Effects of Feeding Betaine on Performance and Hormonal Secretion in Laying Hens

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ABSTRACT

The effects of dietary betaine on performance, blood compositions, hepatic amino acid concentrations and hormonal secretions were examined in laying hens. Egg production was significantly higher in birds fed the 16.5 % protein diet compared to those fed 14.5 % protein diet($p<0.05$), whereas dietary supplementation of betaine did not show any significant effect. The high level of protein and betaine supplementation significantly improved egg weight, egg mass and feed conversion($p<0.05$), while eggshell breaking strength, eggshell thickness and Haugh unit were not influenced by betaine and dietary protein levels. Supplemental betaine did not affect serum total protein, albumin and BUN concentration. However, uric acid concentration significantly increased in 600 ppm betaine-fed groups($p<0.05$). Concentrations of most hepatic amino acid were influenced by increased protein feeding and dietary betaine supplementation. Hormone studies recorded significantly higher serum and hepatocyte IGF-I concentration in 600 and 1,200 ppm betaine treatments ($p<0.05$) compared to those of control group. IGF-I mRNA gene expression of hepatocytes revealed statistically correlated increase in 600 and 1,200 ppm betaine-fed groups compared to the controls($p<0.05$). Serum IGFBP-3 concentration was significantly elevated in 600 ppm betaine treatments However, the secretion of IGFBP-1 in hepatocyte of laying hens fed with 600 and 1,200 ppm of betaine showed a significant decrease compared to the control group ($p<0.05$). Results of these study show that dietary betaine supplementation affects protein and hormone metabolism in laying hens.

▶ **Key words** : Betaine, Blood component, Hepatic amino acid, Hormones, laying hens

INTRODUCTION

Betaine, an amino acid derivative, is a naturally occurring product present in relatively large quantities in sugar beet and aquatic invertebrates, but is not present in significant quantities in most animal feedstuffs(Wang et al., 2004). Chemically, betaine is trimethyglycine and it has been implicated in methionine sparing, osmotic stress protection and fat distribution (Saunderson and Mackinlay, 1990). However, the methionine sparing and fat distribution effects of betaine have been the subject of some controversy.

As a growth hormone that plays major role in vertebrate growth, the insulin-like growth factor-I(IGF-I) is a polypeptide consisting of 70 amino acids and is structurally similar to proinsulin, and it also affects on several important metabolic processes in the growth and differentiation of cells(Froesch et al., 1985; Olivecrona et al., 1999). Although various mechanisms affecting the secretion of IGF-I and IGFBPs have been widely reported in vertebrates, reports on factors affecting the hormonal secretion in poultry have been made scarcely.

Thus, the present study was conducted to investigate the effect of betaine on performance and hormone levels in laying hens.

MATERIALS AND METHODS

ISA-Brown laying hens were individually replaced into cage, and different levels of betaine were fed to the birds. The experimental feed was based upon the corn-soybean meal as a basal diet. Hens were allowed to consume water and feed *ad libitum*, and light was provided for 18 hrs a day. Blood was collected at the end of the experiment and kept under -70℃ until analysis. Hen's productivity, egg quality, blood component, IGF-I, IGF-mRNA, IGFBP-3 and IGFBP-1 concentrations were measured.

RESULTS

Egg production, feed intake, feed conversion and egg quality

Birds fed less 600 ppm in diets with different levels of protein showed no significant difference in egg production, but 16.5% protein-fed groups showed higher egg production than 14.5 % protein-fed groups. However, a protein × betaine interaction showed in egg production when fed low protein diets(14.5 %). It was recorded at 85.1 % in betaine supplementation groups but was 82.0 % in no betaine additional group. The high protein and betaine supplementation diets also improved egg weight, egg mass and feed conversion($p<0.05$), but the feed intake was not influenced by dietary betaine or protein levels(Table1). In addition, there were no significant differences in eggshell breaking strength, eggshell thickness and Haugh unit among the treatments. However, the yolk color index was significantly higher for the 14.5% protein-fed groups than the 16.5 % protein-fed groups(Table2).

Blood composition and Hepatic amino acid concentrations

Blood total protein, albumin, BUN, uric acid and hepatic amino acid concentration illustrated in Tables 3 and 4. Serum albumin concentration was significantly elevated in 18% protein-fed groups compared to those fed the other protein groups ($p<0.05$). Supplemental betaine did not affect serum total protein, albumin and BUN concentration. However, uric acid concentration in control group(2.89 mg/dL) was significantly decreased as compared to 600 ppm betaine-fed groups(3.33 mg/dL) ($p<0.05$). Concentrations of most hepatic amino acids were influenced by increased protein-fed groups and dietary betaine supplementation. Interestingly, low protein diet containing betaine(14 % protein + 600 ppm betaine) showed an increase in methionine aspartate, glutamate, glycine and tyrosine concentrations compared to 14 % protein diets($p<0.05$).

IGF-I secretion and IGF-I mRNA expression pattern in blood and liver

The Figures1 and 2 illustrate IGF-I concentration of laying hens in blood and liver respectively after feeding 0, 300, 600, and 1,200 ppm of betaine. Compared to the IGF-I concentration of the control group, which recorded 12.4±53.67ng/ml, betaine treatment groups illustrated increased IGF-I concentrations by recording 13.24±4.13, 20.67±3.98, and 24.35±4.78 ng/ml, respectively, and such elevated secretion exhibited statistical significance in the treatment fed with 600 and 1,200 ppm of betaine($p<0.05$) compared to the controls. The same pattern of increase in IGF-I concentration, were 34.20±6.57, 48.56±6.57, and 50.45±11.78 ng/ml, in laying hens' liver tissue fed betaine and compared to the control group (31.23±7.45 ng/ml). In addition, this study found statistically significant increase of IGF-I mRNA expression in the treatments fed with 600 and 1,200 ppm of betaine compared to the control(Figure3).

Secretion of blood IGFBP-3 and liver IGFBP-I

The blood IGFBPs presence was identified through the western ligand blotting(WLB) procedures. This procedure, allowed to locate the presence of IGFBP-3, IGFBP-1, 2 and IGFBP-4 bands, and the expression of IGFBP-3was shown to be intensified in betaine fed groups than the control groups (Figure4 A, B). The result of western

Table1. Comparison of productivity in laying hens fed different betaine and protein levels in diets

CP (%)	Betaine (ppm)	Egg production (%)	Egg weight (g)	Egg mass (g/day/hen)	Feed intake (g)	Feed conversion
14.5	0	81.95	60.75	49.90	138.01	2.835
14.5	600	85.06	61.17	52.25	137.65	2.706
16.5	0	88.10	61.84	54.57	137.07	2.533
16.5	600	87.79	63.58	55.88	136.71	2.468
Main effect means						
CP	14.5	83.50[b]	60.96[b]	51.08[b]	137.83	2.770[a]
	16.5	87.94[a]	62.71[a]	55.23[a]	136.89	2.501[b]
Betaine	0	85.02	61.30[b]	52.24[b]	137.54	2.684[a]
	600	86.43	62.38[a]	54.07[a]	137.18	2.589[b]
				P-value		
CP		0.0001	0.0001	0.0001	0.7558	0.0001
Betaine		0.1005	0.0001	0.0001	0.9041	0.0265
CP×Betaine		0.0457	0.0003	0.1099	0.9995	0.4569

[a,b] Means within a column with no common superscripts differ significantly ($p<0.05$).

immunoblotting(WIB) demonstrated significant increase of IGFBP-3 secretion in 600- ppm betaine fed group than the control group (Figure4 C). Through the same WLB procedures, the presence of IGFBPs in laying hens' liver tissues were identified to locate IGFBP-3, IGFBP-1, 2 and IGFBP-4 bands. Among these IGFBP bands, the secretion variation of IGFBP-1, 2 was the most noticeable change. As shown in Figure 5C, the WIB procedures revealed the significant decrease of IGFBP-1 secretion in 600 and 1,200 ppm betaine fed treatment groups than the control groups.

Table2. Comparison of egg qualities in laying hens fed different levels of betaine and protein in diets

CP (%)	Betaine (ppm)	Eggshell breaking strength (kg/cm^2)	Eggshell thickness (㎛)	Haugh unit	Yolk color index
14.5	0	4.65	371	87.77	8.0
14.5	600	4.23	365	87.91	7.8
16.5	0	4.24	369	87.42	7.3
16.5	600	4.24	370	88.14	7.4
Main effect means					
CP	14.5	4.44	368	87.84	7.9^a
	16.5	4.24	369	87.78	7.4^b
Betaine	0	4.44	370	87.59	7.7
	600	4.24	367	88.02	7.6
		p - value			
CP		0.1804	0.5517	0.9579	0.0001
Betaine		0.1659	0.3533	0.6986	0.4967
CP×Betaine		0.1678	0.1665	0.7938	0.1276

^{a,b} Means within a column with no common superscripts differ significantly ($p<0.05$).

Table3. Comparison of blood compositions in laying hens fed different levels of betaine and protein in diets

CP (%)	BET (ppm)	Total protein (g/dL)	Albumin (g/dL)	BUN (mg/dL)	Uric acid (mg/dL)
14.0	0	5.06	1.44	1.63	2.93
14.0	600	5.02	1.46	1.50	3.10
16.0	0	5.18	1.47	1.50	2.80
16.0	600	5.02	1.45	1.75	3.25
18.0	0	5.42	1.53	1.25	2.90
18.0	600	5.76	1.67	2.00	3.75
Main effect means					
CP	14.0	5.04	1.45^b	1.57	3.00
	16.0	5.10	1.46^b	1.63	3.03
	18.0	5.59	1.61^a	1.63	3.33
BET	0	5.22	1.47	1.50	2.89^b
	600	5.27	1.52	1.71	3.33^a
			p - value		
CP		0.3342	0.0401	0.9656	0.5208
BET		0.8871	0.3574	0.2418	0.0450
CP×BET		0.8083	0.4327	0.3244	0.5082

Table4. Comparison of amino acid concentrations in hepatic tissue of laying hens fed diets containing different levels of betaine and protein in diets

CP (%)	Betaine (ppm)	Arg[1]	His	I-le	Leu	Lys	Met	Phe	Thr	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
		Essential amino acid									Non-essential amino acid							
		(%)																
14	0	0.92	0.40	0.54	1.29	1.00	0.27^{b}	0.71	0.67	0.72	0.83	1.25^{b}	0.24^{b}	1.71^{b}	0.66^{b}	0.56^{b}	0.69	0.51^{c}
14	600	1.11	0.49	0.63	1.54	1.19	0.32^{a}	0.84	0.78	0.84	0.99	1.50^{a}	0.27^{ab}	2.01^{a}	0.77^{a}	0.69^{a}	0.80	0.60^{b}
16	0	1.05	0.47	0.62	1.50	1.18	0.32^{a}	0.81	0.78	0.82	0.97	1.43^{ab}	0.28^{a}	1.98^{a}	0.77^{a}	0.70^{a}	0.80	0.59^{b}
16	600	1.18	0.48	0.64	1.56	1.21	0.34^{a}	0.85	0.81	0.86	1.00	1.53^{a}	0.29^{a}	2.09^{a}	0.80^{a}	0.77^{a}	0.83	0.83^{a}
18	0	1.09	0.45	0.62	1.49	1.17	0.33^{a}	0.81	0.77	0.82	0.97	1.49^{a}	0.28^{a}	2.06^{a}	0.79^{a}	0.74^{a}	0.79	0.61^{b}
18	600	1.11	0.45	0.63	1.53	1.19	0.34^{a}	0.83	0.79	0.83	0.99	1.54^{a}	0.31^{a}	2.11^{a}	0.82^{a}	0.78^{a}	0.81	0.61^{b}
SEM		0.03	0.01	0.01	0.03	0.02	0.01	0.01	0.02	0.01	0.02	0.03	0.01	0.04	0.02	0.02	0.02	0.02
Main effect means																		
CP	14	1.00	0.44	0.58	1.41	1.09	0.29^{b}	0.76	0.72	0.77	0.90	1.36	0.26^{b}	1.84	0.71	0.62^{b}	0.74	0.55^{c}
	16	1.11	0.47	0.63	1.53	1.19	0.33^{a}	0.83	0.79	0.84	0.99	1.48	0.29^{a}	2.03	0.79	0.73^{a}	0.80	0.71^{a}
	18	1.10	0.45	0.62	1.51	1.18	0.33^{a}	0.82	0.78	0.82	0.98	1.52	0.29^{a}	2.08	0.80	0.76^{a}	0.81	0.61^{b}
Betaine	0	0.02^{b}	0.44	0.59	1.43^{b}	1.12	0.30^{b}	0.78^{b}	0.74	0.79	0.92	1.39^{b}	0.27^{b}	1.92	0.74	0.66^{b}	0.76	0.57^{b}
	600	0.13^{a}	0.47	0.63	1.54^{a}	1.20	0.33^{a}	0.84^{a}	0.80	0.84	0.99	1.52^{a}	0.29^{a}	2.07	0.80	0.75^{a}	0.81	0.69^{a}
		p-value																
CP		0.2352	0.3730	0.2082	0.2098	0.1731	0.0158	0.1873	0.1493	0.1909	0.1728	0.1382	0.0071	0.0662	0.0370	0.0143	0.1155	0.0001
Betaine		0.0347	0.0885	0.0680	0.0394	0.0692	0.0153	0.0287	0.0577	0.0515	0.0510	0.0247	0.0121	0.0507	0.0540	0.0246	0.0558	0.0001
CP×Betaine		0.4157	0.1413	0.1955	0.2067	0.2360	0.4701	0.2153	0.3577	0.2327	0.2245	0.2995	0.8120	0.3761	0.3702	0.5539	0.3859	0.0002

[1]Arg; Arginine, His; Histidine, I-le; Isoleucine, Leu; Leucine, Lys; Lysine, Met; Methionine, Phe; Phenylalanine, Thr; Threonine, Val; Valine, Ala; Alanine, Asp; Aspartate, Cys; Cystine, Glu; Glutamate, Gly; Glycine, Pro; Proline, Ser; Serine, Tyr; Tyrosine.

[a,b,c] Means within a column with no common superscripts differ significantly ($p<0.05$).

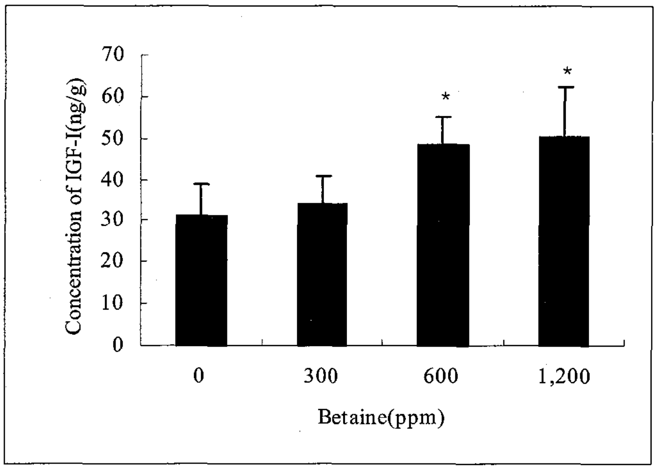

Figure1. The effect of dietary betaine on blood IGF-I secretion in laying hens. Values are expressed as mean SD. *p<0.05, compared to control.

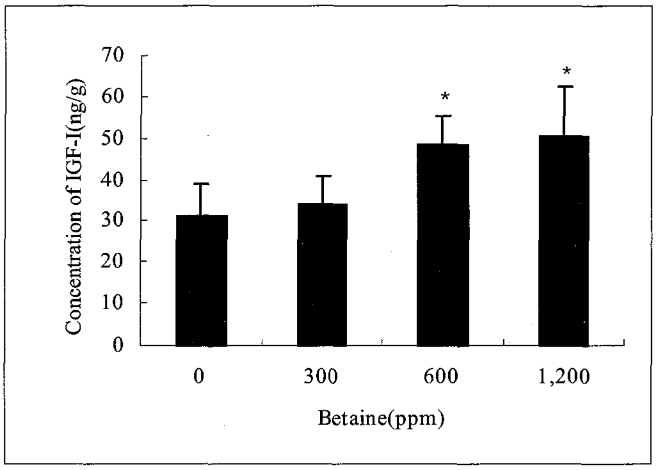

Figure2. The effect of dietary betaine on liver IGF-I secretion in laying hens. Values are expressed as mean SD. *p<0.05, compared to control.

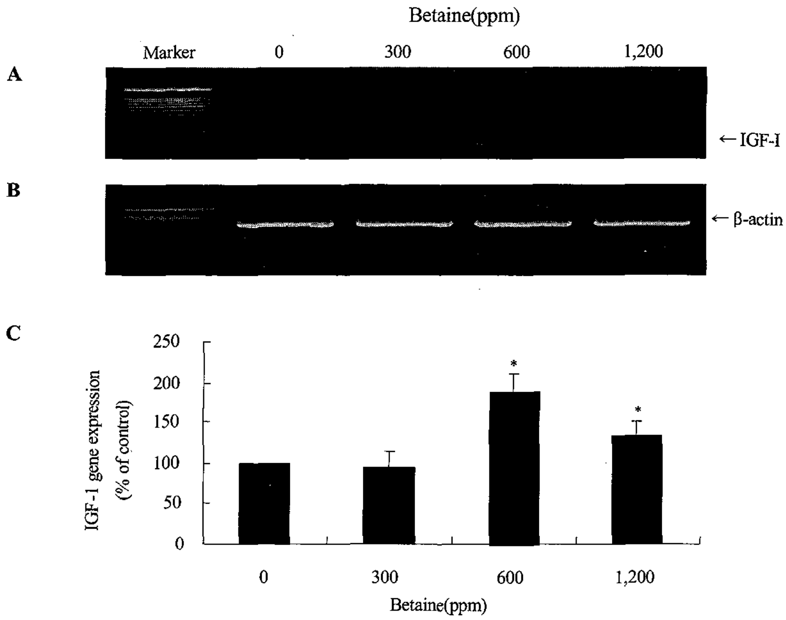

Figure3. The effect of dietary betaine on IGF-I mRNA expression on liver tissue of laying hens. Values are expressed as meanSD. *p<0.05, compared to control.

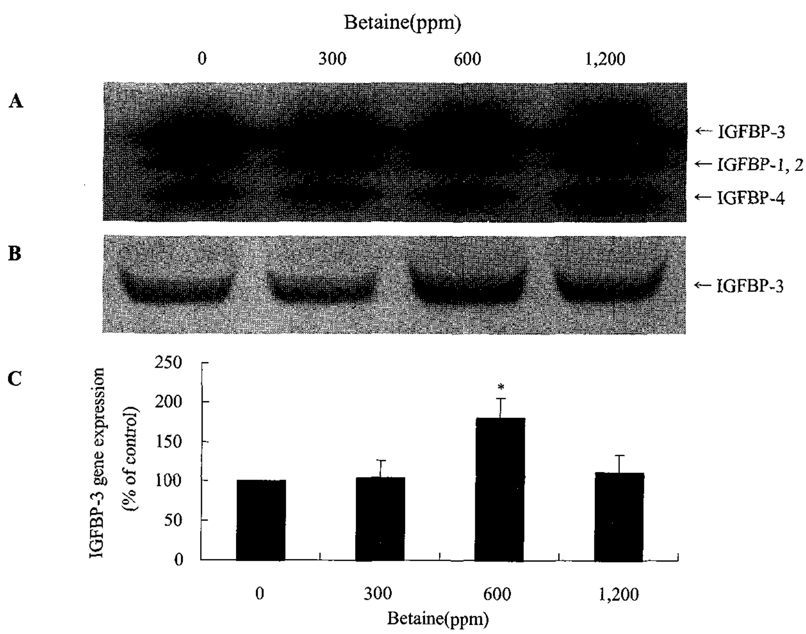

Figure4. The effect of dietary betaine on the secretion of blood IGFBP-3 determined by western immunoblotting (WIB) in laying hens. Values are expressed as mean SD. *p<0.05, compared to control.

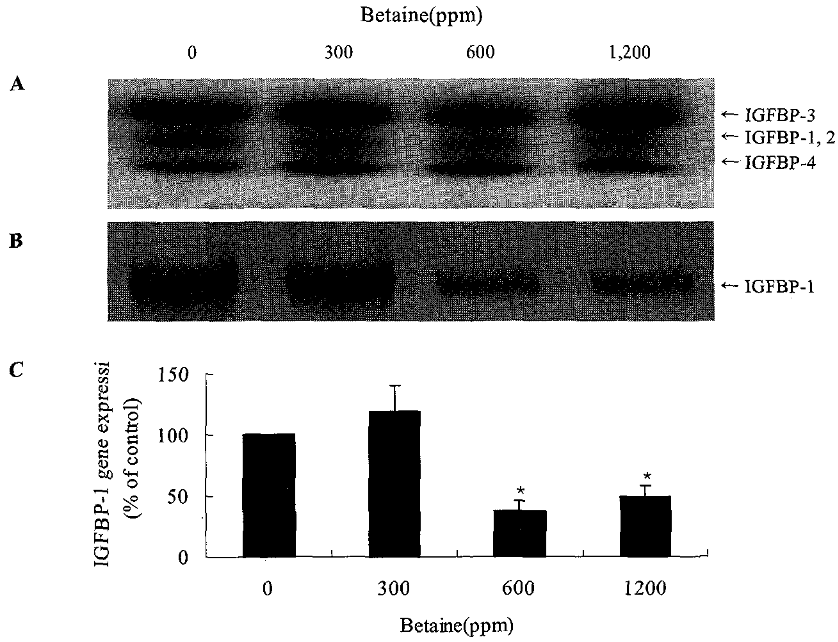

Figure 5. The effect of dietary betaine on the secretion of liver IGFBP-1 determined by western immunoblotting (WIB) in laying hens. Values are expressed as mean SD. *p<0.05, compared to control.

DISCUSSION

As a methyl group supplying source, betaine partially replace requirement for choline and methionine. Although its physiological roles in poultry productivity, protein and lipid metabolism, and immunity increment have been widely reported , the functions on methyl group donation or on the inhibition of lipid deposition are still controversial (Matthews et al., 1998; Pettey et al., 2001). Virtanen and Rosi(1995) reported the improvements in body weight increase, breast meat yield, and in feed requirement by adding betaine in broiler feeds. However, Harms and Russell(2002) reported the no significant effects of betaine on laying hens productivity in terms of egg production and egg weight. Although the present study also did not show any significant difference in egg productivity in betaine fed laying hens, but the betaine feeding exhibited increased egg weight, egg mass and feed conversion compared to the control birds. Also, low protein fed group showed an increase in egg production by addition of 600 ppm betaine. The result seems that betaine is effective in low protein diet than the adequate protein diet in egg production. Based on the observed blood uric acid and hepatic amino acid changes, it is likely that dietary betaine supplementation influences protein metabolism in laying hens.

As an in-vivo metabolite of betaine, glycine increases the internal blood growth hormone levels to play an essential role in controlling the hypothalamic pituitary functions(Kasai et al., 1980). As it is synthesized by the mediation of a growth hormone(GH), IGF-I also has been reported to directly manage the growth and differentiation of tissue by local secretion(Stewart and Rotwein, 1996). However, the feeding of betaine in laying hens' IGFs system of blood and liver has not been carried out conclusively. So, the investigator of the current study performed the study to systematically estimate the effect of betaine feeding on IGFs system in blood and liver of laying hens. The result of the experiment showed the IGF-I concentration of control group to record 12.45±3.67 ng/ml and 31.23±7.45 ng/g in blood and in hepatocytes, respectively. As expected, the IGF-I concentration in blood and in hepatocytes revealed to increase depending on the amount of betaine feeding ($p<0.05$), and the same pattern was found in the expression of mRNA in hepatocytes. The major reason of betaine increasing the IGF-I concentration in laying hens, could be because of the feedback stimulation of glycine, a betaine metabolite which consequently mediates the blood GH secretion in blood of laying hens and might have finally caused to promote such IGF-I secretion variation as has been recorded in the current study. In addition, such blood IGF-I concentration increase could be attributed to the increase of liver IGF-I concentration based upon the findings of Froesch et al. (1985) which have reported that 95 % of blood IGF-I is synthesized in liver, and such IGF-I mRNA expression increase as a major place in synthesizing IGF-I might have played direct role in increasing the blood and liver IGF-I secretion. As the binding proteins to IGF-I, the Insulin like growth factor-binding proteins(IGFBPs), 6 types of these binding proteins have been known, and the most of blood IGFBP-3, upto 80 %, is circulated in the form bound by IGF-I (Hill and Pell, 1998). As the major role of these binding proteins is to transport IGF-I to the target cells, they also have been reported to extend the half-life of IGF-I (Boxter, 1993; Rechler, 1993). Since the information regarding IGFBPs in laying hens is insufficient, such previously reported functions of IGFBPs in other species could not be directly compared to laying hens. However, the increase of IGFBP-3 secretion in rodents and in human has been reported to affect the biological metabolism of estrogen secretion, immunity, and growth hormone increase(Salobir et al., 1996; Amy et al., 1998).Among these IGFBPs, IGFBP-1 has been reported to show significant increase by biological catabolic functions due to stress, infection and immune deficiency, and the increase of GH secretion and immunity increase have been found to suppress the secretion of IGFBP-1(Lee et al., 1993; Jones and Clemmons, 1995). The current study showed the increase of blood IGFBP-3 secretion correlated to the amount of betaine feeding in laying hens, but the secretion of IGFBP-1 in laying hens' liver was found to be decreased. This result could be attributed to the fact that betaine feeding in laying hens might have increased the secretion of blood IGFBP-3, which consequently extends the half-life of blood IGF-I and increase the preservatity to enhance the growth of laying hens and to the differentiation of liver tissue. Through the above pathway, the consumption of betaine correspondingly suppresses the secretion of IGFBP-1 in laying hens' liver by resulting the decrease of catabolic functions, which might have caused to affect the energy conservation. The result of the current experiment revealed an increased blood IGF-I concentration and expression of IGF-I mRNA in the livers, but the secretion of IGFBP-1 in livers was found to be decreased. However, it could be speculated that the increase of blood IGF-I secretion might have been caused by the increased IGF-I mRNA expression in laying hens livers. The results of the current study could be used to investigate the factors affecting the productivity of betaine fed laying hens, and more studies are necessary to investigate the systemic estimation of betaine related to laying hen's productivity.

적요

이 연구는 산란계에서 betaine 첨가 효과를 알아보기 위하여 생산성, 혈액의 성상 및 호르몬의 변화를 조사하였다. 산란율에서 betaine 급여에 의한 차이는 나타나지 않았지만 단백질수준이 낮은 사료에서 betaine이 산란율을 증가시킴을 알 수 있었다. 난중, 산란량 및 사료요구율은 비태인 600 ppm 첨가구에서 개선되었다. 혈중 uric acid 와 간의 아미노산 함량은 betaine 급여구에서 증가하였다. 혈중 IGF-I 농도는 betaine 600과 1,200 ppm 급여 수준에서 현저하게 증가하였으며, 간조직의 IGF-I 농도 또한 혈액의 IGF-I 농도 변화와 동일한 양상으로 betaine 급여구에서 증가하였다. Betaine을 수준별로 급여한 산란계의 WLB을 통해서 혈중에 IGFBPs를 확인한 결과, IGFBP-3, IGFBP-1, 2 및 IGFBP-4 band를 관찰하였으며, IGFBP-3 분비가 betaine 600 ppm 급여구에서 유의하게 증가하였다. 또한 betaine 600, 1,200 ppm 급여구는 대조구에 비하여 IGFBP-1 분비가 유의하게 감소하였다. 이러한 실험 결과, betaine은 산란계의 단백질 대사와 호르몬 변화에 영향을 미칠 수 있는 인자로 사료된다.

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