Biological Forum – An International Journal **7**(1): 1322-1326(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Betaine Hydrochloride in Diet on De novo Lipogenesis in Liver of Broiler

Nima Mosavat*, Mohammad Chamani**, Farhad Foroudi and Ali Asghar Sadeghi*******
**Department of Animal Science, Behbahan Branch, Islamic Azad University, Behbahan, IRAN*
***Department of Animal Sciences, Golpayegan Branch, Islamic Azad University, Golpayegan, IRAN*
****Department of Animal Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, IRAN*
*****Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, IRAN*

(Corresponding author: Nima Mosavat)
(Received 22 March, 2015, Accepted 02 April, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: This study was conducted to investigate the effects of dietary betaine supplementation on de novo lipogenesis in liver of broiler. This study included 160 broilers (Ross 308) conducted in four treatments and four replicates. Each replicate consisted of 10 chicks in a completely randomized design. The diets consisted of the control treatment in which did not use the betaine supplementation. Treatments of the second, third and fourth were containing 0.05, 0.08 and 0.11% betaine hydrochloride, respectively. The results showed that addition of betaine to the diet, tends to reduce the activities of enzymes ACC and FAS, although there was no significant difference (P>0.05). These findings suggest that ACC and FAS activities in broiler, is less responsive to betaine supplementation. So it seems that more research needs to be conducted, taking into account all the factors affecting enzyme activity of FAS and ACC.

Keywords: Betaine hydrochloride, De novo lipogenesis, Liver, Broiler.

INTRODUCTION

Betaine is tri-methylated derivative of glycine, which found in many organisms. It acts in the gastrointestinal tract as an osmoprotective agent and as a methyl group donor in metabolic pathways. Since betaine acts as a methyl group donor, it can partially replace methionine and choline in the diet (Eklund *et al.* 2005). Betaine participates in lipid metabolism and prevents excessive fat accumulation in the liver (Craig 2004; Eklund *et al.* 2005; Simon 1999). In general, the storage of fat in body is caused by the balance between dietary fat absorption, biosynthesis of fatty acids and lipid catabolism through β-oxidation. So, if we assume the same amount of fat absorbed from the diet, the less amount of body's fat may be related to increased catabolism or decreased interior synthesis of fatty acids, or both (Huang *et al.* 2008). Effects of betaine in reducing the accumulation of fat have been studied in poultry (Esteve-Garcia and Mack 2000, McDevitt *et al.* 2000, Saunderson and Mackinlay 1990, Wang *et al.* 2004). Effect of betaine has also been studied on activities and expression of lipogenic enzyme genes. Huang *et al.* (2008) showed that betaine supplementation in the diet reduces the activities of acetyl-CoA carboxylase, fatty acid synthase as well as fatty acid synthase gene expression in pig's abdominal adipose tissue. In a study it was found that betaine decreases activity of carnitine-palmitoyltransferase I in skeletal muscle tissue of pigs (Huang *et al.* 2009). In another study, it was shown that addition of 0.06% betaine in diet decreases lipoprotein lipase activity in abdominal adipose tissue of laying hens (Zou and Lu 2002). De novo lipogenesis occurs in most species and leading to synthesis of fatty acids from non-fat precursors (Ding *et al.* 2012; Leung and Bauman 1975) .In birds; de novo lipogenesis mainly occurs in the liver and appears that the main task of adipose tissue is synthesis and storing of lipid (Griffin *et al.* 1992). Two important enzymes in de novo lipogenesis are acetyl-CoA carboxylase and fatty acids synthase. ACC catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA, and FAS catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA (Kersten 2001). The effects of betaine on abdominal fat reduction in broilers has been studied in many researches, but the basic mechanism for reducing abdominal fat is not yet clear. The main objective of the current study was to investigate the effects of dietary betaine supplementation on de novo lipogenesis in liver of broiler.

MATERIAL AND METHODS

A. Birds and Dietary Treatments

The study was performed with 160 day-old broiler chicks (Ross 308) in a completely randomized design. The experiment was conducted with four treatments and four replications. The chickens were placed based on an average weight equal to the number of 10 chicks in each replication. Water and feed were provided for birds without restriction. Humidity, ventilation, and diets were adjusted according to Ross Catalog 2009. The lighting in the whole rearing period was 23 hours light and 1 h of dark. The diets were fed to chickens at three periods of 0 to 10 (starter), 11 to 24 (grower), and 25 to 42 days (finisher). The Experimental diets were: T1) without the use of supplemental betaine (control); T2) contains 0.05% of the betaine-HCl 98% (equivalent to 0.04 percent of pure betaine); T3) contains 0.08% of the betaine-HCl 98% (equivalent to 0.06 percent of pure betaine); and T4) containing 0.11% of the betaine-HCl 98% (equivalent to 0.08 percent of pure betaine). Ingredients and dietary nutrient analysis are shown in Table 1.

Table 1: Ingredients and nutrients analysis of the experimental diets.

	Starter (0-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Ingredients (%)			
Corn	54.22	60.06	65.84
Oil, vegetable	2	2.3	2
Soybean Meal (44%)	39	33.2	28
DL-Methionine	0.32	0.28	0.23
L-Lysine HCl	0.17	0.15	0.12
L-Threonine	0.08	0.08	0.05
Di Ca-Phosphate	2.2	2	1.8
$CaCO_3$	1.1	1	1
Na-Bicarbonate	0.05	0.07	0.1
NaCl	0.25	0.25	0.25
Vit. & Min. permix[1]	0.5	0.5	0.5
Filler[2]	0.11	0.11	0.11
Totall	100	100	100
Nutrients Analysis			
Crude Protein%	22	20	18
Energy (kcal/kg)	2900	3000	3048
Lysine(SID)%	1.213	1.066	0.926
Methionine(SID)%	0.611	0.549	0.479
Met+Cys(SID)%	0.902	0.817	0.728
Threonine(SID)%	0.789	0.717	0.623
Tryptophan(SID)%	0.238	0.209	0.184
Arginine(SID)%	1.376	1.220	1.081
Calcium%	0.977	0.885	0.831
Ava. Phosphorus%	0.479	0.440	0.403
Sodium%	0.137	0.142	0.149
Chloride%	0.212	0.210	0.205

1- Vitamin & mineral premix (each kg contained): vitamin A, 1800000 IU; vitamin D_3, 400000 IU; vitamin E, 3600 IU; vitamin K_3, 400 mg; thiamine, 360 mg; riboflavin, 1320 mg; niacin, 6000 mg; vitamin B_6, 600 mg; vitamin B_5, 2000; vitamin B_{12}, 3 mg; Folic acid, 200 mg; biotin, 20 mg. Choline 80 g; zinc, 17 g; iron, 10 g; copper, 2 g; manganese, 20 g; selenium, 40 mg; iodine, 200 mg.

2- Filler for T_1 (control) equal to 0.11% sand, for T_2 equal to 0.06% sand + 0.05 percent of Betaine hydrochloride 98%, for T_3 equal to 0.03% sand + 0.08 percent of Betaine hydrochloride 98%, and for T_4 equal to 0.11% Betaine hydrochloride 98%.

B. Sample Collection

At the end of the experiment (42 days), eight birds of each treatment with body weight close to the treatment mean, were selected and killed by cervical dislocation. Their liver were rapidly removed and weighed. The livers were placed in an ice container for subsequent measurement of the enzyme activities.

C. Liver Homogenates

Approximately 5 g of liver tissue was sampled and cut into small pieces, then was placed into tubes containing 15 ml of 0.25 M sucrose in 1mM EDTA-2Na buffer solution, and homogenized. Then was centrifuged (Beckman Instruments Inc., Irvine, CA) at 10000 × g for 10 min under 4°C. The supernatant was centrifuged again at 105000× g for 60 min in 4°C. The resulting supernatant was used to assaying hepatic enzymes (Chen *et al.* 2006).

D. Assay of Enzymes Activity

The activity of FAS (EC 2.3.1.85) was assessed according to the method of Nepokroeff et al. (1975). In this method, in the presence of FAS, malonyl CoA and acetyl CoA oxidize NAPDH and the activity of FAS measures spectrophotometrically by following the oxidation of NADPH at 340 nm. The activity of ACC (EC 6.4.1.2), was assayed according to the method of Qureshi *et al.* (1980) by nmoles of $H14CO_3$- fixed per minute per g liver tissue. The protein concentration in the supernatants was determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

E. Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 16 for windows (SPSS Inc., Chicago, USA). Significant differences at $P< 0.05$ statistical level compared by Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

The effects of dietary betaine supplementation on the acetyl-CoA carboxylase and fatty acid synthase activities in the liver of chickens, is presented in Table 2. Addition of betaine reduced the both enzyme activities, although there was no significant difference between treatments ($P>0.05$).

Table 2: The effect of dietary betaine supplementation on ACC and FAS activities (nmol/min/mg. protein) in liver (Mean ± SE, n = 8).

Treatments	T_1	T_2	T_3	T_4	P- value
ACC	2.28±0.16	2.18±0.15	2.00±0.13	1.75±0.15	0.113
FAS	2.82±0.25	2.69±0.23	2.50±0.26	2.26±0.23	0.421

There is considerable evidence that ACC and FAS have a key role in the regulation of fatty acids biosynthesis in animal tissues. ACC serves as a rate-limiting enzyme of lipogenesis and FAS catalyzes the last step in the lipogenic pathway (Kersten 2001; Numa *et al.* 1971). In chickens, the liver is the main place of de novo fatty acid synthesis (Saadoun and Leclercq 1983; Sanz *et al.* 2000). To our knowledge, little researches have been carried out to study the effect of betaine on lipogenesis in poultry. This study is the first study that investigates the effect of betaine on ACC and FAS activities in broiler's liver. To date, no published data has studied the effect of betaine on enzymes activities of ACC and FAS. In our study, addition of betaine to the diet reduced the enzyme activity of ACC and FAS (compared with control), but the effect was not significant ($P>0.05$). These results consistent with the reports of Xing and Jiang (2012) that showed addition of betaine to the diet of laying hens in 165 and 185 days of age had not significant effects on FAS and ACC gene expression, however, the addition of betaine decreased the expression of these genes in some treatments, compared with the control group. In support of our findings, Lien and Jan (1999) showed that adding choline to the diet of ducks reduced ACC and FAS activities in the liver, but the effect was not significant. In contrast to our results, Huang *et al.* (2008) were indicated that dietary betaine supplementation in pigs decreased significantly the activities of ACC and FAS in subcutaneous adipose tissue. Our findings also inconsistent with results of Xing *et al.* (2011) which is showed the FAS gene expression was significantly reduced in abdominal tissues of broiler chickens at ages 56 and 66 days. Lipogenesis is regulated by an extensive series of interlocking factors such as nutrition, hormones, nuclear transcription factors, and lipogenic enzymes, genetic selection, housing and environmental strategies (Claire D' Andre *et al.* 2013; Girard *et al.* 1994; Huang *et al.* 2008; Wolf 1996). Pothoven and Beitz (1975) reported that acetate availability is effective factor in the concentration of ACC. In support of this, it was shown that content and activities of ACC and FAS in liver increased in broiler chickens by feeding a high-carbohydrate diet (Hillgartner *et al.* 1996; Hillard *et al.* 1980; Teraoka and Numa 1975). It seems that in the chickens, lipogenic enzymes activities are excessive related to the intake of carbohydrates in the diet (Hillard *et al.* 1980). Lipogenesis appears that be sensitive to the insulin/glucagon ratio (Hazelwod 1971). Therefore, nutritional status and dietary energy and metabolic consequences of that (insulin and glucagon) are important factors that determine lipogenesis in the liver of birds (Hillgartner *et al.* 1995; Hillard *et al.* 1980; Leclercq 1984). So, in chick liver the effects of lipotropic, induced from adding of betaine, which is essential to avoid fatty acid synthesis, is much lower than that amount required to reducing the activity of lipogenic enzymes.

This study showed that the addition of betaine to the diet tended to decrease the activities of enzymes FAS and ACC. However, no significant difference was observed ($P>0.05$). Our findings suggest that ACC and FAS activities and de novo lipogenesis in liver of broiler, is less responsive to betaine supplementation. It seems that more research needs to be carried out, taking into account all the factors affecting de novo lipogenesis.

REFERENCES

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* **72**: 248-254.

Chen, K.L., Hsieh, T.Y. and Chiou, P.W.S. (2006). Caponization effects on growth performance and lipid metabolism in Taiwan country chicken cockerels. *Asian Austral J Anim.* **19**(3): 438-443.

Claire D' Andre, H., Paul, W., Shen, X., Jia, X., Zhang, R., Sun, L. and Zhang, X. (2013). Identification and characterization of genes that control fat deposition in chickens. *J Anim Sci Biotechnol*. **4**(1): 43.

Craig, S.A.S. 2004. Betaine in human nutrition. *Am J Clin Nutr*. **80**(3): 539-549.

Ding, F., Pan, Z., Kou, J., Li, L., Xia, L., Hu, S., Liu, H. and Wang, J. 2012. De novo lipogenesis in the liver and adipose tissues of ducks during early growth stages after hatching. *Comp Biochem Phys B.* **163**(1): 154-160.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics. 11: 1-42.

Eklund, M., Bauer, E., Wamatu, J. and Mosenthin, R. (2005). Potential nutritional and physiological functions of betaine in livestock. *Nutr Res Rev.* **18**(1): 31-48.

Esteve-Garcia, E. and Mack, S. (2000). The effect of DL-methionine and betaine on growth performance and carcass characteristics in broilers. *Anim Feed Sci Tech.* **87**(1): 85-93.

Girard, J., Perdereau, D., Foufelle, F., Prip-buus, C. and Ferre, P. (1994). Regulation of lipogenic enzyme gene expression by nutrients and hormones. *FASEB J.* **8**(1): 36-42.

Griffin, H.D., Guo, K.D., Windsor, D. and Butterwith, S.C. (1992). Adipose-tissue lipogenesis and fat deposition in leaner broiler-chickens. *J Nutr*. **122**(2): 363-368.

Hazelwod, R.L. (1971). Endocrine control of avian carbohydrate metabolism. *Poultry Sci.* **50**(1): 9-18.

Hillgartner, F.B., Charron, T. and Chesnut, K.A. (1996). Alterations in nutritional status regulate acetyl-CoA carboxylase expression in avian liver by a transcriptional mechanism. Biochem J. 319(1): 263-268.

Hillgartner, F.B., Salati, L.M. and Goodridge, A.G. (1995). Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiol Rev.* **75**(1): 47-76.

Hillard, B.L., Lundin, P. and Clarke, S.D. (1980). Esentiality of dietary carbohydrate for maintenance of liver lipogenesis in the chick. *J Nutr.* **110**(8): 1533-1542.

Huang, Q.C., Han, X.Y., Xu, Z.R., Yang, X.Y., Chen, T. and Zheng, X.T. (2009). Betaine suppresses carnitine palmitoyltransferase I in skeletal muscle but not in liver of finishing pigs. *Livest Sci.* **126**, 130-135.

Huang, Q.C., Xu, Z.R., Han, X.Y. and Li, W.F. (2008). Effect of dietary betaine supplementation on lipogenic enzyme activities and fatty acid synthase mRNA expression in finishing pigs. *Anim Feed Sci Tech.* **140**(3): 365-375.

Kersten, S. 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep.* **2**(4): 282-286.

Leclercq, B. (1984). Adipose tissue metabolism and its control in birds. *Poultry Sci.* **63**(10): 2044-2054.

Leung, T. and Bauman, D. (1975). In vivo studies of the site of fatty acid synthesis in the rabbit. *Int J Biochem.* **6**: 801 -805.

Lien, T.F. and Jan, D.F. (1999). The effect on the lipid metabolism of Tsaiya ducks when high levels of choline and methionine are added to duck´s diet. *Asian Austral J Anim.* **12**(7): 1090-1095.

McDevitt, R.M., Mack, S. and Wallis, I.R. (2000). Can betaine partially replace or enhance the effect of methionine by improving broiler growth and carcass characteristics? *Brit Poultry Sci.* **41**(4): 473-480.

Nepokroef, C.M., Lakshmanan, M.R. and Porter, J.W. (1975). Fatty acid synthase from rat liver. *Method Enzymol*. **35**, 37-44.

Numa, S., Nakanishi, S., Hashimoto, T., Iritani, N. and Okozaki, T. (1971). Role of acetyl-CoA carboxylase in the control of fatty acid synthesis. *Vitam Horm.* **28**: 213-243.

Pothoven, M.A. and Beitz, D.C. (1975). Changes in fatty acid synthesis and lipogenic enzymes in adipose tissue from fasted and fasted-refed steers. *J Nutr*. **105**(8): 1055-1061.

Qureshi, A.A., Burger, W.C., Prentic, N., Bird, H.R. and Sunde, M.L. (1980). Regulation of lipid metabolism in chicken liver by dietary cereals. *J Nutr.* **110**(3): 388-393.

Saadoun, A. and Leclercq, B. (1983). Comparison of in vivo fatty acid synthesis of the genetically lean and fat chickens. *Comp Biochem Phys B.* **75**(4): 641-644.

Sanz, M.C., Lopez-Bote, J., Menoyo, D. and Bautista, J.M. (2000). Abdominal fat deposition and fatty acid synthesis are lower and β-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J Nutr.* **130**(12): 3034-3037.

Saunderson, C.L. and Mackinlay, J. (1990). Changes in body-weight, composition and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. *Brit J Nutr*. **63**(2): 339-349.

Simon, J. (1999). Choline, betaine and methionine interactions in chickens, pigs and fish (including crustaceans). *World Poultry Sci J.* **55**(4): 353-374.

Teraoka, H. and Numa, S. (1975). Content, synthesis and degradation of acetyl-Coenzyme A carboxylase in the liver of growing chicks. *Eur J Biochem.* **53**(2): 465-470.

Wang, Y.Z., Xu, Z.R. and Feng, J. (2004). The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks. *Anim Feed Sci Tech.* **116**(1): 151-159.

Wolf, G. (1996). Nutritional and hormonal regulation of fatty acid synthase. *Nutr Rev*. **54**(4): 122-123.

Xing, J. and Jiang, Y. (2012). Effect of dietary betaine supplementation on mRNA level of lipogenesis genes and on promoter CpG methylation of fatty acid synthase (FAS) gene in laying hens. *Afr J Biotechnol*. **11**(24): 6633-6640.

Xing, J., Kang, L. and Jiang, Y. (2011). Effect of dietary betaine supplementation on lipogenesis gene expression and CpG methylation of lipoprotein lipase gene in broilers. *Mol Biol Rep.* **38**(3): 1975-1981.

Zou, X.T. and Lu, J.J. (2002). Effects of betaine on the regulation of the lipid metabolism in laying hen. *Agric Sci China.* **1**(9): 1043-1049.