Effect of Gamma Amino Butyric Acid from Germinated Paddy Rice Supplementation on Egg Performance and Qualities

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Abstract

Several physical factors could related to poor egg qualities and loss of production. This study aimed to determine the effects of feed supplemented with γ-amino butyric acid (GABA) from germinated paddy rice (GPR) on egg performance and qualities. A total of 48 laying hens (Ross brown breed), at 34 weeks old, were fed for 49 days. The feeds containing GABA contents of 0 (control), 3.152 and 6.305 mg/kg were fed to chickens, each at four replicates. Hen-day egg production and various egg qualities were assessed. It was found that GABA supplemented feeds improved egg qualities as evidenced by the increased shell strength of the eggs. However, the albumen high and haugh unit of the eggs decreased. Furthermore, GABA supplemented feeds at the studied levels (0-6.305 mg/kg) did not have any effect on hen-day egg production, feed intake and egg weight as well as most of the other egg qualities, including egg width, egg shell weight, albumin weight, yolk weight and yolk ratio. This study suggested that 6.305 mg/kg of GABA from GPR was the optimum level for laying hens to improve egg qualities.

Keywords: Germinated paddy rice, Laying hens, Egg quality, Shell strength

Introduction

Germinated paddy rice (GPR) enhances nutritional qualities. Hydrolytic enzymes and biological components are activated during germination, while starch, polysaccharide, and amino acid are decomposed. The decomposition of polymers in germinated cereal leads to the generation of functional materials. GPR has many nutritional components, including carbohydrates, protein, oil, dietary fiber, vitamin, γ-amino butyric acid (GABA), and γ-oryzanol (Oh *et al*., 2010, p. 113). _Phytonutrients in GPR especially GABA provide beneficial effects on human health, such as decreasing stresses in the brain, helping the anti-hypertensive effect and limiting cancer cell proliferation (Chungcharoen *et al*., 2015, p. 708), regulation of certain cardiovascular functions, controlling heart rate and physiological functions such as neurotransmission, diuretic, and tranquilizing effects and it was recently reported that it also similar role in ion channel signaling in mammals (Lee *et al*., 2020, p. 2; Ramesh *et al*., 2017, pp. 1594-1595). GABA is a kind of inhibitory transmitter compound in vertebrates (Roberts & Frankel, 1950, p. 56) and is widely distributed throughout nature. Currently, it has been suggested that GABA has various effects on animal and plays a great role in regulating appetite and improving nutritional utilization efficiency (Zhang *et al*., 2012, p.141). For example, GABA supplementation resulted in an improved feed intake, growth performance, carcass characteristic and serum parameters in broiler under heat stress (Dai *et al*., 2011, p. 58) Ka *et al*. (2014, pp. 154-164) reported that using GABA from GPR in ration showed positive effects on growth performance and carcass characteristics in broilers and the optimum GABA level was 2 mg/kg. The GABA in feedstuffs was reported to improve laying performance and physical conditions mainly by modulating hormone secretion, enhancing anti-oxidation, immune activity, and maintaining electrolyte balance (Zhang *et al*., 2012, p. 141) GPR could probably be used as an alternative cereal grain source in poultry feedstuffs. Therefore, the present study was carried out to investigate the effects of GABA supplementation in the feeds on laying performance and egg qualities of laying hens.

Materials and Methods

Sample Preparation

Paddy rice of RD 31 variety was obtained from Phitsanulok province (Thailand) and harvested during October 2018. Paddy rice was germinated following the method as described previously (Maisont & Narkrugsa, 2010, p. 914) with some modifications. The schematic diagram of recent trial is demonstrated in (Figure 1)

Paddy Rice
↓
Soak with water (1:2 w/v) at room temperature 12 hrs
↓ Drain water
Incubating at 40°C, 90% RH for 24 hrs
↓ Washing
Drying 50°C, moisture 10%
↓
Ground using mill to pass 80-mesh screen

Figure 1 Experimental schematic diagram of how to prepare GPR

Determination of GABA Content

GABA content was determined using a high-performance liquid chromatography (HPLC; Shimadzu model LC-2010A, Shimadzu Corp., Kyoto, Japan) with a Kromasil 5 µm 100A C18 column (250 mm x 4.6mm). The GABA contents were measured according to the method described previously (Karladee & Suriyong, 2012, p. 14). The ground GPR (3 mg) was dissolved with 80% ethanol in a test tube, shaken thoroughly, and then filtered with a Whatman No. 1 filter paper. The filtered solution was boiled in a water bath (80°C) to evaporate the ethanol. This was followed by addition of 0.5 mL distilled water, and then centrifugation at 10,000 rpm for 10 min. The floating portion on top was aspirated, and 0.2 mL of 0.2 M borate buffer and 1.0 mL of 6% phenol were added. The separation program using 2 mobile phases were applied: A (20 mM ammonium dihydrogen orthophosphate in 15% (v/v) methanol) and B (90% (v/v) acetonitrile in water). The flow rate of the mobile phase was 1 mL/min. Samples were injected at a volume of 1 µL and were detected by absorbance of 263 nm. The oven temperature was maintained at 40°C. The GABA contents were reported as the average value of three replications. It was found that GPR contained GABA at 42.6 mg/kg.

Experimental Diets

Diets included (1) 0% GPR which was the basal diet without GPR or the control sample which contained no GABA (2) 10% GPR which was the basal diet supplemented with 10% GPR and this feed contained 3.152 mg/kg GABA and (3) 20% GPR which was the basal diet supplemented with 20% GPR and this feed contained 6.305 mg/kg GABA. The chemical compositions of GPR were found to be as follows; crude protein (7.48±0.13%), ether extract (1.96±0.12%), crude fiber (12.55±0.40%), ash (4.57±0.03%), nitrogen free extract (67.58±0.44%). Basal diets were formulated to meet or exceed the NRC (1994) and displayed in Table 1.

Table 1 Composition and nutritional level of the experimental diets (% in the feed).

Ingredients	0% GPR	10% GPR	20% GPR
Corn	29.88	29.88	29.88
Paddy	14.80	7.40	-
Germinated Paddy Rice (GPR)*	-	7.40	14.80
Soybean Meal	20.50	20.50	20.50
Pork Meal	10.0	10.0	10.0
Limestone	8.60	8.60	8.60
Palmolive Oil	2.60	2.60	2.60
Dicalcium Phosphate	13.00	13.00	13.00
Premix**	0.30	0.30	0.30
DL-Methionine	0.20	0.20	0.20
Pigments	0.02	0.02	0.02
Salt	0.10	0.10	0.10
Total	100	100	100

* GABA contents in GPR is 42.60 mg/kg contributing to 0, 3.152 and 6.305 mg/kg in the feeds respectively.

** The premix provides the following per kg diet: vitamin A 7000 IU; vitamin D 3 2500 IU; vitamin E 36 mg; vitamin K 32 mg; vitamin B1 2 mg; vitamin B2 5.6 mg; vitamin B6 4 mg; vitamin B12 0.025 mg; nicotinic acid 38 mg; folic acid 1.1 mg; calcium pantothenate 10 mg; biotin 0.16 mg; Cu 10 mg; Fe 80 mg; Mn 100 mg; Zn 60 mg; I 0.55 mg; Se 0.12 mg.

Experimental Design and Animals

A completely randomized design (CRD) was used. A total of 48 laying hens (Ross brown breed), at 34-weeks old were chosen as samples and fed for 49 days. The chickens were divided into three groups with four replicates. Each group was fed with the GABA supplemented diets (GPR 0%, GPR 10% and GPR 20%) respectively. Feed and water were provided ad libitum and the hens in this experiment were acclimatized a 16 h daily photoperiod. The temperature was approximately 25-29°C during experimental period. The layer hens were housed in cages located in a closed poultry house. This work has been carried out at Pibulsongkram Rajabhat University during December 2018- February 2019. All the procedures used in this study have been approved by Animal Care Use Committee of Naresuan University – Thailand (Approval number: NU-AG610914).

Production Performance

Rate of lay was expressed as the average percentage hen-day egg production (HD) based on the average values following the method of Hunton (1995, pp.457-480) as follow equation;

% hen-day egg production (HD) = (number of eggs collected per day/number of hens present that day) x 100.

Egg Qualities

The egg qualities from each replicate of the hens were determined once a week throughout the experimental period. The assessed egg qualities were egg amount, egg height (EH; mm), egg width (Ewd; mm), egg weight (EW; g), shell weight (SW, g), shell thickness (ST, mm), shell strength (SS; gf/m2), albumen height (AH, mm), albumen weight (AW, g), yolk weight (YW, g), yolk Index (YI) and Haugh unit. The egg quality determinator (DET600; NABEL Co., Ltd, Kyoto, Japan) was used. YI was computed as yolk diameter (YD) divided by Yolk Height (YH) and Haugh unit was determined using following equation:

$$\text{Haugh unit} = 100\log\left[H - \frac{\sqrt{G(30W^{0.37} - 100}}{100} + 1.9\right]$$

Where:

G = 32.2

H = Albumen height in mm

W = The weight of whole egg in grams (Ghorbani & Fayazi, 2009, p. 563)

Statistical Analysis

One-way Analysis of Variance (ANOVA) was performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, USA). Differences among the groups were analyzed using the new Duncan's multiple range test (DMRT). Results were expressed in terms of mean ± SD. Probabilities (P<0.05) were also considered to be statistically significant (Duncan, 1995, pp. 1-42).

Results and Discussion

Egg Production Performance

Effects of GABA supplemented feeds on egg production as evaluated by HD, FI and EW showed in Table 2. It was found that the supplemented feeds, at GABA levels used in this study, had no significant (P>0.05) effect on HD, FI and EW. GPR has been previously reported to be used in broilers (Ka *et al*., 2014, pp.154-164). They speculated that supplementation of GABA from GPR at 0-2 mg/kg could not improve the FI in weeks 1 – 4 and as well as the average daily gain (ADG) in weeks 1, 3, 4 and 5. It was believed that egg production performance was not improved in this study because the GABA content in the feeds might be too low (3.152 and 6.305 mg/kg). However, it has been reported that the use of isolated GABA with high concentration (50 mg/kg) improved laying performance, increased egg production and average egg weight (Zhang *et al*., 2012, pp.141-147). Zhang et al. (2012, pp.141-147) reported 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 regulated the utilization of dietary calcium and phosphorus thereby alleviating defected egg caused heat-stress.

Table 2 Effects of GABA supplemented feeds on egg productions after the feeding period of 7 weeks

Feeds	Hen-Day Egg Production (%)^{ns}	Feed Intake (g/head/day)^{ns}	Egg Weight (g)^{ns}
0% GPR	80.77±6.89	103.67±3.18	50.63±8.05
10% GPR	83.83±6.17	108.56±7.14	48.71±2.82
20% GPR	84.62±6.07	108.87±1.51	51.48±1.48

Values are the mean±SD. 0%, 10% and 20% indicate GPR supplementation in the feeds respectively. *P<0.05, **P<0.01; ns, not significant.

Egg Qualities

Egg qualities as evaluated by SS, AH, AW, YW, YI and haugh unit are presented in Table 3. In this study, GABA supplemented feeds significantly (P<0.05) improved shell strength. The values increased from 2,369.15 (control) to 2,661.34 (10% GPR) and 2,641.43 (20% GPR 20%) gf/m^2, respectively. However, they negatively affected albumen height and haugh unit which were similar to the former report of Zhu et al (2015, p. 1010). Zhang et al. (2012, pp.145-146) 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. All these values decreased as the GABA level increased. All the other egg qualities including AW, YW and YI were found to be not significantly (P≥0.05) affected by GABA supplemented feeds. The researchers studied the influence of using the isolated GABA in feed stuff and found that GABA increased shell strength and elevated shell thickness of laying hens (Zhang *et al*., 2012, pp. 141-147) GABA is a major neurotransmitter in the central nervous system of animals, influencing nutrition metabolism. GABA may improve egg qualities by increasing the total protein concentration and modulating the electrolyte balance (Barrio *et al*., 2020, pp. 9-10) Moreover, other components in paddy e.g. calcium and phosphorus may also contribute to the quality of the shell. It has been reported that rice hull silicon plays a major role in improving bone-breaking strength and reducing drip and thawing loss of broiler chickens' breast and thigh muscles (Nakhon *et al*., 2019, pp. 153-155) In addition, rice hull can be used as a natural mineral supplement for feed stuff.

Table 3 Effects of GABA supplemented feeds on egg qualities after the feeding period of 7 Weeks

Feeds	Shell Strength** (gf/m^2)	Albumen** Height (mm)	Albumen Weight (g)	Yolk Weight (g)	Yolk Index	Haugh Unit**
0% GPR	2,369.15±23.78^b	7.75±0.17^a	33.88±8.16	14.67±1.25	1.046±0.04	85.13±0.69^a
10% GPR	2,661.35±22.79^a	6.73±0.14^b	30.13±2.93	14.04±0.75	1.02±0.02	84.48±0.31^b
20% GPR	2,641.43±39.37^a	6.63±0.87^b	31.00±0.82	15.00±0.82	1.01±0.09	84.60±0.14^b

Values are the mean ± SD. 0%, 10% and 20% indicate GPR supplementation in feed stuff, respectively.
*P<0.05, **P<0.01; ns, not significant.

Conclusions

In short, the supplementation of GPR at 0-20% in the feeds for laying hens contributed to the GABA content at the maximum level of 6.305 mg/kg. These GABA levels in the feeds were found to have little effects on egg productions and qualities. The germinated paddy rice (GPR) can be used as an alternative feed ingredient. However, further studies are required in terms of which level of GABA that could significantly improve the egg production and qualities.

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