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Effects of Feeding Betaine on Performance and Blood Hormone in Laying Hens

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산란계에 비태인의 급여가 생산 능력과 혈중 호르몬에 미치는 영향

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ABSTRACT An experiment was conducted to investigate the effects of dietary betaine on egg production, estradiol, progesterone, and melatonin secretion in laying hens. Seventy two ISA-Brown laying hens were placed into individual cage and fed four different levels of betaine (0, 300, 600, 1,200 ppm) in diets for four weeks. Basal diets were mainly mixed with corn-soybean and contained 2,800 kcal/kg ME and 16% CP.

Dietary supplementation of betaine showed significantly higher ($p<0.05$) egg weight and daily eggmass than controls, but did not have consistent influence on the egg production. The serum estradiol was statistically different in betaine treatments compared to that of control ($p<0.05$). No significant changes in serum progesterone concentrations were observed by the addition of betaine in diets. Serum melatonin concentrations also increased with betaine supplementation in diets ($p<0.05$). The results of this study indicate that the dietary betaine may contribute to increase daily eggmass, serum estradiol and melatonin concentration in laying hens.

(Key words : betaine, egg production, estradiol, progesterone, melatonin, laying hens)

Introduction

As of the naturally produced tertiary amine (trimethyl glycine), betaine presents in most of animals and plants, which is synthesized by the oxidation of choline in animal body (Dragolovich, 1994). Although small amounts of betaine is present in animal feeds, betaine has been often applied as a feed additive in poultry farm, and its additive effects have been variously reported (Kidd et al., 1997).

It has been reported to have effective intestinal osmosis controlling effects under high temperature suffering or coccidiosis contaminated broilers, and to decrease effects of methionine of choline supply as the source of methyl group (Kidd et al., 1997; Simon, 1999). Betaine also increased broiler performance along with the quality improvement and enhanced the immunity by decrease the fat deposition (Saunderson and MacKinlay, 1990; Kettunen et al., 2001; Klasing et al., 2001).

The blood concentration of progesterone, luteinizing hormone, estrogen, and androgen in laying hens have been pointed out to be the very important factors in affecting the egg production and its clutch (Sechman et al. 2000). Several researchers reported that the consumption of betaine contributed to increase the concentration of those blood hormones (Yu et al., 2001; Zou and Lu, 2002).

In addition, Shibata *et al.*(2003) reported thyroid removed chicks resulted in activating the betaine homocysteine methyl transferase (BHMT), which suggested the possible roles of betaine in affecting thyroid hormones. Moreover, since betaine plays as a direct methyl donor in the following important chemical pathways of synthesizing protein, creatine, phospholipids, hormone, polyamines, carnitine, adrenaline, and

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DNA methylation, it is highly likely to be related to the synthesis of hormones.

However, there are no study reports yet that reported the relationship between betaine and the secretion of hormones of estradiol, progesterone, and melatonin, which will have a good relationship with the laying hens' productivity. Thus, the current study was conducted to demonstrate the secretion patterns of these hormones and its relationship with performance in laying hens, fed with different levels of betaine in diets.

Materials and Methods

1. Feeding Trial

Seventy two ISA-Brown laying hens of thirty five weeks old were individually assigned with randomly selected 18 hens in each group. Four different levels of betaine (0, 300, 600, and 1,200 ppm) were fed for four weeks. The basal diets were mixed with the corn-soybean, containing 16% CP and 2,800 kcal/kg ME (Table 1). Birds were allowed to consume water and feed *ad libitum*. Day light was up to 18 hrs a day. The blood was collected at the end of the experiment and kept under −70℃ until the analysis.

1) Egg Production, Egg Weight, and Egg Mass

Egg production and weight were measured at the same designated time in everyday. The egg production was calculated by dividing the daily egg number by laying hen numbers, and the daily egg mass was calculated by multiplying the mean daily egg production and mean egg weight.

2) Analysis of Estradiol, Progesterone and Melatonin

(1) Estradiol

The concentration of blood estradiol-17β was measured by following the direct I-estradiol 17β radiommunoassay protocol by using an immuchem estradiol 17β(E_2) kit (ICN Biomedicals, INC. Diagnostics U.S.A), which added the same amount, 100 ul, of blood serum and estradiol-17β standard into the anti-estradiol-17β coated tube, and 1.0 mL of ^{125}I-estradiol-17 β was added to a total tube, which was then shaken gently before incubating for 90 minutes at a 37℃ incubator. All liquid substances in the tube was removed after the incubation before conducting the counting for 60 seconds at a gamma-counter.

(2) Progesterone

The concentration of blood progesterone was measured by following the direct I-progesterone radioimmunoassay pro-

Table 1. Formula and chemical composition of basal diet

Ingredients	(%)
Corn	67.55
Soybean meal	18.28
Corn gluten meal	3.15
Rapeseed meal	0.50
Limestone	8.23
TCP	1.56
Salt	0.34
L-lysine	0.06
DL-methionine	0.03
Vitamin premix[1]	0.20
Mineral premix[2]	0.10
Calculated chemical composition	
ME (kcal/kg)	2,800
CP (%)	16.00
Methionine (%)	0.32
Lysine(%)	0.75
Ca (%)	3.70
P (%)	0.40

[1]Provided per kilogram of diet: vit. A, 5,500IU; vit. D_3, 1,100IU; vit. E, 11IU; vit. B_{12} 0.0066 mg; riboflavin, 4.4mg; niacin, 44mg; pantothenic acid, 11mg (Ca-pantothenate, 11.96 mg); choline, 190.96 mg (choline chloride 220 mg); menadione, 1.1 mg (menadione sodium bisulfite complex, 3.33 mg); folic acid, 0.55 mg; pyridoxine, 2.2 mg(pyridoxine hydrochloride, 2.67 mg); biotin, 0.11mg; thiamin, 2.2 mg(thiamine mononitrate, 2.40 mg); ethoxyquin, 125 mg.
[2]Provided in mg per kilogram of diet; $MnSO_4$, 120; $ZnSO_4$, 100; $FeSO_4$, 60; $CuSO_4$, 10; $Ca(IO_3)_2$, 0.46; $CaCO_3$, min: 150 max: 180.

tocol by using an immuchem progesterone Kit (ICN Biomedicals, INC. Diagnostics U.S.A), which added the same amount, 100 ul, of blood serum and progesterone standard into the anti-progesterone coated tube, and 1.0 mL of ^{125}I-progesterone was added to a total tube with the same procedure of estradiol assay.

(3) Melatonin

For the measurement of blood melatonin concentration, the RIA test kits produced by BuHLMANN LABORATORIES AG (Switzerland). The RIA kit determined the melatonin by using the Kennaway G280 anti-melatonin antibodies comprised by primary and secondary antibodies. The RIA analysis procedure required an initial pretreatment step of preparing a column and conditioning step by adding 1 mL of methanol and distilled water twice, and the following washing step required the addition of 10% 1 mL methanol twice and then, single addition of 1 mL of hexane, and 5 time diluted sample was added before adding 1 mL methanol to extract. After the pretreatment step, the sample was reacted with 100 ul of primary antibody and 100 ul of I-125 for 20±4 hrs, and then 100 ul of secondary antibody was added before conducting the measurement by using a gamma-counter.

2. Statistical Analysis

For the statistical analysis of the data obtained from the present investigation, the analysis of variance (ANOVA) and Student's *t*-test were conducted, and expressed here as mean ±SE values.

Results

Birds fed with different levels of betaine added feeds for four weeks showed no significant difference in egg production between control and betaine treatments groups. However, birds fed 600 and 1,200 ppm betaine revealed to be higher egg production or significantly higher egg weight than control ($p<0.05$). In addition, the daily egg mass of birds treated with betaine recorded to show 59.3±1.54, 60.7±2.05, and 59.7 ±1.25g, respectively, which are significantly higher than 56.8± 1.99g of daily egg mass for control ($p<0.05$) (Table 2). When the blood estradiol concentration of control was 72.8±8.64 ng/mL, birds fed 300, 600, and 1,200 ppm betaine resulted to be statistically significant increase upto 138.4±14.79, 147.28 ±16.12, and 145.01±15.47 ng/mL, respectively ($p<0.05$) (Fig. 1).

There was no difference in blood progesterone of birds fed different levels of betaine in diets (Fig. 2). However, the blood melatonin concentration for control group recorded 39.3±0.10 ng/mL, while the birds fed 300, 600, and 1,200 ppm betaine resulted to record the 45.9±0.08, 52.0±0.09, and 54.5±0.05 ng/mL, respectively ($p<0.05$) (Fig. 3). As the result, the betaine feeding in laying hens was found to have influence on the concentration of blood estradiol and melatonin, and suggested that such hormonal change may be directly related to egg production.

Table 2. Effect of feeding betaine on egg production, egg weight and daily eggmass of laying hens

Betaine (ppm)	Egg production (%)	Egg weight (g)	Daily egg mass
0	92±2.84	61.8±0.07	56.8±1.99
300	90±2.26	66.2±0.16*	59.3±1.54*
600	93±2.81	65.0±0.16*	60.7±2.06*
1,200	94±1.97	63.4±0.12*	59.7±1.25*

* Means with the different superscripts within a column differ significantly ($p<0.05$).

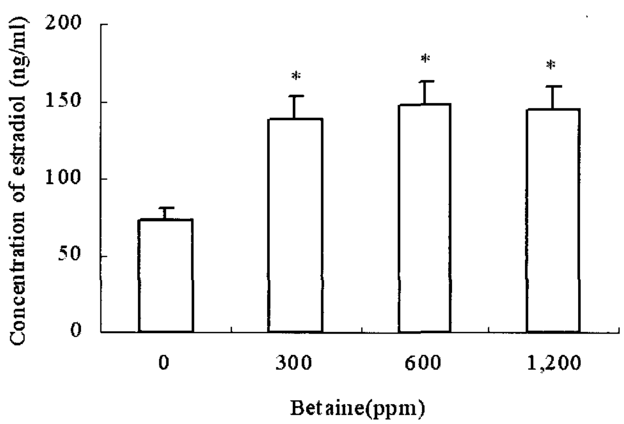

Fig. 1. Effect of feeding betaine on blood estradiol content in laying hens. Values are expressed as mean±SE.
* $p<0.05$, compared to control.

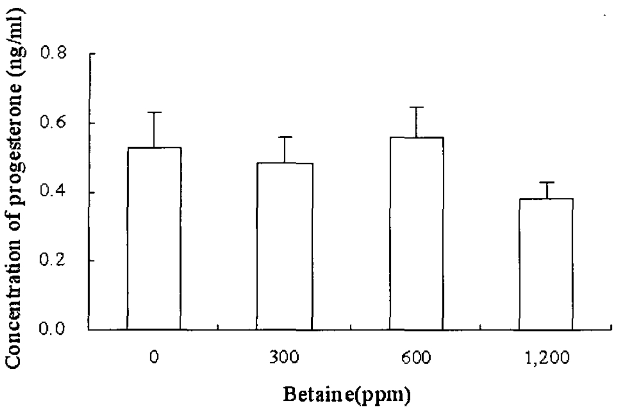

Fig. 2. Effect of feeding betaine on blood progesterone content in laying hens. Values are expressed as mean±SE.
*$p<0.05$, compared to control.

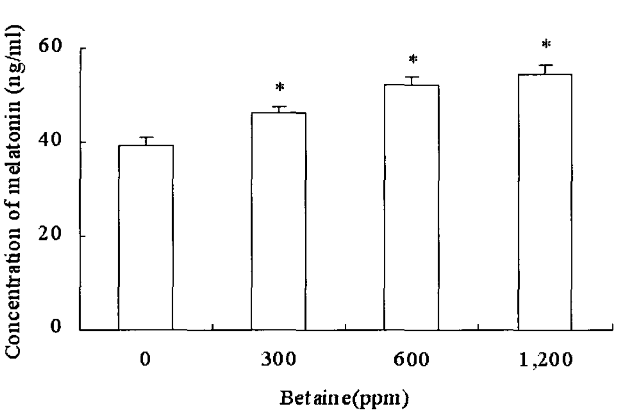

Fig. 3. Effect of feeding betaine on blood melatonin in laying hens. Values are expressed as mean±SE.
*$p<0.05$, compared to control.

Discussion

As a methyl group supplying source, betaine partially replaces the requirement for choline and methionine in animals. Although its physiological roles in poultry production, protein and lipid metabolisms, 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). Furthermore, it is scarce to find any study reports regard on the secretion pattern change of estradiol, progesterone, and melatonin in laying hens by betaine feeding. Since a methyl group is related with protein and hormone synthesis, the present comparison study was conducted to review the changing patterns of estradiol, progesterone, and melatonin secretion by betaine feeding in laying hens to demonstrate the correlationship between hormone secretion pattern and egg production. Virtanen and Rosi (1995) reported the improvements in body weight increase, breast meat yield, and feed conversion by adding betaine in broiler feeds.

However, Harms and Russell (2002) reported that no significance was found in effects of betaine on egg production and weight in laying hens. Although the present study did not show any significant difference in egg production in birds fed betaine, feeding betaine concentration more than 600 ppm exhibited increased egg production compared to control. Egg weight and daily egg mass increased uniformly in all betaine addition groups. The result of this experiment was similar to the previous report of Park et al. (2005), which reported no statistical difference in egg production between beatine treatments and control, but increased egg weight and daily egg mass in 600 ppm betaine treatment. As of an osmosis controlling substance, betaine has been reported to influence on nutrient digestibility due to its roles in promoting cell growth, existence, and cell vitality.

The feeding betaine improved methionine, lysine and protein digestibility in coccidiosis contaminated broilers (Remus et al., 1995; Augustine and Danforth, 1999). Although it is controversial due to the uncorrespondence of several reports in egg weight, the reason of egg weight increase by betaine feeding in present study mainly found from the role of betaine in increasing bioavailability of intestinal amino acids to increase the albumen production of egg. Thus, it is considered to require carrying out more in depth studies by segmenting betaine concentration.

As Lunenfeld et al. (1982) reported estradiol's important role in the continuous growth of follicle, increasing receptors of follicle stimulating hormone and luteinizing hormone in follicle, and increasing the reactivity to gonadotropic hormone. Wingfield et al. (1989) demonstrated that estradiol would be highly related with production in laying hens. In addition, Lang et al. (1984) reported the role of progesterone in promoting the ovulation in laying hens, and stimulating the secretion of luteinizing hormone. At the current study, the blood estradiol concentration was significantly increased by the increase of dietary betaine feeding in laying hens, whereas the concentration variation of progesterone by feeding betaine was not observed.

The probable reason of increasing egg production in laying hens by feeding betaine may be related with the increase of estrogen in blood, while no variation of progesterone concentration related with ovulation was found from the present study. Such inconsistency is considered to be revealed by conducting further studies. As of a neuronal hormone synthesized and secreted from pineal gland, melatonin secretion is controllable by the amount of light, and it was previously reported to have relationship in animal propagation, secretion, temperature maintenance, time and seasonal periodic changes, and immune response (Pang and Ralph, 1975; Brzezinski, 1997). In addition, melatonin is responsible for controlling the systems of hypothalamus, pituitary, and gonadotropin to regulate reproductive physiology (Reiter, 1995). Thus, the concentration of blood melatonin is suggested to be related with egg production in laying hens (Ryu and Kang, 1997).

In addition, glycine, the metabolite of betaine was reported to regulate gonadotropic hormone, which will have effects on activating the function of hypothalamic-pituitary (Kasai et al., 1980). Finkelstein et al. (1983) reported that betaine and choline activate the secretion of BHMT in liver, and correspondingly, the secreted BHMT affects the hormone secretion. The present study demonstrated that the feeding betaine in laying hens increased not only the blood estrogen concentration, but also melatonin concentration.

The probable reason for this could be found from a consideration that betaine acted on neuronal tissues of brain and pituitary to stimulate the secretion of gonadotropin releasing hormone (GnRH) which correspondingly increased the secretion of estradiol and melatonin. In conclusion, the present study resulted to show increase of estradiol and melatonin secretion by betaine feeding in laying hens to increase the egg weight and egg mass.

적 요

이 연구는 산란계 사료에 비태인의 첨가가 산란율, 에스트라다이올, 프로제스테론, 멜라토닌의 분비에 미치는 영향을 구명하고자 실행하였다. 사양 실험은 산란 피크에 도달한 35주령 이사브라운 72수를 개체별 케이지에 배치하여 CP 16%, ME 2,800 kcal/kg의 기초 사료에 비태인을 0, 300, 600, 1,200 ppm 수준으로 첨가하여 4주간 급여하였다.

비태인 첨가구에서 난중과 1일 산란양은 대조구에 비하여 현저하게 증가하였지만($p<0.05$), 산란율에서 일관성은 없었다. 혈청의 에스트라다이올은 비태인처리구에서 대조구와 매우 다르게 나타났다($p<0.05$). 혈청내 프로제스테론은 처리구간에 차이가 없었으며, 멜라토닌은 비태인 처리구에서 대조구에 비하여 유의적으로 높게 나타났다($p<0.05$). 본 연구 결과 산란계 사료에 비태인의 첨가로 1일 산란양, 난중이 증가하였으며, 혈청내 에스트라다이올과 멜라토닌 함량이 높게 나타났다.

(색인어 : 비태인, 산란율, 에스트라다이올, 멜라토닌, 산란계)

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