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Effect of dietary inclusion of *Pleurotus tuber-regium* on performance and intestinal morphology of growing rabbits

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***Target Audience:** Animal Nutritionists, Rabbit farmers, Animal Scientist*

Abstract

The Pleuroteus mushroom has immense growth and health promoting potentials. However, reports on its utilization in the diet of the rabbit is sparse. Therefore, a 56-day feeding trial was conducted to investigate the effects of dietary inclusion of Pleurotus tuber-regium sclerotium powder (PTRSP) in growing rabbits. Forty–eight crossbred rabbits aged between 7 and 9 weeks with average initial weight of 700±25 g were used for the study. The rabbits were allotted to 4 experimental diets containing 0.0, 25.0, 50.0 and 75.0 g/kg PTRSP, respectively. Each treatment had 4 replicates with three rabbits each in a completely randomised design. Growth performance, serum biochemistry and intestinal morphology were determined. The weight gain and feed conversion ratio were better (P<0.05) in rabbits fed diet containing 50.0g/kg PTRSP. Rabbits fed diet containing 75.0 g/kg PTRSP had lower (P<0.05) serum cholesterol, creatinine and alanine transaminase and higher (P<0.05) total glucose concentration compared to those receiving other experimental diets. Rabbits fed diet with 50.0g/kg PTRSP had higher (P<0.05) caecal apical width than those fed diet containing 0.0 g/kg. Inclusion of 50.0 g/kg of PTRSP in the diets of rabbits is beneficial for improved growth, cholesterol metabolism and intestinal morphology without any adverse effects.

***Key words:** Mushroom; Growth; Gut morphology; Serum measurements*

Description of Problem

Rabbit is an important livestock specie kept as pet and laboratory model worldwide. Under intensive system of management, rabbits are usually fed concentrates diets that are usually too low in fibre and too high in protein, fat and carbohydrate. Movement is also largely restricted. Lack of sufficient fibre in diet, combined with the stress of restricted movement, has been identified as a significant cause of morbidity and mortality in young rabbits (1). Many of the diseases commonly seen in rabbits can be directly attributed to, or associated with, the feeding of an inappropriate diet and this could be largely prevented (2). Thus, feeding an appropriate diet to rabbits under intensive system of management is critical to the growth and health status of the animal.

The fruiting body of *Pleuroteus tuber regium* (PTR) is rich in protein, while sclerotium is rich in fibre and non-starch polysaccharides mainly β-glucans which has pharmacological activity (3-4). The sclerotium of PTR contains a good amount of protein (12-18%), carbohydrate (63-65%), fat (1-4%) and fibre (0.25-10%). It is also a rich source of Potassium, calcium, magnesium, sodium and phosphorous (ranging from 0.028 to 0.223 %), vitamins and phytochemicals (5). It also contains essential micronutrient such as manganese and cobalt and cadmium (6). The essential nutrients in PTR exerts antioxidant hypocholestrolaemic, anti-lipidaemic, and

antihyperglycaemic properties (7,8,9). The PTR mushrooms are considered as functional foods or nutraceuticals because they provide both health and nutritional benefits and may exert immense growth promoting and health improving potentials in livestock animals. The reports of different researchers (10,11,12,13) indicated that the inclusion of edible mushrooms in the diet may result in increased body weight, improved feed conversion ratio and gut morphology, lowered total serum cholesterol and exert antioxidant-protective activity in different livestock specie. However, reports on the utilization of PTR in rabbits are rare; hence this study seeks to investigate the impact of dietary inclusion of PTRSP on growth performance, intestinal morphology and some serum biochemical parameters in growing rabbits.

Materials and methods

Cultivation of *Pleurotus tuber-regium* and processing of *Pleurotus tuber-regium* sclerotium powder

The sclerotia of PTR grown on rice husk and sawdust substrate were obtained from the mushroom unit of the Bioresources Development Centre, Isanlu, Kogi state, Nigeria. Harvested sclerotia were sun dried for 3 days, chopped into small pieces and milled into fine powder (less than 1 mm in particle size) using a Binatone dry blender. A portion was removed for chemical analyses while the rest was used for the feeding trial.

Experimental diets

Four experimental diets containing varying inclusion levels of PTRSP were formulated to meet the nutritional requirement for growing rabbits (14). Diet 1 contained 0 g kg^{-1}, while diets 2, 3 and 4 contained 25.0, 50.0 and 75.0 g/kg PTRSP, respectively. The chemical composition of the PTRSP used to formulate the diets and ingredient and proximate and mineral composition of the four experimental diets used in this study are presented in Tables 1 and 2 respectively.

Determination of chemical composition of PTRSP and experimental diets

The parameters determined for proximate analyses include ash, moisture, crude protein, fat, fibre and carbohydrate. All of these were determined following the methods described by AOAC (15). Total calcium and magnesium contents were determined by EDTA versanate complexometric titration method as described by Harbourne (16) while sodium and potassium contents were determined by flame photometry as described by Onwuka (17). Other metal contents (Zn, Fe, Cu and Mn) were determined by the atomic absorption spectrophotometric technique as described by Onwuka (2005). Phytochemical analysis was done to determine total alkaloids, saponins and flavonoids using the gravimetric method (18).

Experimental animals and experimental design

Forty-eight healthy, cross-bred 7-9 weeks old weaner rabbits with an average initial weight of 700±25 g was obtained from the animal house of Bioresources Development Centre Idofin-Isanlu, Kogi state, Nigeria. They were housed in wire mesh cages (3 rabbits per hutch) and allowed seven days of acclimatisation to cage. The rabbits were allotted on weight equalisation basis to the experimental diets 1, 2, 3, and 4 containing 0, 25.0, 50.0 and 75.0 g/kg PTRSP, respectively. Each dietary treatment was replicated 4 times with each replicate having 3 rabbits in a completely randomised design. Feed and water were offered to the rabbits *ad libitum*. The feeding trial lasted for 56 days In line with the ethics regulations stated in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of

Health, all the rabbits received humane care during the experiments (19).

Data collection

Data was collected daily on feed intake per pen, weight gain was monitored weekly and mortality records were kept. On the 56th day of the feeding trial blood samples from 2 rabbits per replicates were collected into tubes without anticoagulants for the analysis of serum biochemical parameters. Blood samples were centrifuged at 3,000 rpm for 10 minutes to obtain the serum which was stored at 4^{0} C. Total protein (TP), Albumin, globulin, total cholesterol, triglycerides high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine were determined using standardised procedures of the commercial Randox kit (20).

Histomorphological parameters

On the 56th day of the feeding trial, animals were slaughtered after their weights were taken. The heart, stomach kidney, spleen, and liver of the rabbits were harvested and weighed. The percentage weights of the harvested organs relative to the carcass were determined. Small pieces of the caecum, duodenum and liver tissues were collected in 10% formaline buffer for proper fixation. The tissues were embedded in paraffin wax, histological sections of 5–6 μm in thickness were made and stained with hematoxylin and eosin for examination (21).

Statistical analysis

Data collected were laid out in a completely randomised design and subjected to a one-way Analysis of Variance using general linear model (GLM) procedures within SAS software of SAS (22). Statistical differences amongst means were separated using Duncan Multiple Range Test and significant means were separated at 95% confidence interval.

Results

Table 1 shows the percentage chemical composition of *Pleuroteus tuber regium* sclerotium powder. Macro-minerals present in PTRSP were; Calcium, Magnesium, Potassium, Sodium, Iron and Phosphorous while trace elements detected included; Copper, Zinc and Manganese. Antinutrients present in the PTRSP were; phytate, tannin, cyanide and alkaloid.

Data on some performance characteristics of rabbits fed diets containing varying inclusion level of PTRSP are presented on Table 3. There were significant differences ($P<0.05$) in the final live weight (FLW), weight gain, feed intake, feed conversion ratio, cost of feed per kg gain, in rabbits fed the various experimental diets. Rabbits fed diet containing 50.0 g/kg PTRSP had higher ($P < 0.05$) live weight, weight gain, and lower ($P < 0.05$) FCR compared to rabbits on the control diet and other dietary treatments. Values of feed intake and FCR were improved ($P < 0.05$) following increasing dietary inclusion of PTRSP (up to 50.0 g/kg). The cost of feed significantly ($P<0.05$) increased with increasing dietary inclusion of PTRSP however, cost of feed per kg weight gain in rabbits fed diets containing 25.0 and 50.0 g/kg PTRSP were comparable to those fed the control diet while those of rabbits fed 50 g/kg PTRSP diet was lower.

Table 1: Chemical composition of *Pleurotus tuber regium* sclerotium powder

Proximate composition (% dry matter)	
Moisture	13.5
Crude Protein	7.28
Crude fat	0.91
Crude fibre	9.58
Ash	3.60
Cabohydrate	65.57
Mineral composition (%dry matter)	
Calcium	0.458
Magnesium	1.32
Potassium	5.12
Sodium	0.843
Copper	0.0415
Zinc	0.0163
Manganese	0.00172
Iron	0.275
Phosphorous	4.15
Anti-nutritional compositions (% dry matter)	
Phytate	0.027
Tannin	10.06
Cyanide	0.77
Alkaloid	3.3

Table 2: Ingredient and proximate compositions of experimental diets

	Treatments and PTRSP inclusion levels			
Ingredient (g/kg)	1 (0.0)	2 (25.0)	3 (50.0)	4 (75.0)
PTRSP	0.00	25.0	50.0	75.0
Maize offal	100.0	75.0	50.0	25.0
Maize	310.0	310.0	310.0	310.0
Full fat Soya	100.0	100.0	100.0	100.0
Groundnut cake	50.0	50.0	50.0	50.0
Wheat offal	300.0	300.0	300.0	300.0
Rice husk	100.0	100.0	100.0	100.0
Oyster shell	10.0	10.0	10.0	10.0
Bone meal	20.0	20.0	20.0	20.0
Premix	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0
Total	1000	1000	1000	1000
Proximate composition (%Dry matter)				
Crude protein	14.3	14.0	13.9	135
Crude fat	5.14	5.07	5.00	4.93
Crude fibre	9.47	9.25	9.03	8.80
Oyster shell	10.0	10.0	10.0	10.0
Bone meal	20.0	20.0	20.0	20.0
Premix	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0
Total	1000	1000	1000	1000
Proximate composition (% Dry matter)				
Crude protein	14.3	14.0	13.9	135
Crude fat	5.14	5.07	5.00	4.93
Crude fibre	9.47	9.25	9.03	8.80
Calcium	0.01	0.01	0.01	0.01

Table 3 : Performance of growing rabbits fed diets containing *Pleuroteus tuber regium* sclerotium powder

	Experimental diets					
Parameter	1	2	3	4	SEM	P-value
Initial weight (g)	715	718	723	723	2.28	0.69
Final live weight (g)	1175^{b}	1077^{b}	1531^{a}	1182^{b}	50.3	0.001
Total weight gain (g)	460^{b}	359^{b}	808^{a}	459^{b}	51.1	0.002
weight gain (g/day)	8.21	6.41	14.43^{a}	8.20	0.85	0.002
Total Feed intake (g)	4270^{a}	2609^{b}	4027^{a}	3635^{a}	167	0.000
Feed Conversion ratio	9.26^{b}	7.27^{b}	4.98^{a}	7.92^{b}	2.13	0.005
Cost/kg feed(₦/kg)	84.4^{d}	85.9^{c}	87.4^{b}	88.9^{a}	0.35	<0.001
Cost of feeding (N/rabbit)	360^{a}	224^{b}	352^{a}	323^{a}	14.4	<0.001
Cost of feed ₦/kg (1000g) gain	638^{a}	684^{a}	458^{b}	762^{a}	37.3	0.018

^{abc} means along the same row with different superscripts are significantly different at (P<0.05), SEM: Standard error of mean
₦ = naira

Table 4: Effects of dietary *Pleuroteus tuber regium* sclerotium powder on serum biochemical measurements in growing rabbits

	Experimental diets					
Parameter	1	2	3	4	SEM	P-value
Total protein (g dl^{-1})	9.02^{a}	8.17^{ab}	7.58^{b}	7.12^{b}	0.25	0.003
Albumin (g dl^{-1})	5.47^{a}	5.11^{ab}	4.47^{b}	4.31^{b}	0.16	0.062
Globulin (g dl^{-1})	3.46	3.06	3.11	2.81	0.13	0.115
Total cholesterol (mg dl^{-1})	66.4^{ab}	76.6^{a}	71.3^{a}	60.1^{b}	2.07	0.019
Triglyceride (mg dl^{-1})	66.1^{ab}	69.1^{ab}	72.3^{a}	61.4^{b}	1.64	0.420
High density lipoprotein (mg dl^{-1})	38.7^{b}	49.7^{a}	42.8^{ab}	34.4^{b}	1.95	0.020
Low density lipoprotein (mg dl^{-1})	14.4	13.1	14.0	13.5	0.33	0.542
Very Low density lipoprotein (mg dl^{-1})	13.2^{ab}	13.8^{ab}	14.5^{a}	12.3^{b}	0.33	0.060
Glucose (g dl^{-1})	117^{b}	118^{b}	113^{b}	131^{a}	1.84	0.002
Creatinine (µl^{-1})	1.18^{b}	1.46^{a}	1.22^{b}	0.84^{c}	0.05	<0.001
Aspartate aminotranferase (µl^{-1})	52.1^{ab}	58.4^{a}	50.0^{b}	52.7^{ab}	1.27	0.055
Alanine aminotranferase (µl^{-1})	49.7^{a}	45.0^{b}	47.7^{ab}	37.3^{b}	1.63	0.015
Alkaline Phosphatase (µl^{-1})	73.6^{a}	71.0^{ab}	62.3^{b}	71.8^{ab}	7.76	0.062

^{abc} means along the same row with different superscripts are significantly different at (P<0.05), SEM: Standard error of mean N= naira

Table 4 presents the result on serum biochemical indices of the rabbits fed experimental diets. A decrease (P < 0.05) in the serum TP was observed following increasing dietary levels of PTRSP. Rabbits fed diets containing Increasing levels of PTSP (up to 50.0 g/kg) in the diet had higher (P<0.05) serum total cholesterol and HDL compared to those on the control diet and 75.0g/kg. Highest (P < 0.05) glucose concentration and lowest (P < 0.05) creatinine value were recorded in rabbits fed diets containing 75.0 g/kg PTRSP. Rabbits fed diets containing varying inclusion levels of PTRSP (up to 75.0 g/kg) showed reduced (P < 0.05) alanine aminotransferase in comparison to those on control diet.

The relative organ weights and histomorphological measurements taken from the rabbit are presented in Table 5. Inclusion of PTRSP did not affect ((*P* >0.05) the weights of organs except those of liver, spleen and duodenum. Rabbits fed diet containing PTRSP (up to 75.0 g/kg) had smaller liver and duodenum weight compared to those on control diet with no PTRSP. The caecal apical

width of rabbits on 50.0g/kg was higher (P<0.05) than those of other dietary treatments. Caecal basal width of rabbits on 75.0 g/kg was same as that of the control while those on 25 and 50.0 g/kg was lower (P<0.05), Caecal Laminal propial depth of rabbits with dietary PTRSP was lower (P<0.05) than the control.

Table 5: Histomorpholgical measurements in rabbits fed diets containing Pleuroteus tuber-regium sclerotia powder

	Experimental diets					
Parameter	1	2	3	4	SEM	P-value
Live weight (g)	1204	1223	1308	1204	25.6	0.320
Relative weights of organs (%)						
Kidney	0.59^{a}	0.59^{a}	0.79^{a}	0.56^{b}	0.002	0.561
Liver	3.41^{a}	2.43^{b}	2.50^{b}	2.15^{b}	0.15	0.001
Spleen	0.04^{b}	0.07^{a}	0.05^{a}	0.03^{b}	0.003	0.040
Heart	0.25	0.26	0.29	0.24	0.007	0.871
Stomach	2.50^{ab}	2.53^{ab}	2.05^{b}	3.48^{a}	0.21	0.180
Duodenum	2.39^{a}	1.37^{b}	1.48^{b}	1.15^{b}	0.12	0.015
Small intestine	1.66	2.19	1.67	2.16	0.11	0.252
Caecum	7.82^{ab}	8.96^{a}	8.12^{ab}	7.13^{b}	0.32	0.189
Caecum *(μm)*						
Villi height	700^{b}	635^{c}	850^{a}	825^{a}	46.0	0.044
Apical width	70.0^{b}	65.0^{b}	340.0^{a}	60.0^{b}	48.3	0.049
Basal width	250^{a}	145^{c}	230^{b}	250^{a}	16.2	0.042
Laminal propial depth	50.0^{a}	45.0^{ab}	35.0^{c}	40.0^{bc}	1.90	0.002
Duodenum*(μm)*						
Villi height	400	480	425	480	32.9	0.588
Apical width	35.0	40.0	45.0	40.0	2.82	0.486
Basal width	70.0	95.0	90.0	90.0	6.60	0.403
Laminal propial depth	45.0	65.0	80.0	85.0	20.2	0.051

^{abc} means along the same row with different superscripts are significantly different at (P<0.05), SEM: Standard error of mean

Discussion

The chemical composition of the PTRSP used in the current study revealed that they are as nutritive as those reported by earlier researches (23-24). The crude protein content compares well with those of PTRS reported by (25) and those of conventional feed ingredients such as maize (7-9%) and maize bran (8-10%). Carbohydrate yield of 655.7 g kg^{-1} DM is also higher than values (568.0, 221.0 g kg^{-1} DM) reported earlier (5, 25-26). These differences in observation may be attributed to the source of PTR and different growth medium used in the different studies. The PTR used for this study was cultivated and harvested fresh from our laboratory while those in the former study were grown in the open field and purchased from the market. The crude fibre content (95.8 g kg-^{1}) was higher than values of PTRSP reported by Ikewuchi and Ikewuchi, (5), and those of maize, soya bean meal. It compares well with those of commonly used fibre sources in rabbit's diet such as maize bran (90. 0 g kg-^{1}) and wheat offal (85. 0 g kg-^{1}). The chemical profile suggests that the incorporation of *P. tuber-regium* sclerotia in diet could be beneficial in improving growth rate, aiding bowel movements as well as reducing digestive disorders and incidence of enteritis and diarrhoea in rabbits (27-28). Low crude fat contents seen in Table 2 are in sync with the observation of Akindahunsi and Oyetayo (26) and value obtained was lower than those of most conventional feed ingredients in rabbit feed production. Macrominerals (K, Ca, Mg, Na, P) content of

the PTR was higher than those reported by (24) This variation in macromineral contents could be attributed to differences in cultivation medium, as well as the methods of processing and analysis (29). The result of anti- nutrient composition revealed low value of tannins, phytate and hydro cyanide in proportions not high enough to constitute human/animal poison (30).

Improved growth response in terms of live weight, weight gain, feed intake and feed conversion ratio observed in rabbits fed diet containing 50.0 g/kg PTRSP over those on other dietary treatments (0.0, 25.00 and 75.0 g/kg PTRSP). These observations thus suggest that this inclusion levels are beneficial as growth promoter to the rabbits. Decline in feed intake in rabbits placed on diet 4 may suggest increasing dietary levels of anti-nutrients. Anti-nutrients have been reported to reduce feed acceptability by binding feed protein to the salivary gland and epithelium of the mouth (30). Observations from cost benefit analysis suggests that inclusion of PTRSP in the diet of rabbit does not lead to an increase in cost of production and inclusion of 50 g/kg PTRSP may result in a lowering of the cost of production.

All serum biochemical parameters measured in this study (with the exception of albumin concentrations in rabbits fed diets 1 and 2) fell within the range of normal reference values for rabbits as reported by (32) Lower total cholesterol observed in rabbits fed diet containing 75.0 g/kg PTRSP compared with those on other dietary treatments and in albino mice [24]. Higher HDL values observed in rabbits fed with 25.0 g/kg and 50.0 g./kg compared to those on control and 75.0 g/kg suggests that the former levels of inclusion of PTRSP is more beneficial with respect to improving cholesterol metabolism in the rabbits. Higher glucose levels seen in rabbits fed with 75.0 g/kg PTRSP compared to those on other dietary treatments may be an indication of improved carbohydrate utilisation as values were within reference range for rabbits (26, 27). Comparable creatinine values in rabbits fed with 75.0 g/kg PTRSP. These observations seem to indicate diet 3 (50 g/kg) did not impart any oxidative stress in the kidney and an increasing level of dietary mushroom to 75.0 g/kg might have helped in combating renal oxidative stress. No differences were seen in the activities of AST and ALP amongst the treatment groups. These observations thus suggest that the various inclusion rates of PTRSP did not pose any health challenge or trigger any damage in the liver and the kidneys of the rabbits (32). Lower values found in rabbits fed with varying levels of PTRSP in comparison to the control suggests that the levels used in this study were optimal for the health of the rabbits

Comparable relative weights of heart, stomach, small intestine and kidney are in agreement with the observations of (24) who found that dietary inclusion of PTR did not affect the organ weight of albino mice. The influence of dietary iinclusion of PTRSP on liver weight runs contrary to the findings of (24) who found no difference in liver weights of rats fed diets incorporated with 5 and 10% PTR. Differences in observation may be due to the fact that rabbits in the current study were fed lab-cultivated PTRSP while rats in the former study were fed naturally grown PTRSP purchased from the market. Since the spleen filters out microorganisms, food directly impacts the spleen. And changes in the spleen weight often reflect alterations in the immune system. An increase in spleen weight often suggests the presence of anti-proteolytic substances in the diets (33). Hence, reduced spleen weight following increasing inclusion levels of PTRSP in the current study suggests the absence or reduction of anti-proteolytic substances and consequentially an improved immune system. The trend of reduction in liver and spleen weight seen in rabbits of PTRSP

diet indicates that diets influencing liver also affect spleen health (34)

The villi of the absorptive epithelium play key roles in the final stages of nutrient digestion and assimilation (35). Rabbits fed with increasing PRTSP levels (50.0 and 75.0 g/kg) had higher caecal and duodenal villi height in comparison with the control. This observation thus suggested increased surface area capable of greater absorption of available nutrients (36) in theses group of rabbits. Earlier research works (37-38) have demonstrated that inclusion of phytogenic substances in the diets of livestock animals may lead to an improvement in the gut functions causing increased, unchanged or decrease intestinal segments. Thus enhanced development of gut (seen as increased caecal villi height and apical) following dietary inclusion of varying levels of PTRSP in the current study could imply greater efficiency in the utilization of feed as a result of increasing enzyme digestion and surface area for absorption of nutrients (38).Thus the results of the current study are in line with those of (39) who reported that dietary supplementation of 0.2 g/kg pure curcumin derived from turmeric in a corn-soybean based diet affected villi height and width of the small intestine in Arbor acre broiler chickens Consequently, changes in intestinal morphology can explain the observed beneficial effects on productivity and feed conversion ratio in this study

Conclusion and Applications

1. Inclusion of 50.0 g/kg PTRSP in the diet of growing rabbit had positive effects on the growth performance, cholesterol metabolism and intestinal morphology compared to those fed 0.0, 25.0 and 75.0 g/kg PTRSP diets.
2. Dietary Inclusion of 75.0 g/kg PTRSP improved glucose metabolism and reduced serum creatinine
3. The inclusion of varying levels of PTRSP (up to 75.0 g/kg) lowered alanine aminotransferase in comparison to those on control diet.
4. It is therefore concluded that dietary inclusion of PTRSP (up to 75.0 g/kg) does not impart any adverse effects on the growing rabbits and its use should be further exploited by farmers and nutritionists

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