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Effects of dietary gamma-aminobutyric acid on egg production, egg quality, and blood profiles in layer hens

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ABSTRACT: Hy-line brown commercial layers (32 weeks old) were used to investigate the effects of GABA (gamma-aminobutyric acid) derived from *E. coli* strains on productivity, egg quality, and blood profile. In total, 288 birds (1946 ± 0.5 g) were fed four different levels of GABA (0, 25, 50, and 100 ppm), in a basal diet based on corn-soybean meal, for five weeks. Egg production, egg weight, and egg mass during weeks 32 to 36 showed significant improvement, as dietary GABA was increased from 0 to 100 ppm (linear, $P < 0.05$). Additionally, GABA supplementation was associated with increased eggshell breaking strength and albumen height (quadratic and linear, $P < 0.05$). Blood variables, such as white blood cells, red blood cells, lymphocyte, cortisol, epinephrine, and norepinephrine concentrations, were not influenced by addition of GABA to the diet; however, the haptoglobin concentration decreased significantly (linearly) and the IgG concentration increased (quadratically) in the GABA-fed groups ($P < 0.05$). These results suggest that diets containing GABA may beneficially affect productivity, egg quality, serum haptoglobin, and IgG concentrations in layers.

Keywords: gamma-aminobutyric acid; egg production; egg quality; blood profile; layer

Gamma-aminobutyric acid (GABA) is a non-essential amino acid that is found widely in nature including in bacteria, yeasts, plants, and animals. It is biosynthesised through the α-decarboxylation of glutamic acid, catalysed by a glutamate decarboxylase (Chung et al. 2009). GABA not only plays the role of a principal inhibitory neurotransmitter in the central nervous system, but also exhibits various nutritional and pharmacological functions, such as the induction of diuresis, a blood pressure-lowering effect, promotion of the absorption of metal ions, tranquilising effects, protecting the liver against alcohol damage, and immunomodulatory effects (Omori et al. 1987; Oh and Choi 2000; Adeghate and Ponery 2002; Kimura et al. 2002; Jin et al. 2013).

To date, there have been several studies on GABA production by fermentation methods using bacteria, fungi, and yeast (Smith et al. 1992; Lu et al. 2008). Several *Escherichia coli* strains have been reported to convert L-glutamate to GABA and the isolation of these strains has been studied for industrial purposes.

GABA has shown potential benefit in animals for use as a feed additive. In the livestock sector, it has been demonstrated that feeding GABA improves feed intake and weight gain in growing pigs and weanling pigs, and decreases sow weight loss in lactation (Fan et al. 2007; Liang et al. 2009; Yang et al. 2009). Wang et al. (2013) reported that GABA has beneficial effects such as improvement of feed intake, lactation performance, and animal health in dairy cows during early lactation. Dai et al. (2011) demonstrated that dietary GABA exerted stress-relaxation functions, helping to prevent heat stress-related symptoms in growth performance and carcass traits in broilers.

Although some evidence for GABA as a dietary supplement has been confirmed, few data are available, in particular with regard to the biological properties of GABA in layers. Thus, the objective of our study was to collect information on GABA as a feed ingredient by measuring laying performance, egg quality, and blood profiles in layers that

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were fed diets that included GABA produced by an *Escherichia coli* strain.

MATERIAL AND METHODS

Experimental birds and design. All animal-based procedures in this study were approved by the Animal Care and Use Committee of Dankook University.

In total, 288 Hy-line brown commercial layers were assigned randomly to four treatment groups, with 12 replicates of six hens in each treatment, from 32 to 36 weeks of age. Six layers were housed per 114 × 50 × 40 cm wire cage, and were subjected to a photoperiod of 16 h light and 8 h dark/day. The experimental diet was fed in a mash form, and was a corn-soybean meal, as a basal diet (Table 1). Feed and water were provided *ad libitum*. Dietary treatments were as follows: (1) control (no GABA, basal diet), (2) 25 ppm GABA (control + 25 ppm GABA), (3) 50 ppm GABA (control + 50 ppm GABA), and (4) 100 ppm GABA (control + 100 ppm GABA). The GABA was provided by FEEDUP Co. (Nonsan, Korea). It was produced from the GABA-producing *E. coli* E11, which was isolated from soil for GABA production. *E. coli* E11 was found to have high GABA-producing ability and glutamate decarboxylase activity.

Table 1. Basal diet composition (as-fed basis)

Ingredients (%)	
Corn	56.28
Soybean meal, 46% CP	15.53
Wheat grain	10.00
Corn gluten meal, 60% CP	2.00
Wheat bran	5.00
Tallow	1.70
Limestone	7.52
Dicalcium phosphate, 18% P	1.37
Salt	0.30
DL-Met, 50%	0.10
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Calculated energy content	
ME (kcal/kg)	2700
Analyzed nutrient content (%)	
CP	17.04
Ether extract	3.98
Lys	0.78
Met + Cys	0.63
Ca	3.45
Total P	0.61

[1]Provided per kilogram of diet: 12 500 IU vitamin A, 2500 IU vitamin D_3, 13 IU vitamin E, 2 mg vitamin K_3 1 mg vitamin B_1, 5 mg vitamin B_2, 1 mg vitamin B_6, 0.04 mg vitamin B_{12}, 0.9 mg folic acid, 55 mg niacin, 14 mg Ca-pantothenate, and 0.1 mg D-biotin

[2]Provided per kilogram of diet: 50 mg Mn (as MnO_2), 620 mg Zn (as $ZnSO_4$), 5 mg Cu (as $CuSO_4 \cdot 5H_2O$), 40 mg Fe (as $FeSO_4 \cdot 7H_2O$), 0.3 mg Co (as $CoSO_4 \cdot 5H_2O$), 1.5 mg I (as KI), and 0.15 mg Se (as $Na_2SeO_3 \cdot 5H_2O$)

Egg production, egg quality, and blood sample preparation. Egg production and egg weights were recorded daily, while feed consumption was measured at the end of the experiment (36 weeks). Egg mass output was measured using hen-day egg production and egg weight records. Feed conversion ratio was calculated as feed consumption per hen divided by egg mass per d per hen. Thirty six eggs per treatment (three eggs/cage) were collected randomly at 36 weeks, and used to determine the egg quality. Haugh units, albumen height, and yolk colour were determined, using an egg multi tester (Touhoku Rhythm Co., Ltd., Tokyo, Japan). Eggshell breaking strength was evaluated using an Eggshell force gauge, model II (Robotmation Co., Ltd., Tokyo, Japan), and eggshell thickness was measured using a dial pipe gauge (Ozaki Mfg. Co., Ltd., Tokyo, Japan).

At the end of the experiment, blood samples were taken from the jugular vein (24 layers in each treatment (two layers/cage) to determine haematological and biochemical indicators. For WBC, RBC, and lymphocytes, ~3 ml of blood in tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) were analysed immediately after collection. Blood samples for measurements of haptoglobin, IgG, cortisol, epinephrine, and norepinephrine were collected randomly into serum separator tubes (Becton Dickinson Vacutainer Systems). The blood samples were centrifuged (3000 × *g*, 20 min, 4 °C) within 1 h of collection and after centrifugation blood plasma was obtained and serum was separated from the blood clot at room temperature for a few hours, and then stored at –70 °C until assayed.

Blood profiles. Blood cell counts (white blood cells, WBC, red blood cells, RBC, and lymphocytes)

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Table 2. The effect of dietary gamma-aminobutyric acid (GABA) on productivity in layers[1]

	GABA (ppm)				SEM[2]	*P*-value	
	0	25	50	100		linear	quadratic
32–36 week							
Egg production (%)	93.5	95.2	95.4	97.1	0.48	< 0.001	0.931
Egg weight (g)	62.2	64.3	65.9	66.8	0.15	< 0.001	0.348
Egg mass (g/day/hen)	58.2	61.2	62.9	64.9	0.35	< 0.001	0.643
Feed intake (g)	127	125	131	130	0.79	0.536	0.943
Feed conversion (g : g)	2.18	2.04	2.08	2.00	0.02	0.279	0.558

[1]Each mean represented by 72 birds per treatment (n = 72)
[2]Standard error of the mean

in whole blood were analysed using an automatic blood analyser (ADVIA 120, Bayer, NY, USA). Serum haptoglobin concentrations were assayed using an enzyme-linked immunosorbent assay kit (TP801; Tri-Delta Diagnostics, Inc., Morris Plains, NJ, USA). The serum IgG levels were assessed using an automatic biochemistry blood analyser (Hitachi 747, Hitachi, Tokyo, Japan). Serum cortisol concentrations were assayed using a standardised solid phase radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The determination of serum epinephrine (EPI) and norepinephrine (NE) was performed using an ion-exchange purification procedure followed by high-performance liquid chromatography (HPLC) with electrochemical detection, as described by Hay and Mormède (1997). Briefly, the samples were loaded on the cationic columns, and the catecholamines were eluted using boric acid. The eluates were assayed with HPLC with electrochemical detection with an applied potential of +650 mV. The intra-assay coefficient of variations (CV) for NE and EPI were 7.0% and 7.1%, respectively. The inter-assay CVs were 6.5% and 11.6%, respectively.

Statistical analysis. Data were statistically analysed by ANOVA, using the GLM procedure of the SAS program, for a randomised complete block design. Mean values and standard error of means (SEM) are reported. Orthogonal polynomial contrasts were conducted to measure the linear and quadratic effects for increasing GABA levels on all measurements. Statements of statistical significance are based on $P < 0.05$.

RESULTS

Egg production performance

Layers fed with diets supplemented with GABA during weeks 32 to 36 showed significant differences in egg production, egg weight, and egg mass compared with the control treatment, as dietary GABA increased from 25 to 100 ppm (linear, all $P < 0.001$). However, there was no significant difference in feed intake or the feed conversion ratio of layers fed with different levels of GABA in the diet (Table 2).

Table 3. The effect of dietary gamma-aminobutyric acid (GABA) on egg quality in layers[1]

	GABA(ppm)				SEM[2]	*P*-value	
	0	25	50	100		linear	quadratic
Eggshell breaking strength (kg/cm^2)	4.28	4.48	4.46	4.44	0.08	0.112	0.041
Eggshell thickness (mm^{-2})	44.1	44.7	45.1	45.0	0.60	0.356	0.684
Haugh units	89.3	91.2	91.2	91.8	1.23	0.246	0.666
Albumen height (mm)	8.16	8.58	8.62	8.78	0.25	0.007	0.362
Yolk colour	6.16	6.32	6.20	6.66	0.27	0.336	0.636

[1]Each mean represented by 36 eggs per treatment (n = 36)
[2]Standard error of the mean

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Table 4. The effect of dietary gamma-aminobutyric acid (GABA) on blood profiles in layers[1]

	GABA (ppm)				SEM[2]	*P*-value	
	0	25	50	100		linear	quadratic
WBC (K/μl)	311	318	324	329	14.3	0.656	0.978
RBC (M/μl)	2.41	2.24	2.37	2.33	0.04	0.786	0.512
Lymphocyte (%)	54.3	55.1	57.0	58.5	1.94	0.438	0.938
Haptoglobin (μg/ml)	150	123	110	108	5.52	0.014	0.289
IgG (μg/ml)	488	540	543	683	36.64	0.044	0.545
Cortisol (ng/ml)	2.74	2.54	2.62	2.56	0.15	0.385	0.814
Epinephrine (pg/ml)	91.5	87.9	82.1	74.2	4.82	0.280	0.854
Norepinephrine (pg/ml)	179.3	153.2	143.5	141.8	9.85	0.204	0.563

[1]Each mean represented by 24 birds per treatment (n = 24)

[2]Standard error of the mean

Egg quality

Eggshell breaking strength was found to increase (quadratically) compared with the control group (P = 0.048). Also, there was a linear effect on albumen height as dietary levels of GABA increased (P = 0.007, Table 3). There was no significant difference in eggshell thickness, Haugh units, or yolk colour of layers fed with different levels of GABA in the diet.

Blood profiles and haptoglobin

Serum levels of IgG were elevated in the GABA treatment group compared with the control group (linear, $P < 0.044$). Haptoglobin concentrations were lower in birds fed the GABA supplemented-diet, compared with birds fed the control diet as dietary GABA increased (linear, P = 0.014). However, GABA treatment had no significant effect on WBC, RBC, or lymphocytes. Also, serum hormone levels (cortisol, epinephrine, and norepinephrine) were not affected by the addition of GABA (Table 4).

DISCUSSION

In this study, the addition of 0.25 to 100 ppm GABA resulted in dose-dependent increases in egg production, egg weight, and egg quality. Since there is currently a lack of useful information regarding GABA in performance and egg quality in layers, the present study cannot fully explain this improvement in egg production and egg quality. However, the increased laying performance evoked by dietary GABA in this study may be related to previously described neuroendocrine system-mediated effects on nutrient metabolism (Zhang et al. 2012). It was reported that inclusion of GABA in the layer diet not only alleviated stress in hens under heat stress but also played a role in appetite regulation and improvement of nutrient utilisation. In a recent study, Zhu et al. (2015) suggested the possibility that GABA-producing microorganisms could increase the utilisation of dietary calcium and phosphorus, thereby improving egg quality. Additionally, GABA possesses many anti-oxidant activities (Huang et al. 2011; Chen et al. 2013), which may result in improved performance. Zhang et al. (2012) also observed that increasing supplementation of GABA increased the level of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and decreased the level of malondialdehyde (MDA). Thus, the increase of nutrition utilisation and antioxidant activities in response to GABA are likely the main cause of the improvements in laying performance and egg quality in layers. Our findings indicate that GABA had a direct beneficial effect on laying performance and egg quality, especially, which manifested as a significant effect on egg production, egg mass, egg weight, eggshell breaking strength, and albumen height.

Epinephrine and norepinephrine are the main neurotransmitters of the sympathetic nervous system (McCorry 2007), and cortisol secretion is partly controlled by GABA (Rosmond et al. 2002). To evaluate the effect of GABA on the functional ability of the sympathetic nervous system, epinephrine, norepinephrine and cortisol concentrations were evaluated in the serum of layers, but were not found to differ to levels in the controls. Thus, our results indicate that

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the GABA had no direct effect on the sympathetic nervous system in comparison with the controls.

Haptoglobin is an acute phase protein that is rapidly increased in the blood by disease-causing agents and many physiological changes and plays an important role in immunity. Koutsos et al. (2006) observed that lipopolysaccharide (LPS) challenge in chicks resulted in inflammatory immune responses, as indicated by elevated levels of haptoglobin. GABA has some positive effects on cellular immune function, such as activation or suppression of cytokine secretion, modification of cell proliferation and even cell migration (Jin et al. 2013). The levels of lipopolysaccharide-induced tumour necrosis factorα and inflammatory cytokines (interleukin (IL)-1β, IL-6) in serum were significantly decreased by GABA at 80 mg/kg under beak-trimming stress as reported by Xie et al. (2013). The results of our study were similar to the previous report of Zhang et al. (2012), which documented an increase in immunity (serum IgG and IgA) between GABA treatments and control. Serum haptoglobin concentrations were reduced in GABA-supplemented birds, compared with the control, although the layers in this study were not subjected to stress. Additionally, in our study, an increase in serum IgG concentrations were observed with GABA supplementation, showing the opposite pattern to serum haptoglobin concentrations. Thus, it might be expected that GABA may have modulatory effects on humoral immune responses by activating IgG and decreasing haptoglobin, although no significant difference was observed in WBC or lymphocyte counts between the GABA treatments and control.

CONCLUSION

The present study shows that GABA is a valuable natural feed ingredient for layers, and in particular, exerts beneficial effects with respect to egg production, egg mass, egg weight, eggshell breaking strength, albumen height and serum IgG concentrations, as well as a lowering of serum haptoglobin concentrations.

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