Effect of Lactofeed probiotic and different sources of fat on performance, carcass characteristics and lipid parameters in broiler chickens

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Journal of Livestock Science (ISSN online 2277-6214) 8: 143-149
Received on 4/4/2017; Accepted on 27/6/2017

Abstract
This study was done for evaluating the effect of Lactofeed probiotic and different sources of fat on performance, carcass characteristics and lipid parameters in chickens. For this purpose, a total of 240 male chickens of strain Ross 308, in a completely randomized design, were divided into 6 treatments with 4 replicates (10 birds per replicate). The experimental diets included: (1) basal diet (control); (2) basal diet + 3% animal fat from tallow; (3) basal diet + 3% plant oil from soybean; (4) basal diet + probiotic; (5) basal diet + (probiotic + 3% animal fat from tallow) and (6) basal diet + (probiotic + 3% plant oil from soybean). The effects of fat and its type on weight gain and conversion ratio were significant (P<0.05), and the breast growth in chickens was higher in soybean oil diets. The results showed that the weights of carcass, breast and thigh were the lowest in the chickens related to the mixed fat and probiotics (P<0.01 and P<0.05). The weight of liver and heart was higher in fat-included diet (P<0.05). The type of fat and probiotics had no significant effect on triglyceride, LDL and VLDL (P>0.05). The results showed that the use of probiotics in fat-included diet has a negative effect on performance and carcass characteristics.

Key Words: broiler chickens; carcass; lipid parameters; probiotic; soybean; triglyceride.

Introduction

The increase in the digestion and absorption of nutrients in poultry is of great importance; one of the things that are very important in this area is the microbial flora of the digestive system of poultry (El-Husseiny et al., 2002). The effect of probiotics on the health of the host animal is positive and useful. These microorganisms play a role in improving microflora by limiting the activity of pathogenic bacteria and thus reduce feed conversion ratio (Panda et al., 2000). Also, different sources of fat are usually used in the diet in order to increase the energy density and the palatability of poultry diets. Since the amount of energy from fats is 2.25 times more than carbohydrates, they are considered as high-energy components in the diet. The formation of micelles is necessary for fat absorption in the intestine. One of the basic constituents of micelles is bile salts and their presence is essential for the digestion and the absorption of fats (Vieira et al., 2002).

Most of digestive system bacteria can change the structure of bile salts through the Deconjugation, Dehydration and Dehydrogenation. The bacteria such as *Bifidobacteria* and *Clostridia* have the hydrolyzing enzymes for bile salts. *Lactobacilli* in the small intestine also hydrolyze bile salts. *Lactobacilli* are the main cause of the hydrolysis of bile salts in the digestive system. The lactic acid-producing bacteria such as *Lactobacilli* and *Bifidobacteria* are used in making probiotics. These bacteria are involved in bile salt hydrolysis. The use of probiotics in the diet increases the small intestine bacteria population and this may cause reducing the digestibility of fat in diet. Since the compositions of fats in herbal and animal sources are very different, therefore, it seems that the effect of probiotics on their usability and especially formation of micelles is different (Tannock et al., 1989). Nowadays, due to the restriction of the use of antibiotics as additives in poultry diets, much research is in progress to identify suitable replacement for them (Mohnl, 2006). Therefore, this study was to compare the effects of using probiotics and the type of dietary fat source on performance, carcass characteristics and lipid parameters in broiler chickens.

Material and Methods

Chickens, diets and management

The experiment, in a completely randomized design on 240 1-day-old male chicks of strain Ross 308 with 6 treatments and 4 replicates, in Farajpoor farm was conducted in starter period (1-10 day), grower period (11-28) and finisher period (29-42). Each replication included 10 chicks. The lighting, temperature and air conditioning program used was consistent with the specifications in the Ross-308 lineage manual during the experimental period (Aviagen, 2010).

The basal diet based on corn and soybean meal was balanced. Experimental diets included: (1) basal diet (control); (2) basal diet + 3% animal fat from tallow; (3) basal diet + 3% plant oil from soybean; (4) basal diet + probiotic; (5) basal diet + (probiotic + 3% animal fat from tallow) and (6) basal diet + (probiotic + 3% plant oil from soybean). The experimental diets were formulated by using (Aviagen, 2010) (Tables 1, 2 and 3) and User Friendly Feed Formulation Done Again (UFFDA) software. During the period, all conditions were similar for chickens, and the feeding diets were *ad libitum* in the whole period.

Performance

The following growth performance variables were evaluated: body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). The birds were weighed on the first day of the experiment, then weighed weekly throughout the remaining experimental period (7 to 42 d of age). Feed consumption was provided weekly and the leftover fed was weighed weekly for calculating the feed conversion ratio.

Carcass characteristics

At the end of the experiment (42 days), a bird weighing close to the average weight of group was selected and slaughtered from each replicate. The weight of carcass, breast, thighs, liver, heart and abdominal fat were determined.

Lipid parameters

At day 21 of each replicate, a bird was selected and blood samples were collected from the wing veins. Blood samples were centrifuged, and the serum was separated in Pars Lab. Then, the amounts of triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) of blood serum samples were determined by an enzymatic method. Enzymatic Method is for the analysis of serum by UV-Visible Spectroscopy.

Statistical analysis

Analysis of the obtained data was conducted by SAS software in a completely randomized design (SAS, 2003). Means difference was compared by Duncan's multiple range test at 5% level.

Table 1: Ingredients and calculated analyses of the basal diets in starter

Ingredients (%)	Starter (0-10 days old)					
	T1	T2	T3	T4*	T5*	T6*
Corn grain	62.25	54.5	53	62.25	54.5	53
Soybean meal	34.8	35	35	34.8	35	35
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Fish powder	0.43	1.5	1.5	0.43	1.5	1.5
Calcium carbonate	0.75	0.75	0.75	0.75	0.75	0.75
Dicalcium phosphate	0.8	0.8	0.8	0.8	0.8	0.8
Salt	0.22	0.22	0.22	0.22	0.22	0.22
DL-methionine	0.18	0.18	0.18	0.18	0.18	0.18
DL-lysine	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin and mineral permix[1]	0.50	0.50	0.500	0.50	0.50	0.500
Filler[2]	-	3.48	4.98	-	3.48	4.98
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	2953	2953	2953	2953	2953	2953
CP (%)	21.02	21.02	21.02	21.02	21.02	21.02
Methionine (%)	0.34	0.34	0.34	0.34	0.34	0.34
Lysine (%)	1.15	1.15	1.15	1.15	1.15	1.15
Methionine + Cysteine (%)	0.69	0.69	0.69	0.69	0.69	0.69

[1] Each kg (DM basis) of vitamin and mineral premix contained: vitamin A: 11000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K: 4 mg; vitamin B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg and Selenium: 0.25 mg. [2] Inert filler used to complete diet formulations to 100%. * Probiotic added 400 g/ton in starter period. T1: basal diet (control); T2: basal diet + 3% animal fat from tallow; T3: basal diet + 3% plant oil from soybean; T4: basal diet + probiotic; T5: basal diet + (probiotic + 3% animal fat from tallow) and T6: basal diet + (probiotic + 3% plant oil from soybean) ME: metabolizable energy and CP: crude protein.

Table 2: Ingredients and calculated analyses of the basal diets in grower

Ingredients (%)	Grower (11-28 days old)					
	T1	T2	T3	T4*	T5*	T6*
Corn grain	67.56	59.32	58.10	67.56	59.32	58.10
Soybean meal	28.98	29	29	28.98	29	29
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Fish powder	0.15	1.45	1.63	0.15	1.45	1.63
Calcium carbonate	1.10	1.10	1.10	1.10	1.10	1.10
Dicalcium phosphate	1.16	1.16	1.16	1.16	1.16	1.16
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.21	0.21	0.21	0.21	0.21	0.21
DL-lysine	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin and mineral permix[1]	0.500	0.500	0.500	0.500	0.500	0.500
Filler[2]	-	3.92	4.96	-	3.92	4.96
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	2973	2973	2973	2973	2973	2973
CP (%)	18.77	18.77	18.77	18.77	18.77	18.77
Methionine (%)	0.31	0.31	0.31	0.31	0.31	0.31
Lysine (%)	0.97	0.97	0.97	0.97	0.97	0.97
Methionine + Cysteine (%)	0.67	0.67	0.67	0.67	0.67	0.67

[1] Each kg (DM basis) of vitamin and mineral premix contained: vitamin A: 11000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K: 4 mg; vitamin B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg and Selenium: 0.25 mg. [2] Inert filler used to complete diet formulations to 100%. * Probiotic added 400 g/ton in starter period. T1: basal diet (control); T2: basal diet + 3% animal fat from tallow; T3: basal diet + 3% plant oil from soybean; T4: basal diet + probiotic; T5: basal diet + (probiotic + 3% animal fat from tallow) and T6: basal diet + (probiotic + 3% plant oil from soybean) ME: metabolizable energy and CP: crude protein.

Table 3: Ingredients and calculated analyses of the basal diets in finisher

Ingredients (%)	Finisher (28-42 days old)					
	T1	T2	T3	T4*	T5*	T6*
Corn grain	71.96	63.63	62.33	71.96	63.63	62.33
Soybean meal	25	25	25	25	25	25
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Fish powder	-	1.32	1.55	-	1.32	1.55
Calcium carbonate	1.04	1.04	1.04	1.04	1.04	1.04
Dicalcium phosphate	1.1	1.1	1.1	1.1	1.1	1.1
Salt	0.24	0.24	0.24	0.24	0.24	0.24
DL-methionine	0.15	0.15	0.15	0.15	0.15	0.15
DL-lysine	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin and mineral permix[1]	0.500	0.500	0.500	0.500	0.500	0.500
Filler[2]	-	4.01	5.08	-	4.01	5.08
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	3012	3012	3012	3012	3012	3012
CP (%)	17.33	17.33	17.33	17.33	17.33	17.33
Methionine (%)	29.8	29.8	29.8	29.8	29.8	29.8
Lysine (%)	0.86	0.86	0.86	0.86	0.86	0.86
Methionine + Cysteine (%)	0.60	0.60	0.60	0.60	0.60	0.60

[1] Each kg (DM basis) of vitamin and mineral premix contained: vitamin A: 11000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K: 4 mg; vitamin B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg and Selenium: 0.25 mg. [2] Inert filler used to complete diet formulations to 100%. * Probiotic added 400 g/ton in starter period. T1: basal diet (control); T2: basal diet + 3% animal fat from tallow; T3: basal diet + 3% plant oil from soybean; T4: basal diet + probiotic; T5: basal diet + (probiotic + 3% animal fat from tallow) and T6: basal diet + (probiotic + 3% plant oil from soybean) ME: metabolizable energy and CP: crude protein.

Results

Body weight gains in the treatments 2 and 5 (Table 4) were decreased compared to the control (P<0.05), but in treatment 3 (Table 4), body weight gain (BWG) was increased compared to the control. Feed intake and feed conversion ratio in treatment 3 (Table 4) were significant compared to all treatments (P<0.05). Although the treatment 4 (Table 4) was not significant in comparison with the control (P>0.05), but was numerically higher than all other treatments.

Table 4: The effects of Lactofeed probiotic and different sources of fat on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)

Treatment	1 to 42 day		
	WG	FI	FCR
Basal diet (control)	$2246.55^{b}\pm29.401$	$3946.10^{ab}\pm45.871$	$1.75^{ab}\pm0.040$
Basal diet + 3% animal fat from tallow	$2142.39^{c}\pm48.543$	$3875.13^{b}\pm20.719$	$1.81^{a}\pm0.042$
Basal diet + 3% plant oil from soybean	$2384.22^{a}\pm10.329$	$3683.23^{c}\pm23.225$	$1.54^{c}\pm0.008$
Basal diet + probiotic	$2305.47^{ab}\pm14.080$	$3966.95^{a}\pm19.858$	$1.72^{b}\pm0.018$
Basal diet + (probiotic + 3% animal fat from tallow)	$2112.37^{c}\pm14.162$	$3865.20^{b}\pm21.206$	$1.82^{a}\pm0.018$
Basal diet + (probiotic + 3% plant oil from soybean)	$2245.21^{b}\pm26.677$	$3926.45^{ab}\pm6.835$	$1.74^{ab}\pm0.018$
SEM	21.583	21.674	0.021
P-value	0.0001	0.0001	0.0001
CV	4.721	2.738	6.169

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means and CV: coefficient of variation.

The weights of liver and heart in the treatments 2, 3, 5 and 6 (Table 5) were significantly increased than the control (P<0.05). The weight of abdominal fat for none of the treatments was significant compared to the control group (P>0.01). The weights of carcass and thighs in treatment 5 (Table 5) were significant compared to all the treatments and were lowest in numbers (P<0.01 and P<0.05). Breast weight in the treatments 2 and 5 (Table 5) was significant with the control and the lowest value was in the treatment 5 (Table 5) (P<0.05).
The amount of cholesterol in the treatments 2, 3, 5 and 6 (Table 6) was significant compared to control and the lowest value was for treatment 6 (Table 6) (P<0.05). The value of Triglyceride, LDL and VLDL was not significant in any of the treatments compared to the control (P>0.01). HDL levels in all groups except the control group 5 (Table 6) were significant compared to the control (P<0.05). Highest HDL level was related to the treatment in which soybean oil was used (Table 6).

Table 5: The effects of Lactofeed probiotic and different sources of fat on carcass characteristics

Treatment	**Carcass**	**Breast**	**Thigh**	**Liver**	**Heart**	**Abdominal fat**
Basal diet (control)	1495.25[a]±82.570	707.75[a]±42.476	608.75[abc]±25.782	43.80[d]±1.339	10.66[d]±0.346	13.52[a]±0.518
Basal diet + 3% animal fat from tallow	1407.25[a]±46.808	592.50[bc]±13.665	526.00[c]±17.855	49.30[b]±1.46	17.40[a]±0.288	12.75[a]±0.530
Basal diet + 3% plant oil from soybean	1506.75[a]±38.581	762.50[a]±41.332	677.75[a]±37.981	48.32[bc]±0.942	15.02[b]±0.248	11.70[a]±0.409
Basal diet + probiotic	1467.25[a]±44.924	673.50[ab]±36.218	576.75[bc]±25.352	45.25[cd]±1.380	10.30[d]±0.242	9.82[a]±0.801
Basal diet + (probiotic + 3% animal fat from tallow)	1150.50[b]±48.737	532.50[c]±45.115	400.00[d]±34.317	59.55[a]±0.521	12.06[c]±0.116	10.62[a]±0.400
Basal diet + (probiotic + 3% plant oil from soybean)	1497.50[a]±21.914	727.00[a]±21.637	617.00[ab]±8.396	51.42[b]±1.071	11.50[c]±0.324	10.50[a]±0.405
SEM	38.028	20.953	20.698	1.244	0.541	0.511
P-value	0.03	0.0018	0.0001	0.0001	0.0001	0.1973
CV	13.112	15.417	17.861	11.305	20.689	20.243

The means within the same column with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means and CV: coefficient of variation.

Table 6: The effects of Lactofeed probiotic and different sources of fat on lipid paramers Table 6: The effects of Lactofeed probiotic and different sources of fat on lipid paramers

Treatment	1 to 21 day				
	Cholesterol	Triglyceride	HDL	LDL	VLDL
Basal diet (control)	116.00[a]±3.240	105.50[a]±4.112	49.00[a]±2.581	19.75[a]±1.258	10.40[a]±1.174
Basal diet + 3% animal fat from	91.75[b]±4.888	112.00[a]±4.743	33.80[c]±1.356	16.25[a]±1.683	13.50[a]±1.707
Basal diet + 3% plant oil from	83.75[bc]±1.493	102.75[a]±5.452	42.52[b]±1.645	14.87[a]±1.080	11.00[a]±1.414
Basal diet + probiotic	116.00[a]±3.439	104.50[a]±3.752	41.75[b]±1.652	18.50[a]±1.040	12.92[a]±1.540
Basal diet + (probiotic + 3% animal fat from tallow)	85.75[bc]±2.212	109.00[a]±4.490	51.75[a]±1.794	15.37[a]±0.750	14.75[a]±2.174
Basal diet + (probiotic + 3% plant oil from soybean)	79.50[c]±2.327	98.25[a]±3.816	38.00[bc]±1.581	12.60[a]±1.701	9.90[a]±0.672
SEM	3.318	1.844	1.431	0.945	0.660
P-value	0.0001	0.3598	0.0001	0.2966	0.2076
CV	24.277	8.577	16.383	28.559	26.776

The means within the same column with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means and CV: coefficient of variation.

Discussion

Efficiency of metabolizable energy in fats is higher because of low heat increment. So it is expected that the chickens of fat-included treatment have higher performance. Effects of fat source type used in diet show their positive effect especially soybean oil on the performance. Favorable effect of fats especially soybean oil have been reported on body weight gain of birds (Scaife et al., 1994). Performance improvement from using fat-included diets is related to the appropriate influence of fats on the animal's feed intake and better use of diet energy. Better influence of vegetable oils than animal oils (such as soybean oil) on broiler performance is due to the high proportion of unsaturated- to saturated-fatty acids and also the superiority of micelles formation because of the creation of monoglycerides after its hydrolysis in the intestine (Scott et al., 1982). The results of a study showed that unsaturated vegetable oils are less excreted by feces, and they subsequently produce more metabolizable energy than animal fat. This may justify the superiority of soybean oil than animal fat on body weight gain (Zollitsch et al., 1996).

Ketels and DeGroote (1989) showed that adding soybean oil in poultry diet causes the decrease of feed intake and improving conversion ratio. As animal feeding is controlled via the fat in intestine, the fat-included treatment chickens are fed sooner and because soybean vegetable oil is better absorbed among the fats, it shows better growing efficiency and conversion ratio. According the results, the best conversion ratio was observed for the treatment of 3% soybean oil. Improvement of conversion ratio due to the use of fat is probably because of the better use of dietary energy of 3% soybean oil. Better use of this diet energy may be because of the reason that adding fat to diet causes slower pass of nutrients in digestive system and subsequently the digestion and absorption of nutrients is performed with higher efficiency (Dersjant Li and Peisker, 2005).

Atteh and Leeson (1983) stated that the use of soybean oil in the diet causes weight gain for poultry breasts that is identical with the results of this research. The researchers said that the use of fat in poultry diet due to the high reduction in feed passing results in better digestion and absorption of nutrients, and amino acids are in better position to improve carcass weight. Soybean oil is also better absorbed than animal fat in the diet that will increase carcass weight gain (Ajuyah et al., 1991).

In this experiment, the effects of fat and probiotic supplementation in diet were significant on weight gain, carcass weight, breast and thigh weights in the whole course of treatment. The performance of birds and carcass weight related to lacto-feed and fat treatment (animal or soybean oil) was lower. The role of intestinal microbial flora (especially *Lactobacilli* and *Bifidobacteria*) has been reported in the analysis of bile acids (Feighner and Dashkevicz, 1987). In preparing the probiotics (including lacto-feed) the mentioned species were used. Therefore the slowdown of growth caused by the use of protexin in fat diets can be related to the decrease of fat digestion from hydrolysis of bile acids by bacteria in protexin and the lack of energy supply necessary for the growth (Soomro et al., 2002).

The results showed that liver weight in fat-included (animal or vegetable) diets was higher compared to the control. But the weight of the liver in fat- and probiotics- included diets was observed above all others (Scaife et al., 1994). Bile acids are needed for fat digestion and absorption in the intestine that are made in the liver. Having fat in the diet needs to produce more bile that must be made by liver; as the result, the activity of the liver is increased and its size gets larger. On the other hand, in probiotic-included diets, bile acids are deconjugated by bacteria (especially *Lactobacilli*) and their absorption in the intestine is decreased, and they are excreted by feces. The increasing need to production of bile acids in liver to compensate the amount excreted by feces causes the increased activity of the liver and its bigger size (Dora et al., 2003).

In a research to investigate the effect of fat on heart, realized that the amount of fat in the heart of the birds that received fat was significantly higher than the birds that didn't receive. Consequently, their heart was heavier than the birds that didn't receive fat (Zanini et al., 2006). Using fat had no significant effect on abdominal fat. The most important feeding factor that can affect abdominal fat is diet energy level and energy to protein ratio in diet, and with regard to the equal energy and also equal energy to protein ratio, no significant difference was observed between the treatments (Latour et al., 1994).

The researchers found that the use of animal fat in poultry diet increases cholesterol. The increase of cholesterol increases the values of VLDL and LDL in blood and decreases HDL level the one that affects opposite to LDL. The results of this test are consistent with previous reports (Huang et al., 2007).

Lower level of cholesterol and HDL in the blood of probiotic-included treatments birds can be from the probiotic effect in microbial flora increase of the intestine. This microbial flora causes the decrease in digestion and absorption of fats by biological changes in bile acids and disorder in their liver-intestinal cycle (Ali et al., 2001).

Conclusion

Using soybean oil in boiler feed is suggested according the results and with regard to the importance of feed conversion ratio and also carcass quality. Also, because of the negative effect of existing bacteria in Lactofeed probiotics on the digestion and absorption of fat, its use in fat-included diets is not suggested because of performance reduction.

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