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PLEUROTUS OSTREATUS POLYPHENOLS EXTRACT BASED DIETS ENHANCED HEPATIC CHOLESTEROL METABOLISM RELATED GENE EXPRESSION IN WEANED RABBITS

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ABSTRACT: *Mycelium of oyster mushroom (Pleurotus ostreatus) contain active flavonoids that have antioxidant and suppressive cholesterolemic effects. This study was conducted to explore the mechanism and effects of dietary Pleurotus ostreatus phenolic supplementation on levels of cellular cholesterol deposition and metabolism in weaned rabbits. Twenty four (24) weaned rabbits were randomly divided into three groups with eight (8) replicates per group in a complete randomized design experiment. Rabbits were fed control diet (basal) and/or a control diet supplemented with 450 mg/kg or 900 mg/kg P. ostreatus phenolic extracts (POPE) for seven weeks. Blood and liver samples were collected to determine serum adiponectin and hepatic cholesterolic related genes. 3-hydroxyl-3 methyl-glutaryl coenzyme A reductase (HMG-CoAR) and cholesterol 7 α-hydroxylase (CTP7A1) mRNA levels and sterol regulatory element binding transcription factor 1(SREBF1) were down regulated while, Low-density lipoprotein receptor (LDL-R) and sterol co enzyme A desaturase gene (SCD) were up regulated to decrease fat mass, serum triglycerides (TG), serum total cholesterol and low density lipoprotein cholesterol (LDL-C) levels in rabbits on 450 and 900mg/kg P. ostreatus phenolic extract supplementation diets. Serum adiponectin concentration and High density lipoprotein cholesterol (HDL-C) increased from 22.5 to 32.09 and 1.21 to 1.56 mmol/L, respectively for control and POPE based diets. The results suggested that dietary supplementation of P. ostreatus extract at 900 mg/ kg diets could improve lipid metabolism in rabbits by regulating hepatic cholesterol metabolism gene expression and optimizing fatty acid uptake and synthesis.*

KEYWORDS: weaned rabbits, cholesterol, P.ostreatus, phenolic extract, lipids

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INTRODUCTION

The increase in figures of human that come down with cardiovascular diseases (CVD) have become a serious health challenge. In 2018, approximately, 18.9 million people has been reported to die of CVDs and its related ailments, accounting for about 31% of all global death (Sun et al, 2019). In Literatures, one of the primary predisposing factor of CVD is atherosclerosis, an arteritic inflammatory disease caused by the deposition of cholesterol and fibrous materials in the arterial walls and muscles use often as meat, which forms lesions (Sebastian et al, 2017). Blood total cholesterol (T-CHO), triglycerides (TG) and lipoproteins are the key pathogenic factors of atherosclerotic and CVDs if not in the right proportion (Xiaojiao et al, 2019). Serum fatty deposits inside the arterial walls thus narrow the arteries. From the forgoing, there is the need for blood constituent's analysis to readily determine the clinical, health status and meat nutritional quality.

The consumption of meat, majorly from Poultry (animal protein) is on the increase and their nutritional quality is of importance in determining value and their demands (acceptance). Meat quality however, depends on the composition of feed that is supplied to animals, capable of being converted to lean or low fat muscles (Agbana et al, 2022). The use of phenol extracts from most non-conventional feedstuffs as additives in feeds has proven to improve the meat quality of birds by reducing both back and abdominal fat (Chuang et al, 2020; Ogino et al, 2017).

Most feeds in Nigeria, are produced from non-conventional feedstuffs. The use of non-conventional feedstuff in feed as either energy source or additive therefore, should be backed up by assessment of the health status of the animals because some are known to affect blood parameters. A readily available and fast means of assessing the clinical and nutritional health status of animals in feeding trial may be the use of blood analysis (Daramola et al, 2015). Therefore, blood constituent analysis is imperative considering that many by-products are now used to feed livestock as alternatives to expensive feedstuffs. The search for cheaper alternative and safe feed ingredients continue to be the interest of most animal nutritionist. As such, normal serum cholesterol level should be of interest, since consumers are becoming more health conscious by limiting their cholesterol intake.

LITERATURE /THEORETICAL UNDERPINNING

Epidemiological studies have shown also that low density lipoprotein-cholesterol (LDL-C) bombarded by free radicals is positively linked to the risk of atherosclerotic CVDs (Leontowicz et al, 2022). Thus, blood lipids abnormality that occurs when there is imbalance in levels of TG, T-CHO and LDL-C has been implicated in the etiology of CVDs.

Similarly, cholesterol, one of the three major classes of lipid; though produced and used by animal cells is insoluble in water and is transported by blood plasma within protein particles known as lipoprotein (Alejo et al, 2018). These lipoproteins can either be very low in density (VLDL), low in density low lipoprotein (LDL), intermediate in density lipoprotein (IDL) or high in density low protein (HDL). High levels of lipoprotein other than HDL may be indispensable for the synthesis of bile, sex hormone, steroids, cortisone and Vitamin D, needed by all cells of the body during metabolism but, could act in synergy with triglycerides to cause CVDs (Assmann and Schulte, 2009). The body manufactures about 800 - 1000 mg of total

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cholesterol in the liver (Zhu et al, 2022). However, the optimum daily serum level should not exceed 200 mg/dl and the recommended daily cholesterol intake should not exceed 200mg or 25 % of polyunsaturated fat per energy in take (Xianyong et al, 2015). Cholesterol is transported in the blood plasma in the form of lipoprotein and the greater proportion of about 100 - 180 mg/dl of it is associated with the low density lipoprotein (LDL) which is linked to CVDs. Smaller amounts (40-70 mg/dl) are associated with High density lipoprotein (HDL) and least is associated with very low density lipoprotein (VLDL) of about 10 - 30 mg/dl (Zimmermann et al, 2014). The body obtained cholesterol majorly from diets and also synthesized de novo. As such, researchers have suggested an examination and formulation of diets that are low in cholesterol level.

Many studies have shown that extract of most tropical fruits, fibres and vegetables contain a variety of polyphenols or antioxidants that are low in fats and capable of reducing oxidative damages, lipid peroxidation and serum lipoid cells (Zimmermann et al, 2014), that could subsequently, reduced the risk of CVDs. Normal lipid metabolism plays an important role in maintaining lipid homeostasis in the body and reducing trans-fat while, disorder in lipid metabolism has been implicated in the risk of dyslipidaemia (Assmann and Schulte, 2009). Pleutorus ostreatus extract though, from fungi has been reported as an antioxidants that is capable of chelating free radicals and decreasing oxidative stress in animal cells (Chuang et al, 2020). In vivo, it could enhances Nrf-2 and glutamate–cysteine ligase catalytic (GCLC) expression, improve lipid metabolism and meat quality of broilers (Chuang et al, 2020).

Furthermore, it has been reported that dietary P. ostreatus polyphenols had an effect on reducing lipid deposition and blood cholesterol levels in laying hens (Wang et al, 2018). However, the mechanism for these effects are not clear because of the interaction of genes and combinations of environmental factors (stress, breed difference, management) with diets. Animal nutrigenomics studies is therefore an attempt at this. Hence, it is essential to elucidate the mechanism by which P. ostreatus polyphenols modulate cholesterol deposition and metabolism. This study investigated the effect of dietary P .ostreatus polyphenols extract supplementation (nutrients) on hepatic cholesterol metabolism related gene expression in weaned rabbits.

METHODOLOGY / MATERIALS AND METHODS

Experimental site

The study was conducted at the Rabbitry unit of the Research farm of Department of Animal Health and Production Technology, Kogi State Polytechnic, Itakpe Campus, which lies in the Guinea Savanna of coordinates Latitude 7.6384 ^{0} N and Longitude 6.335 ^{0} E. The temperature throughout the year ranges from 18.87 ^{0}C to 34.4 ^{0}C with an average of 26.64 ^{0}C and the average annual rainfall is 1280 mm (https://www.mindat.org/loc.3983 html).

Sample preparation

Samples of Mushroom (Pleurotus ostreatus) were obtained from the field at season. They were sorted into pilei and stipes, washed, sun dried, mixed and milled into fine powder.

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Extraction of polyphenols

It was done according to the procedure adopted by Monika et al, (2016). Briefly, 100 g of the powdered mushroom samples was mixed with100 ml of 80 % methanol. Samples were sonicated (shaken) in a K260 shaker for 8 hrs. Centrifuge at 3000 rpm with a universal 320R centrifuge and then filtered through Whatman No. 2 paper. The extraction was repeated twice, and both supernatants were mixed and evaporated at 40^0C to dryness using Buchi Rotavapor R-205 (UK). The obtained residues were weighed and stored at -12^0C before use.

Experimental Animals, diets and management

A total of twenty four (24) mixed breed bucks weighing averagely 258 ± 5.0g were obtained and allowed to acclimatize for seven days before being assigned randomly to three (3) dietary treatment groups denoted as G1, G2 and G3 in a completely Randomized design (CRD) experiment with eight (8) pairs per group for 2 months ad libitum feeding trial. The rabbits in G1 had a basal diet without supplementation, rabbits in G2, and G3 had diets containing basal diet supplemented with either of 450 mg/kg and 900 mg/kg P. ostreatus polyphenol extract. Prior feeding trial, bucks were dewormed with piperazine by oral administration against internal parasites and Ivomec was administered subcutaneously against external parasite. The basal diet was formulated according to National Research Council (NRC) standard to meet the nutrient requirements for weaned rabbits. Composition of the diets and nutrient levels are presented on Table 1.

Table 1: Composition and Nutrient contents of Basal diet

Ingredients	**%**
Maize	45.00
Maize bran	10.00
Wheat offal	15.00
Soya bean meal	13.00
Beniseed	10.00
Blood meal	03.00
Bone meal	02.00
Limestone	01.00
Salt	00.30
Vitamin premix	00.50
Mineral premix	00.20
Total	100.00
Calculated	
Crude protein	20.00

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Crude ash	05.30
Ether extract	08.66
Crude fibre	04.67

[1]Vitamin premix content per kg diet: Vit. A, 15,000 iu, Vit. D3, 3000 iu; Vit. E, 30mg, Vit. K3, 4 mg, thiamine, 3 mg; riboflavin, 8 mg; pyridoxine, 5 mg; Vit. 12, 25 µg; Ca-pantothenate, 19mg; niacin, 50 mg; folic acid, 1.5 mg and biotin, 60 µg.
[2] Mineral premix content per kg diet: Co ($CoCO_3$), 0.255 mg; Cu ($CuSO_4.5H_20$), 10.8 mg; $FeSO_4.H_20$, 90 mg; Mn ($MnSO_4.H_20$), 90 mg; Zn (ZnO), 68.4 mg; Se (Na_2SeO_3), 0.18 mg.

Sample collection

At the 49th day of the trial, five rabbits per treatment were slaughtered after whole blood collection by being anesthetized with chloroform. About twenty milliliters (20 ml) of blood samples was collected from the pre cava vein of rabbits which were fasted overnight, in vacuum tubes, left to rest for more than 30 minutes and then centrifuged at 5000 x g for 10 minutes to separate the blood cells and serum. The serum was collected and then frozen at -20^{0}C.

5g of liver sample were excised from the left side of the carcass and was frozen in liquid nitrogen immediately before it was stored at - 80 ^{0}C.

Determination of serum Lipid profile

Total serum cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDLC) were quantified using an automatic biochemical analyzer (TBA-125FR, Toshiba, Japan) and respective assay kits. The adiponectin (ADPN) in rabbit's serum were also measured following manufacturer's protocol. The protein analytes bind to the antibody and the enzyme–linked immunosorbent assay (ELISA) reader measured absorbance at key wavelengths.

Determination of hepatic cholesterol related genes

Hepatic cholesterolic gene expression was determined according to the procedure of Xu et al, (2019), briefly, total RNA in 100 mg liver samples each was extracted by Trizol reagent. After measurement of total of total RNA's concentrations, 1000 ng of total RNA from each sample was reverse transcribed to cDNA by a prime Script RT reagent kit with gDNA eraser.

Real- time quantitative PCR was performed using SYBR select master mix (Applied Biosystems). The relative expression of the target gene was calculated by the $2^{-\Delta\Delta Ct}$ method and β - actin was used as the house keeping gene (Livak and Schmittgen, 2001).

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA). Statistically significant ($P< 0.05$) means were compared and separated by Duncan's multiple range test using SPSS software (Duncan, 1955).

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The Primer sequence of each Gene according to Genbank or other research.

Gene name	Primer sequence	Genbank No
β-actin	F:5'-CTGGCACTAGACACAATGAATG-3'	X 00182.1
	R:5'-ACATCTGCTGCAGTAACCCGTA-3'	
HMG-CoAR	F:5'-GGTCCCGAATGAATGCCCTTGA-3'	NM_001122988
	R:5'-ACCGTTCTCCTGGCTCTTTGGG-3'	
CYP7A1	F:5'-TATGGCACGATGCACATCCAGATG-3'	NM_0010053572
	R:5'-GGGCAAGGCAGATCTCTTCCAA-3'	
LDL-R	F:5'- CAGCACCCAGACTCAAGACGCA-3'	NM_001206354
	R:5'-CTACCCCCAACAGATCTCATAAT-3'	
SCD	F:5'-CAGCAAGAACCAGACACCAAGC-3'	XM_00116324
	R:5'-CCAGGCTTGGTTCTGATCGGAGA-3'	
ATB	F:5"-TGGGGACTGTTTTGATTGCACGA-3'	NM-001166262.1
	R:5'-CTCCCAGTCAGGGTTGCAGCA-3'	

RESULTS AND FINDINGS

Serum Lipid profile.

Serum adiponectin (ADPN) and High density lipoprotein cholesterol (HDL-C) contents significantly ($P<0.05$) increased in rabbits fed P. ostreatus polyphenol extract (POPE**)** based diets and statistically higher in 900 mg/kg supplemented diet as shown in Table 1. Serum total cholesterol, Low density lipoprotein cholesterol (LDL-C), Sterol regulatory element binding transcription factor 1 (SREBF1) and ratio of LDL-C/HDL-C also significantly ($P< 0.05$) decreased with increasing levels of POPE supplementation in diets of rabbits. In comparison to the control, both 450mg/kg and 900 mg/kg POPE supplemented diets recorded a decrease in serum triglycerides (TG).

Hepatic cholesterolic Gene expression

As shown in Table 2, the mRNA expressions of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoAR) and cholesterol 7 α- hydroxylase (CTP7A1) decreased significantly ($P<0.05$) in both 450 and 900 mg/kg POPE supplemented diets than in the control group while, low density lipoprotein receptor (LDL-R), and sterol co-enzyme A desaturase gene (SCD) significantly ($P < 0.05$) increased in 900 mg/kg POPE group, but statistically similar to 450 mg/kg group compared to control group.

DISCUSSION

Lipid profile

Adiponectin is an important adipocytokine produced primarily to regulate cellular adipocyte metabolism (Zhu et al, 2022). In line with this view, the increase in serum concentration of

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adiponectin in POPE diets observed in this study might confirmed that POPE supply altered lipid metabolism in growing rabbits. High levels of serum non-lipoprotein (HDL-C) also recorded in this study for rabbits on POPE based diets suggested protective nature, better lipid metabolism and healthy lean meat of rabbits (Xianyong et al, 2015).

The reduction in serum concentration of LDL-C suggested reduced fat accretion and coordinated regulation of metabolism of fat for lean tissue gain in carcass of rabbits on POPE based diets. These results supported evidences that shown that most plant extracts improved lipid metabolism in farm animals (Zhu et al, 2022; Xianyong et al, 2015; Abdelatty et al, 2019), which was probably associated with higher content of polyphenols and flavones in them (Zhu et al, 2022). Similarly, these results are in agreement with those of Abdel-moneim et al. (2015) who found that LDL-C decrease significantly in hypercholesterolomic rats due to feeding on orange peels. The highest value recorded for LDL-C (3.00) in control group however, falls within normal and safer range (2.6 – 3.2 mmol/L).

The reduction in serum total cholesterol, total triglycerides and LDL-C/HDL-C in rabbits on POPE diets suggested that P.ostreatus polyphenol extracts initiate lipid transport in blood and tissue thus leading to the reduction of the cholesterol levels. The results were in accordance with that of Xiaojiao et al. (2019), who found that, diets rich in monounsaturated rape seed oil decreases triglyceride and high cholesterol level in the blood of hypocholesterolemic rats. Though, this findings are not in line with the results observed by Abdelatty et al. (2019) who reported no significant differences for these lipid biochemical parameters studied in rabbits. The discrepancies in results might be due to differences in breeds of studied animals and diets effect.

Gene expression

To examine the mechanism of hepatic cholesterol lowering effect of POPE based diets on rabbits, HMG-CoAR, CYP7A1, SREBF1 and LDL-R mRNA expression levels were determined. LDL-R has been implicated in the absorption of cholesterol, as they transfers cholesterol into liver cells by binding to LDL to synthesize steroid hormone as well as bile salt. The increase in LDL-R gene in rabbits on POPE diets suggest efficient liver detoxification of unneeded cholesterol (Zimmermann et al, 2014). Similarly, plasma transmembrane protein, could have mediated the uptake of LDL-cholesterol, a substrate for steroid hormone production which binds with other lipoproteins (Sun et al, 2019). LDL-R sit on the outer surface of many cells, where they pick up LDLs circulating in the blood stream and transport them in the cells. POPE based diets might have up regulated genes that promote cellular LDL catabolism to release cholesterol that is quickly used up, stored or removed from the body.

Substantial evidence revealed that HMG-CoA reductase catalyzes de novo cholesterol synthesis and is a transcriptional target of SREBP in response to low cholesterol levels (Alejo et al, 2015). High levels of intermediate lipid species in cholesterol synthesis trigger the binding of HMG-CoA reductase to INSIG which interactions promotes the degradation of HMG-CoA reductase. Therefore, HMG-CoA, a restriction enzyme for synthesis of cholesterol could have been up regulated by POPE in rabbits to optimally regulate the levels of expression of cholesterol in rabbits (Leontowicz et al, 2022).

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CYP7A1 plays a major role in transformation of cholesterol to bile, and it's over expression in liver has been reported to accelerate hepatic cholesterol catabolism (break down) and increase bile acid excretion (Ogino et al, 2017). The observed findings in this study was similar to those reported by Xu et al.(2019) who reported a cholesterolic-lowering effect of dietary apple polyphenols supplementation in weaned piglets.

IMPLICATION TO RESEARCH

The study elucidates the roles of bioactive compounds found naturally in P. ostreatus in reducing cholesterol synthesis and enhancing cholesterolic metabolism towards optimizing of nutrients in diets for production of lean fat meat in rabbits. These phytochemical can modify gene transcription and translation, which can alter biological responses, metabolism processes and synthesis of cholesterol via regulating gene expression patterns.

This information can act as biomarkers for feed preparation for animal nutritionists and prognosis prediction tools. Thus, extracts kept cholsterolic response genes switched down so that healthy meat in the form of low fat can be achieved.

CONCLUSION

Dietary POPE Supplementation released nutrients that decreased serum mRNA expressions of HMG-CoAR, CYP7A1, and increased LDL-R mRNA expression, suggesting that hepatic cholesterol- lowering effect of POPEs might be as a result of decrease in cholesterol synthesis and increase in absorption of cholesterol. Specifically, dietary POPE supplementation reduced hepatic lipid deposition that subsequently, modulate the expression of cholesterolic genes. However, the underlying mechanism for cholesterol lowering effect of POPE is not exhaustively studied and requires further investigation.

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TABLES

Table 1: Serum lipid profile of rabbits fed P.ostreatus polyphenol extract (POPE) based diets.

Parameters	G1	G2	G3	SEM
Adiponectin, µg/mL	22.50 ± 0.08^{c}	30.05± 0.11^{b}	32.09 ± 0.06^{a}	0.05
Total cholesterol, mmol/L	4.38 ± 0.89^{a}	2.78 ± 0.31^{b}	1.80 ± 0.01^{c}	0.05
Triglycerides, mmol/L	6.41 ± 0.49^{a}	4.06 ± 0.08^{b}	2.05 ± 0.50^{b}	0.10
HDL-C, mmol/L	1.21 ± 0.01^{c}	1.42 ± 0.01^{b}	1.56 ± 0.01^{a}	0.20
LDL-C, mmol/L	3.00 ± 0.01^{a}	2.40 ± 0.02^{b}	2.00 ± 0.02^{c}	0.01
LDL-C/HDL-C	0.34 ± 0.44^{a}	0.26 ± 0.13^{b}	0.15 ± 0.24^{c}	0.10

a, b, c

Values with different letters within a column are significantly P < 0.05 different.

\able 2: mRNA expression levels in rabbits fed P.ostreatus polyphenol extract based diets.

Parameters	G1	G2	G3	SEM
HMG-CoAR	1.00 ± 0.89^{a}	0.76 ± 0.30^{b}	0.78 ± 0.01^{b}	0.05
CYP7A1	1.00 ± 0.49^{a}	0.62 ± 0.08^{b}	0.64 ± 0.50^{b}	0.03
SREBF1	1.00 ± 0.05^{b}	1.20 ± 0.05 ^{a}	1.31 ± 0.05^{a}	0.06
LDL-R	1.00 ± 0.04^{b}	1.13 ± 0.01^{a}	1.21 ± 0.03^{a}	0.02

a, b, c

Values with different letters within a column are significantly P < 0.05 different.

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