FUDMA Journal of Agriculture and Agricultural Technology
ISSN: 2504-9496
Vol. 8 No. 1, June 2022: Pp.158-163

https://doi.org/10.33003/jaat.2022.0801.030

BIODEGRADED MANGO PEEL BASED DIETS ALTERS LIPOGENIC GENES AND mRNA EXPRESSION IN GROWING RABBITS.

***Agbana J.A[1], Amana, O. C[2], Ajagbe, D.A[2], Okpanachi, U[3]**

[1]Department of Animal Health& Production, Kogi State Polytechnic, P.M.B 1101, Itakpe
[2]Department of Animal Production, Kogi State University, P.M.B 1004, Anyigba
[3]Department of Animal Production, University of Jos, Plateau State
Correspondence author : *Jagbanah@gmail.com ;+234(0)7068200397

ABSTRACT

This study investigated the effect of bio fermented mango peel meal (FMPM) on lipid metabolism – related gene expression in liver tissues and abdominal adipose tissue in weaned rabbits. Twenty (20) weaned New Zealand white rabbits were randomly assigned to four dietary groups with five (5) rabbits in each group in a completely randomized design experiment. The diets had a control(G1) supplemented with unfermented mango seed meal, an experiment diet of mango peel fermented by either of *P. ostreatus*, (G2) and *P. rivulosis* (G3) and combinations of *P. ostreatus* plus *P. rivulosis* 50:50 (G4).The results showed that FMPM supplementation down regulated the mRNA expression of fatty acid synthesis (FAS) and acetyl CoA carboxylase (ACC) in liver tissues and the lipoprotein lipase (LPL) expression in abdominal fat tissues ($P< 0.05$). The mRNA expression of perixisomeproliferators activated receptor - alpha (PPAR-α) compared to control group, was up regulated in the livers of rabbits on fermented diets ($P<0.05$). However gene expressions of PPAR-γ and L-FABP liver fatty acid binding protein were not altered ($P> 0.05$) in rabbits fed FMPM supplemented diets. These findings indicated that FMPM regulated lipid metabolism by either increasing or decreasing the expression of lipogenic genes and altering multiple pathways in the liver. This information provided new essential information and demonstrated great potential inherent in nutrigenomics in researching nutrients added to livestock diets.

Keywords: Bio fermented; gene expression; liver; lipid; abdominal fat tissues

INTRODUCTION

Fat provides 2.25 form of energy in feed, improve palatability and limit dustiness in feed. However, excessive fat content in feeds which is stored in the abdomen and other lipid response organ is a major concern in livestock production. Lipid metabolism is associated with the nutritional value of meat,yield of lean meat and consumers preference or acceptability of meat (Duarte *et al*, 2013). Lean meat yield has high nutritional value and it is most preferred by consumers (Niu *et al*, 2018). Therefore, efforts geared toward reducing fat content and deposition area priority for livestock producers.

With the increase in knowledge of nutrigenomics (study that show relationship between nutrients, diets and genes), that reveals the potentials to develop more sustainable approaches to formulate specific targeted diets to adequately meet the requirements of different classes of livestock and suit their performances in response to environmental influence, Animal nutritionists have made attempts at using precise nutrition strategies to pacify this problem.
However, nutrigenomics rely on omic technologies(proteomics, genomics,metabolomics),that allows large amount of data relating to gene variants to identify and measure many bioative molecules that simultaneously affect dietary exposure.Therefore, these results indicate the potential of specific diet and dietary supplements to prevent excessive fat deposition in carcasses of some farm animals. One of such supplement is fermented mango peel meal (FMPM).Mango peel meal, an energy source with huge potential but, scantly utilized as feedstuff in most livestock diets owing to it high fibre content and presence of tannin, a toxic polyphenol, has good nutritive value (Ojokoh, 2007).

Kuijk *et al* (2016) proposed solid state fermentation (SSF) techniques as the only method that best degrade fibre and adopts low cost approaches that are not hazardous to animals, people and the environment. SSF provides many essential nutrients like amino acid, vitamins, small size peptides and oligosaccharides (Sharma &Arora, 2015).
White rot fungi can degrade lignin without altering cellulose and hemicelluloses contents of substrates and can serve as microbial feed addictive for preventing disease (Niu *et al*, 2018). *Pleurotus ostreatus* and *Poria rivulosis* were used in mango peel meal (MPM) fermentation by Agbana *et al*

(2021) who reported a significant decrease in free tannin content ($P<0.05$) of mango peel fermented with two fungi strains. Little information exists on the influence of fermented mango peel meal on lipid metabolism in rabbits.

The liver and abdominal tissues are the most important organ for lipid metabolism (fatty acid synthesis) and the adipose tissue is the primary site of fat storage as triglycerides (Huang *et al*, 2013). However, fat deposition results in complex interactive processes that involve both genetics and environmental factors (Nie *et al*, 2019). Metabolomics, comprehensively reveal the principles of changes and mechanism of the bio system affected by exogenous substances that provides better understanding of effects of organic feed on livestock (Ji *et al*, 2018). It has been used to research the metabolism of adipose tissues in chickens. Therefore, this study investigates the effect of dietary supplementation of fermented mango peel meal (MPM) on lipid-related gene expression in tissues and liver metabolomics.

MATERIALS AND METHODS

Mango peels was obtained at season from Anyigba District (Lat. 7^0 6' and Long. 6^0 43'E), Kogi State and the strains *P. ostreatus and P. rivulosis* was obtained from the culture bank of the Department of microbiology,Kogi State University, Anyigba. These were maintained on potato dextrose agar plates (PDA).

Fungi Species and Spore Preparation

P. Ostreatus and pora rivulosis were sub cultured on potato dextrose Agar (PDA) plates, incubated at 25^0 C for 48 hours until mycelia colonized most of the plates surface as described by *Pinto et al* (2012). This is to maintain fungi viability.

Spore suspension of each fungus was prepared by taken a nip from 2 days grown culture with a sterile wire loop and suspending it in 10ml of suspension medium (Peptone dextrose 4% M/V). Fungi suspension was diluted to a concentration of 4.0×10^6 spore/ml for each fungus.

Preparation of Fermented Mango Peel Meal (FMPM)

The milled substrate (Mango peels) to be fermented was sterilized and autoclaved at 121^0C for 10 minutes. About 500g of the treated substrates was kept in sixteen (16) sterilized volumetric flasks, moistened and inoculated each with spores suspension of either of *P. ostreatus, P. rivulosis* and combinations of *P. ostreatus and P. rivulosi*s (50:50) at seeding rate of 8.4×10^6 spores/g. The volumetric flasks were then covered with sterilized cotton wool and incubated at 30^0c for 48hrs in an incubator.The action of the fungi was terminated by oven drying at 50^0c after 72 hours. The unfermented MPM (Control) undergoes the same treatment but, was inoculated with saline culture medium. The chemical composition of unfermented and fermented MPM by *P. ostreatus, P. rivulosi*s and combination of *P. ostreatus + P. rivulosis* (50:50) were analyzed according to the AOAC (2003) and free tannin content was determined by the standard method of Makkar (1999).

Animal Management and Experimental Diets

A total of twenty (20) New Zealand White male rabbits weighing averagely 358 ± 5.0g were obtained and allowed to acclimatize for seven days before being assigned randomly to four (4) dietary treatment groups namely: G1, G2, G3 and G4 in a completely Randomized design (CRD) experiment with five (5) pairs per group for 2 months feeding trial. The rabbit in G1 were raised on a normal diet without fungi action, rabbits in G2, G3 and G4 had diets containing either spores of *P. ostreatus, P. rivulosis* and combination of *P. ostreatus and P. rivulosis* at 50:50. Prior feeding trial, bucks were deworned with piperazine by oral administration against internal parasites and Ivomec was administered subcutaneously against external parasite. Composition of the diets and nutrient levels are presented on Table 1.

Growth Performance

Record of weight gain (g/rabbit), feed intake (g/rabbit) and feed conversion ratio were obtained weekly for 8 weeks.

Average daily weight gain was calculated by dividing the total weight gain by 56 days. Weekly feed intake was measured as the difference between the feed offered and left over. Total feed supply – total leftover/ total number of rabbits per treatments.

FCR = Feed intake/weight gain, where weight gain is equal to final weight – initial weight (g).

Sample Collection

At the termination of the feeding trial, rabbits were anesthetized with chloroform and sacrificed. 5g liver tissue samples and abdominal fat each were carefully excised and immediately frozen in liquid nitrogen and stored at-80^0c for analysis of mRNA expression.

Table 1-Ingredients and compositions of experimental diets

Ingredients	G_1	G_2	G_3	G_4
Maize	45.45	45.50	45.58	45.59
Maize bran	10.00	10.00	10.00	10.00
Wheat bran	17.00	17.00	17.00	17.00
Soybean meal	12.55	12.50	12.42	12.41
Blood meal	3.00	3.00	3.00	3.00
Unfermented Mango Peel Meal	10.00			
Fermented Mango Peel Meal		10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Vitamin Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Crude protein	18.52	18.56	18.56	18.58
Crude ash (%)	5.32	5.75	5.76	5.85
Ether extract(%)	0.70	0.71	0.72	0.74
Free tannin (Mg/Kg)	124.54	45.85	43.97	32.97

RNA Isolation and Real-time Quantitative Polymerase Chain Reaction (qPCR) Analysis

The total RNA of the liver and abdominal fat was isolated using Trizol reagent (Invitrogen,USA) according to the manufacturer's protocol, and the RNA concentration were measured using the NanoDrop ND – 1000 spectrophotometer. All isolated RNA samples were then adjusted to the same concentration and then reverse transcribed to cDNA with a prime script TM RT reagent kit (Thermo scientific, cat. #K1624) according to the manufacturer instructions. A real – time qPCR analysis was carried out in a 7500 real time PCR analyzer using a SYBR – Green PCR master mix (Applied Biosystem, TM Cat. #1306409), to assay the relative quantitative mRNA expression of genes in the liver of rabbits. The PCR program was performed for 5 mins at 95^0C, followed by 38 cycles of 94^0C (20 Secs), 55^0C for 30 Secs, 72^0C (20 secs), and 72^0 C for 5 mins. Gene primers of fatty acid synthesis (FAS), acetyl CoAcarboxylase (ACC), Lipoprotein lipase (LPL), Peroxisome proliferator – activated receptor (PPAR-α), peroxisome proliferator – activated receptor γ(PPAR-γ) and β – actin were designed as described by Wu (2012), and the liver fatty acid – binding protein (L-FABP) were designed using online NCBI primer design software and synthesized from GeneLink™. All samples were analyzed in triplicate, and the results are expressed as $2 - ^{\Delta\Delta CT}$, according to the method of Livak and Schmittgen (2011).

Primers details for the analysis of lipid-related metabolism genes expression

Gene	Genbank number	Primer Sequence (5'-3')
FAS	NM_206155	Forward: TCAGGTGTTCTGGAATGCAA
		Reverse: AATCCTGGTGGGCAATCGTAG
LPL	NM_206158	Forward: AGTCAGAGGTGAAGTCAGGCGAAAC
		Reverse: CTGCTCCAGGCACTTCACAAATC
L-FABP	AF_480899	Forward: ATGAGCTTCACTGGAAAGTACGAG
		Reverse: TCTTGATGTCCTTACCCTTCTGG
ACC	NM_206138	Forward: TGCACTGGAACTGGATGATAGTGA
		Reverse: CACGATGTAGGCACCAAACTTGA
PPAR-α	XM_10010776	Forward: TGCACTGGAACTGGATGATAGTGA
		Reverse: TCCTACATTTACAAGACCAGGAACGA
PPAR-γ	XM_10010770	Forward: TGTGAAGTTCAACGCACTGGAATTA
		Reverse: GGAGCTCCAAAGCTTGCAACA
β –actin	XM_206318	Forward: ATTGTCCACCCGAAATGCTTC
		Reverse: AAATAAAGCCATGCCAATCTCGTC

Statistical Analysis

Data obtained for body weight, lipid content and mRNA expression were analyzed using SPSS 18.0 statistical software (SPSS Inc. Chicago, USA) in a one way analysis of Variance at significant level of 0.05. Differences among means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Table 1 shows data on the chemical composition of unfermented MPM and MPM fermented by *P. ostreatus, P. rivulosis and P. ostreatus + Pora rivulosis.* Fermented MPM based diets had higher crude protein and crude ash contents than unfermented. Free tannin concentration was reduced by 63.2% in G2 (From 124.54 mg/kg to 45.85 mg/kg), 65% (124.54 mg/kg to 43.97 mg/kg) in G3 and 73.53% (124.54 mg/kg to 32.97 mg/kg) in G4 compared to unfermented mango peel meal based diets. The increase in crude protein and crude ash content in biofermented MPM based diets over the control diet (G1) might be due to the presence of nitrogen fixing bacteria in the mash which might have increase microbial nitrogen during fermentation due to increased production of single cell protein (Ojokoh,2007) and the presence of mycelium of fungi grown on the substrates. Probably,the micro organisms have ability to synthesis and secrete some extracellular enzymes that degrade cellulolytic materials during fermentation thus, enhancing the release of nutrients in to diets (Niu *et al*, 2015). The result is consistent with the findings of *Zhang et a*l (2018).Similar results were reported by *Tang et al* (2012), who observed that Cotton Seed Meal fermented by *Bacillus subtilis* had lower free gossypol content and higher crude protein level to unfermented cotton seed meal (CSM). The concentration of free tannin observed in this study was less than 30 mg/kg reported in gossypol based diets, and had no effect on rabbit health. The reduction in free tannin levels in biofermented diets observed in this study could be attributed to processing effect as studies with chicks, rats and rabbits revealed that microbial fermentation may be effective in reducing extractable tannin contents and detoxifying tannin in diets (Agbana *et al*, 2021, Duarte *et al*, 2013., Niu *et al*, 2015).

Table 2 shows data on growth performance of rabbits fed bio fermented mango peel meal based diets. The results revealed that average daily body weights, final live weight, final feed intake were significantly ($P<0.05$) affected by diets. The final body weight gain of treatments G_2, G_3 and G_4 were significantly ($P<0.05$) different from the control (G_1); they had higher weights. This revealed that the use of white rot fungi to biodegrade the PMP improved the weight gained by rabbits as this is also evidently revealed in the average daily weight gain. Thus, suggesting better utilization of diets by rabbits and effective release and absorption of crude protein and carbonhydrate that are essential for growth, thereby improving feeding efficiencies, bioavailability of chelated amino acids and complexes of mineral elements (Tang *et al*, 2012).This observation however, is contrary to the findings of *Nie et al*,(2019) who observed no significant changes ($P<0.05$) in body weight and feed intake among birds on fermented FCSM diets and unfermented CSM diets. But, in line with the report of Cha *et al,(2018)* who observed an increase in weight gain of adult birds on biofermented based diets. The discrepancies observed in these reports may be as a result of variations in geographical locations of experimental, breeds and specie of animals difference.

The improvement in feed consumption in treatments G_2 to G_4 than G_1 may be due to improvement in palatability, texture and digestibility of the fermented mango peel based diets. Probably, the transit time of the feed in the gastro intestinal tract is reduced. Besides, there is addition of vitamin B complex during biodegradation by fungal, which increases the appetite for food by the rabbits just as vitamin B complex does for man and also reduces the bitter taste associated with astrigent nature of tannin based diets (*Duarte, et al, 2013*).

Table 2: Growth Performance of Rabbit Fed Degraded and Un degraded Mango Peel Meal

Parameter(s)	G_1	G_2	G_3	G_4	SEM
Average initial live weight(g/r)	650.20	660.35	642.36	640.79	22.3 NS
Average final live weight (g/r)	1987.16^{c}	2341.41^{a}	2221.31^{b}	2201.41^{b}	14.01 *
Average daily body weight (g/r)	47.74^{c}	56.47^{a}	52.82^{b}	52.17^{c}	2.32 *
Average daily feed intake (g/r)	110.41^{d}	132.33^{a}	120.41^{b}	117.21^{c}	0.56 *
Average final feed intake (g/r)	4307.52^{c}	5001.92^{a}	4643.8^{b}	4553.64^{b}	0.01 *
Feed conversion ratio	2.16	2.13	2.09	2.07	0.01 NS

a, b, c

Mean values on the same row with different superscripts differs significantly $P < 0.05$

Effect of FMPM on lipid relative mRNA expression in liver and abdominal fat tissue

Hepatic acetylCoA caboxylase (ACC) and fatty acid synthesis (FAS) in treatment groups Significantly ($P < 0.05$) decreased compared with that in the control group (Table 3).

The down – regulated mRNA expression may possibly be as a result of decrease in fatty acid synthesis in rabbits on fermented MPM diets as ACC, a limiting enzyme of lipogenesis as been reported to play a key role in the rate-limiting and regulation of fatty acid synthesis that catabolises acetylCoA to generate malonly COA (*Richard et al, 2018*). Similarly, FAS has been confirmed as a catayst involved in the conversion of Malonly COA to palmitate and also a gene critical in the control of lipogenesis (*Joseph et al*, 2018). Therefore, ACC and FAS have higher correlation for lipogenesis among lipogenic genes (*Huang et al*, 2013).

Expression levels of L-FABP, PPAR-∝ and LPL genes in liver tissues of rabbits on FMPM diets also showed a significant change ($P < 0.05$) between groups.The up regulated expression of PPAR – ∝ gene and LPL gene observed in this study indicates that fatty acid β oxidation may be higher in rabbits fed MPM fermented by *P. ostreaus + P. rivulosis* which suggests that the combined fermentation of MPM enhances triglyceride hydrolysis, generating fatty acids and glycerol for energy supply (*Nie et al*, 2019). This is consistent with the observation that fatty acidβ-oxidation increases by up regulating the transcription levels of PPAR-∝. The obvious up regulation of hepatic PPAR-∝ and LPL may be associated with better gain-to-feed ratio and growth rate when Mango Peel Meal (MPM) was added to the diets (*Nie et al*, 2019).

Table 3: Relative Gene Expression of mRNA in the Liver Tissue & Abdominal Tissue of Rabbit fed FMPM

Gene(s)	G_1	G_2	G_3	G_4
Liver tissue(s)				
FAS	1.0^{a}	0.30^{b}	0.20^{c}	0.10^{d}
ACC	1.0^{a}	0.50^{b}	0.40^{c}	0.20^{d}
L-FABP	1.0^{d}	1.20^{c}	1.50^{b}	1.55^{a}
PPAR-α	1.0^{d}	1.30^{c}	1.50^{b}	1.80^{a}
LPL	1.0^{a}	1.20^{b}	1.40^{c}	1.60^{d}
Abdominal tissue(s)				
LPL	1.0^{a}	0.50^{b}	0.2^{c}	0.2^{c}
PPAR-γ	1.0	0.90	0.80	0.80^{NS}
L-FABP	1.0	0.90	0.80	0.70^{NS}

a, b, c

Mean values on the same row with different superscripts differs significantly $P < 0.05$

Fermented mango peels (FMPM) supplemented diets significantly ($P < 0.05$) decreased LPL mRNA level in abdominal fat content. This down regulated level may be associated with a decreased fatty acid synthesis and fat deposition in the abdomen of the rabbits. On the contrary, *Zhang et al*, (2018) observed no alteration in hepatic LPL gene expression in broilers fed fermented cotton seed meal (FCSM).This difference might be as a result of variations in strain of experimental animals, breeds difference and feeding regimes. This transcription level of PPAR-γ and L – FABP in abdominal fat tissue was not significantly ($P > 0.05$) influenced by fermented mango peel meal (FMPM) supplementation as such, FMPM supplementation did not alter adipocyte differentiation through the expression of PPAR-γ gene. These results were consistent with the observations of *Zhang et al*, (2018), *Nie et al.,* (2019) and *Huang et al.,* (2013).

Generally, the variations in the expression levels of all lipid related genes in rabbits feed FMPM supplemented diets may be attributed to the nature of metabolites (exoenzymes, vitamins, organic acids), probiotics types present in fermented substrates and unknown active substances which may regulate the lipid metabolism of farm animals.

CONCLUSION

The result of this study has shown that dietary supplementation of Mango Peel Meal fermented with *P. ostreatus + P. rivulosis* reduced the levels of abdominal fat and liver fat by down-regulating the expression of hepatic lipogenetic genes (ACC and FAS) and LPL in abdominal fat tissue. However, the regulation of lipid metabolism remains a complex processes involving multiple pathways and

mechanisms yet to be fully unravelled. This discovery may provide new essential information which has great potential in nutrigenomics for researching nutrients often added to livestock feeds.

REFERENCES

Agbana, J.A, Osebeyo, E.S, Enefola, M.O and Oyewole, B.O. (2021). Enhancement of the nutritional value of mango peels with selected white rot fungi for use as livestock feed. *Proc.3rd Int. Conf. Biotechnology Society of Nig., South West Zone,* Ibadan. P.135.

AOAC (Association of Official Analytical Chemists) 2003, official methods of Analysis, 18thEdit. AOAC,Washington D.C, USA.

Cha,Y.S, Kim, S.R, Yang, J.A. (2018). Fermented soybean based red pepper paste, decreases visceral fat and improves blood lipids profiles in over weight adults. *Nutr.Metab.*,**10**(1):24.

Daurte,K.F, Junqueira,O.M, Borges, L.L and Rodriguez, E. (2013).Performance, carcass traits and body composition of broilers fed different linseeds oil levels between 21 and 56 days of age.*Brazillian J Poultry Sci*, **15**:55-60.

Huang, T, Zhang,Y, Zhou,Y, Zhang, Z, Xie, Z. (2013). Green tea polyphenols alleviate obesity in broiler chickens through the regulation of fat-metabolism related genes and transcription factor expression. *J Agric and Food Chem*. **61**:8565-8572.

Ji, B, Ernest,B, and Gooding, J.R. (2018).Transcriptomic and metabolomic profiling of chicken adipose tissue in response to insulin neutralization and fasting. *BMC Genomics***13**(2):441.

Joseph,S.B, Laffitte,B.A, Patel,P.H, Watson,M.A and Matcukuma, K.E. (2018).Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biological Chem*. **277**:11019-11025.

Kuijk Van, S.J.A, Baars, J.J.P, Hendricks, W.H, Cone, J.W. (2016).Effects of particle size and amount of inoculum on fungal treatment of wheat straw and wood chips. *J. Anim. Sci, Biotech.,* **7:**39.

Livak, K.J and Schmittgen,T.D. (2011). Analysis of relative gene expression data using real – time quantitative PCR method. *Methods* **25**:402-408.

Macdonald, P, Edwards, R.A, Morgan, C.A. (2007). Animal Nutrition. Seventh Edition, Prentice Hall Print. Pp.54-98.

Makkar,H.P.S. (2019). Plant toxins and detoxification methods to improve feed quality. *Journal of Animal Sci,* **12**:467-480.

Nie, C.X, Zhang, W.J, Ge, W.X, Liu,Y.F and Liu, J.C. (2019). Effect of cotton seed meal fermented with *Yeast* on the lipid related gene expression in broiler chickens. *Brazillian J Poultry Sci,* **57**:57-63.

Niu, C.X, Koh, J.H., Yu, R.W and H.J, Suh (2015) Biological activities of *saccharomyces cerevisae* and fermented rice bran as feed additives. *Lett. Appl. Microbiol*.,**35**(1):47-51.

Ojokoh, A.O. (2007). Effect of fermentation on the chemical composition of mango peels. *African J of Biotechnology.* Vol.**6** (16):1979-1981.

Richards,M.P., Poch, S.M, Coon, C.N., Ashwell, C.M and McMurty, J.P. (2018) Feed restriction Significantly alters lipogenic gene expression in broilers breeder chickens. *J .Nutr.***133**:707-715.

Sharma, R.K, and D.S Arora. (2015). Fungal degradation of lignocellulosic residues: an aspect of improved nutritive quality. *Crit. Rev. Microbiol.***41**:52-60.

Tang, J.W, Sun,H.,Yao,X.H, Feng, J .(2012). Effects of replacement of soybeans meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow feathered broilers. *Asian – Australasian J Animal Sci*. **25**:393-400.

Wu, X.H. (2012).Effect and regulation of Linseed oil on meat quality and fat metabolism of broilers. Ph.D Thesis, Univ. North West, A & F, Yangling, China.

Zhang, B.,Yang, X, and Long, L. (2018) Effects of dietary lipids and *clostridium butyricum* on serum lipids and lipids - related gene expression in broiler chickens. *Animal* **5**:1909-1915.