Effect of oral administration of *Pleuroteus tuber-regium* extract on growth, serum metabolites and histomorphological changes in growing rabbits

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Abstract

The effects of oral administration of aqueous extract of *Pleurotus tuber-regium sclerotium* (PTRSE) in drinking water on growth, serum analytes and histomorphology parameters were examined using 72 cross bred growing rabbits. Eighteen rabbits each were allotted to 4 treatments with; 0, 500, 750 and 1250 mg PTRSE per liter (mg/l) in drinking water per day. The experiment lasted for 60 days. Data collected were subjected to one-way analysis of variance (ANOVA). The results revealed an improvement in the live weight ($P < 0.01$), weight gain (Linear (L), Quadratic (Q): $P < 0.01$), feed conversion ratio (L, Q:P < 0.01), water intake (L, Q:P < 0.01) and extract intake (L, Q:P < 0.01) following increasing levels of PTRSE in drinking water. Oral application of 750 mg/l PTRSE in drinking water resulted in reduced ($P < 0.01$) serum total cholesterol, low-density lipoprotein, very low-density lipoprotein, creatinine and alkaline phosphatase and increased cecal villi height (L, Q:P < 0.05) and apical width (Q:P < 0.05). Pathological indicators suggested damages to hepatic tissues in rabbits on 1250 mg/l PTRSE. In conclusion, the oral application of 750 mg/l PTRSE in drinking water can improve the growth, cholesterol profile and absorptive capacity in growing rabbits without imposing any detrimental effect.

Introduction

In West Africa, *Pleurotus tuber-regium* (PTR) is a mushroom specie of high economic value. It is in demands for culinary, medicinal and bioremediation purposes (Oyetayo 2011). Of all the parts of PTR, greater interest is placed on the sclerotium amongst the indigenous people. Researches (Huang et al. 2014; Nworu et al. 2014, Oghenemaro 2017) has shown that PTR sclerotium has hematinic, immunostimulant, anticancer, anti-inflammatory, hypolipidemic, hepatoprotective and antihyperglycemic properties Research has also proven its ability to promote the growth of beneficial bacteria, inhibit the proliferation of harmful microbes, enhance immunity and stimulate the growth and proliferation of the absorptive cells in the gut (Sethiya 2016).

The nutraceutical and growth promoting potential of PTR has been largely attributed to its phytochemical constituents and significant amount of non-starch polysaccharides (e.g β -glucan) found in its cell wall. Solvent extraction of raw plants is usually done to optimize the concentration of bioactive components (Aziz et al. 2003). However, researches have shown that the bioactivities of extract vary according to extraction methods used (Hayouni et al. 2007; Asif and Kaleem 2018). Many bioactive compounds have been identified in mushroom extracts and are available for use as dietary supplements (Jyotika et al. 2013). Hence this study was designed to investigate the effect of the aqueous extract of *Pleurotus tuber-regium sclerotium* (PTRSE) and its effects on growth and some physiological and biochemical parameters in growing rabbits.

Materials And Methods

Pleurotus tuber-regium extract preparation

Indigenous edible Nigerian *Pleurotus tuber-regium* was sourced from the Bioresources Development Centre Isanlu, Kogi State, Nigeria. The sclerotia's dark brown skin was removed while the white tissue was cut into small pieces and pulverized (> 0.5 mm) with a dry electric mill. An aqueous decoction of PTRSE was prepared according to the method described by Poonam et al. (2011). The PTR powder (PTRSP) was added into the water at a ratio of 1:15 w/v. The solution was allowed to cool and then filtered. The filtrate was concentrated and dried in a rotary evaporator maintained at 45 °C and stored in air-tight containers at room temperature.

Phytochemical screening of PTRSP and PTRSE

Samples of PTRSP and freshly prepared PTRSE were collected and screened for phytochemical constituents. Quantitative estimation of the total phenol was determined by the Folin – Ciocalteu reagent method as described by Malick and Singh (1980). Flavonoid concentration was determined and expressed as mg of rutin equivalent (Helmja et al. 2007). Alkaloid concentration was determined according to the procedure described by Harbone (1984). The tannin contents were determined using Folin Denis Reagent as described by Makkar et al. (1993).

Experimental animals and experimental design

This experiment was carried out at the Animal House of the Bioresources Development Centre, Isanlu, Kogi State, Nigeria. The rabbits were housed individually in separate hutches measuring 60 cm × 45 cm × 45 cm under controlled light cycle (12 h light/12 h dark). After a 7-day acclimatization period, 72 cross-bred rabbits with a weight range of 715 and 725 g were allotted on a weight equalization basis to 4 treatment groups. Eighteen rabbits each were allotted to 4 treatment groups; with 0, 500, 750 and 1250 mg PTRSE per liter (mg/l) in drinking water per day. The extract concentration used in the current study were based on the reports of Okolo et al. (2017) who established that no mortality or toxic signs occurred in Sprague Dawley rats orally administered 50–5000 mg kg⁻¹ b.w of *Pleuroteus tuber regium*. The required amount of concentrated PTRSE was measured and reconstituted in appropriate volume. Fresh reconstituted drinking water was offered in a pottery on a daily basis to the rabbits. Throughout the 60-day experimental period, the animals were maintained on concentrate ration, formulated to meet the nutrient requirement of growing rabbits (Table 1)

Data collection

Data was collected on feed intake per pen daily, weight monitored weekly, and mortality records kept. The left-over water was measured the next morning to determine the water and extract intake.

Blood Collection and Serum Chemistry analyses

On day 60 of the experiment, blood was collected from the ear and/or lateral saphenous veins of 9 rabbits per treatment (36 rabbits in all) for serum biochemical analyses. The activities of alkaline phosphatase (ALP), total protein, albumin, total cholesterol, triglycerides high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and blood urea nitrogen (BUN) were determined according to the methods described by Reitman and Frankel (1957). Commercial laboratory test kits (Randox laboratories UK) and a UV Vis Spectrophotometer (721 (D), PEC Medical, USA) was used to carry out these analyses.

Histomorphological parameters

On the 60th day of the experiment, a total of 24 rabbits comprising 6 rabbits per treatment, 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% formalin 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 gut measurements and histopathological examinations

Statistical analysis

Data collected were subjected to a one-way analysis of variance (ANOVA) test in a completely randomized design. Statistical analysis was carried out using the 9.1 version of the SAS software (SAS, 2000). A Duncan, multiple range test was done at 95% confidence interval to separate statistically significant means. Polynomial contrast (Linear (L), quadratic (Q) and cubic (C)) was also applied to determine the effect of dosage of PTRSE.

Results

Figure 1 shows the concentration of phytochemicals present in both PTRS powder and extract. Phytochemicals with antioxidant properties were detected in varying concentrations in both PTRSP and PTRSE. The phytate, tannin, alkaloid and phenol contents of the extract were higher than those of the powder. However, the flavonoid content of the extract was lower than that of the powder. Cyanide, an important antinutrient was also detected in minute amounts in in both powder and extract.

Table 2 shows the performance and serum biochemical parameters in the experimental rabbits. The live weight ($P < 0.01$) and weight gain (L, Q:P < 0.01) increased following increasing levels of extract in drinking water. In a similar vein, FCR reduced (L, Q:P < 0.01). The highest values for live weight and weight gain were recorded in rabbits administered 750 mg/l PTRSE while the least values were seen in the control group. Feed intake and FCR in rabbits on 750 mg/l were similar to those on 1250 mg/l PTRSE. A corresponding decline in feed intake (L:P < 0.05 , Q:P < 0.01), followed increasing levels of PTRS extract in drinking water. Water intake ($P < 0.05$) in rabbits on 500 mg/l and 750 mg/l PTRSE were higher than those of the control group. As the extract's levels in drinking water increased, extract intake increased (L, Q:P < 0.01). Percentage mortality in all treatments were less than 5%.

In comparison with the control and other treatment groups, serum albumin concentration was cubically lower ($P < 0.05$) while globulin was higher (L:P < 0.05 , Q:P < 0.01) in rabbits on 750 mg/l PTRSE. Serum total cholesterol in rabbits administered varying levels of extract in drinking water was higher ((L:P < 0.05 , Q:P < 0.01), Triglyceride was quadratically lower ($P < 0.05$), HDL and LDL were higher ($P < 0.01$) while VLDL was quadratically lower ($P < 0.05$) than those of the control group. An increase (L:P < 0.05 , Q:P < 0.01) in glucose concentration was observed following increasing levels of extract in drinking water and rabbits on 1250 mg/l PTRSE had the highest value while the control group had the least. Serum creatinine in rabbits on varying levels of PTRSE was cubically lower ($P < 0.05$) than those of the control and rabbits on 750 mg/l PTRSE had the lowest value. Serum ALT was quadratically lower ($P < 0.01$) in rabbits on 500 mg/l while ALP was lowest (L, Q:P < 0.01) in rabbits on 750 mg/l PTRSE when compared to those of control and other treatment groups.

Table 3 shows the relative organ weights and intestinal measurements in growing rabbits orally administered with aqueous extracts of PTRS in drinking water. The relative weight of kidney and cecum were cubically higher ($P < 0.01$) in T2 while those of small intestine were cubically lower ($P < 0.01$) in T3 when compared with the control and those allotted to other treatment groups. There were no differences ($P > 0.05$) in the relative weights of liver, spleen, heart, stomach and duodenum. Cecal weight in rabbits on 750 mg/l PTRSE was cubically higher ($P < 0.01$) than those on other treatment groups. Cecal villi height in rabbits on 750 and 1250 mg/l PTRSE was higher (L, Q:P < 0.05) while apical width in rabbits on 750 mg/l PTRSE was quadratically higher ($P < 0.05$) than those of the control group. A non-significant ($P > 0.05$) increase in duodenal villi height was also observed following increasing levels of extracts in drinking water.

Pathology indicators suggest the worst liver tissue damage occurred in rabbits on 1250 mg/l (Fig. 5). and the least occurred in those on 750 mg/l PTRSE (Fig. 3). The histopathological evaluation of the hepatic tissue samples was based on the pattern and severity of the injury. The experimental rabbits showed signs of non-alcoholic fatty liver disease (NAFLD) as seen with the different degrees of micro vesicular steatosis observed in rabbits on 0, 500 and 750 mg/l PTRSE (Fig. 2–5). There are no necrotic zones, necroinflammation, cholestatic patterns of liver injury in all the groups. However, a focal area of hemorrhagic lesion was seen in rabbits on 1250 mg/l PTRSE (Fig. 5).

Discussion

Higher phytochemical contents found in PTRSE in comparison to PTRSP is an indication that hot water extraction resulted in the optimization of the concentrations of these bioactive compounds. This in line with the reports of Jo et al. (2012). Lower flavonoid content in PTRSE is an indication that hot water extraction may have led to the degradation of this phytochemical. This observation agrees with the reports of Elhamirad and Zamanipoor (2012) who found that many antioxidants exhibiting high preventive activity at storage temperatures, rapidly break down and lose their effectiveness when exposed to elevated temperatures. Therefore, for optimization of flavonoid extraction, other formal extraction methods that do not involve elevated temperature should be explored. Antinutrients (tannin, cyanide and phytate) detected in both powder and extract occurred at minimal dose considered to be non-toxic to the rabbits.

The improved growth found in rabbits on PTRSE compared to control agrees with the report of Guo et al. (2004) who showed that mushroom extract increases body weight gain and lowers FCR in broilers. Decline in feed intake amongst rabbits administered PTRSE suggest that PTRSE is an appetite suppressant in agreement with the result of Hess et al. (2017) who found that mushroom extract increased satiety and reduced prospective food intake in human subjects. The opposite trend in water intake against declining FCR seen in rabbits on PTRSE is similar to the observations of Chikumba, and Chimonyo (2014) in chickens. Percentage mortality in all rabbits were less than 5% and increases in hepatic enzymes were not reflected. This indicates that PTRSE (up to 1250 mg/l) do not impose detrimental effects on the rabbits. This observation is in sync with the reports of Okolo et al (2017) in rats orally administered 50–5000 mg kg⁻¹ b.w of *Pleurotus tuber regium*.

Improved cholesterol metabolism seen in rabbits administered PTRSE contrasts with the results of Ijeh et al. (2009). The author found that dietary incorporation of PTR at 5 and 10% had no significant hypolipidaemic effects in albino mice. These differences in observations may be due to differences in the mode and route of administering the PTRSE. In the former study PTRSP was incorporated into diet while extract was added to water in the current study. This observation suggest that the extraction of the whole plant and the route of administration (via drinking water) led to the release of more bioavailable phytochemicals, consequently improving cholesterol metabolism. Reduced serum enzyme concentration on PTRSE administration to rabbits suggests that increasing levels of extract in drinking water did not cause any injury to the growing rabbit's tissue. Most values were not higher than those of the control and were within the range previously reported for normal rabbits (1.78-9.8 mmol/l for AST, 1.4-16.6 mmol/l for creatinine, 0-10.0 IU/l for ALT, and 4-20 IU/l for ALP (Harcourt-brown 2002;; Archetti et al. 2008). These results suggests that the dosage of PTRSE used in the current study is not harmful to the liver.

Relative weight in most of the visceral organs among rabbits on varying levels of PTRSE and control suggest there was no hepatic injury and this corresponds with the liver enzymes result earlier reported in the current study. The highest cecal weight in rabbits on 750 mg/l PTRSE is an indicator of improved feed motility and digestibility (Hulls et al., 2012). Increasing values of villi height and apical width observed in this study indicate that increasing levels of PTRSE in drinking water of growing rabbits enhances the intestinal absorptive surface and this corresponds to greater absorption of available nutrients and improved growth (Yasar and Forbes 1999; Panda, et al. 2015)

The different degrees of micro-vesicular steatosis seen in experimental rabbits are likely caused by obesity and that caged animals lack sufficient exercise. Over time, NAFLD has the propensity to culminate in Non-alcoholic steatohepatitis (NASH) and chronic hepatitis characterized by cellular, congestion, and fibrosis. group might be suggestive of vascular injury. The focal area of haemorrhagic lesion seen in rabbits on 1250 mg/l PTRSE is a sign of Drug-induced liver injury (DILI) but further investigation is required since the level of hepatic enzymes does not suggest so (Kleiner 2017).

It was concluded that the oral administration of 750 mg of the aqueous extract of *Pleurotus tuber-regium* per litre of drinking water can improve the growth, cholesterol profile and nutrient absorptive capacity of growing rabbits without imposing any detrimental effect.

Declarations

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Author's contributions

SA, AB, SO, TM conceived and designed research.

SA, SK, AA, BB and D conducted experiments.

TM and SO contributed new reagents or analytical tools.

SA analyzed data.

SA, AB, SO and TM wrote the manuscript.

All authors read and approved the manuscript.

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Code availability: Not applicable

Ethics approval: All procedures performed in this study were done following the institution's ethical standards or practice at which the studies were conducted. The experiment was performed in accordance with the ethical

standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate: Not applicable

Consent for publication: All authors have given their consent for their name to be included in this publication

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Tables

Table 1 Ingredient and nutrient composition of the diet

Items	(g/kg)
Maize offal	100.0
Maize	310.0
Full fat Soya	100.0
Groundnut cake	50.0
Wheat offal	300.0
Rice husk	100.0
Oyster shell	10.0
Bone meal	20.0
Premix	5.0
Salt	5.0
Total	1000
Proximate composition (% Dry matter)	
Crude protein	15.3
Crude fat	5.14
Crude fibre	12.47
Calcium	0.01

Premix composition per Kg diet: vit A: 4000000IU, vit D: 800000IU, vit E: 40000mg, vit K₃: 800mg, vit B₁: 1000mg, 6000mg, vit B₆: 5000mg, vit B₁₂: 25mg, Niacin: 6000mg, Panthothenic acid: 2000mg, Folic acid: 200mg, Biotin: 8mg, Man 300000mg, Iron: 8000mg, Zinc: 20000mg, Cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline: 800000mg.

Table 2 Performance characteristics and serum biochemical measurements in experimental rabbits administered varying levels of PTRSE in drinking water.

Parameter	Amount of PTRSE in drinking water in mg/l				SEM	Probability		
	0	500	750	1250		Linear	Quadratic	Cubic
Initial weight (g/rabbit)	718.03	725.09	725.29	721.56	1.71	NS	NS	NS
Final live weight (g/rabbit)	1284.33 ^d	1562.14 ^c	1810.75 ^a	1723.16 ^b	42.55	**	NS	**
Weight gain (g/rabbit/day)	9.44 ^d	13.95 ^c	18.09 ^a	16.69 ^b	0.70	**	**	**
Feed intake (g/rabbit/day)	71.17 ^a	67.08 ^{ab}	64.00 ^{bc}	61.41 ^{bc}	1.03	**	**	**
Feed conversion ratio	7.54 ^a	4.81 ^b	3.54 ^c	3.68 ^c	0.342	**	**	*
Water intake (ml/rabbit/day)	157.17 ^b	193.17 ^a	183.03 ^a	150.24 ^b	4.88	**	NS	NS
Extract intake (mg/l/rabbit/day)	0.000 ^d	0.086 ^c	0.135 ^b	0.178 ^a	0.014	**	**	**
Mortality (%)	1.67 ^b	2.20 ^b	3.33 ^a	3.33 ^a	0.228	**	NS	NS
Total protein (g/dl)	9.02	9.13	8.75	9.09	0.12	NS	NS	NS
Albumin (g/dl)	5.47 ^a	5.34 ^a	4.51 ^b	5.72 ^a	0.14	NS	NS	*
Globulin (g/dl)	3.46 ^b	3.79 ^{ab}	4.24 ^a	3.38 ^b	0.12	*	*	*
Total cholesterol (mg/dl)	52.91 ^c	93.07 ^a	66.36 ^b	62.56 ^{bc}	3.48	**	*	**
Triglyceride (mg/dl)	79.42 ^a	62.20 ^b	66.13 ^b	61.89 ^b	2.08	NS	*	**
High density lipoprotein (mg/dl)	26.85 ^c	52.84 ^a	38.73 ^b	35.11 ^b	2.35	**	NS	**
Low density lipoprotein (mg/dl)	10.18 ^c	27.79 ^a	14.40 ^b	15.07 ^b	1.41	**	**	**
Very low density lipoprotein (mg/dl)	15.88 ^a	12.44 ^b	13.23 ^b	12.38 ^b	0.42	NS	*	**
Glucose (mg/dl)	117.36 ^b	120.20 ^b	123.7 ^b	138.40 ^a	2.09	**	*	NS
Creatinine (mg/dl)	1.18 ^a	0.853 ^{ab}	0.66 ^b	1.14 ^{ab}	0.09	NS	*	NS
Aspartate transaminase (Iu/L)	52.07	51.87	50.04	51.29	0.79	NS	NS	NS
Alanine amino transferase (Iu/L)	49.67 ^a	37.87 ^b	44.89 ^a	44.89 ^a	1.30	NS	**	*
Alkaline phosphatase (Iu/L)	73.58 ^a	68.67 ^a	58.53 ^b	70.27 ^a	1.79	**	**	*

^{a-d} Means along the same row with different superscripts are significantly different at P<0.05 and P<0.01

* P<0.05 ** P<0.01 NS not significant

Table 3 Organ relative weights and intestinal histomorphometry measurements in rabbits administered varying levels of PTRSE in drinking water.

Parameter	Amount of PTRSE in drinking water in mg/l					Probability		
	0	500	750	1250	SEM	Linear	Quadratic	Cubic
Weight (g)	1163.20 ^{ab}	1035.45 ^b	1254.05 ^a	1059.45 ^b	32.66	NS	NS	NS
Length (%)	0.60 ^b	0.69 ^a	0.58 ^b	0.65 ^{ab}	0.02	NS	NS	*
Width (%)	3.41 ^{ab}	3.36 ^{ab}	3.76 ^a	2.51 ^b	0.19	NS	NS	NS
Depth (%)	0.04	0.04	0.04	0.05	0.002	NS	NS	NS
Volume (%)	0.25	0.25	0.27	0.25	0.007	NS	NS	NS
Area (%)	2.50	3.81	2.50	2.83	0.30	NS	NS	NS
Volume (%)	2.39	1.77	1.94	2.00	0.15	NS	NS	NS
Intestine (%)	1.66 ^a	0.97 ^b	2.31 ^a	1.83 ^a	0.17	NS	NS	**
Length (%)	7.82 ^{bc}	6.19 ^c	10.00 ^a	7.82 ^{bc}	0.50	NS	NS	**
Weight (μm)	700.00 ^b	625.00 ^b	990.00 ^a	1100.00 ^a	61.65	**	*	**
Width (μm)	70.00 ^b	70.00 ^b	100.00 ^a	50.00 ^b	6.53	NS	*	*
Depth (μm)	250.00	200.00	215.00	200.00	8.90	NS	NS	NS
Proximal depth(μm)	50.00	40.00	40.00	40.00	2.18	NS	NS	NS
Volume								
Weight (μm)	400.00	410.00	450.00	500.00	27.74	NS	NS	NS
Width (μm)	35.00	30.00	35.00	35.00	11.25	NS	NS	NS
Depth (μm)	70.00	60.00	75.00	70.00	4.09	NS	NS	NS
Proximal depth(μm)	45.00	50.00	40.00	55.00	4.15	NS	NS	NS

^{a-c} Means along the same row with different superscripts are significantly different at P<0.05 and P<0.01

* P<0.05 ** P<0.01 NS not significant

Figures

Figure 1

Quantitative assay of the Phytochemical constituents of PTR powder and extract

X40

X100

X400

X400

X400

X400

Figure 2

Liver plates show mild to moderate disseminated microvesicular steatosis (green arrows), congestion (blue arrows), mild periportal infiltration by inflammatory cells (black arrows) and mild infiltration of zone 2 by inflammatory cells (dotted arrow).

X40

X100

X400

X400

X400

X400

Figure 3

Liver plates show marked to severe disseminated microvesicular steatosis (green arrows), mild periportal steatosis (black arrows) and focal congestion (blue arrows).

Figure 4

Liver plates shows moderate to marked disseminated microvesicular steatosis (green arrows) and focal area of periportal infiltration by inflammatory cells (black arrows).

X40

X100

X400

X400

X400

X400

Figure 5

Liver plates show periportal infiltration by inflammatory cells (black arrows), portal fibrosis (dotted arrow) and focal area of hemorrhagic lesion (blue arrows).