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Non Renseigné, Norhani Abdullah, Syed Jalaludin, Michael C.V.L. Wong, Yin Wan Ho. Effects of *Lactobacillus* feed supplementation on cholesterol, fat content and fatty acid composition of the liver, muscle and carcass of broiler chickens. *Animal Research*, 2006, 55 (1), pp.77-82. hal-00890029

HAL Id: hal-00890029

<https://hal.science/hal-00890029>

Submitted on 11 May 2020

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Effects of *Lactobacillus* feed supplementation on cholesterol, fat content and fatty acid composition of the liver, muscle and carcass of broiler chickens

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(Received 14 June 2004; accepted 2 August 2005)

Abstract – An experiment was conducted to study the effects of feed supplementation with a mixture of *Lactobacillus* cultures (LC) on cholesterol, fat and fatty acid composition in the liver, muscle and carcass of broiler chickens. One hundred and thirty-six, one-day-old male broiler chicks (Avian-43) were assigned randomly to two dietary treatments: (i) a basal diet (control), and (ii) a basal diet + 0.1% LC. The cholesterol contents of the carcass and liver but not the muscle, were significantly ($P < 0.05$) lower in LC-fed broilers. The fat contents of the liver, muscle and carcass were also significantly ($P < 0.05$) lower in the LC-fed broilers when compared to the control broilers. Supplementation of LC in the broiler diets significantly ($P < 0.05$) reduced the oleic acid (C_{18:1}) levels of the liver, muscle and carcass but the arachidonic acid (C_{20:4}) level was significantly ($P < 0.05$) increased in the liver only. Supplementation of LC also increased the total polyunsaturated fatty acids (PUFA) in the liver. The results of the present study indicate that LC reduces the fat content of the liver, muscle and carcass of broiler chickens, but it has very little potential to modify the fatty acid composition.

***Lactobacillus* / cholesterol / fat / fatty acids / chicken**

Résumé – Effets de l'addition de lactobacilles dans l'aliment de poulets de chair sur les teneurs en cholestérol, graisse et acides gras du foie, du muscle et de la carcasse. Une expérience a mesuré les effets de l'addition d'un mélange de cultures de lactobacilles (LC) dans l'aliment de poulets de chair sur la composition en cholestérol, en graisse et en acides gras du foie, du muscle et de la carcasse. Cent trente six poussins mâles de 1 jour (Avian-43) ont été répartis au hasard dans deux lots recevant soit un régime témoin non supplémenté soit le même régime + 0,1 % de LC. Les concentrations en cholestérol de la carcasse et du foie mais pas celles du muscle, étaient significativement ($P < 0,05$) plus faibles chez les poulets consommant LC par rapport aux témoins. La supplémentation de LC

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dans les aliments a réduit significativement ($P < 0,05$) la teneur en acide oléique ($C_{18:1}$) dans le foie, le muscle et la carcasse mais celle en acide arachidonique ($C_{20:4}$) n'était significativement augmentée ($P < 0,05$) que dans le foie. La supplémentation en LC a également augmenté la teneur du foie en acides gras polyinsaturés (PUFA). Les résultats de la présente étude suggèrent que LC réduit la teneur en graisse du foie, du muscle et de la carcasse des poulets de chair mais a une action limitée sur la composition en acides gras.

lactobacille / cholestérol / graisse / acides gras / poulet de chair

1. INTRODUCTION

Probiotics or direct-fed microbials are live microbial supplements which beneficially affect the health of the host animal by improving its intestinal microbial balance [8]. In recent times, with the renewal of interest in the use of probiotics, development of more effective probiotic preparations, which comply with the various probiotic characteristics, has been found to improve performance in poultry [9, 11]. Several of our studies have consistently shown that the growth performances of broilers supplemented with a probiotic containing 12 *Lactobacillus* cultures are significantly improved [12–14, 24]. The present study was conducted to determine the effects of this *Lactobacillus* probiotic on cholesterol, fat content and fatty acid composition of the liver, muscle and carcass of broiler chickens.

2. MATERIALS AND METHODS

2.1. Chickens and diets

One hundred and thirty-six, one-day-old Avian-43 male broiler chicks were obtained from a commercial hatchery. The chicks were weighed individually, wing banded and assigned randomly to two dietary treatments. Each dietary treatment had four replicate cages with 17 chicks per cage and the cages were randomized with respect to the dietary treatments. Each cage (0.9 m length \times 0.6 m width \times 0.5 m height) was provided with a self-feeder and waterer. Feed was provided ad libitum in a mash form throughout the experimental period.

Table I. Composition of the basal diets.

Ingredient (g·kg ⁻¹ unless otherwise stated)	Starter (1 to 21 d)	Grower (22 to 42 d)
Ground yellow corn	538.9	603.0
Soyabean meal (44.6% CP)	361.9	318.6
Fish meal	30.0	30.0
Palm oil	37.3	24.4
60% choline chloride	2.5	2.0
Trimix ¹	1.0	1.0
Salt (NaCl)	2.0	1.0
Antioxidant	0.125	0.125
DL-methionine	1.775	0.375
Limestone	13.0	13.0
Dicalcium phosphate	11.5	6.5
Total	1000.0	1000.0
Calculated analysis (g·kg ⁻¹ except energy)		
Crude protein	220.0	199.9
Crude fat	63.1	52.2
Crude fibre	38.0	36.5
Calcium	10.2	9.0
Available phosphorus	4.5	3.5
Metabolizable energy (MJ·kg ⁻¹)	13.06	13.06

¹ Trimix (per kg Trimix): iron 100 g; manganese 110 g; copper 20 g; zinc 100 g; iodine 2 g; selenite 0.2 g; cobalt 0.6 g; antioxidant (santoquin) 0.6 g; folic acid 0.33 g; thiamin 0.83 g; pyridoxine 1.33 g; biotin 2% 0.03 g; riboflavin 2 g; cyanocobalamin 0.03 g; D-calcium pantothenate 3.75 g; niacin 23.3 g; retinol 2000 mg; cholecalciferol 25 mg; α -tocopherol 23 000 mg IU.
CP = crude protein.

The dietary treatments consisted of a basal diet (control) and basal diet + 0.1% mixture of 12 *Lactobacillus* cultures (LC). The basal diet (Tab. I) was a corn-soyabean diet formulated to meet nutrient requirements

[17]. The 12 *Lactobacillus* cultures (which belong to four species) and the method of preparation as a feed supplement were the same as those described by Jin et al. [10, 12]. The viability of LC was checked biweekly to ensure that the viability of the cultures remained at 1×10^9 viable cells per gram. The viable LC cells were incorporated into the basal diet daily at feeding time. The experiment was carried out for 42 days.

2.2. Sampling procedure

At the end of the experiment (42 days of age), eight chickens (2 chickens per cage) from each treatment (4 cages) were randomly selected, weighed and sacrificed. The carcasses were opened and the livers and the breast muscles (without skin) were removed and frozen at -20°C . The remaining carcasses were eviscerated, weighed, and frozen at -20°C until being used for analyses. The frozen carcasses were thawed for 24 h at 4°C prior to autoclaving. Each carcass was autoclaved for 1 h [23], and then homogenized in a Waring commercial blender¹. The homogenate was freeze-dried² to a constant weight and re-ground with a domestic blender³ to give a homogenous consistency prior to chemical analysis.

2.3. Chemical analysis

Fat, cholesterol and fatty acid composition of the carcass, liver and muscle samples were determined. Fat contents of the carcass, liver and muscle samples were measured by extraction in a Soxhlet apparatus⁴ with petroleum ether [1].

Total lipids were extracted from the carcass (about 1 g), liver (about 2 g) and muscle (about 4 g) with 40 mL chloroform:methanol (2:1, vol/vol) in a 50 mL ground-glass

extraction flask according to the method of Folch et al. [4]. In order to determine the cholesterol content, the lipids were further saponified with ethanolic KOH to remove the fatty acids. Cholesterol was then extracted with n-hexane and measured using the method of Rudel and Morris [19]. For fatty acid determination, extracted lipids were transmethylated with BF_3 and methanolic KOH [1]. Fatty acid profiles were determined by gas chromatography⁵. Aliquots of 2 μL were injected into a fused-silica capillary column (30 m \times 250 μm , inside diameter)⁶. The injector temperature was programmed at 220°C and the detector at 220°C . The column temperature was set at the range of $100\text{--}190^\circ\text{C}$ with temperature programming at the rate of $5^\circ\text{C}\cdot\text{min}^{-1}$ increments for optimal separation. The identification of peaks was made by comparison with the retention time of the authentic fatty acid methyl ester standard⁷. Quantification was made using an internal standard (heneicosanoic acid; $\text{C}_{21:0}$)⁸ added to the initial sample prior to transmethylation. An HP Chemstation software⁹ was used to integrate peak areas and fatty acid values were expressed as weight percentages.

2.4. Statistical analysis

Treatment effects were compared using analysis of variance and treatment means were separated using the least significance difference. Computation was done by using the SAS program [21].

3. RESULTS

The average body weights of the control and LC-fed chickens sampled for the analysis of the body composition were 2195 g and 2296 g respectively. The cholesterol contents in the carcass and liver of LC-fed broilers were significantly ($P < 0.05$) reduced

¹ Waring Products Division, New Hartford, Connecticut 06057.

² Labconco Corporation Kansas City, Missouri 64132.

³ Braun GmbH, Kronberg, Germany.

⁴ Soxhlet System HT1043 Extraction Unit.

^{5, 9} Hewlett Packard Co., Wilmington, DE 16808-1610.

⁶ Supelco Park, Bellefonte, PA.

^{7, 8} Sigma-Aldrich, St. Louis, MO63178.

Table II. Effects of *Lactobacillus* cultures on cholesterol and fat contents of carcass, liver and muscle of broiler chickens at 42 d of age¹.

	Control	LC	SEM
Cholesterol (mg·100g ⁻¹)			
Carcass	103.79 ^a	90.25 ^b	3.81
Liver	276.58 ^a	225.03 ^b	10.65
Muscle	59.48 ^a	57.38 ^a	1.55
Fat content (%)			
Carcass	14.06 ^a	13.29 ^b	0.18
Liver	3.50 ^a	2.88 ^b	0.09
Muscle	0.89 ^a	0.70 ^b	0.03

Means within a row with no common letters differ significantly ($P < 0.05$).

¹ Control=basal diet; LC=basal diet + 0.1% mixture of 12 *Lactobacillus* cultures.

by 13 and 19%, respectively, when compared to the control broilers (Tab. II). However, the cholesterol content of the muscle was not affected by the supplementation of LC to the broilers. The fat contents in the carcass, liver and muscle were significantly

($P < 0.05$) decreased in LC-supplemented broilers as compared to the control broilers (Tab. II). The fat contents in the liver and muscle were not as high as that in the carcass.

The major fatty acid compositions of the total lipids from the carcass, muscle and liver of broilers are presented in Table III. There were significantly ($P < 0.05$) lower levels of oleic acid (C_{18:1}) in the carcass, muscle and liver but significantly ($P < 0.05$) higher levels of arachidonic acid (C_{20:4}) in the liver of the LC-supplemented broilers when compared to the control broilers. Broilers supplemented with LC also had significantly ($P < 0.05$) lower levels of total monounsaturated fatty acids (MUFA) in the carcass and liver than the control birds. However, there were no significant differences in the total saturated fatty acids (SFA) in the carcass, liver and muscle of the control and LC-fed broilers. The level of total polyunsaturated fatty acids (PUFA) was significantly ($P < 0.05$) higher in the liver of broilers supplemented with LC but there were no significant differences in the levels

Table III. Effects of *Lactobacillus* cultures on the fatty acid composition of carcass, liver and muscle of broiler chickens at 42 d of age¹.

Fatty acid	Carcass			Liver			Muscle		
	Control	LC	SEM	Control	LC	SEM	Control	LC	SEM
	—% of total fatty acid—			—% of total fatty acid—			—% of total fatty acid—		
Myristic acid (C _{14:0})	3.13	3.27	0.07	1.50	1.53	0.21	1.69	1.93	0.07
Palmitic acid (C _{16:0})	49.16	49.57	0.54	42.19	40.21	0.74	39.69	40.74	0.53
Palmitoleic acid (C _{16:1})	8.38	8.34	0.59	4.55	3.80	0.48	5.02	5.05	0.53
Stearic acid (C _{18:0})	4.57	4.69	0.24	15.09	15.99	0.37	7.07	7.14	0.31
Oleic acid (C _{18:1})	31.15 ^a	29.08 ^b	0.59	20.38 ^a	17.36 ^b	0.72	25.42 ^a	23.14 ^b	0.55
Linoleic acid (C _{18:2})	1.85	2.81	0.41	11.91	13.49	0.57	16.60	17.42	0.43
Linolenic acid (C _{18:3})	0.15	0.18	0.04	0.20	0.38	0.11	0.48	0.55	0.03
Arachidonic acid (C _{20:4})	0.07	0.09	0.01	3.16 ^b	5.48 ^a	0.21	2.72	2.79	0.29
ΣSFA	56.86	57.53	0.49	58.78	57.74	0.92	48.45	49.81	0.51
ΣMUFA	39.53 ^a	37.41 ^b	0.58	24.93 ^a	21.16 ^b	1.07	30.44	28.18	0.78
ΣPUFA	2.07	3.08	0.40	15.27 ^b	19.35 ^a	0.83	19.32	20.20	0.58

Means within a row for carcass, liver or muscle, with no common letters differ significantly ($P < 0.05$).

¹ Control = basal diet; LC = basal diet + 0.1% mixture of 12 *Lactobacillus* cultures.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

of PUFA in the carcass and muscle between the treatments. The lowest level of PUFA was observed in the carcass. The supplementation of LC in broiler diets did not affect the levels of linolenic acid (C_{18:3}) or the saturated fatty acids (C_{14:0}, C_{16:0} and C_{18:0}) in the liver, muscle and carcass.

4. DISCUSSION

In this study, broilers fed LC were found to have lower cholesterol contents in the carcass and liver. This result lends support to the finding of our earlier study in which LC-fed broilers had significantly lower serum cholesterol level than the control broilers [12]. Significant reductions of cholesterol in the liver and carcass of broilers fed *Bacillus subtilis* have been reported by Santoso et al. [20]. Other workers have also reported reductions of cholesterol in the muscle and liver of lambs fed *L. acidophilus* [16], and in the liver of rats fed a mixture of bacteria and yeast [5]. Based on the studies to date, the mechanism(s) responsible for the cholesterol-lowering effect of probiotics remains unclear, but it has been suggested that the effect could be obtained through retarded cholesterol synthesis and increased degradation of cholesterol [5]. The hepatic synthesis of bile acids from cholesterol is the major route of cholesterol excretion [22]. Certain lactic acid bacteria have the ability to produce bile salt hydrolase enzyme, which deconjugates bile salts [15], resulting in greater fecal excretion of bile acids [2]. To re-establish the enterohepatic circulation of biliary acids, the liver would partition more cholesterol into the bile and less into the bloodstream and this may cause a loss of cholesterol from the tissues [18]. The 12 *Lactobacillus* cultures (LC) used as a probiotic in the present study have been found to produce bile salt hydrolase and exhibit deconjugating activity of bile salts (unpublished data), which could have contributed to an increase in excretion of cholesterol and reduced cholesterol in chickens.

Supplementation of LC to broilers significantly reduced the fat contents of the liver, muscle and carcass. Santoso et al. [20] also found that supplementation of *B. subtilis* in broiler diets significantly ($P < 0.05$) reduced carcass fat deposition and triglyceride levels in the serum, liver and carcass, and suggested that *B. subtilis* could significantly decrease the activity of acetyl-Co A carboxylase which catalyses the rate-limiting step in fatty acid biosynthesis.

In the present study, supplementation of LC was more effective in reducing the monoenoic acid (oleic acid) than the saturated fatty acids. This suggests that supplementation of LC may reduce the synthesis or absorption of oleic acid. A reduced level of oleic acid has also been observed in the liver of rats fed a mixture of probiotics [7]. Davis and Boogaerts [3] demonstrated that oleic acid is the most potent fatty acid in enhancing triglyceride synthesis in the cells. However, the mechanism by which oleic acid activates the triglyceride secretion remains unclear. Among the different tissues examined, only the liver PUFA was significantly ($P < 0.05$) improved with the supplementation of LC. The increased level of arachidonic acid (C_{20:4}) in LC-fed broilers observed in this study was in agreement with the findings of Fukushima and Nakano [5, 6] and Fukushima et al. [7] in which significant increased levels of arachidonic acid (C_{20:4}) were found in the liver and serum of rats supplemented with a mixture of probiotics. Fukushima et al. [7] suggested that the increased levels of arachidonic acid were due to the $\Delta 6$ -desaturase activity in the liver microsomes of the probiotic-fed animals. Supplementation of LC in broiler diets had no significant effects on the fatty acid composition of muscle tissues, with the exception of a significantly ($P < 0.05$) lower level of oleic acid. The change in the composition of fatty acids in muscle tissue lipid was less susceptible than that in the liver or carcass.

In conclusion, the results from the present study demonstrated that LC supplementation

to broilers reduced the fat contents in the carcass, muscle and liver, and cholesterol in the carcass and liver. Supplementation of LC also increased the total PUFA in the liver but has very little effect on the fatty acid composition of the muscle.

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