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Research Article

EFFECT OF HIGHER DIETARY L-ARGININE SUPPLEMENTATION ON LIPOGENIC GENE EXPRESSION IN BROILER CHICKEN BY RT-PCR

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Abstract- A total of 150 day old Cobb 400 broiler chicks were randomly allocated to 5 treatment groups *viz*., T_1(100 % of arginine requirement), T_2 (125 % arginine), T_3 (175 % arginine), T_4 (125 % arginine for finisher period only) and T_5 (175 % arginine for finisher period only). The aim of this study was to evaluate the effect of dietary Arginine supplementation over the mRNA expression level of lipogenesis genes in broilers. The mRNA expression levels of lipogenesis gene were analyzed by RT-PCR. Gene expression of the two lipogenic genes *viz*., fatty acid synthase and lipoprotein lipase were decreased in the abdominal fat tissue by addition of arginine and 75 % more arginine from 0 to 42 days (T_3) showed the highest reduction than other supplemented groups (T_2,T_4 and T_5). Dietary arginine supplementation also decreased the gene expression of FAS in liver. Reduction was highest in T_3 compared to other supplemented groups (T_2, T_4 and T_5).

Key Words: Broiler chicken, L-arginine, Lipogenic gene expression, RT-PCR, Adipose tissue

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Introduction

Human health and animal production is greatly relied on lean meat than fat accumulated meat. Moreover, this has a great impact on reducing feed efficiency of broilers. Arginine supplementation reduced the fat mass and enhanced the expression of key genes responsible for glucose, fatty acid oxidation and increased meat production in poultry. Dietary arginine supplementation increased mRNA levels for FAS in muscle, while decreasing those for LPL, glucose transporter-4 and ACC-α in adipose tissue, which indicated that arginine differentially regulates expression of fat metabolic genes in skeletal muscle and white adipose tissue, therefore favoring lipogenesis in muscle but lipolysis in adipose tissue [3]. Dietary L-Arginine supplementation in the diets of broiler chickens showed effective reduction of abdominal fat deposition due to reduced hepatic FAS gene expression. Increasing dietary protein level in the diets of broiler chickens to 26.6 %, 23.5 % and 20.7 % in the starter, grower, and finisher phases led to a reduction in total carcass fat deposition [9]. Moreover, increasing dietary CP level from 17 % to 23 % in fat and lean broiler chickens from 21 to 63 days of age caused a significant reduction in abdominal fat deposition [5]. These can be evaluated through lipogenic gene expression. Therefore, the present study was designed to evaluate the effect of different levels of arginine on lipogenic gene expression in broiler chicks.

Materials and Methods

The experiment used completely randomized design consisting of 5 dietary treatments with 3 replicates consisting 10 chicks per replicate. Two types of diets were used over the period of experiment; starter diet was used from 1 to 21 days and finisher diet till the end of the experiment (22-42 days). The control diet consisted (T_1) of 100 % arginine for starter and finisher period as per the [6] requirement and four treatment groups with 125 % (T_2) and 175 % (T_3) arginine level compared to control during both starter and finisher periods and 125 % (T_4) and 175 % (T_5) arginine level during finisher period only compared to control. The required amount of L- arginine (free base) extra pure was purchased from Sisco Research Laboratory (SRL). The experimental diet was formulated as per [1]. The feed ingredient composition (%) of the broiler chicken starter diet (0-3 weeks) and finisher diet (4-6 weeks) were depicted in the [Table-1] and [Table-2] respectively. On 42nd day of feeding experiment, birds were slaughtered by kosher method and samples of breast muscle, liver and abdominal fat tissues were collected from 10 birds contributing 2 birds per treatment. RNA isolation was done according to the procedure [2]. Complementary DNA (cDNA) was synthesized from the isolated RNA by using the standard cDNA synthesis kit. The quality of cDNA was assessed by measuring absorbance at 280 nm and 260 nm, followed by electrophoresis through 1.25% agarose gels. Real-time PCR analysis was performed for the expression of lipogenic genes (FAS in abdominal adipose tissue, liver and muscle and LPL in abdominal adipose tissue and muscle) and β-Actin gene was considered as the housekeeping gene. Primers used for RT-PCR are presented in [Table-3].

Gene expression was measured by 7500 Fast Real Time PCR (Applied Biosystems Inc, CA, USA) with SYBR Premix Ex Taq™ (Perfect Real Time, Takara, Shiga, Japan). Forty cycles of amplification were performed. The thermal cyclic conditions used in real time PCR was as follows: 1 cycle at 95 °C for 15 seconds, and 40 cycles at 95 °C for 15 seconds and 60 °C for 30 seconds. The comparative Ct value method was used to determine expression levels of target genes among the control and treated with reference to β-Actin gene. The 2-ΔΔCT method was used to calculate relative changes in gene expression determined.

Results

The relative expression of mRNA of fatty acid synthase and lipoprotein lipase

gene as a result of arginine supplementation in broiler chicken is depicted in [Table-4] and [Fig-1] for FAS gene and LPL gene in [Table-5]and [Fig-2].

Table-1 *Feed ingredient composition (%) of the broiler chicken starter diet (0-3 weeks)*

Ingredient	T_1	T_2	T_3	T_4	T_5
Maize grain	53.00	53.00	53.00	53.00	53.00
Soyabean meal	33.68	33.68	33.68	33.68	33.68
Dry fish	5.00	5.00	5.00	5.00	5.00
Palm oil	4.00	4.00	4.00	4.00	4.00
Mineral mixture*	2.00	2.00	2.00	2.00	2.00
Common salt	0.25	0.25	0.25	0.25	0.25
L – Lysine	0.09	0.09	0.09	0.09	0.09
DL – Methionine	0.20	0.20	0.20	0.20	0.20
L – Arginine	0.00	0.39	1.17	0.00	0.00
Feed additive**	0.61	0.61	0.61	0.61	0.61
Saw dust	1.17	0.78	0.00	1.17	1.17
Calculated nutrient density					
ME (kcal / kg)	3086	3086	3086	3086	3086
Crude protein (%)	22.22	22.22	22.22	22.22	22.22
Calcium (%)	1.01	1.01	1.01	1.01	1.01
Available phosphorous (%)	0.53	0.53	0.53	0.53	0.53
Lysine (%)	1.30	1.30	1.30	1.30	1.30
Methionine (%)	0.58	0.58	0.58	0.58	0.58
Arginine (%)	1.56	1.95	2.73	1.56	1.56

***Mineral mixture:** each kg of diet supplied with 6.40 g Calcium, 1.20 g Phosphorous, 55 mg Manganese, 2 mg Iodine, 52 mg Zinc, 2 mg Copper and 20 mg Iron,
****Feed Additives** : Vitamin AB_2D_3K – 0.03 %, Ultra Vit–M – 0.05 %, Coccidiostat – 0.050 %, Perivac plus (each 200 g contains Vitamin E 20 g, Biotin 160 mg, Selenium 50 mg and carrier) – 0.050 %, Choline chloride – 0.40 %, Probiotic – 0.03 %.

Table-2 *Feed ingredient composition (%) of the broiler chicken finisher diet (4-6 weeks)*

Ingredient	T_1	T_2	T_3	T_4	T_5
Maize grain	57.00	57.00	57.00	57.00	57.00
Soya bean meal	28.00	28.00	28.00	28.00	28.00
Dry fish	5.50	5.50	5.50	5.50	5.50
Palm oil	5.50	5.50	5.50	5.50	5.50
Mineral mixture*	2.00	2.00	2.00	2.00	2.00
Common salt	0.25	0.25	0.25	0.25	0.25
DL – Methionine	0.18	0.18	0.18	0.18	0.18
L – Arginine	0.00	0.34	1.02	0.34	1.02
Feed additive**	0.55	0.55	0.55	0.55	0.55
Saw dust	1.02	0.68	0.00	0.68	0.00
Calculated nutrient density					
ME (kcal / kg)	3228	3228	3228	3228	3228
Crude protein (%)	20.02	20.02	20.02	20.02	20.02
Calcium (%)	1.01	1.01	1.01	1.01	1.01
Available phosphorous (%)	0.54	0.54	0.54	0.54	0.54
Lysine (%)	1.12	1.12	1.12	1.12	1.12
Methionine (%)	0.54	0.54	0.54	0.54	0.54
Arginine (%)	1.37	1.71	2.40	1.71	2.40

***Mineral mixture:** Each kg of diet supplied with 6.4 g Calcium, 1.2 g Phosphorous, 55 mg Manganese, 2 mg Iodine, 52 mg Zinc, 2 mg Copper and 20 mg Iron.
****Feed Additives** : Vitamin AB_2D_3K – 0.01 %, Ultra Vit-M – 0.01 %, Coccidiostat – 0.050 %, Perivac plus (each 200 g contains Vitamin E 20 g, Biotin 160 mg, Selenium 50 mg) – 0.050 %, Choline chloride – 0.40 %, Probiotic – 0.03 %

Table-3 *The sense and antisense primer sequences of fatty acid synthase, lipoprotein lipase and reference genes used for real time PCR*

Gene	Orientation	Primers sequences (5′ → 3′)	Product size (bp)	Reference
β actin	Forward	TGCGTGACATCAAGGAGAAG	300	Ebrahimi *et al.*(2014)
	Reverse	TGCCAGGGTACATTGTGGTA		
FAS	Forward	GGAGTCAAACTAGTTATCCATGGCC	423	
	Reverse	AAAGGAGATTCCAGCATCGTGCAGC		
LPL	Forward	ATGAAGAGGGGCTACGAGGT	150	
	Reverse	CCCATTCCATAACAGCCAAG		

FAS – Fatty Acid Synthase, LPL – Lipoprotein Lipase

Table-4 *Effect of dietary L-arginine supplementation on fatty acid synthase (FAS) gene expression in broiler chicks**

Gene	Tissue type	T_1	T_2	T_3	T_4	T_5
FAS	Liver	1	0.60	0.40	0.70	0.56
	Abdominal fat	1	0.65	0.47	0.77	0.60
	Breast muscle	1	1.50	2.21	1.37	1.96

*Mean of 3 samples

Table-5 *Effect of dietary L - arginine supplementation on lipoprotein lipase (LPL) gene expression in broiler chicks*

Gene	Tissue type	T_1	T_2	T_3	T_4	T_5
LPL	Abdominal fat	1	0.50	0.34	0.66	0.41
	Breast muscle	1	1.70	2.30	1.65	2.05

*Mean of 3 samples

Gene expression of the two lipogenic genes in the abdominal fat tissue including fatty acid synthase (FAS) and lipoprotein lipase (LPL) were decreased by arginine treatment and 75 % more L – arginine from 0 to 42 days (T_2) showed the highest decrease than other supplemented groups. Dietary arginine also decreased the gene expression of FAS in liver. Decreases were highest in T_3 compared to other arginine supplemented groups (T_2, T_4 and T_5).

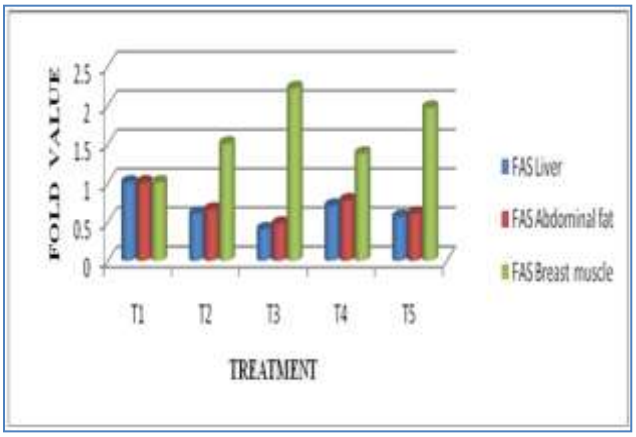

Fig-1 The relative expression of mRNA of fatty acid synthase (FAS) gene as a result of arginine supplementation in broiler chicks

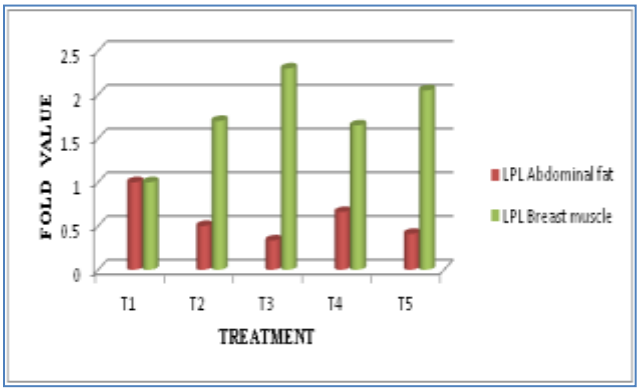

Fig-2 The relative expression of mRNA of lipoprotein lipase (LPL) gene as a result of arginine supplementation in broiler chicks

Discussion

In this study, lipogenic gene, FAS mRNA expression in liver and abdominal fat were decreased while increased in breast muscle tissue in the excess arginine supplemented groups irrespective of period of supplementation. Adipose tissue accumulation is determined by the balance between lipogenesis and lipolysis / fatty acid oxidation. High expression of FAS in muscle tissue was responsible for increasing its fat content [7]. Accordingly, dietary arginine inclusion at 0.25 % reduced abdominal fat deposition by suppressing hepatic FAS mRNA expression [4]. Lipoprotein lipase (LPL) has a crucial role in uptake and partitioning of fatty acids by adipose tissue and skeletal muscle. Arginine supplementation decreased mRNA concentrations of LPL in abdominal fat tissue which indicates a lower entry of fatty acid to the fat tissue. On the other hand, arginine supplementation increased mRNA concentration of LPL in muscle which indicates increased intake of lipids in muscle tissue [3]. Arginine supplementation differentially regulates the expression of lipogenic genes in skeletal muscle and white adipose tissue in a way that favors lipogenesis in muscle but lipolysis in adipose tissue [8]. Therefore, arginine, by regulating gene expression of LPL, changes the partitioning of lipids in a way to increase intramuscular fat deposition, while reducing white fat deposition in chickens.

Conclusion

The results revealed that adding 75% more arginine than the recommended level to the broiler chicken diet either from day old or from the finisher period significantly increased lipogenic gene expression in muscles, while decreasing those in adipose tissue and liver. Excess arginine supplementation to the broiler chicks, decreased the body fat synthesis and increased the intra muscular fat synthesis by altering the lipogenic gene expression with significant increased serum protein levels.

Application of research

1. By using L-arginine in the broiler diet we can reduce the fat deposition in the abdominal region of the broiler.
2. In the poultry meat, we can have better breast rather abdominal fat deposition.

Research Category: Poultry nutrition, Broiler chicks lipid metabolism modulator.

Abbreviations

ME: Metabolisable energy
LPL: lipoprotein lipase
FAS: Fatty acid synthase

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