the T_1, T_2, T_3 and T_4 respectively. T_1 serves as the control. The rabbits were fed twice daily, in the morning and evening, water was given *ad libitum* to the rabbits. After 30 days, blood samples were collected from 12 rabbits (6 does and 6 bucks) in each treatment group for serum biochemical evaluation. The results showed that there were significant differences ($P<0.05$) among the treatment groups in the serum biochemical parameters: urea (significant difference ($P<0.05$) between rabbits on T_1, T_2, T_3 and those on T_4); Aspartate transaminase (significant difference ($P<0.05$) between rabbits on T_2 and T_1; between T_3 and those on T_1 and T_4); Alanine transaminase (significant difference ($P<0.05$) between rabbits on T_1, T_3, T_4 and those onT_{20}); and glucose (significant difference ($P<0.05$) between rabbits on T2 and those on T_1, T_3, T_4), except Alkaline phosphatase and cholesterol which were similar ($P>0.05$) among the treatment groups. The results of this study indicate that inclusion of ginger rhizome meal in the diets of the rabbits up to 5% improved their serum biochemical indices.

Key words: Blood, feed, evaluation, gender, rabbits, trial.

INTRODUCTION

The common rabbit (*Oryctologus cunniculus*) is a species of rabbits native to south Western Europe (spain and Portugal) and north west Africa (Hoffman and Smith, 2005). The New Zealand white rabbit is an exotic breed that weighs about 4.1 to 4.5 kg at maturity. They have white fur that covers every part of the body with shiny eyes; they are commercial meat type and reach puberty at about 5 to 6 months of age (Egu, 2016).

The rapid increase in the world population in general and in the Nigeria population in particular has aggravated the animal protein deficiency. Food and Agricultural Organization (2006) estimated the average animal protein consumption in Nigeria to be 7.4 g per capita/day as compared with 38 g per capita/day of animal protein consumed in South Africa. The increasing demand for animal protein indicates the need to intensify livestock production. Poultry production which was the quickest way to bridge this animal protein deficiency gap was observed to be characterized by high cost of production due to high cost of feeds which accounts for more than 70 to 80% of the total production costs (Akinmutimi, 2001; Ojebiyi et al., 2006). As a result of this high cost of production in the poultry sector, most of the livestock producers are shifting to other farm animals where the cost of production is low. The rabbit was identified to possess the potential of becoming an important source of animal protein with its ability to utilize forage and cheap feedstuff efficiently.

Ginger is an underground rhizome of the plant *Zingiber officinale* belonging to the family *Zingiberaceae*, it is considered a common constituent of diet worldwide (Bartley and Jacobs, 2000; Zhang et al., 2009). Medicinal herbs such as garlic (*Allium sativum*) and ginger (*Zingiber officinale*) have been reported to possess antibacterial, antiseptic, anti-inflammatory, anti-parasitic and immunomodulatory properties and are used for treatment of disorders of the gastrointestinal tract such as indigestion, constipation, dyspepsia, nausea and vomiting as well as rheumatism and diabetes. (Muhammad et al., 2009; Zhao et al., 2011; Afzal et al., 2001; Zhang et al., 2009; Ogbuewu et al., 2014).

Ginger extract possesses anti-oxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals (Muhammad et al., 2009). Arshad et al. (2014) reported that numerous active ingredients are present in ginger including terpenes and oleoresin which are called ginger oil. Ginger also constitutes volatile oils approximately 1 to 3% and non-volatile pungent components oleoresin (Zick et al., 2008). The major identified components from terpene are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol (Hazan et al., 2012) and lipophilic rhizome extracts yielded potentially active gingerols which can be converted to shogaols and paradol (Arshad et al ., 2014). The extracts of ginger has been reported to have multiple pharmacological effects which include inhibition of prostaglandin, thromboxane and leukotrienes synthesis, inhibition of cold and platelet aggregation, cardio tonic effects, gastro-intestinal actions, thermogenic and antibiotic activities as well as digestive stimulants (Guyer, 2003). Studies have shown that ginger can be used as a natural growth promoter as it enhanced immune functions and favoured meat quality in animal (Okoye et al., 2006).

Serum biochemical analysis is used to determine the heart condition, liver and kidney functions as well as to evaluate protein quality and amino acid requirements in animals (Harper et al., 1999; Etim and Oguike, 2011). This study was carried out to determine the influence of graded levels of ginger rhizome meal on serum biochemical indices of New Zealand White rabbits and to compare this influence on their sex difference.

MATERIALS AND METHODS

Location of study

The experiment was conducted at the Rabbitory Unit of the Teaching and Research Farm of the Faculty of Agriculture, Abia State University, Umudike location near umuahia, Nigeria. This is within the south east Agro ecological zone of Nigeria and lies within latitude 5˚ 29' N and longitude 7˚ 33' E and at an altitude of 122 m (400 feet) above sea level. The area has an annual ambient temperature of 25 3˚C, relative humidity between 65 to 89%, annual rainfall 2000 to 2484 mm and the soil is sandy loamy with average pH 5.5 (Adiele et al., 2005).

Experimental animals and their management

Forty eight sexually matured male and female New Zealand White rabbits aged 7 to 8 months were used for this experiment. The rabbit bucks have an average weight of 1200.00 g whereas the rabbit does have an average weight of 1250.00 g. Each animal was housed in a clean, well ventilated single hutch of 50 x 50 cm^2 with wire mesh floor and wooden frames. They were acclimatize for 2 weeks and fed with commercial concentrate diet (Top Grower Mash) containing 16.00% crude protein and 2450 Kcal/kg metabolizable energy. Each hutch was tagged for easy identification. The animals were dewormed before the commencement of the experiment. The experimental period lasted for complete 30 days.

Processing of ginger rhizome meal

Fresh rhizomes of ginger (*Zingiberofficinale*) were sourced from Ogbete market in Enugu, Enugu State, Nigeria. The fresh ginger rhizomes were washed in water to remove dirt. Their sizes were reduced, sun dried, milled to fine powder, sieved using 2.5 mm test sieve and formulated into diet.

Experimental diets and design

Four experimental diets namely T_1, T_2, T_3 and T_4 were formulated. T_1 had no ginger rhizome meal hence serves as the control (0%) while T_2, T_3 and T_4 contained 5, 10 and 15% ginger rhizome meal respectively. Freshly cut forage consisting of *Panicum maximum, Pennisetum purpureum* and *Centrosema pubescens*was fed as basal diet. The animals were fed 700 g of forage and 1kg of concentrate twice daily. Water was provided *ad libitum* and multivitamin drugs were given to them through drinking water to boost their immunity against infections. The chemical composition of the forages and the nutrient composition of the experimental diet are shown in Tables 1 and 2 respectively. A randomized complete block design was used to assign the animals to the experimental diets such that there were 12 rabbits (6 bucks and 6 does) replicated three times with 4 rabbits (2 does, 2 bucks) per replicate on each diet. The rabbits were fed the test diets for 30 days after which blood samples were collected and evaluation carried out.

Blood collection and serum biochemical evaluation

Four (4) rabbits (2 does, 2bucks) from each replicate, making a total of 12 rabbits (6 does, 6 bucks) per treatment were selected for bleeding. 3 ml of blood was

Table 1. Chemical composition of the forages (%).

Parameters	CP	PM	PP
Dry matter	73.38	85.30	73.69
Crude protein	25.20	9.79	7.87
Ether extract	3.34	1.20	2.59
Ash	7.89	8.10	8.51
Crude fibre	8.10	8.70	8.50
Nitrogen free extract	33.53	41.60	42.40
ME (MJ/Kg DM)	0.93	1.46	1.20

PM, *Panicum maximum,* PP, *Pennisetum purpereum,* CP, *Centrosema pubescens,* ME, metabolizable energy, MJ/Kg, DM, mega joule/kilogram dry matter.

Table 2. Composition of experimental diets.

Ingredients	T_1	T_2	T_3	T_4
	0%	5%	10%	15%
Maize	40	40	40	40
Ginger meal	-	5	10	15
Wheat offal	15	10	5	-
P.K.C	14	14	14	14
S.B.M	22	22	22	22
Fish meal	2	2	2	2
Oyster shell	2.5	2.5	2.5	2.5
Bone meal	3.0	3.0	3.0	3.0
Methionine	2.5	2.5	2.5	2.5
Lysine	2.5	2.5	2.5	2.5
Vitamin / Premix	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5
Mycofix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated analysis				
Crude protein %	20.01	19.79	19.57	19.36
ME (Kcal / kg)	2,747.62	2,771.67	2,808.84	2,846.01

ME, Metabolizable energy, Kcal/Kg, kilocal/kilogram, PKC, Palm kernel cake, SBM, Soyabean meal.

aspirated from the marginal ear vein of each rabbit using needle and syringe for serum biochemical evaluation. They were transferred into Plain bottles and allowed to coagulate. The coagulated blood was subjected to standard methods of serum separation and the harvested sera were used for evaluation of serum biochemical parameters: urea, cholesterol and glucose were determined according to the methods described by Baker and Silverton (1986). Aspartate transaminase, Alanine transaminase and Alkaline phosphatase activities were determined using spectrophotometric method as described by Rej and Hoder (1983). The standard flame photometry method using Gallenkamp analysis was used to determine serum calcium.

Data Analysis

Data obtained on serum biochemical indices of the male and female rabbits were subjected to analysis of variance (ANOVA) with Dunnett's control test using the SPSS (1996) package. Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (2002). Statistical differences between sexes were determined by students't-test. The analysis is considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The results of serum biochemical parameters of New

Table 3. Serum biochemical parameters of New Zealand white rabbits fed graded levels ginger rhizome meal.

Parameters	Dietary Levels (%)				SEM
	T_1	T_2	T_3	T_4	
	0.0	5.0	10.0	15.0	
Urea (mmol/l)	12.42[a]	12.42[a]	12.04[a]	10.34[b]	0.41
AST (iu/l)	24.68[c]	34.90[ab]	38.96[a]	26.89[bc]	2.98
ALT (iu/l)	31.35[a]	24.13[b]	28.89[a]	28.96[a]	1.31
ALP (iu/l)	48.12	56.30	44.25	51.38	7.77
Cholesterol (mg/dl)	2.13	2.46	2.63	1.47	0.33
Glucose (mmol/l)	102.22[b]	139.83[a]	110.45[b]	91.15[b]	8.33
Calcium (mmol/l)	14.22[a]	13.54[a]	13.23[a]	11.99[b]	0.38

[a,ab,b,bc,c]:Means within the same row with different superscripts are significantly (P<0.05) different. SEM, Standard Error of means, ALP, Alkaline phosphatase, ALT, Alanine transaminase, AST, Aspartate transaminase.

Zealand White rabbits fed graded levels of ginger rhizome meal are shown in Table 3. There were no significant differences (P>0.05) among the treatment groups in the mean values of Alkaline phosphatase (ALP) and cholesterol, but there were significant differences (P<0.05) among the treatment groups in the mean values of urea, Aspartate transaminase (AST), Alanine transaminase (ALT), glucose and calcium

Urea values obtained in this study ranged from 19.34 to 12.42 mmol/l. Rabbits on T_1 and T_2 had the highest urea value of 12.42 mmol/l and this differed significantly (P<0.05) from rabbits on T_4. There were no significant differences (P>0.05) among rabbits on T_1, T_2, and T_3 in urea values. The least value of 10.34 mmol/l in urea was observed in rabbits on T_4.. The result followed a decreasing trend from T_1 to T_4. The result of this study agreed with the normal reference range of 10.00 to 33.00 mmol/l reported by University of Pennsylvania School of Veterinary Medicine (2002) and Merck (2012). The normal blood urea values obtained in this trial were indication that the amino acid of the test diets was balanced. It has been reported that serum urea content depends on both the quantity and quality of protein supplied in the diet (Iheukwumere and Herbert, 2002).

Aspartate transaminase (AST) values ranged from 24.68 to 38.96 iu/l. Rabbits in T_3 had the highest value of 38.96 iu/l and this differed significantly (P<0.05) from rabbits on T_1 and T_4 which were similar (P>0.05) to each other in AST value. Rabbits on T_4 were similar (P>0.05) to those on T_2 in AST value. Similarly, there was no significant difference (P>.05) between rabbits on T_3 and T_2 in AST values. The result followed an increasing trend from T_1 to T_3 and later decreased significantly in T_4. The values of AST obtained in this study were within the normal range of healthy rabbits 10 to 120 iu/l as reported by University of Pennsylvania School of Veterinary Medicine (2002). However, the values of AST obtained in this study were higher than the range of 7.73 to 16.0 iu/l reported by Ahamefule et al., (2006). They fed weaner rabbits sundried, ensiled and fermented cassava peel based diets. This disparity may not be unconnected to age and nutritional status of the rabbits. Analysis of liver enzymes (AST, ALT, and ALP) activities give valuable diagnostic information for a number of disease conditions.

Alanine transaminase (ALT) values ranged from 24.13 to 31.35 iu/l. The highest value of 31.35 iu/l was recorded in rabbits on T_1 and this differed significantly (P<0.05) from rabbits on T_2 .There were no significant differences (P>0.05) among rabbits on T_1, T_3 and T_4 in ALT values. The least ALT value of 24.13 iu/l was observed in rabbits on T_2. The values of ALT obtained in this study agreed with the normal range of 10.00 to 45.00 (iu/l) reported by Merck (2012). However, the values of ALT obtained in this study disagreed with the range 9.17 to 12.80 iu/l reported by Ahamefule et al. (2006), they fed rabbits with sundried, ensiled and fermented cassava peel based diets. The normal value of ALT obtained in this study indicates that the activities of osteoblast were not affected because the blood level of ALT is usually a good indicator of the rate of bone formation.

Alkaline phosphate (ALP) values ranged from 44.25 to 56.30 iu/l. The highest numerical value of 56.30 iu/l was recorded in rabbit on T_2 followed by T_4 (51.38 iu/l), T_1 (48.12 iu/l) and T3 (44.25 iu/l) which had the least value in ALP. The values of ALP obtained in this study agreed with the range of 17.00-192.00 (iu/l) reported by (Merck, 2010). The ALP values obtained in this study did not follow any given pattern. Moreso, the values obtained differ with the range 5.92 to 8.38 iu/l reported by Ewuola et al. (2010), who fed weaner rabbits using dietary prebiotics and probiotics. The disparity may not be unconnected with the differences in physiological factors of these rabbits. Alkaline phosphatase assay is useful in the diagnosis of obstructive liver diseases (Murray et al., 2003). The liver is the organ involved in the detoxification of xenobiotics and other harmful chemicals that gain entrance into the body (Murray et al., 2003). An increase in alkaline phosphatase, alanine transaminase and aspartate transaminase values would signify necrosis or

Table 4. Effect of sex on serum biochemical parameters of New Zealand white rabbits fed graded levels of ginger rhizome meal.

Parameters	Male	Female	SEM
Urea (mmol/l)	12.66[a]	10.94[b]	0.28
AST (iu/l)	32.98	29.74	2.11
ALT (iu/l)	31.31[a]	25.36[b]	0.93
ALP (iu/l)	52.91	47.11	5.49
Cholesterol (mg/dl)	1.88	2.46	0.23
Glucose (mmol/l)	138.19[a]	83.63[b]	6.22
Calcium (mmol/l)	13.07	13.42	0.27

[a,b]:Means within the same row with different superscripts are significantly (P<0.05) different. SEM, Standard Error of means. ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphates.

myocardial infarction which are all indicators of drug toxicity or harmful chemicals in the body (Nelson and Cox, 2005).

Serum cholesterol values ranged from 1.47 to 2.63 mg/dl. Rabbits on T_3 recorded the highest numerical value of 2.63 mg/dl in serum cholesterol followed by T_2 (2.46 mg/dl), T_1 (2.13 mg/dl) and T_4 which had the least value of 1.47 mg/dl in serum cholesterol. The values increased progressively from T_1 to T_3 and got reduced at T_4. As dietary levels of ginger increased from 5 to 10%, the cholesterol value increased. Mufwa et al. (2011) and Mohammed et al. (2014) reported higher values (33.12 to 40.00 mg/dl) and (48.44 to 49.11 mg/dl) respectively. The values of cholesterol obtained in this study were slightly above the range of 0.30 to 2.10 reported by Merck (2012). It could be that ginger rhizome meal (5 to 10%) increased metabolism and efficient utilization of fat that led to increased cholesterol values in the serum. Cholesterol in the serum has been associated with the quantity and quality of fat supplied in the diet (Esonu et al., 2001).

Glucose values ranged from 91.15 to 139.83 mmol/l. Rabbits on T_2 recorded the highest glucose value of 139.83 mmol/l and this differed significantly (P<0.05) from rabbits on T_1 (102.22 mmol/l), T_3 (110.45 mmol/l) and T_4 (91.15 mmol/l) which were similar (P>0.05) to each other in glucose values. The glucose values obtained in this study did not agreed with the findings of Ewuola et al. (2012) who reported a higher glucose range of 114.00 to 142.00 mmol/l when fed rabbit with graded levels of *Moringa oleifera* leaf meal. The result agreed with the range of 80 to 150 mmol/l reported by Mufwa et al. (2011) and University of Pennsylvania School of Veterinary Medicine (2012). Glucose is one of the metabolites measured as an indicator of the energy status of animals. Normal glucose levels in rabbits indicate adequate synthesis in the liver from propionate metabolism as the major glucose precursor (Sowande et al., 2008). The study also indicates that there was no wasting or catabolism of muscle tissue and that rabbits were not surviving at the expense of body reserve.

Serum calcium values ranged from 11.99 to 14.22 mmol/l. Rabbits on T_1 had the highest value of 14.22 mmol/l in serum calcium and this differed significantly (P<0.05) from rabbits on T_4 which had the lowest value of 13.23mmol/l. There were no significant differences (P>0.05) among rabbits on T_1 T_2, and T_3 in serum calcium values. The values decreased progressively from T_1 to T_4. The serum calcium values obtained in this study agreed with the result of Mohammed et al. (2014) in rabbits, and were within the normal calcium range (8.00 to 15.50) reported by University of Pennsylvania School of Veterinary Medicine (2002). However, calcium values obtained in this study were higher than the range of 3.01±0.09 to 2.50±3.72 reported by Ozkan et al. (2012) in New Zealand White rabbits. The serum calcium plays a vital role in bone formation and structure as they constitute the major mineral content that helps in the formation and strengthening of bones. It also mediates in excitation and contraction of muscle fibre (Banerjee, 2007).

The results of serum biochemistry of male and female New Zealand White rabbits fed graded levels of ginger rhizome meal are shown in Table 4. There were significant differences (P<0.05) between the treatment groups in urea, ALT and glucose. Conversely, there were no significant differences (P>0.05) between the treatment groups in AST, ALP, cholesterol and calcium. The results also revealed that all the serum biochemical indices of the male rabbits were higher than the female rabbits except for cholesterol and calcium. The result agreed with the range reported by University of Pennsylvania School of Veterinary Medicine (2002), Ozkan et al. (2012) and Merck (2012). Biochemical components are sensitive to elements of toxicity and also can be used to monitor protein quality of feeds. It also signifies proper functioning of some internal organs.

Conclusion

The results of this study have shown that ginger rhizome

meal can bring about improvement of serum biochemical parameters of the pubertal male and female rabbits at the inclusion level of 5%.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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