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Research Article

Dosage Effects of *Lactococcus lactis* ssp. *lactis* 2 as a Probiotic on the Percentage of Carcass, Abdominal Fat Content and Cholesterol Level in Broilers

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Abstract

Objective: This study was conducted to determine the dosage effects of the lactic acid bacteria (LAB) *Lactococcus lactis* ssp. *lactis* 2 as a probiotic on the percentage of carcass, abdominal fat content and cholesterol level in broilers. **Methodology:** *Lactococcus lactis* ssp. *lactis* 2 was isolated from broiler faeces. The microbe count was 1.3×10^8 CFU g⁻¹. The treatments in this study consisted of the following groups, P0: Without administration of the probiotic, P1: With the administration of 0.01% zinc bacitracin antibiotic, P2: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 0.5% (6.5×10^8 CFU kg⁻¹), P3: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 1% (1.3×10^9 CFU kg⁻¹) and P4: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 1.5% (1.95×10^9 CFU kg⁻¹). **Results:** This study showed that administration of the LAB *Lactococcus lactis* ssp. *lactis* 2 with a 0.5% (6.5×10^8 CFU kg⁻¹), 1% (1.3×10^9 CFU kg⁻¹) and 1.5% (1.95×10^9 CFU kg⁻¹) dose did not influence abdominal fat content, however, probiotic administration reduced cholesterol in breast meat and thigh meat and reduced fat in breast meat compared to the antibiotic and control treatments. Administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1.5% dose (1.95×10^9 CFU kg⁻¹) resulted in a higher percentage of carcass and lower percentage of thigh meat cholesterol compared to the 0.5% (6.5×10^8 CFU kg⁻¹) and 1% (1.3×10^9 CFU kg⁻¹) doses. **Conclusion:** The administration of *Lactococcus lactis* ssp. *lactis* 2 did not affect abdominal fat content but reduced breast meat cholesterol and thigh meat cholesterol. The 1.5% dose (1.95×10^9 CFU kg⁻¹) produced a higher percentage of carcass and lower thigh meat cholesterol than did the other doses.

Key words: Abdominal fat, broiler, carcass, cholesterol, *Lactococcus lactis* ssp. *lactis* 2

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The development of the livestock subsector has the purpose of producing animals to meet dietary protein needs both in quality and quantity. One step that can be taken in an effort to fulfil protein needs, especially animal protein, is the development of broiler farming. In an effort to spur growth and prevent disease in broilers, the use of antibiotics in the diet has become essential. However, in early 2006, the use of antibiotics was prohibited by the European Union as a disease prophylactic (antimicrobial growth promoters) in animal feed, because continuous antibiotic use can lead to the accumulation of antibiotic residues in livestock in the muscles, liver and other organs. If this product is consumed by humans, it also causes health problems.

The development of food safety requirements has limited the use of antibiotics because in addition to the positive properties related to the suppression of pathogenic bacterial infections, antibiotics can also kill beneficial gastrointestinal tract microbes¹. Therefore, to suppress the use of antibiotics, one alternative is to use probiotics that can replace the function of antibiotics in the feed as drivers of growth.

Generally, the probiotic widely used in chickens is a type of LAB that can increase growth and efficiency in food use without absorption of probiotics in the body, thus there is no residue in the livestock body and meat cholesterol is also reduced².

The study conducted by Daud *et al.*³ showed that administration of 0.2% probiotic in broiler feed did not significantly change the percentage of carcass, meat fat content or abdominal fat content but did reduce cholesterol levels in the breast. Afriani⁴ showed that the addition of probiotics to broiler feed did not improve the efficiency of feed use or the fat content of the meat but did reduce meat cholesterol. These differences can be caused by several factors, namely, the strains of bacteria in the probiotics and dose administered to livestock.

One LAB with potential for use as a probiotic is *Lactococcus lactis* ssp. *lactis* 2, which was isolated from the faeces of broilers in a series of previous studies, tested *in vitro* and qualified to serve as a probiotic⁵. The administration of *Lactococcus lactis* ssp. *lactis* 2 in this study aimed to provide a basis for the development of methods for reducing and suppressing the use of antibiotics to improve the productivity and quality of broiler meat.

MATERIALS AND METHODS

The broilers used in this study were Lohmann 202 strain day-old chicks (DOC). All DOC had relatively similar weights

Table 1: Composition of feed ingredients and food substances in the feed treatments

Food ingredients (%)	P0	P1	P2	P3	P4
Corn flour	57	57	57	57	57
Rice bran	8	8	8	8	8
Fish flour	12	12	12	12	12
Soybean meal	17	17	17	17	17
Coconut meal	6	6	6	6	6
Total	100	100	100	100	100
Dried culture LAB (%)	-	-	0.5**	1**	1.5**
Zinc bacitracin (%)	-	0.01**	-	-	-
Crude protein (%)	22.4	22.4	22.4	22.4	22.4
Crude fat (%)	4.8	4.8	4.8	4.8	4.8
Crude fibre (%)	5.2	5.2	5.2	5.2	5.2
Ca (%)	0.96	0.96	0.96	0.96	0.96
P (%)	0.69	0.69	0.69	0.69	0.69
Energy metabolism (kcal kg ⁻¹)	2916.9	2916.9	2916.9	2916.9	2916.9

**Feed additives (%)

with a mean of 44.93 g±1.23. The experiments used a complete randomized design (CRD) with 5 feed treatments. Each treatment included four replications and each replication consisted of 5 chickens for a total of 100 chickens. Feed and water were provided *ad libitum* during the study. The composition of feed is listed in Table 1. *Lactococcus lactis* ssp. *lactis* 2 was isolated from broiler faeces in a series of previous studies and tested *in vitro* for qualification to serve as a probiotic⁵. *Lactococcus lactis* ssp. *lactis* 2 probiotic flour was produced by freeze-drying with the addition of skim milk powder as a cryoprotectant. The *Lactococcus lactis* ssp. *lactis* 2 probiotic in this research contained 1.3×10⁸ CFU g⁻¹ of microbes. Treatments in this research consisted of P0, without the administration of probiotic, P1: With the administration of zinc bacitracin antibiotics (0.01%), P2: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 0.5% dose (6.5×10⁸ CFU kg⁻¹), P3: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1% dose (1.3×10⁹ CFU kg⁻¹) and P4: With the administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1.5% dose (1.95×10⁹ CFU kg⁻¹).

The percentage of carcass and abdominal fat were measured at the end of the study and serum cholesterol levels, HDL (high density lipoprotein) and LDL (low density lipoprotein) in the blood were measured at the end of observation period (i.e., in the 5th week). Cholesterol levels were analysed using the cholesterol oxidase/peroxidase aminophenazone phenol (CHOD-PAP) method. The breast and thigh meat fat content of the broilers was analysed based on the Soxhlet extraction method⁶.

Statistical analysis: The results were analyzed using one-way analysis of variance and a p<0.05 was considered statistically significant. The treatment effects were evaluated by orthogonal contrast.

Table 2: Carcass and abdominal fat percentage in broilers under a variety of treatments for 35 days

Carcass (%)	Carcass (%)			p-value
	P0	P1	P2, P3 and P4	
Contrast of P0 Vs P2, P3 and P4	66.75		69.14	0.002
Contrast of P1 Vs P2, P3 and P4		67.8		0.057
Contrast of probiotic:				
Linear				0.032
Quadratic				0.916
Abdominal fat (%)				
Contrast of P0 Vs P2, P3 and P4	3.23		2.78	0.206
Contrast of P1 Vs P2, P3 and P4		2.79	2.78	0.977
Contrast of probiotic:				
Linear				0.455
Quadratic				0.068

P0: Without the administration of probiotic, P1: The administration of zinc bacitracin antibiotics at 0.01%, P2: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic with 0.5% dose (5 g kg⁻¹-6.5×10⁸ CFU kg⁻¹), P3: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1% dose (10 g kg⁻¹-1.3×10⁹ CFU kg⁻¹), P4: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1.5% dose (15 g kg⁻¹-1.95×10⁹ CFU kg⁻¹)

RESULTS AND DISCUSSION

The percentage of carcass and abdominal fat in broilers:

The carcass percentages of the broilers after the various treatments are listed in Table 2. The average percentage of carcass in broilers from the control group was lower (p = 0.002) than the *Lactococcus lactis* ssp. *lactis* 2 treatment group and the average percentage of carcass for broilers in the antibiotic treatment group was not different (p = 0.057) from broilers in the *Lactococcus lactis* ssp. *lactis* 2 treatment groups. There was a positive correlation between of *Lactococcus lactis* ssp. *lactis* 2 dose (p = 0.032) and carcass percentage. The percentage of carcass obtained in this research was approximately 66.75-69.89%. Bell and Weaver⁷ reported that the carcass percentage of broilers varied between 65-75% of body weight.

The percentage of abdominal fat did not differ among the five treatments and this was expected because the consumption levels of feed did not differ and the energy content of feed was similar among the treatments. Energy consumption was also similar for all treatments, so energy intake did not exceed the needs of the broilers, thus there was no excessive energy intake to eventually be deposited in the form of body fat. The average percentage of abdominal fat of broilers in this research was approximately 2.36-3.30% and was within the normal range. In contrast, Adriani *et al.*⁸ reported that adding up to 2% probiotic (mixed *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) significantly affected abdominal fat content. This occurs because bioactive substances such as bacteriocin improve the metabolism of carbohydrates and fat in the body.

Table 3: Serum cholesterol, excreta cholesterol, breast meat cholesterol and thigh meat cholesterol of broilers under a variety of treatments for 35 days

Description	Treatments			
	P0	P1	P2, P3 and P4	p-value
Blood cholesterol (mg/100 mL)				
Contrast of P0 Vs P2, P3 and P4	125.50		107.58	0.021
Contrast of P1 Vs P2, P3 and P4		112.75	107.58	0.471
Contrast of probiotic:				
Linear				0.476
Quadratic				0.514
Excreta cholesterol (mg/100 g)				
Contrast of P0 Vs P2, P3 and P4	48.79		61.76	0.013
Contrast of P1 Vs P2, P3 and P4		51.46	61.76	0.041
Contrast of probiotic:				
Linear				0.977
Quadratic				0.805
Breast meat cholesterol (mg/100 g)				
Contrast of P0 Vs P2, P3 and P4	4.00		3.15	0.021
Contrast of P1 Vs P2, P3 and P4		4.03	3.15	0.017
Contrast of probiotic:				
Linear				0.280
Quadratic				0.524
Thigh meat cholesterol (mg/100 g)				
Contrast of P0 Vs P2, P3 and P4	5.11		4.16	0.008
Contrast of P1 Vs P2, P3 and P4		4.83	4.16	0.050
Contrast of probiotic:				
Linear				0.022
Quadratic				0.293

P0: Without the administration of probiotic, P1: The administration of zinc bacitracin antibiotics at 0.01%, P2: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 0.5% (5 g kg⁻¹ - 6.5×10⁸ CFU kg⁻¹), P3: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1% dose (10 g kg⁻¹-1.3×10⁹ CFU kg⁻¹), P4: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1.5% dose (15 g kg⁻¹-1.95×10⁹ CFU kg⁻¹)

Cholesterol in the blood, excreta, breast meat and thigh meat of broilers:

The statistical results in Table 3 show that broiler cholesterol levels in the control group were higher (p = 0.021) than *Lactococcus lactis* ssp. *lactis* 2 treatment group, but cholesterol levels in the antibiotic treatment group did not differ from those of the *Lactococcus lactis* ssp. *lactis* 2 treatment group (p = 0.471), further, the dose of *Lactococcus lactis* ssp. *lactis* 2 did not show a linear (p = 0.476) or quadratic (p = 0.514) relationship with blood cholesterol level.

Excreta cholesterol levels in the control group were lower (p = 0.013) than the *Lactococcus lactis* ssp. *lactis* 2 groups and excreta cholesterol levels in the antibiotic treatment group were lower (p = 0.041) than the *Lactococcus lactis* ssp. *lactis* 2 groups. However, the level of *Lactococcus lactis* ssp. *lactis* 2 did not show a linear (p = 0.977) or quadratic (p = 0.805) response to increasing excreta cholesterol levels.

Cholesterol level in breast meat of the control group was higher ($p = 0.021$) than the *Lactococcus lactis* ssp. *lactis* 2 treatment group, but cholesterol levels in the antibiotic treatment group were not different ($p = 0.071$) from the cholesterol levels of the *Lactococcus lactis* ssp. *lactis* 2 treatment group. Cholesterol level in thigh meat of the control group was higher ($p = 0.008$) than the *Lactococcus lactis* ssp. *lactis* 2 treatments group and thigh meat cholesterol level in the antibiotic treatment was higher ($p = 0.050$) than in the treatments with *Lactococcus lactis* ssp. *lactis* 2.

Increased doses of *Lactococcus lactis* ssp. *lactis* 2 did not result in a decreasing linear response ($p = 0.022$) with respect to cholesterol levels in thigh meat. A quadratic response was not seen and the level of *Lactococcus lactis* ssp. *lactis* 2 was not identified as a factor affecting cholesterol in breast and thigh meat. Therefore, it can be assumed that the increase in the level of *Lactococcus lactis* ssp. *lactis* 2-1.5% within the experimental interval (i.e., from 0.5%) still results in decreased thigh meat cholesterol.

The decrease in blood cholesterol level and increase in excreta cholesterol followed by the decrease in breast and thigh meat cholesterol level showed that the presence of *Lactococcus lactis* ssp. *lactis* 2 probiotic in feed significantly contributed to the decrease of cholesterol level through assimilation of cholesterol by the LAB *Lactococcus lactis* ssp. *lactis* 2.

Under certain conditions, the cholesterol levels exceeded normal and various processes can be activated to compensate for this excess cholesterol. First, cytosolic HMG-CoA synthase and microsomal 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase are inhibited either independently or in a coordinated manner depending on the supply of free fatty acids in the cell. Second, the catabolism rate of cholesterol can increase due to the stimulation of α -hydroxylase activity. Third, the activity of acyl-coenzyme A (CoA): Cholesterol acyltransferase (ACATs) is stimulated so that excess cholesterol is altered by free fatty acids into an ester compound which is then stored in the cytoplasm. Fourth, biosynthesis of the receptor can be reduced so that the LDL-making process is reduced in cells. Fifth, more cholesterol transported into the membrane results in a higher degree of regularity in the double-lipid layer of the membrane such that membrane permeability increases and the process of lipoprotein (LDL) entry increases. Sixth, the process of cholesterol release will increase due to the increase of very low-density lipoprotein (VLDL) from liver cells or high density lipoprotein (HDL) from the peripheral blood cells.

Cholesterol assimilated by LAB is not absorbed by the intestines and is expelled together with faeces incorporated

with the LAB, which is then detected in excreta⁹. Therefore, more cholesterol incorporated by the LAB means higher detected cholesterol levels in the excreta of the broilers and this will directly impact (i.e., decrease) the amount of cholesterol in the blood. Assimilation of cholesterol in the intestinal tract by bacteria prevents absorption of cholesterol by the body and blood and results in reduced blood cholesterol.

In addition to affecting cholesterol assimilation in the intestines, another suspected mechanism of cholesterol decrease is bile salt hydrolase enzyme activity by *Lactococcus lactis* ssp. *lactis* 2 through the deconjugation of bile salts. The deconjugation of bile salts increases deconjugated bile acids, which cannot be absorbed by the small intestine, the amounts of bile acids returning to the liver will thus be reduced and to balance the level of bile acids, the body will utilize cholesterol because it is a precursor for the manufacture of bile acids in the liver. This cycle continues so that the catabolism of cholesterol becomes more rapid and finally reduces the accumulation of cholesterol. This process reduces cholesterol on the whole¹⁰⁻¹³.

The average blood cholesterol of broilers in this study was categorized as normal with a range of approximately 103-125.5 mg/100 mL. According to Basmacioglu and Ergul¹⁴, the average blood cholesterol in broilers was 52 mg/100 mL to 148 mg dL⁻¹. Differences in the amount of blood cholesterol depend on the food provided, age and gender. A decrease of blood cholesterol after treatment with *Lactococcus lactis* ssp. *lactis* 2 was followed by an increase in HDL cholesterol and a decrease in LDL cholesterol in blood.

Based on the results of the statistical analysis (Table 4), HDL levels among the five treatments did not differ, but LDL levels in the treatment with *Lactococcus lactis* ssp. *lactis* 2 were lower ($p = 0.001$) than the control group and an increase in the level of probiotic did not produce a linear or quadratic response with respect to HDL or decrease in LDL. However, the average broiler HDL values in the treatment with *Lactococcus lactis* ssp. *lactis* 2 were higher than the control group and antibiotic treatment group. The increase of HDL in this study was followed by a decrease of LDL.

Breast meat fat and thigh meat fat in broilers: Based on Table 5, the breast fat content in the control group was higher ($p = 0.005$) than the *Lactococcus lactis* ssp. *lactis* 2 treatment group and fat content of the breast in the antibiotic treatment group was higher ($p = 0.003$) than the *Lactococcus lactis* ssp. *lactis* 2 treatment group. This indicates a better fat content of breast meat after treatment with *Lactococcus lactis* ssp. *lactis* 2 than in the control and antibiotic treatment groups.

Table 4: Broiler HDL and LDL under a variety of treatments for 35 days

Description	Treatments			p-value
	P0	P1	P2, P3 and P4	
HDL (mg/100 mL)				
Contrast of P0 Vs P2, P3 and P4	62.00		70.67	0.092
Contrast of P1 Vs P2, P3 and P4		69.75	70.67	0.851
Contrast of probiotic:				
Linear				0.357
Quadratic				0.365
LDL (mg/100 mL)				
Contrast of P0 Vs P2, P3 and P4	56.25		30.00	0.001
Contrast of P1 Vs P2, P3 and P4		34.50	30.00	0.496
Contrast of probiotic:				
Linear				0.853
Quadratic				0.847

P0: Without the administration of probiotic, P1: The administration of zinc bacitracin antibiotics at 0.01%, P2: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 0.5% dose (5 g kg^{-1} - $6.5 \times 10^8 \text{ CFU kg}^{-1}$), P3: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a 1% dose (10 g kg^{-1} - $1.3 \times 10^9 \text{ CFU kg}^{-1}$), P4: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic with 1.5% dose (15 g kg^{-1} - $1.95 \times 10^9 \text{ CFU kg}^{-1}$)

Table 5: Thigh meat and breast meat fat of broilers under a variety of treatments for 35 days

Description	Treatments			p-value
	P0	P1	P2, P3 and P4	
Breast meat fat (%)				
Contrast of P0 Vs P2, P3 and P4	1.44		0.81	0.005
Contrast of P1 Vs P2, P3 and P4		1.49	0.81	0.003
Contrast of probiotic:				
Linear				0.198
Quadratic				0.646
Thigh meat fat (%)				
Contrast of P0 Vs P2, P3 and P4	2.17		1.41	0.127
Contrast of P1 Vs P2, P3 and P4		2.07	1.41	0.182
Contrast of probiotic:				
Linear				0.228
Quadratic				0.790

P0: Without the administration of LAB, P1: The administration of zinc bacitracin antibiotics of 0.01%, P2: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 0.5% (5 g kg^{-1} - $6.5 \times 10^8 \text{ CFU kg}^{-1}$), P3: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 1% (10 g kg^{-1} - $1.3 \times 10^9 \text{ CFU kg}^{-1}$), P4: The administration of *Lactococcus lactis* ssp. *lactis* 2 probiotic at a dose of 1.5% (15 g kg^{-1} - $1.95 \times 10^9 \text{ CFU kg}^{-1}$)

Khaksefidi and Rahimi¹⁵ reported that the breast meat fat content was lower (1.99%) ($p < 0.05$) in probiotic-fed chickens than in the control treatment (3.95%). The increase in the level of *Lactococcus lactis* ssp. *lactis* 2 did not show a linear ($p = 0.198$) or quadratic ($p = 0.646$) relationship with decreased breast fat content.

The percentage of thigh fat in the control group was not different ($p = 0.127$) from that in the *Lactococcus lactis* ssp.

lactis 2 and antibiotic treatments ($p = 0.182$) and did not differ from the *Lactococcus lactis* ssp. *lactis* 2 treatments. The increased dose of *Lactococcus lactis* ssp. *lactis* 2 did not show a linear ($p = 0.228$) or quadratic ($p = 0.790$) relationship with decreased thigh fat content. Although the fat content of thigh meat did not differ significantly among treatments, the fat content in broilers after treatment with *Lactococcus lactis* ssp. *lactis* 2 was lower (1.41% on average) than in the control group (2.17% on average) and the antibiotic treatment (2.07% on average). This result was similar to results reported by Endo and Nakano¹⁶, who showed that the addition of probiotics including species of *Bacillus*, *Lactobacillus*, *Streptococcus*, *Clostridium*, *Saccharomyces* and *Candida* to broilers diets decreased cholesterol concentrations in thigh meat and increased linolenic acid and the unsaturated, saturated fatty acid ratio in pectoral and thigh meat. Khaksefidi and Rahimi¹⁵ also reported that the fat content of thigh meat was lower (4.87%) ($p < 0.05$) in probiotic-fed chickens than in control chickens (7.06%).

Statistically, the increase in the dose of *Lactococcus lactis* ssp. *lactis* 2 did not show a linear or quadratic relationship with the decrease in breast and thigh meat fat content, however, numerically, the dose of *Lactococcus lactis* ssp. *lactis* 2 that was added to the broiler feed was able to decrease the fat content in breast meat and thigh meat in 35 days. The decrease in meat fat content (breast fat and thigh fat) is assumed to be a result of the decrease in cholesterol levels in the blood. Breast meat fat content in broilers in this study was approximately 0.6-1.49% and thigh fat content was approximately 0.99-2.17%.

CONCLUSION

- The administration of *Lactococcus lactis* ssp. *lactis* 2 at a dose of 0.5% ($6.5 \times 10^8 \text{ CFU kg}^{-1}$), 1% ($1.3 \times 10^9 \text{ CFU kg}^{-1}$) and 1.5% ($1.95 \times 10^9 \text{ CFU kg}^{-1}$) did not affect abdominal fat content in broilers but did decrease breast meat cholesterol and thigh meat cholesterol, probiotic treatment also decreased breast meat fat better than the antibiotic treatment and control treatment.
- The administration of *Lactococcus lactis* ssp. *lactis* 2 at a dose of 1.5% ($1.95 \times 10^9 \text{ CFU kg}^{-1}$) produced a higher percentage of carcass and lower thigh meat cholesterol compared to the 0.5% ($6.5 \times 10^8 \text{ CFU kg}^{-1}$) and 1% ($1.3 \times 10^9 \text{ CFU kg}^{-1}$) doses.

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