

Original study

Intermittent feeding programme and addition of *Bacillus subtilis* based probiotics to the diet of growing broiler chickens: Influence on growth, hepatic enzymes and serum lipid metabolites profile

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Abstract

This study was conducted to examine the effects of Intermittent feeding programme (IFP) and *Bacillus subtilis*-based probiotic (BSP) addition in diet on liver malic enzyme and isocitrate dehydrogenase activity, lipid metabolism and performance in broiler chickens. Five hundred and four one-day old male broiler chicks were randomly allocated in four experimental treatments (T1, T2, T3 and T4). Groups T1 (control diet) and T3 (*Bacillus subtilis*-based probiotic diet) were fed *ad libitum*, whereas groups T2 (control diet) and T4 (*Bacillus subtilis*-based probiotic diet) served as IFP from day 8 to the end of the experiment. The data on initial body weight, weekly feed consumption and body weight gain were recorded up to six weeks of age. Carcass composition, blood parameters and hepatic enzyme activity were measured at the end of the experiment. Although body weight gain was not significantly different among any of the treatments, the birds raised under IFP consumed significantly ($P<0.05$) lower feed (207 g) and utilized their feed more efficiently (1.78) than those of the control group fed *ad libitum* (1.84). Carcass weight as a percentage of live weight was not affected by probiotic supplementation on the diet, but IFP significantly reduced ($P<0.05$) broiler carcass weight. However, the liver malic and isocitrate dehydrogenase enzyme activity was not significantly different between IFP and BSP groups. All serum lipid metabolites concentration decreased ($P<0.05$) with probiotic treatment. It may be concluded that dietary supplementation with

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BSP may influence the pathway of fat metabolism through promotion and/or suppression of serum lipid metabolites.

Keywords: Intermittent feeding program, *Bacillus subtilis*, isocitrate dehydrogenase enzyme, malic enzyme, broiler

Abbreviations: AFP: abdominal fat pad, BSP: *Bacillus subtilis*-based probiotic, HDL: high density lipoprotein, ICD: isocitrate dehydrogenase, IFP: intermittent feeding programme, LDL: low density lipoprotein, MAE: malic enzyme, NADPH: nicotinamide adenine dinucleotide phosphate, NEFA: non-esterified fatty acid, TG: triglyceride, VLDL: very low density lipoprotein

Introduction

Utilization of successful genetic selection techniques in order to rapid growth and efficient feed conversion rate in commercial broiler chickens has resulted in different metabolic disorders such as hyperphagia, obesity (Yang *et al.* 2010) and leg problems (Wijtten *et al.* 2010). *Ad libitum* feeding ensures a high feed intake and over-feeding can induce *de novo* synthesis of fatty acids in liver and lipogenesis (Hermier *et al.* 1997). The increase of fat deposition in carcass effectively decreases the carcass quality. However, the high growth under *ad libitum* feeding is occasionally associated with increasing mortality and severe leg problems. Feed restriction in poultry has been commonly used to control the body weight (Fassbinder-Orth & Karasov 2006) and alleviate metabolic disorders such as ascites (Özkan *et al.* 2006) and leg problems (Wijtten *et al.* 2010, Saffar & Khajali 2010). Also the effect of feed restriction on lipid metabolism was documented in studies of some research groups (Lippens *et al.* 2009, Yang *et al.* 2010). Feed restriction is classified into two forms including a quantitative feed restriction where the birds' access to the feed physically declines during certain times of the day and a qualitative feed restriction, where birds' full access to particular nutrients decreased through the provision of a feed diluted with inert fibres (Fanooci & Turki 2010). According to the previous investigations, the response of the bird to a feed restriction programme is related to feeding strategies (Urdaneta-Rincon & Leeson 2002), severity of restriction (Lippens *et al.* 2009) and genetic make-up (Saleh *et al.* 2005). Therefore, usage of an appropriate method of feed restriction for broilers is difficult (Lippens *et al.* 2009).

Probiotics have been defined as live microbial feed additive that in adequate amounts of usage can beneficially influence intestinal microflora balance and improve animal performance (Xiangqian *et al.* 2008, Mountzouris *et al.* 2010). Today, addition of probiotic in diet is a common strategy for animal nutrition, because probiotic is a natural growth promoter instead of feed antibiotic (Rekiel *et al.* 2007). Adding probiotic in the diet improves the animal immune system and the host's resistance to enteric pathogens, affects intestinal morphology as well as intestinal function, feed conversion, weight gain and performance of birds (Dalloul *et al.* 2003, Chichlowski *et al.* 2007, Vila *et al.* 2009, Ohh 2011). Li *et al.* (2006) also reported that addition of *Bacillus subtilis* culture to the layer diet decreased cholesterol concentration in egg yolk.

Nicotinamide adenine dinucleotide phosphate (NADPH) is an essential component for *de novo* synthesis of fatty acids in the liver (Xu *et al.* 2003). Nicotinamide adenine dinucleotide

phosphate is produced by the activity of malic enzyme (MAE) and isocitrate dehydrogenase (ICD) in the liver (De Beer *et al.* 2007, Xu *et al.* 2003). Thus, better insight into these liver enzymes is extremely important to understand the main biological role of these enzymes in lipid metabolism of broiler birds.

While lipogenesis and export of lipid in the liver is related to the nutrient status of the diet (Yang *et al.* 2010), the effect of intermittent feeding programme (IFP) and/or addition of *Bacillus subtilis*-based probiotics (BSP) on liver enzymes activity is poorly understood. The main objective of the current study was to investigate the effect of IFP in the presence or absence of BSPs in diet on liver MAE and ICD enzymes activity, lipid metabolism and broiler performance.

Materials and Methods

Birds, feeding and housing

This experiment was conducted on five hundred and four one-day old male broilers consisting of four treatments and six replicates. All male broilers were obtained from the hatchery of Arian breeding centre (Babol, Iran) and were randomly assigned into one of the four treatments on the basis of equal body weight (21 birds kept on each replicate). Four treatments included T1): the control group was a corn-soybean based commercial diet fed *ad libitum*; T2): a group was fed similar to the control diet but subjected to IFP; T3): a group was fed a control diet containing BSP and T4): a group was fed the same diet as group 3 and subjected to IFP. All dietary treatments were formulated to meet the Arian broilers' requirements as well as NRC (1994) recommendation for starter (1 to 14 d), grower (15 to 28 d) and finisher (29 to 42 d) of growth periods (Table 1). Intermittent feeding programme in treatment 2 and 4 was applied from day 8 to the end of the experiment with 5 feeding times per day. Feeding was done from 1 to 2, 6 to 7, 11 to 12, 16 to 17 and 21 to 22 each day. The level of 50 mg of BSP, that was commercially available as Calsporin (Calpis Co., Ltd. Tokyo, Japan), was supplemented per kg of the basal diet for broilers in group 3 and 4. Before addition of probiotic to the experimental diets, culturing techniques were used for enumeration of *Bacillus subtilis* in trypticase soy agar (Merck, Darmstadt, Germany) after dilution in sterile saline (Leuschner *et al.* 2003). Results were expressed as log₁₀ colony forming units per gramme of probiotic product. Broilers were reared on wood shavings litters and assigned to a clean floor pen (2 m²) with one hanging tube feeder and four nipple drinkers. The lighting schedule, the temperature and healthy programme throughout the experiment were provided according to the Arian broiler breeder management guidelines. For inhibition of cross contamination of probiotic between experimental pens, the treatment units were separated by plastic sheets (Aliakbarpour *et al.* 2012). Chickens were weighed weekly to estimate their growth. To determine daily feed intake by each pen the uneaten feed was discarded and fresh feed was replaced in feeders at the end of each day. Feed conversion ratio was calculated weekly.

Table 1
Feed composition and ingredients of experiment basal diets

Component	Starter (1 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 42 d)
Ingredient, %			
Corn	57.1	62.4	67.6
Soybean meal	37.0	32.0	27.0
Vegetable fat	1.25	1.70	1.58
DL-Met	0.29	0.23	0.22
L-Lys HCl	0.07	0.03	0.09
Theronin	0.05	0.03	0.04
Dicalcum phosphate	1.85	1.61	1.51
Limestone	1.15	0.98	0.95
NaCl	0.32	0.28	0.28
Soduim Bicarbonate	0.15	0.1	0.1
CholinCholoride	0.17	0.14	0.14
Vitamin permix1	0.3	0.25	0.25
Mineral permix2	0.3	0.25	0.25
Calculated analysis			
ME, kcal/ kg	2 907	3 003	3 050
Crude protein, %	22.13	20.09	18.21
Ca, %	1.04	0.90	0.85
Avp, %	0.71	0.65	0.61
Met, %	0.58	0.50	0.47
Lys, %	1.15	1.00	0.92
Thr, %	0.76	0.67	0.62

¹The vitamin mix provided the following (per kg of diet): thiamine mononitrate, 2.5 mg; nicotinic acid, 45 mg; riboflavin, 6 mg; d-calcium pantothenate, 15 mg; vitamin B12 (cobalamin), 0.025 mg; pyridoxine hydrochloride, 3 mg; d-biotin, 0.15 mg; folic acid, 1.5 mg; choline chloride, 840 mg; cholecalciferol, 4 000 IU; trans-retinyl acetate, 10 000 IU; all-rac- α -tocopheryl acetate, 55 IU; ethoxyquin, 1.25 mg. ²The trace mineral mix provided the following (per kg of diet): manganese (manganese oxide), 120 mg; iron (ferrous sulphate), 40 mg; zinc (zinc oxide), 100 mg; copper (copper sulphate), 16 mg; iodine (calcium iodate), 1.25 mg; selenium (sodium selenate), 0.3 mg.

Carcass composition

At the end of the experiment, six chickens per replicate were randomly selected and slaughtered for carcass analysis. The heads, feathers, feet and viscera were removed after slaughter. Then, abdominal fat pad was removed and weighed. Carcass weight was calculated as the percentage of body weight (Petek *et al.* 2005) and the relative percentage of abdominal fat pad, wing, thigh, breast and back for individual broilers were estimated as percentage of carcass weight.

Liver enzyme assays

After slaughter, livers were stored at -70°C prior to analysis. The liver supernatants were prepared according to the methodology of Ochoa (1955) and Plant (1962). Subsequently, the activities of MAE and ICD enzymes were evaluated according to Geer *et al.* (1980) and the procedures recommended by the manufacturer of the kits (BioVision, USA, Cat no.K756-100), respectively.

Blood biochemical parameters assays

One bird from each pen was randomly chosen at the end of the experiment and blood samples were taken via the wing vein and centrifuged at $2000 \times g$ for 30 min. Then, serum was separated and stored at -20°C . Serum level of triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were estimated according to the procedures recommended by the manufacturer of the specific kits (Pars Azmun Company, Tehran, Iran). Total non-esterified fatty acid (NEFA) was determined using NEFA kit (Randox Laboratories Ltd, United Kingdom, Lot number 350FA). Analyses for serum parameters were conducted with an automated clinical chemistry analyser (Hitachi 911, Tokyo, Japan). Very low density lipoprotein (VLDL) was calculated according to Rifai *et al.* (1992).

Statistical analyses

The effects of BSP and IFP on liver enzymes, blood parameters and growth performance were determined in a 2×2 factorial arrangement. Before all data were analysed using the GLM procedures of SAS (SAS Institute Inc. 2003), differences between means were examined using Duncan's multiple range test and the statements of statistical significance were based on $P < 0.05$.

Results

Growth performance

Data on body weight gain, feed intake and feed conversion ratio of experimental groups throughout the experiment are summarized in Table 2. The feed intake significantly decreased ($P < 0.05$) due to IFP at 8 to 42 days of age. However, it was not affected by IFP between 35 to 42 days of age. This feeding procedure significantly reduced body weight at 8 to 21 days of age ($P < 0.05$) but there was no main effect of feeding strategies on body weight gain at 22 days of age to the end of the experiment. Similarly, IFP significantly improved feed conversion ratio between 8 to 42 day of age ($P < 0.05$).

Addition of probiotic did not significantly affect body weight gain or feed intake during rearing period ($P > 0.05$), but between 36 to 42 days of age feed conversion ratio was significantly improved by probiotic addition ($P < 0.05$). No interaction was found between BSP and IFP on growth performance in this experiment.

Determination of carcass cut yield

In this study, weight of wing, abdominal fat pad, thigh, breast and back which was presented as percentage of the live body weight, did not differ significantly ($P > 0.05$) among the treatments (Table 3). There was a significant decrease in carcass weight, wing and back based on body weight percentage due to feeding strategies ($P < 0.05$). The carcass composition parameters were not significantly affected by BSP ($P > 0.05$). At the present experiment, no interaction was found between BSP and IFP on carcass composition.

Table 2
Effects of intermittent feeding program and probiotic on feed intake, body weight gain and feed conversion rate

		Feed intake, g						Body weight gain, g						Feed conversion rate					
FI	Pro	0-7 d	8-21 d	22-35 d	36-42 d	8-42 d	0-7 d	8-21 d	22-35 d	36-42 d	8-42 d	0-7 d	8-21 d	22-35 d	36-42 d	8-42 d			
Add lib	-	149.97	1 049.60	2 190.40	1 327.45	4 567.44	120.02.	671.52	1 199.07	574.53	2 444.50	1.25	1.57	1.83	2.34	1.87			
IFP	-	149.59	959.21	2 112.62	1 298.87	4 370.70	119.34	642.67	1 198.75	598.93	2 440.35	1.25	1.49	1.76	2.19	1.79			
Add lib	+	150.76	1 027.83	2 200.56	1 329.05	4 557.43	121.39	677.19	1 202.32	626.87	2 506.36	1.24	1.52	1.83	2.13	1.82			
IFP	+	152.84	936.88	2 096.84	1 305.56	4 339.28	121.26	646.12	1 166.18	632.86	2 445.15	1.26	1.45	1.80	2.06	1.78			
SEM		0.574	6.952	11.759	12.267	21.468	0.740	3.494	7.308	12.373	9.431	0.005	0.011	0.008	0.036	0.006			
Main effect means																			
FI																			
Add lib		150.37	1 038.71 ^a	2 195.48 ^a	1 328.25	4 562.43 ^a	120.70	674.36 ^a	1 200.69	600.70	2 475.43	1.26	1.54 ^a	1.83 ^a	2.23	1.84 ^a			
IFP		151.21	948.05 ^b	2 104.73 ^b	1 302.21	4 354.99 ^b	120.30	644.39 ^b	1 182.46	615.90	2 442.75	1.25	1.47 ^b	1.78 ^b	2.13	1.78 ^b			
Pro																			
-		149.78	1 004.40	2 151.51	1 313.16	4 469.07	119.68	656.10	1 198.91	586.73	2 442.43	1.25	1.53	1.80	2.26 ^a	1.83 ^a			
+		151.80	982.35	2 148.70	1 317.30	4 448.35	121.32	661.65	1 184.24	629.87	2 475.75	1.25	1.49	1.82	2.10 ^b	1.80 ^b			
Probability																			
FI		-	<0.0001	0.0010	0.3013	0.0001	-	0.0004	0.2268	0.5461	0.0985	0.3493	0.0067	0.0057	0.1653	0.0001			
Pro		0.0930	0.1285	0.9060	0.8676	0.6347	0.2817	0.4943	0.3275	0.0967	0.0925	1.0000	0.0675	0.2043	0.0318	0.0173			
FI x Pro		-	0.9842	0.5874	0.9184	0.8056	-	0.8406	0.2348	0.7138	0.1459	0.5302	0.9140	0.2835	0.5741	0.2092			

Means with different superscripts within the same column differ significantly ($P \leq 0.05$). FI: feeding strategies, Pro: probiotic

Table 3
Effects of intermittent feeding programme and probiotic on carcass composition and blood parameters of broilers

FI	Pro	Carcass	AFP	Relative to live body weight, %				Blood parameters					VLDL, mg/dl	NEFA, mm/l
				Wing	Thigh	Breast	Back	Chol, mg/dl	TG, mg/dl	HDL mg/dl	LDL, mg/dl			
Add lib	-	70.07	1.32	7.90	20.55	21.58	15.83	139.50	74.40 ^a	49.83	72.83	14.88 ^a	0.70 ^a	
IFP10	-	68.35	1.22	7.70	20.52	20.90	15.37	134.33	50.20 ^b	40.33	77.83	10.57 ^b	0.75 ^a	
Add lib	+	69.65	1.13	8.02	20.25	21.72	15.70	113.33	41.67 ^b	40.00	63.50	8.33 ^b	0.42 ^b	
IFP	+	68.52	1.20	7.73	20.55	21.25	15.27	105.83	39.17 ^b	40.17	57.67	7.83 ^b	0.37 ^b	
SEM		0.20	0.04	0.06	0.12	0.15	0.11	4.79	2.08	2.26	3.50	0.47	0.03	
Main effect means														
FI														
Add lib		69.86 ^a	1.23	7.96 ^a	20.53	21.65	15.77 ^a	126.42	58.03 ^a	44.92	68.17	11.61 ^a	0.56	
IFP		68.43 ^b	1.21	7.72 ^b	20.40	21.08	15.32 ^b	120.08	44.68 ^b	40.25	67.75	9.20 ^b	0.56	
Pro														
-		69.21	1.27	7.80	20.53	21.24	15.60	136.92 ^a	62.30 ^a	45.08	75.33 ^a	12.73 ^a	0.73 ^a	
+		69.08	1.17	7.88	20.40	21.48	15.48	109.58 ^b	40.42 ^b	40.08	60.58 ^b	8.08 ^b	0.39 ^b	
Probability														
FI		0.0023	0.8231	0.0469	0.5699	0.0708	0.0458	0.5159	0.0045	0.3141	0.9532	0.0193	0.9424	
Pro		0.7621	0.1894	0.5184	0.5699	0.4319	0.5871	0.0098	<0.0001	0.2817	0.0481	<0.0001	<0.0001	
FIxPro		0.4823	0.2709	0.7188	0.4786	0.7229	0.9379	0.9042	0.0170	0.2976	0.4484	0.0575	0.4446	

Means with different superscripts within the same column differ significantly ($P \leq 0.05$). FI: feeding strategies, Pro: probiotic, AFP: Abdominal fat pad, Chol: Cholesterol, intermittent feeding programme

Liver enzymes and blood parameters assay

In this study, ICD and MAE activity did not differ significantly among the treatments ($P>0.05$, data not shown). Feeding strategy did not have an effect on serum metabolites related to fat metabolism such as cholesterol, NEFA and lipoproteins HDL and LDL (Table 3), but there was a significant decrease in TG and VLDL by feeding strategy ($P<0.05$). In this experiment, there was a significant decrease in TG, cholesterol, NEFA and lipoproteins LDL and VLDL due to BSP ($P<0.05$), but there was no main effect of probiotic on serum HDL ($P>0.05$). No interaction was found between BSP and IFP on liver enzymes and blood parameters except for plasma TG concentration.

Discussion

There are contradictory reports on the effect of feed restriction on broiler performance. Some researchers had reported no significant difference in body weight of broilers when they received intermittent feeding and broilers fed *ad libitum* (Saffar & Khajali 2010, Svihus *et al.* 2010). However, Petek *et al.* (2005) demonstrated that IFP effectively decreased body weight. In this experiment, IFP decreased body weight gain at the early phase of age (8 to 21 days) ($P<0.05$) but between the 22 to 42 days of age body weight gain was not affected by IFP ($P>0.05$). Various factors such as timing, severity and duration of the restriction, gender and age of the parent stock, feed texture (Lippens *et al.* 2009), genetic make-up (Saleh *et al.* 2005) and feeding strategies (Urdaneta-Rincon & Leeson 2002) can influence growth performance of feed-restricted birds. It has been reported that under feed restriction, the digestive system probably indicates an adaptive response (Özkan 2010) and birds are able to use the crop and proventriculus-gizzard for feed storage in long periods of feed deprivation (Buyse *et al.* 1993). The crop is used as an intermediate storage organ in broiler chickens (Svihus *et al.* 2010). Therefore, these birds can maintain their growth rate during meal feeding. Changes in the enzymatic or absorptive capacity of the gastrointestinal tract or changes in post-absorptive processes can change feed efficiency (Fassbinder-Orth & Karasov 2006). The improvement in feed efficiency observed in feed-restricted chickens was probably attributed to reduced overall maintenance requirements, improvement of chemical digestion and nutrient absorption in the broiler gut (Pineiro *et al.* 2004). The results of our experiment showed that intermittent-fed birds were able to adapt to the feeding strategy for improving feed conversion and nutrient requirements, because they had similar body weight gain with control birds from 22 days of age to the end of the study.

In previous studies, researchers indicated that addition of probiotic can stimulate growth and improve feed conversion ratio in broilers (Onderci *et al.* 2008, Bansal *et al.* 2011). In this study, a BSP diet did not affect body weight gain and feed intake but improved birds feed conversion ratio ($P<0.05$) from 36 days of age to the end of the experiment. It was documented that probiotic can improve intestinal microbiota, the integrity of the intestinal mucosal barrier, digestive and immune function of intestine and broiler health (Tellez *et al.* 2006, Mountzouris *et al.* 2010). There are conflicting reports on the effects of probiotic application in poultry industry. Different factors such as bird age, overall farm hygiene, environmental stress, hydrophobicity of the probiotic bacterial cell surface, composition of microbial species, concentration of bacteria in probiotic and dosage, methods of using, frequency

of application, the combination of probiotics and synergistically acting components are effective on probiotic efficacy (Karimi *et al.* 2008, Awad *et al.* 2009, Flint & Garner 2009, Mountzouris *et al.* 2010).

Lipids and especially triglycerides may be stored in adipocytes, hepatocytes and growing oocytes in the birds (Hermier 1997). Deposition of fat in the abdominal area of the body is not suitable for customers. Reduction of deposition of lipids and increase of lipolysis is a main goal to improve the feed conversion and product cost. Environmental stress, energy status and hormonal stimuli can affect lipogenesis in birds (Lee *et al.* 2009). In the present study, there was no significant difference in AFP content as percentage of body weight among the treatments (Table 3). Similarly, Urdaneta-Rincon & Leeson (2002), Saleh *et al.* (2005), Lippens *et al.* (2009) and Saffar & Khajali (2010) reported that feed restriction had no effect on abdominal fat deposition. Although, some previous investigation has shown that feed restriction resulted in obesity and an increase in abdominal fat in broilers (Zhan *et al.* 2007, Wijtten *et al.* 2010). On the other hand, some reports showed that feed restriction decreased fat pad or abdominal fat in broilers (Santoso 2001, Moritz *et al.* 2005).

In our experiment, a significant decrease in carcass, wing and back weight (as percentage of the live body weight) was observed due to the IFP ($P < 0.05$). This result is not in agreement with the report of Petek *et al.* (2005), who concluded that intermittent feeding had no significant influence on carcass weight based on percentage of body weight. Researchers indicated that bird response to feed restriction could be influenced by various factors such as different feeding strategies applied (Urdaneta-Rincon & Leeson 2002), restriction programmes, duration of feed restriction (Khetani *et al.* 2009) and the excess of energy consumed (Tahmoorespur *et al.* 2010).

Usage of probiotic in animal diet improved meat quality (Ko *et al.* 2008, Zhou *et al.* 2010). In the present experiment, we studied the effect of probiotic on broiler carcass composition to evaluate the quality of bird yields. The findings of our study showed that the usage of the BSP at a dose of 50 mg per kg diet did not affect Arian male broilers abdominal fat pad and other carcass cut yields. Our findings are in agreement with the results of Pelicano *et al.* (2003), who indicated that probiotics inclusion of bacillus, *Lactobacillus* or *Saccharomyces cerevisiae*, had no effect on abdominal fat and carcass yields.

Nicotinamide adenine dinucleotide phosphate is an essential component for *de novo* synthesis of fatty acids in the liver, which is produced by the activity of ICD, MAE and glucose-6-phosphate dehydrogenase in the cytosol (Xu *et al.* 2003). Malic enzyme (EC 1.1.1.40) catalyses the oxidative de-carboxylation of malate to pyruvate and CO_2 while converting NADP^+ to NADPH (Goodridge *et al.* 1989). Glucose is a mediator for transcription of fatty acid synthesis and MAE (Hillgartner & Charron 1998). The MAE can produce most of the need for NADPH for the process of *de novo* synthesis of fatty acids and production of palmitate in birds (Goodridge *et al.* 1989, Hillgartner & Charron 1998). Palmitate is the precursor molecule for the production of other fatty acids (Adams & Davis 2001). Isocitrate dehydrogenase (EC 1.1.1.42) catalyses the oxidative decarboxylation of isocitrate to α -keto-glutarate and CO_2 , simultaneously generating NADPH from NADP^+ (De Beer *et al.* 2007). Xu *et al.* (2003) reported that a decline in total activities of the enzymes glucose-6-phosphate dehydrogenase, MAE, ICD and lipoprotein lipase in the fat reduces the deposition of subcutaneous fat. There are reports that indicate feed restriction can influence enzymatic activity in lipid metabolism.

Santoso (2001) reported that hepatic acetyl-CoA carboxylase activity was reduced in early feed-restricted broiler and resulted in reduction of final body fat deposition. According to previous investigations, nutritional conditions such as starvation (Goodridge *et al.* 1989), feeding a low-carbohydrate, high-fat diet and feeding high-carbohydrate for a long duration, low-fat diet (Hillgartner & Charron 1998) and dietary protein (Adams & Davis 2001) can affect the activity of fatty acid synthesis and MAE in birds. Under the condition of our experiment, IFP and BSP did not affect the liver MAE and ICD activity.

Lipoproteins are assembled and secreted to the bloodstream by the liver and intestine. Liver synthesizes HDL and VLDL and it is the major site for synthesis of cholesterol and phospholipids. Triglyceride is the major product of liver *de novo* synthesis (Hermier *et al.* 1997). Very low density lipoproteins and HDL are main carriers of TG and cholesterol, respectively. The nature of lipid deposition in the body is related to the availability of TG and their transportation by VLDL. Lipoprotein lipase catalyses the breakdown of TG from portomicrons and VLDL to free fatty acid and glycerol (Chen *et al.* 2005). Then the increase of free fatty acids in blood can induce deposition of lipid in muscle and adipose tissue (Xu *et al.* 2003). Although Yang *et al.* (2010) reported that feed restriction can affect lipid metabolism by catalysing tissue lipids to provide body energy, which increased serum total cholesterol level, there are different reports on the effect of feed restriction on blood lipid metabolites. Zhan *et al.* (2007) reported serum free fatty acid and VLDL to be slightly increased, whereas serum TG decreased ($P < 0.01$) in feed-restricted compared to *ad libitum* birds. Concentrations of total cholesterol and HDL cholesterol in serum were significantly higher in early feed restriction group at 14 d compared to *ad libitum* bird ($P < 0.05$) as documented by Yang *et al.* (2010). According to the study of Santoso (2001), the concentration of serum TG was significantly reduced in the early feed restriction group ($P < 0.01$), whereas cholesterol concentration was not affected ($P > 0.05$). In our experiment, although TG and VLDL were decreased by IFP, serum level of HDL, cholesterol, total NEFA and LDL were not significantly affected by IFP ($P > 0.05$).

Cenesiz *et al.* (2008) found that addition of kafir (includes *Lactobacillus*, *Streptococcus* and yeast species) to drinking water could reduce serum cholesterol and lipid levels in broilers. Fazeli *et al.* (2010) showed that addition of *Lactobacillus plantarum* to mice diet reduced serum cholesterol, LDL and TG. Cavallini *et al.* (2009) reported that addition of *Enterococcus faecium*-based probiotic to a rabbit diet decreased the blood TG. Dibaji *et al.* (2012) found that the use of symbiotic at a certain concentration in diet could significantly reduce the LDL and HDL to LDL ratio in serum but did not affect HDL, VLDL, TG and cholesterol levels. At this experiment, BSP addition decreased serum lipid metabolites except for HDL and there was no IFP effect in birds receiving BSP in the diet. According to previous investigations, some mechanisms are hypothesized for the effect of probiotic microorganism on lipid metabolism. They can be listed as: posing bile salt hydrolase activity and precipitation of cholesterol by some microorganisms such as *Lactobacillus*, *Bifidobacterium* (Sudha *et al.* 2009), incorporation of cholesterol into or binding to bacterial microorganism (Liong & Shah 2005) and making of short-chain fatty acids by probiotic bacteria (Ooi & Liong 2010).

In conclusion, the results of this study indicated that IFP or BSP did not affect hepatic malic and isocitrate dehydrogenase enzymes activity and abdominal fat content measured as percentage of live body weight. However, IFP significantly decreased feed intake and improved feed conversion ratio which is economically important in broiler breeding industry.

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