



Effect of γ -Aminobutyric Acid-producing *Lactobacillus* Strain on Laying Performance, Egg Quality and Serum Enzyme Activity in Hy-Line Brown Hens under Heat Stress

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ABSTRACT: Heat-stress remains a costly issue for animal production, especially for poultry as they lack sweat glands, and alleviating heat-stress is necessary for ensuring animal production in hot environment. A high γ -aminobutyric acid (GABA)-producer *Lactobacillus* strain was used to investigate the effect of dietary GABA-producer on laying performance and egg quality in heat-stressed Hy-line brown hens. Hy-Line brown hens (n = 1,164) at 280 days of age were randomly divided into 4 groups based on the amount of freeze-dried GABA-producer added to the basal diet as follows: i) 0 mg/kg, ii) 25 mg/kg, iii) 50 mg/kg, and iv) 100 mg/kg. All hens were subjected to heat-stress treatment through maintaining the temperature and the relative humidity at $28.83 \pm 3.85^\circ\text{C}$ and 37% to 53.9%, respectively. During the experiment, laying rate, egg weight and feed intake of hens were recorded daily. At the 30th and 60th day after the start of the experiment, biochemical parameters, enzyme activity and immune activity in serum were measured. Egg production, average egg weight, average daily feed intake, feed conversion ratio and percentage of speckled egg, soft shell egg and misshaped egg were significantly improved ($p < 0.05$) by the increasing supplementation of the dietary GABA-producer. Shape index, eggshell thickness, strength and weight were increased linearly with increasing GABA-producer supplementation. The level of calcium, phosphorus, glucose, total protein and albumin in serum of the hens fed GABA-producing strain supplemented diet was significantly higher ($p < 0.05$) than that of the hens fed the basal diet, whereas cholesterol level was decreased. Compared with the basal diet, GABA-producer strain supplementation increased serum level of glutathione peroxidase ($p = 0.009$) and superoxide dismutase. In conclusion, GABA-producer played an important role in alleviating heat-stress, the isolated GABA-producer strain might be a potential natural and safe probiotic to use to improve laying performance and egg quality in heat-stressed hens. (**Key Words:** γ -Aminobutyric Acid, *Lactobacillus*, Heat Stress, Laying Performance, Egg Quality)

INTRODUCTION

The negative effect of heat-stress on the welfare of domestic animals is a matter of grave concern (Attia et al., 2009). Heat-stress could reduce feed intake (Rhoads et al., 2009), increase maintenance requirements (Fox and Tylutki, 1998) and decrease diet digestibility (Ole Miaron and Christopherson, 1992). For broilers, heat-stress significantly

decreases the activity of intestinal amylase (Feng et al., 2012) and causes a sharp decline of the number of lymphocytes and plasma cells in the jejunum (Quinteiro-Filho et al., 2012). In addition, heat-stress also affects growth rate, metabolism, immunity and reproduction of animals with an immeasurable impact (Attia et al., 2011; Bloemhof et al., 2012; Nesamvuni et al., 2012). Although remarkable advances of feeding animals under high-density conditions have been widely applied (Attia et al., 2006; Al-Harhi, 2014), heat-stress remains a costly issue for the animal production, especially for poultry as they lack sweat glands. Therefore, alleviating heat-stress is necessary for ensuring animal production in hot environments.

Gamma-aminobutyric acid (GABA), a four-carbon nonprotein amino acid, functions as an inhibitory

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transmitter compound in the central nervous system and some non-neuronal tissues of adult mammals (Watanabe et al., 2002). In recent years, it has been found to be helpful in alleviating heat-stress. The GABA is not only involved in the regulation of a variety of behavioral and physiological response (Sliwowska et al., 2006), but also induces a decrease of body core temperature and an increase of feed intake in chicks in a hot environment (Tajalli et al., 2006; Miyazawa et al., 2012). Furthermore, GABA is beneficial to the performance of chicks through alleviating the negative effects of heat-stress (Dai et al., 2011; Zhang et al., 2012). GABA producing microorganisms (such as lactic acid bacteria [LAB], fungi and yeast) has attracted attention as a natural and safety strategy for alleviating heat-stress in livestock. Lactic acid bacteria are one of the most commonly used microorganisms in food fermentation, and *Lactobacillus* species are the main GABA-producing LAB. The GABA producing *Lactobacillus brevis* (*Lb.* *paracasei*) was firstly isolated from funazushi (Komatsuzaki et al., 2005). Afterward, GABA-producing *Lb. buchneri* (Cho et al., 2007) and *Lb. brevis* (Kim et al., 2007) were isolated from kimchi. Li et al. (2008) isolated a high GABA-producer *L. brevis* NCL912 and developed a fed-batch fermentation process for efficient synthesis of GABA (Li et al., 2010). The above studies imply that the microbial production of GABA, especially by LAB strains, has received wide attention. However, few studies have been conducted using a GABA-producing strain to alleviate heat-stress in hot summer.

In the present study, we hypothesized that a GABA-producing strain of LAB would have a beneficial effect on heat-stress and in mitigating heat-stress caused damage. Thus, to test this hypothesis, an isolated GABA-produced LAB strain from our laboratory was used to investigate its effect on laying performance, egg quality and immune activity in Hy-Line brown hens under heat-stress.

MATERIALS AND METHODS

Production of γ -aminobutyric acid-producing lactic acid bacteria

The GABA-producing LAB strain was isolated from acidophilus milk by our laboratory. Acidophilus milk was separately inoculated into polypepton yeast extract glucose broth and individual colonies were isolated and purified (Choi et al., 2006). According to the amount of GABA produced, the best isolates for GABA-producing LAB were identified using 16S-rDNA sequence analysis. After 24 h of incubation at 39°C, bacterial cells in 0, 25, 50, and 100 mL culture broth of the LAB strain were harvested by centrifuging at 25,000×g (Heraeus, Biofuge22R, Hanau, Germany). The harvested cells were suspended and washed well with 10 mL distilled water, and then re-centrifuged at

25,000×g, and this procedure was repeated three times. The obtained cells were re-suspended in 10 mL distilled water and mixed well with 800 mg skimmed milk powder. The mixed materials were first freeze-dried at -20°C for 2 h and then freeze-dried at -80°C for 14 h using freeze dryer (HuiChengJia instrument technology Co. Ltd., Beijing, China) to produce 0, 25, 50, and 100 mL/800 mg freeze-dried GABA-producer.

Animals and management

All animals involved in this study maintained under the guidelines of the Anhui Sciences and Technology University Animal Care and Use Committee (Fengyang, China). A total of 1164 Hy-Line brown hens at 280 days of age were obtained from the animal farm of Anhui Science and Technology University (PR China). All hens were provided with free access to clean water and feed, indoor ventilation and lighting, regular cleaning and disinfection of the corresponding environment. Diets were formulated following the nutrient requirement recommendations of NRC (1994) (Table 1), while the ambient temperature was kept at 28.83±3.85°C (surpassing the optimal ambient temperature range 15°C to 23°C for laying), and the relative humidity was maintained in the range from 37.2% to 53.9% during the entire experiment. Under the above temperature and humidity conditions, the hens would be considered as heat-stressed to a certain extent.

Experiment design

The 1,164 Hy-Line brown hens were randomly divided

Table 1. Composition and nutrient content of the basal diet (as fed)

Items	
Ingredients (%)	
Corn	63.0
Soybean meal	20.0
Bran	3.0
Limestone	9.0
Premix ¹	5.0
Total	100.0
Analyzed nutrient content	
Metabolic energy (MJ/kg) ²	10.98
Crude protein (%)	15.30
Calcium (%)	3.49
Available phosphorus (%)	0.36
Lysine (%)	0.72
Methionine (%)	0.34
Methionine plus cysteine (%)	0.59

¹ Premix, providing Cu 12.5 mg, Fe 82.5 mg, Se 0.4 mg, Zn 87.5 mg, I 0.4 mg, Mn 64.0 mg, vitamin A 11,000 IU, vitamin D 3,100 IU, vitamin E 18 mg, vitamin K 2.5 mg, vitamin B₁ 2.0 mg, vitamin B₂ 6.0 mg, vitamin B₆ 3.6 mg, vitamin B₁₂ 19 mg, nicotinic acid 27.0 mg, calcium pantothenate 12.0 mg, folic acid 0.8 mg, and biotin 130 mg/kg basal diet.

² Calculated.

into 4 groups based on the amount of GABA-producer supplied along with the basal diet with each group consisting of three replicates with 97 hens. The amounts of freeze-dried GABA-producer supplied with the basal diet among the 4 experimental groups were as follows: i) 0 mg/kg, ii) 25 mg/kg, iii) 50 mg/kg, and iv) 100 mg/kg. A small amount of the basal diets was first mixed with the respective amount of freeze-dried GABA-producer to make a small batch, and the remaining basal diet was added to obtain a homogeneous mixture. The feeding experiment was performed for 60 days after a 15-d adaptation period. The experiment was carried out commencing August 7, 2013 in the animal farm of Anhui Science and Technology University, PR China.

Laying performance measurement

The laying rate, egg weight, egg mass and feed intake of each group were recorded daily to calculate egg production (laying hens/total hens×100, %), average egg weight (AEW), feed conversion ratio (total feed intake/total egg mass) and average daily feed intake (ADFI). Meanwhile, the numbers of spot eggs, soft eggs, deformed eggs and sand eggs from each group were also recorded daily to calculate the corresponding index percent (%). At the 30th and 60th day after the experiment started, three hens were randomly selected from each replicate and were slaughtered. The whole blood was set aside for approximately 20 min and then centrifuged with 3,000×g at 4°C for 10 min (Heraeus, Biofuge22R, Germany) and then the serum was stored at -20°C for examining serum biochemical parameters, enzyme activity and immune function.

Egg quality assay

Thirty-one eggs from each group were collected at the 10th, 20th, 30th, 40th, 50th, and 60th day of the feeding experiment and stored at 4°C until analyses (less 1 wk). Shape index, eggshell thickness, shell strength, shell weight, albumen height, haugh unit, yolk color and weight were determined (Kirunda et al., 2001) using an egg quality determinator (DET6000, NABEL Co., Ltd, Kyoto, Japan).

Serum biochemical parameters

The levels of calcium, phosphorus, potassium, sodium, glucose, blood urea nitrogen, cholesterol, total protein and albumin (ALB) in hen serum were measured by Nanjing Jian-cheng Bioengineering Institute assay kits (Nanjing, China) following the manufacturer's instructions.

Enzyme activities

The activities of serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), alkaline phosphatase (ALP) and alanine

aminotransferase (ALT) activities were determined by corresponding kits following the manufacturer's instructions. All kits were purchased from Nanjing Jian-cheng Bioengineering Institute (Nanjing, China).

Statistical analysis

Data in the experiment were analyzed using general linear model (GLM) procedure of SAS (2004) according to the following model as equation (1):

$$Y_{ijk} = \mu + T_i + H_j + D_k + THD_{ijk} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the response variable, μ is the overall mean, T_i is the fixed effect of treatment ($i = 1$ to 4), H_j is the random effect of hens ($j = 1$ to 1,164), D_k is the random effect of feeding experiment day ($k = 1$ to 60), THD_{ijk} is the interaction between treatment i and day k in hen j , and ε_{ijk} is the residual error. Orthogonal polynomial contrasts were performed to determine linear and quadratic effects within freeze-dried GABA-producing LAB bacteria addition treatments. Least square mean and standard error of the means (SEM) were calculated with least square mean statement of the GLM procedure. The replication was used as the unit in analyzing the feeding experimental data. A p value <0.05 was considered significant, whereas $0.05 \leq p$ value <0.10 was present as tendency.

RESULTS

Effect of dietary supplement γ -aminobutyric acid-producing strain on laying performance

As shown in Table 2, increasing dietary supplementation of freeze-dried GABA-producing strain F6 linearly improved egg production (0.32%, 1.76%, and 3.21%; $p < 0.001$), AEW (0.35%, 0.32%, and 1.57%; $p < 0.001$), and ADFI (1.07%, 2.37%, and 2.38%; $p < 0.001$). Compared with the control group, feed conversion ratio and percentage of speckled eggs, soft shell eggs and misshaped eggs from hens fed supplemental freeze-dried GABA-producing strain diet were significantly decreased by 2.35% ($p < 0.001$), 8.57% ($p = 0.015$), 19.36% ($p < 0.001$) and 42.46% ($p < 0.001$), respectively. In addition, calcium coated eggs were quadratically reduced ($p = 0.048$).

Effect of supplementation of γ -aminobutyric acid-producing strain on egg quality

In Table 3 it can be seen that shape index (1.53%, 1.53% and 3.07%; $p < 0.001$), eggshell thickness (3.22%, 3.22% and 6.45%; $p = 0.011$), eggshell strength (1.75%, 4.77% and 5.77%; $p = 0.001$) and eggshell weight (0.62%, 3.76% and 6.42%; $p = 0.025$) were increased linearly with increasing supplemental dietary freeze-dried GABA-producing strain. Freeze-dried GABA-producing strain

Table 2. Effect of freeze-dried γ -aminobutyric acid-producing strain on laying performance in heat-stressed Hy-Line brown hens

Item	Dosage of GABA producing <i>Lactobacillus</i> strain (mg/kg)				SEM	p-values		
	0	25	50	100		ANOVA	Linear	Quadratic
Egg production (%)	87.05 ^c	87.33 ^c	88.59 ^b	89.85 ^a	0.341	<0.001	<0.001	0.029
AEW (g)	62.07 ^b	62.29 ^b	62.27 ^b	63.05 ^a	0.083	<0.001	<0.001	<0.001
Egg mass	54.03 ^b	54.40 ^b	55.16 ^b	56.65 ^a	0.208	<0.001	<0.001	<0.009
ADFI (g/day)	109.63 ^b	110.81 ^{ab}	112.23 ^a	112.24 ^a	0.692	0.027	0.004	0.407
Feed conversion ratio ¹	2.03 ^a	2.04 ^a	2.03 ^a	1.98 ^b	0.005	0.002	<0.001	0.008

GABA, γ -aminobutyric acid; SEM, standard error of the mean; ANOVA, analysis of variance; AEW, average egg weight; ADFI, average daily feed intake.

¹ Feed conversion ratio = ADFI/egg mass.

^{a,b,c} Within a row, means that do not have a common superscript letter differ significantly.

supplementation had no significant effect on albumen height, Haugh unit, yolk color, and yolk weight of hens.

Effect of γ -aminobutyric acid-producing strain supplementation on serum parameters

In hens fed freeze-dried GABA-producing strain supplemental diets, serum calcium was respectively increased quadratically by 5.93%, 10.78% and 11.65% ($p = 0.040$), while the concentration of phosphorus showed linear effect (13.585%, 17.93% and 27.17%; $p = 0.026$), glucose exhibited linear influence (1.90%, 17.12% and 12.00%; $p = 0.029$), total protein (1.97%, 8.09% and 5.47%; $p = 0.043$) and ALB (1.37%, 10.07% and 3.32%; $p = 0.012$) were linearly improved (Table 4). Furthermore, serum concentration of potassium ($p = 0.070$) and sodium ($p = 0.087$) tended to increase linearly in hens supplied with freeze-dried GABA-producing strain, whereas serum concentration of cholesterol decreased linearly (4.77%, 9.55% and 13.69%; $p = 0.001$). No significant difference in blood urea nitrogen was observed among different diet groups ($p > 0.100$).

Activity of serum enzymes in heat-stressed hens fed

experimental diets

Table 5 shows the activity of serum enzymes in experimental hens. With increasing supply of dietary freeze-dried GABA-producing strain, activities of GSH-Px (2.33%, 25.98% and 16.12%; $p = 0.009$) and SOD (4.47%, 7.15% and 6.14%; $p = 0.002$) were linearly increased. While MDA activity (13.12%, 20.58% and 24.30%; $p < 0.001$) was reduced and ALP activity tended to increase linearly ($p = 0.056$). The activity of LDH, CPK, and ALT was not significantly affected with increased dietary freeze-dried GABA-producer.

DISCUSSION

Generally, approximately 2% of all chicken eggs have some defect, ranging from minor, barely noticeable faults to downright alarming deformities. There are two important factors causing deformity: One is disturbance or stress during calcification process and the other is poor nutrition (for example, low or excess calcium in the hen's diet). In the study, it was noticed that an improvement in the percentage of speckled eggs, soft shell eggs, misshaped eggs and calcium coated eggs in supplemental freeze-dried GABA-

Table 3. Effect of freeze-dried γ -aminobutyric acid-producing strain on egg quality in heat-stressed Hy-Line brown hens

Item	Dosage of GABA producing <i>Lactobacillus</i> strain (mg/kg)				SEM	p-values		
	0	25	50	100		ANOVA	Linear	Quadratic
Speckled egg (%)	19.52 ^a	19.37 ^a	17.00 ^b	17.17 ^b	0.370	<0.001	<0.001	0.676
Soft shell egg (%)	2.22 ^a	1.86 ^{ab}	1.74 ^b	1.77 ^b	0.132	0.030	0.015	0.145
Misshaped egg (%)	0.84 ^a	0.60 ^b	0.37 ^c	0.48 ^{bc}	0.075	<0.001	<0.001	0.334
Calcium coated egg (%)	1.88 ^a	1.53 ^b	1.66 ^{ab}	1.86 ^{ab}	0.131	0.206	0.919	0.048
Shape index	1.30 ^c	1.32 ^b	1.32 ^b	1.34 ^a	0.003	<0.001	<0.001	0.724
Eggshell thickness (mm)	0.31 ^b	0.32 ^{ab}	0.32 ^{ab}	0.33 ^a	0.003	0.073	0.011	0.839
Eggshell strength (kgf/m ²)	3.98 ^c	4.05 ^{bc}	4.17 ^{ab}	4.21 ^a	0.046	0.013	0.001	0.690
Eggshell weight (g)	6.38 ^b	6.42 ^{ab}	6.62 ^{ab}	6.79 ^a	0.124	0.130	0.025	0.617
Albumen height (mm)	7.07	7.10	6.92	6.95	0.233	0.933	0.608	0.708
Haugh unit	81.02	84.12	83.82	83.32	1.478	0.467	0.337	0.246
Yolk color	8.22	8.82	8.85	8.82	0.219	0.001	0.401	0.389
Yolk weight (%)	25.21	25.82	25.93	25.69	0.249	0.147	0.478	0.440

GABA, γ -aminobutyric acid; SEM, standard error of the mean; ANOVA, analysis of variance.

^{a,b,c} Within a row, means that do not have a common superscript letter differ significantly.

Table 4. Effect of freeze-dried γ -aminobutyric acid-producing strain on parameters of serum in heat-stressed Hy-Line brown hens

Item	Dosage of GABA producing <i>Lactobacillus</i> strain (mg/kg)				SEM	p-values		
	0	25	50	100		ANOVA	Linear	Quadratic
Calcium (mmol/L)	6.40 ^b	6.78 ^{ab}	7.09 ^a	6.58 ^{ab}	0.204	0.129	0.357	0.040
Phosphorus (mmol/L)	1.84 ^b	2.09 ^{ab}	2.17 ^{ab}	2.34 ^a	0.147	0.290	0.026	0.789
Potassium (mmol/L)	6.09	6.41	6.78	6.80	0.290	0.148	0.070	0.605
Sodium (mmol/L)	151.5	153.0	156.0	155.8	1.99	0.326	0.087	0.680
Glucose (mmol/L)	8.41 ^b	8.57 ^b	9.85 ^a	9.42 ^{ab}	0.409	0.065	0.029	0.481
Cholesterol (mmol/L)	3.14 ^a	2.99 ^{ab}	2.84 ^{bc}	2.71 ^c	0.089	0.015	0.001	0.904
Total protein (mg/mL)	50.75 ^b	51.75 ^{ab}	54.86 ^a	53.53 ^{ab}	1.186	0.099	0.043	0.337
Albumin (g/L)	18.95 ^b	19.21 ^b	20.86 ^a	19.58 ^{ab}	0.499	0.061	0.012	0.136
Blood urea nitrogen (mmol/L)	2.66	2.16	2.83	2.50	0.392	0.669	0.925	0.231

GABA, γ -aminobutyric acid; SEM, standard error of the mean; ANOVA, analysis of variance.

^{a,b,c} Within a row, means that do not have a common superscript letter differ significantly.

producing strain diet-fed hens and this may due to the improvement of the GABA-producer. Zhang et al. (2012) demonstrated that GABA not only alleviated stress in hens but also played a great role in regulating appetite and improving nutrition utilization efficiency. The resulting higher concentrations of serum calcium and phosphorus that were observed suggested that a GABA-producer could regulate the utilization of dietary calcium and phosphorus thereby alleviating defected egg caused heat-stress. Mashaly et al. (2004) and Al-Harhi (2014) noted that high ambient temperature has a detrimental effect on laying performance, feed intake and feed conversion efficiency. Therefore, such high ambient temperature might cause a decline in activity of digestive enzymes (e.g. amylase, lipase, and trypsin) in the alimentary canal (Belay and Teeter, 1993; Larbier et al., 1993; Attia et al., 2011). However, GABA has been reported to alleviate heat stress through improving the digestive enzyme activities. Zhang et al. (2012) demonstrated that dietary GABA in Roman hens could increase amylase, lipase and trypsin activities in the gastrointestinal tract, thereby increasing egg production, AEW and ADFI. They observed an increased egg production, AEW and ADFI and improved feed conversion ratio with increasing freeze-dried GABA-producing stain of

dietary GABA content. In contrast, the present results indicated that GABA-producing strain of LAB might improve laying performance by alleviating heat-stress.

The basic composition of egg includes protein and yolk. Zhang et al. (2012) noted a significant increase in albumen height, haugh unit, yolk color, and yolk weight in Roman hens at 50 mg/kg of dietary GABA content. In contrast, the present study demonstrated that GABA-producer had no significantly effect on albumen height, haugh unit, yolk color, and yolk weight. However, the improvement in egg quality in terms of shape index, shell thickness, strength, and weight with increasing dietary GABA-producer content was observed in the present study. Puthongsiriporn et al. (2001) reported that depressed eggshell thickness and shell strength results in a poorer egg shell quality and shelf-life. Being important components of eggshells, calcium and phosphorus play a crucial role in eggshell weight, shell thickness and shell strength. We also found an increase of absorption and utilization of the dietary mineral substances, especially calcium and phosphorus and this might be the reason for eggshell improvement. The present results are consistent with the report by Zhang et al. (2012), who reported that GABA could modulate the electrolyte balance.

Calcium, phosphorus, potassium, and sodium are not

Table 5. Effect of freeze-dried γ -aminobutyric acid-producing strain on enzyme activity of serum in heat-stressed Hy-Line brown hens

Item	Dosage of GABA producing <i>Lactobacillus</i> strain (mg/kg)				SEM	p-values		
	0	25	50	100		ANOVA	Linear	Quadratic
GSH-Px (μ mol/L)	234.54 ^b	240.01 ^b	295.48 ^a	272.37 ^{ab}	13.075	0.011	0.009	0.287
SOD (U/mL)	184.38 ^b	192.63 ^a	197.58 ^a	195.71 ^a	2.458	0.005	0.002	0.052
MDA (nmol/L)	7.24 ^a	6.29 ^b	5.75 ^b	5.48 ^b	0.307	0.003	<0.001	0.282
LDH (U/mL)	2076.0	1927.3	1841.3	1980.7	130.33	0.642	0.342	0.494
CPK (U/mL)	2161.5	2389.7	2176.7	2189.2	175.87	0.772	0.870	0.546
AP (U/mL)	372.83	384.17	456.0	522.5	57.53	0.254	0.056	0.636
ALT (U/mL)	13.33	13.16	12.16	10.66	1.385	0.518	0.161	0.635

GABA, γ -aminobutyric acid; SEM, standard error of the mean; ANOVA, analysis of variance; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; AP, alkaline phosphatase; ALT, alanine aminotransferase.

^{a,b} Within a row, means that do not have a common superscript letter differ significantly.

only involved in the synthesis of eggshells, but also play an important role in modulating the serum electrolyte balance (Hodges, 1969; Viveros et al., 2002). It was observed that freeze-dried GABA-producer improved concentration of serum calcium, phosphorus, potassium and sodium and this result might be related to transportation and absorption by GABA. Chen et al. (2013) noted that GABA could increase the activity of Ca^{2+} - Mg^{2+} -adenosine and Na^{+} - K^{+} -adenosine, benefiting the transportation of mineral elements. The ALP is present in different tissues of the body, and its activity reflects the absorption function of intestine (Sanderson and He, 1994). Increasing ALP activity and higher concentrations of calcium, phosphorus, potassium and sodium in GABA diets suggested that GABA-producer benefits transportation and absorption of mineral element, especially calcium and phosphorus.

A lack of disaccharidases can result in the inhibition of complete decomposition of carbohydrates, as well as in the absorption of monosaccharides conversion and utilization (Karamouz et al., 2011). In this context, Chen et al. (2013) found that while heat-stress inhibits the activity of sucrase and maltase in the intestinal mucosa of chickens, GABA alleviates this inhibition and this might explain the linear increase in serum glucose by freeze-dried GABA-producer in the present study. In addition, serum levels of protein and ALB were increased presumably of due to the GABA-producer functioning as the protein source. A decreased cholesterol level in hens was observed, and this result was consistent with the previous reports by Liang and Shah (2005) and Nguyen et al. (2007) that demonstrated a reduction in host cholesterol levels by LAB (Ooi and Liang, 2010).

Freeman and Crapo (1982) noted that heat-stress could stimulate the release of corticosterone and catecholamines and initiate lipid peroxidation in cell membranes, including membranes of T and B lymphocytes, leading to over-production of oxygen free radicals OH and O_2 (Slater, 1984). GSH-Px and SOD, as important components of the radical scavenging system, are involved in the process of antioxidation (Attia et al., 2006; Wei et al., 2011). They specifically catalyze reduction of glutathione (GSH) to clear H_2O_2 and reduce the generation of lipid peroxides, thus protecting the structure and functions of cell membrane. Huang et al. (2011) demonstrated that GABA might improve GSH-Px and SOD activities in pigeons. The possible explanation for above result is that the increase in glutamate level (an important raw material for the synthesis of GSH) was a result of an increased GABA content increasing the activity of the antioxidation enzyme. In this study, it was observed that increase in the level of GSH-Px and SOD was consistent with the observation of Chen et al. (2013), who reported that GABA could enhance the activities of GSH-Px and SOD in heat-stressed chickens.

Therefore, the present experimental results suggested that a GABA-producer could improve activity of GSH-Px and SOD as well as GABA.

Malondialdehyde is a product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage. In the present study, decreased MDA was obtained with increasing dietary GABA-producer, which might be attributed to the increased anti-oxidation GSH-Px and SOD activity. Similar results were reported by Baydas et al. (2005), Williams et al. (2006) and Attia et al. (2009), who observed that the decreased level of MDA was always accompanied by high activity of antioxidation enzymes.

The activity of ALP reflects the absorption function of the intestine (Sanderson and He, 1994), especially absorption of Ca and P. In the present study, the activity of ALP tended to increase, suggesting that dietary GABA-producer could improve absorption of mineral elements, which can explain the increase in serum Ca, P, K, and Na, and thus increased eggshell quality.

The enzyme LDH is of medical significance as it is found almost in all body tissues (such as blood cells and heart muscle). It also acts as a marker for common injuries and diseases, because LDH is released during tissue damage. Creatine kinase, also known as CPK or phospho-creatine kinase, is an enzyme expressed by various tissues and cell types. Creatine kinase catalyzes the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate, therefore ATP can be generated from phosphocreatine and ADP. Generally, low levels of ALT are normally found in the blood. However, when the liver is damaged or diseased, it releases ALT into the bloodstream, which increases the ALT level in blood. In the present study, LDH, CPK, and ALT numerically decreased with increasing dietary GABA-producer, suggesting that GABA-producer could alleviate injuries of heat-stress to some extent. Nutritional regulation of GABA might be the reason for the above observation. Chan and Suk (2004) noted that GABA indirectly converted into glutamine in the intestine and glutamine was the major energy source of epithelial cells and lymphocytes and the nitrogen source for cell proliferation and differentiation. However, we did not obtain any significant benefit in hens from dietary freeze-dried GABA-producer due to the relative content of GABA in GABA-producer.

In conclusion, the present study demonstrated that the supplementation of dietary freeze-dried GABA-producer to heat-stressed Hy-Line brown hens could increase activity of antioxidation enzymes, modulate the electrolyte balance and improve feed intake of hens. Therefore, the improved immune function, antioxidation enzyme, electrolyte balance and feed intake ultimately resulted in the improvement of laying performance and egg quality. The present

experimental result indicated that GABA-producer plays an important role in alleviating heat-stress. Thus, the isolated GABA-producer, which has a high ability to produce GABA, could be used as a natural and safe probiotic for improving laying performance and egg quality in heat-stressed hens.

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