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Modulatory effects of probiotic *Lactobacillus acidophilus* on expression of lipolytic genes, carcass traits and growth performance in New Zealand white rabbit

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ABSTRACT

This research was undertaken to determine the impact of probiotic supplementation on the genetic expression of lipoprotein lipase (LPL), hepatic lipase (HL), low-density lipoprotein receptor (LDLr), and cholesterol ester transfer protein (CETP) in the liver of New Zealand white rabbit, carcass traits and growth efficiency. Thirty male New Zealand white rabbits eight weeks old were allocated into two groups. Each group had 3 replicates, each with 5 rabbits. The first group was given a basal diet. The second group was fed the basal diet with probiotic "Lacto biotech" supplemented in water (1gm/liter). The experiment lasted for eight weeks. The obtained results revealed that dietary supplementation of probiotic significantly ($P<0.05$) decreased the expression of the LPL gene, a non-significant decrease in HL and CETP genes and LDLr gene expression increased significantly in the liver compared to the control group. Supplementation of probiotic showed no significant effect on carcass traits however improved growth performance in rabbits. In conclusions, probiotic supplementation downregulates LPL and upregulates LDLr genes with a slight decrease in HL and CETP gene expression. With no marked effect on carcass traits, enhance body weight and body weight gain.

1. INTRODUCTION

Since there is a severe lack of animal protein available for human consumption in underdeveloped nations, many of them grow rabbits for meat. It has been suggested that rabbit meat is a good source of animal protein. Because it is slimmer than that of other animals and includes higher levels of protein, vitamin B, and low levels of fat, cholesterol, and sodium, rabbit meat is highly valued for human consumption (Ezema and Eze, 2014).

Probiotics are now widely utilized as growth stimulants, the fact that probiotics don't leave behind any residues in animal production or cause antibiotic resistance when consumed (Tufarelli et al., 2017). Also, they have a favorable impact on rabbits' health, feed intake, growth performance, and growth conversion ratio (Bhatt et al., 2017a).

Probiotics are bacteria that offer the host health benefits when taken in sufficient amounts they can be utilized to promote gut health and treat dysbiosis (Lau et al., 2017). The growth of *E. coli* 0157:H7 in the rabbits' intestines was successfully stopped by probiotics (Geetanjali et al., 2017). Among the microorganisms typically used as probiotics are lactic acid bacteria, including *lactobacilli*, *enterococci*, *bifidobacteria*, and yeasts (Linaje et al., 2004).

Through the creation of an advantageous environment, probiotics increase nutrient intake, improve intestinal digestion and increase energy utilization in rabbits (Pogány Simonová et al., 2009; Amber et al., 2014).

In addition to producing short chain fatty acids (SCFAs) like acetate, butyrate, and propionate that the colonocytes and intestinal flora need as fuel, probiotic bacteria also produce vitamins K and B2 (Ohira et al., 2017).

The lactic acid bacteria (LAB) group includes lactobacilli, which are fermenting, Gram-positive, non-pathogenic, and non-toxic bacteria. They are helpful for food fermentation since they are connected to the formation of lactic acid from carbohydrates (Gu and Roberts, 2019).

Improvements in the digestibility of energy and the majority of analytical fractions (dry matter, crude protein, and crude fiber) were obtained while using *Lactobacillus acidophilus* (Amber et al., 2004). Maintaining a substantially improved gastrointestinal health and gut environment that promote growth with an effective feed conversion rate (FCR) may lead to a higher digestibility of crude protein and fiber components (Combes et al., 2013).

The growth performance of weaned rabbits is improved by *lactobacillus acidophilus* caused due to higher nitrogen retention and nutritional digestibility in rabbits (Phuoc and Jamikorn, 2017).

Oral paste, water feed additives, microbial cells, microbial cultures, and microbial metabolites are all examples of probiotic products (Geetanjali et al., 2017).

Basic probiotic actions include preventing pathogen adherence, producing antimicrobial substances like bacteriocins and defensins, competitively excluding harmful bacteria, enhancing barrier function, lowering luminal pH, and modulating the immune system. By preventing dangerous microorganisms, probiotics help to improve health conditions (Maldonado Galdeano et al., 2019).

In many bacteria, the ribosome produces bacteriocins, which are bioactive antimicrobic peptides that bind to the cells of

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pathogenic microorganisms by piercing their phospholipid membranes. The primary mechanism of bacteriocin-mediated pathogen reaction involves the penetration of pathogenic bacteria through the cytoplasmic membrane, which results in the suppression of DNA and RNA synthesis and cell leakages (van Zyl et al., 2020). Bacteriocins can fight antibiotic-resistant bacteria and limit the capacity of pathogen cells to colonize the GIT (Kuebutornye et al., 2020; Lajis, 2020).

Although the molecular basis of probiotics' actions is mostly unknown, they can be categorized into four groups(Rijkers et al., 2010). First, direct interaction with the gut microbiota and enzymatic activities of probiotics within the gut lumen. Second, interaction with the gut mucus and epithelium, including barrier effects, digestive processes, the mucosal immune system, and the enteric nervous system. Third, signaling to the host beyond the gut to the liver, the systemic immune system, and other potential organs. Fourth, interaction with the host's extraintestinal tissues.

So, this study aims to determine the effect of probiotic on the expression of some lipolytic genes (Lipoprotein lipase (LPL), Hepatic lipase (HL), low-density lipoprotein receptor (LDLr), and Cholesteryl ester transfer protein (CETP)) and to clarify the impact of probiotic on carcass traits and also Evaluate how probiotics affect growth performance.

2. MATERIAL AND METHODS

2.1. Rabbits, Management, and housing:

The current study was undertaken at Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University under guidelines of institutional Animal Care and Use Committee Research Ethics Broad (No. BUFVTM 02-12-20). Thirty male New Zealand white rabbits (eight weeks old, about 1200 gm body weight) were used in this study. The rabbits were distributed randomly into two groups, each group containing three replicates of five rabbits. The house was clean, disinfected, and well-ventilated with proper environmental temperature, and lightening was provided for 16:8 hours of darkness throughout the experimental period. The rabbits were housed in wire mesh cages with identical housing and care practices, and feed was applied twice a day and water was available all the time by nipple system.

2.2. Groups and treatment:

The probiotic "Lacto biotech®" is produced and exported by Mycrofeed Srl, Italy, and obtained by Cairomed Pharmaceuticals Company.

Rabbits were randomly distributed into two groups:

Group I: supplemented with the basal diet as shown in table 1

Group II: supplemented with the basal diet + probiotic (1gm per liter of drinking water).

Rabbits allowed an adaptation period for two weeks with a gradual change in diet to the experimental diet, the trial lasted for eight weeks.

2.3. Determination of lipogenic genes expression:

2.3.1. Sample collection:

Twelve representative rabbits (chosen at random as three rabbits per replication) had been slaughtered for sampling on day 56 after the experiment's start. For further investigation, liver samples were obtained and stored at -80°C.

2.3.2. RNA extraction and Real-time PCR for lipolytic gene expression:

Trizol Reagent was used following the manufacturer's instructions to extract total RNA (Invitrogen, Korea). Spectro Star Nanodrop (BMG Lab Tec. GmbH, Germany) measured the concentration and purity of RNA at 260/280 nm absorbance. Then, about 2 µg of total RNA were reverse transcribed into cDNA using Applied Biosystems' 2X Reverse Transcriptase Master Mix under the manufacturer's instructions. Primer designs were supported by the NCBI Primer-BLAST software. Table 2 illustrates primers used for quantitative real-time PCR (qRT-PCR).

Real-Time PCR Quantitative Analysis:

The Applied Biosystem 7500 Fast Real-time PCR, USA, was used to quantify the mRNA. The quantitative PCR was conducted using the SYBER Green Master Mix in a 20 µL reaction mixture (TOPreal TM qPCR 2X PreMIX). The initial activation (3 min/95 °C), denaturation (3 sec/95 °C), and annealing/extension (30 sec/60 °C) were used to justify the cycling condition, and 40 cycles were used in total, according to Zhao et al. (2009). The GAPDH gene served as the standard for all gene expression levels. Utilizing the $2^{-\Delta\Delta Ct}$ technique, gene expression has been compared and quantified (Livak and Schmittgen, 2001).

Table 1 Ingredients and nutritional composition of basal diet.

Ingredients	Amount (kg/ ton)	%
Berseem hay 16%	303.00	30.30
Wheat bran	250.00	25.00
Soybean meal 46	175.00	17.50
Yellow corn	136.00	13.60
Fennel hay	50.00	5.00
Molasses	30.00	3.00
Glutafeed	27.00	2.70
Limestone	10.60	1.06
Monosodium phosphate	8.25	0.83
Salt	3.50	0.35
Vitamin, mineral premix	3.00	0.30
Bi sodium carbonate	1.90	0.19
Anticoccidial	1.00	0.10
Antimycotoxin	0.50	0.05
D-L methionine	0.25	0.03
Nutrients chemical composition		
Component	Value	Unit
Digested energy	2,600.92	Kcal / kg
Crude protein	17.99	%
Crude fiber	13.48	%
Lysine	0.97	%
Methionine + cystine	0.60	%
Calcium	1.10	%
Total phosphorus	0.70	%
Chloride	0.23	%
sodium	0.20	%

2.4. Determination of carcass traits:

- The hot carcass weight is the weight of the animal after slaughtering including the head, thoracic viscera (heart and lungs), liver, and kidneys were weighed.
- Commercial carcass (includes head, thoracic viscera, liver, and kidneys).

Table 2 Primers used for qRT-PCR

Gene name	Primer sequence (5´- 3´)	Expected product size	Accession number
GAPDH	F- GCCGCTTCTTCTCGTGCAG	145	L23961
	R- ATGGATCATTGATGGCGACAACAT		
LPL	F- ACAAGAGAGAACCAGACTCCAAC	216	ENSOCUT00000008235
	R- TCAGACTTCCAGCAATGCCAG		
HL	F- CTACATCAGCGGAAAGCACA	241	AF041202
	R- GAGCTCCAGGAAGTGACAGC		
LDLr	F- TGCACTCCATCTCCAGCATC	264	M11501
	R- TCTTCTCGCACCAGTTCACC		
CETP	F-AGCTCTTCACAAACTTCATCTCCTTC	206	M27486
	R- CTTGTGATGGGACTCCAGGTAGG		

(GAPDH refers to Glyceraldehyde 3-phosphate dehydrogenase, LPL refers to Lipoprotein lipase, HL refers to hepatic lipase, LDLr refers to low-density lipoprotein receptor and CETP refers to Cholesteryl ester transfer protein).

- Reference carcass (no head or viscera) was weighed.
- Percentages of the head, thoracic viscera, liver, and kidneys were calculated relative to the commercial carcass weight.
- Percentages of commercial and reference carcass weights relative to slaughter weight (dressing percentage) were also computed (Wang et al., 2016).

2.5. Growth parameters:

2.5.1. Body weight (BW):

Weekly weight measurements of the rabbits were taken individually, and the results were utilized to calculate growth rates (Bhatt et al., 2017b).

2.5.2. Body weight gain (BWG):

The difference in body weight between two consecutive weights was subtracted to determine the body weight gain each week.

2.6. Statistical analysis:

The statistical software tool SPSS was used for data analysis (version 21; SPSS Inc., Chicago, IL, USA). The results achieved were found by the independent sample T- test study to be mean ± SE. Meaningful significance ($P<0.05$).

3. RESULTS

The effect of probiotic on lipolytic gene expression was shown in fig 1. The expression of the LPL gene significantly decreased in the probiotic supplemented group compared to the control group. HL and CETP genes expression showed a non-significant decrease in the probiotic supplemented group compared to the control group. The expression of LDLr gene increased significantly in probiotic supplemented group compared to control group.

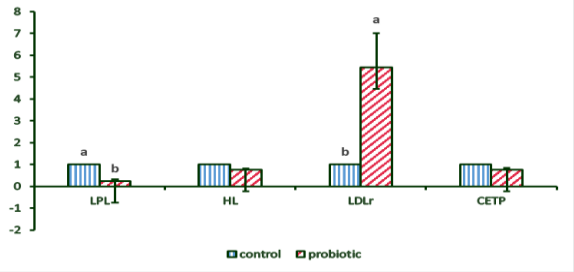

Fig. 1 Effect of probiotic on lipolytic gene expression. (GAPDH refers to Glyceraldehyde 3-phosphate dehydrogenase, LPL refers to Lipoprotein lipase, HL refers to hepatic lipase, LDLr refers to low-density lipoprotein receptor and CETP refers to Cholesteryl ester transfer protein).

Table 3 shows the result of probiotic supplementation on carcass traits. There was no significant difference between the probiotic supplemented group and the control group.

Table 4 shows that there was a significant improvement in Body weight (BW) and Body weight gain (BWG) in the probiotic supplemented group relative to the control group.

Table 3 Effect of the probiotic supplement on carcass traits.

	Control group	Probiotic supplemented group
Hot carcass weight (gm)	1354.44 ± 42.63	1335.00 ± 24.22
Carcass dressing%	1062.22 ± 47.30	972.33 ± 28.92
Head weight(gm)	130.22 ± 3.43	123.33 ± 3.84
Heart weight(gm)	6.44 ± 0.24	6.22 ± 0.22
Lung weight(gm)	9.44 ± 0.41	10.56 ± 0.53
Liver weight(gm)	52.33 ± 2.72	45.00 ± 0.88
Kidney weight(gm)	14.11 ± 1.06	17.56 ± 1.89
Commercial carcass weight (gm)	210.44 ± 5.17	198.22 ± 6.71
Reference carcass weight (gm)	946.00 ± 50.57	858.33 ± 29.16
Head (%)	62.30 ± 1.24	63.22 ± 0.70
Lung (%)	4.50 ± 0.20	5.42 ± 0.29
Liver (%)	25.86 ± 0.94	22.97 ± 0.90
Kidney (%)	7.33 ± 0.55	7.50 ± 0.55

Values were expressed as means ± standard error.

Table 4 Effect of the probiotic on BW & BWG.

	Parameter	Control group	Probiotic supplemented group
Body weight (BW) (gm/rabbit)	Initial weight	1271.89 ± 45.56	1275.89 ± 43.96
	1st week	1456.44 ± 37.14	1482.11 ± 31.08
	2nd week	1578.22 ± 27.01	1618.00 ± 24.11
	3rd week	1691.44 ± 25.55	1700.11 ± 20.24
	4th week	1698.44 ± 24.40	1747.33 ± 15.14
	5th week	1725.22 ± 20.66	1760.33 ± 22.69
	6th week	1800.33 ± 25.67	1859.11 ± 13.14
	7th week	1826.89 ± 31.77	1873.11 ± 15.91
	8th week	1841.67 ± 19.88^{b}	1954.44 ± 23.90^{a}
Body weight gain (BWG) (gm/rabbit)	1st week	184.56 ± 18.28	206.22 ± 29.32
	2nd week	121.78 ± 16.04	135.89 ± 13.98
	3rd week	113.22 ± 17.19	82.11 ± 10.78
	4th week	7.00 ± 6.47	47.22 ± 12.27
	5th week	26.78 ± 15.72	13.00 ± 18.41
	6th week	75.11 ± 13.92	98.78 ± 22.12
	7th week	26.56 ± 18.04	64.78 ± 24.26
	8th week	14.78 ± 22.95^{b}	81.33 ± 22.60^{a}
	Final BWG	569.78 ± 39.72	678.56 ± 39.92

Values (mean ± standard error) with different letters within the same raw significantly different $p\leq0.05$.

4. DISCUSSION

The current study showed that gene expression of LPL in the probiotic supplemented group was significantly lower than in the control group. This finding is in agreement with Wang et al. (2017), who reported that the gene expression of LPL in the *Lactobacillus johnsonii* group was significantly decreased in comparison to the control group. Also Karimi et al. (2017) observed that single or multiple species probiotic treatment significantly down-regulated the LPL gene expression. It can be said that probiotic supplementation increased Peroxisome proliferator-activated receptor gamma (PPARG) expression, which in turn up-regulates the expression of Angiopoietin-like 4 (ANGPTL-4). When ANGPTL-4 is upregulated, LPL is downregulated, which lowers TG levels (Karimi et al., 2017).

Expression of the HL gene in the current study showed a slight decrease in probiotic supplemented group compared to the control group, as *Lactobacillus acidophilus* boosted HDL and lowered LDL in experimental mice as a result of probiotics (Jouybari et al., 2009). The fact that there is a direct correlation between LDL and hepatic lipase and an inverse relationship with HDL suggests that elevated hepatic lipase plays a role in developing an atherogenic profile (Miksztowicz et al., 2012).

Expression of LDLr gene significantly increased in the probiotic supplemented group when compared to the control group. This result was similar to Palaniyandi et al. (2020) who found that expression of LDLr gene in the liver was greater group supplemented with probiotics. Also (Tamtaji et al., 2019) found that there is significant upregulation in LDLr gene expression in patients supplemented with selenium and probiotic compared with only selenium supplemented patients. This result disagreed with (Borzabadi et al., 2018) who reported that probiotics didn't affect LDLr gene expression. A drop in serum LDL levels results from increased hepatic LDLr expression, which also causes the liver to absorb more plasma LDL cholesterol (Palaniyandi et al., 2020), As a result of probiotics, *Lactobacillus acidophilus* increased HDL and decreased total and LDL in experimental mice (Jouybari et al., 2009).

There was a slight decrease in CETP gene expression in the probiotic supplemented group compared to the control group, as HDL was increased with *Lactobacillus acidophilus*, but LDL was decreased (Jouybari et al., 2009). Increased CETP activity is hypothesized to be linked to decreased HDL levels (Barter, 2011).

The study showed that supplementation of probiotics had no significant effect on carcass traits. These findings are similar to those (Bhatt et al., 2017c), who reported that Probiotics

had no noticeable impact on the carcass characteristics and no adverse effects of *Lactobacillus acidophilus* supplementation on carcass traits. Also similar to El-Badawi et al. (2017), who said that Supplementing with good bacteria had no impact on the carcass features, flesh-to-bone ratio, or chemical makeup of the meat from New Zealand white rabbits. Also Sherif (2017) said that There were no notable consequences. A rabbit's carcass characteristic is caused by a feed supplement. These findings disagreed with Fathi et al. (2017), who reported that Probiotic supplementation had a substantial impact on carcass characteristics, carcass weight, dressing percentage, and cuts of the mid and rear parts as a proportion of live body weight. In comparison to the other dietary groups, the rabbits given a diet high in probiotics showed a considerably ($P \leq 0.001$) greater dressing percentage.

The study showed that supplementation of the probiotic resulted in a significant improvement in growth efficiency compared to the control group. Bhatt et al. (2017b) reported that *Lactobacillus acidophilus* group's rabbits had higher cumulative body weight (BW) than the control group. Also Fathi et al. (2017) found that Probiotics can be added to feed to enhance growth performance, carcass weight, and meat quality. Also Bhatt et al. (2017c) found that in growing rabbits, *L. acidophilus* supplementation improved feed conversion ratio, weight gain, and nutrient consumption and digestion.

5. CONCLUSION

We can conclude that the probiotic supplementation downregulates LPL and upregulates LDLr genes with a slight decrease in HL&CETP gene expression, with no marked effect on carcass traits, and enhance body weight and body weight gain.

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