

Effect of dietary γ -aminobutyric acid on performance parameters and some plasma metabolites in Cherry Valley ducks under high ambient temperature

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(Received 2 Aug 2012; revised version 17 Mar 2013; accepted 15 Apr 2013)

Summary

Gamma aminobutyric acid (GABA) is one of the inhibitory neurotransmitters that may have the ability to relieve the intensity of stress. Heat stress remains a major threat for duck production in summer in most areas of China. The current study was conducted to investigate the effect of dietary GABA on performance parameters and plasma metabolites of heat exposed ducks. Two thousand Cherry Valley ducks (19 d) were randomly divided into two groups, each group with five replicates. One group was fed basal diet and the other fed basal diet with 100 mg/kg GABA for 42 days. The ambient temperature from day 19 to 42 was $29.7 \pm 3.5^\circ\text{C}$. Growth performance and rectal temperature were determined on days 19, 32 and 42. Plasma samples were collected at day 42. The results showed that dietary GABA had no significant effect on ducks' rectal temperature but decreased the feed intake ($P < 0.05$). No differences were observed on survival rate, body weight, and dressing percentage. Gamma aminobutyric acid decreased feed conversion ratios ($P < 0.05$) and plasma glucose concentration ($P < 0.05$). However, GABA increased plasma concentrations of triglyceride and free fatty acids ($P < 0.05$). No differences of GABA concentrations were found in the blood, heart, liver, and kidney between the two groups, but was increased in the brain of GABA group ($P < 0.05$). These findings suggest that 100 mg/kg dietary GABA decreased feed intake and increased fat mobilization in ducks exposed to high ambient temperature, which might attenuate the adverse effects of heat stress on duck production.

Key words: Cherry Valley ducks, Feed intake, γ -aminobutyric acid, Heat stress

Introduction

High ambient temperature results in reduced feed intake, egg production, egg quality and immunity in poultry (Sahin *et al.*, 2003; Rahimi and Khaksefidi, 2006). Ducks, like most birds and animals, are more suited to staying warm than staying cool. However, the optimal room temperature for housed Peking ducks ranges from 10 to 15°C . Panting occurs when the ambient temperature is over 25°C (Van Der Meulen and den Dikken, 2004). Temperatures higher than 29°C resulted in heat stress in ducks (Hagan and Heath, 1976; Hester *et al.*, 1981). Heat stress in ducks may have adverse effects, starting

with a lack of weight gain (in meat-type birds), or a reduction in or cessation of egg-laying.

Compared with broiler and layers, there is relatively less information about how to counteract heat stress in ducks. In traditional raising systems, ducks have had free access to bathing water, which resulted in heat-alleviating effect on the birds. After it was realized that water for swimming is not absolutely necessary in ducks (Lee *et al.*, 1991; Huang *et al.*, 1993), ducks have started to be raised in conditions without water bathing, even in cages equipped with nipple-drinkers or cut-in-half pipes. Better egg production and feed-conversion is effectively obtained in such breeding

systems (Lee *et al.*, 1991), but heat stress will become inevitable in new intensive raising regimes without water bathing for ducks.

Gamma aminobutyric acid (GABA) is a four-carbon non-protein amino acid that has been conserved from bacteria to plants and vertebrates (Bouché and Fromm, 2004). Gamma aminobutyric acid is present in relatively high concentrations in various hypothalamic nuclei (Frosini *et al.*, 2002). Gamma aminobutyric acid plays an important role in mammalian central thermoregulation (Jha *et al.*, 2001). Furthermore, GABA has been proposed to be released at higher amounts into the extracellular space of brain tissues during heat stress (Frosini *et al.*, 2002). Gamma aminobutyric acid and GABA-agonists may induce hypothermia partly mediated by the activation of bicuculline-insensitive GABA-receptors, whereas those of both GABAA and GABAB receptors (two main classes of GABA receptors) antagonists induce hyperthermia (Serrano *et al.*, 1985). Hu *et al.* (2008) showed that 10 mg/kg of GABA supplement into the basal diets of finishing pigs could increase the activity of glutathione peroxidase and superoxide dismutase, as well as decrease the concentration of malondialdehyde, cortisol, and corticosterone. Dietary GABA offered a potential nutritional strategy to prevent heat stress related depression in performance and carcass characteristics of laying hens (Zhang *et al.*, 2011) and broiler chickens (Zhu *et al.*, 2009; Dai *et al.*, 2011; Wang *et al.*, 2011).

To our knowledge, the effect of GABA on feed intake and growth performance in ducks has not yet been established. The aims of the present study were to investigate the effects of dietary GABA supplementation on the performance and plasma metabolites of Cherry Valley ducks under high ambient temperature. Furthermore, the GABA residues in blood and main organs were determined.

Materials and Methods

Animals, experimental protocol and sampling

A total of 2000 one-day-old Cherry

Valley ducks were used in the present study. The ducks were raised in the same regime on the floors without water bathing until 19-day-old when the ambient temperature was around 29°C ($29.7 \pm 3.5^\circ\text{C}$) in summer season (August to mid September in China).

The ducks were randomly divided into two groups with 5 replicates at 19-day-old. Twenty percent sampling of whole birds for each group showed that there was no significant difference between the initial body weights of ducks among the experimental groups (0.95 ± 0.02 kg vs. 0.98 ± 0.04). Then one group was fed the basal diet containing 100 mg/kg (Dai *et al.*, 2011) GABA (Tiancheng Biotechnology, Jinan, China) and the other was fed the basal diet alone (Control). Maize and miscellaneous meal-based diets were formulated according to the NRC (1994) (Table 1). The feed and water were provided *ad libitum*. The study was approved by the Shandong Agricultural University, Taian, China and conducted in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, P. R. China).

At day 42, blood samples (n=20) were drawn from the wing vein using a heparinised syringe within 30 s and collected in ice-cold tubes. Plasma was obtained after centrifugation at $400 \times g$ for 10 min at 4°C and was stored at -20°C for further analysis of glucose, triiodothyronine (T3), free fatty acid (FFA), triglyceride (TG), and uric acid (UA) by methods as follows. Immediately after the blood sample was obtained, the birds were sacrificed by exsanguination and the samples of the heart, liver, kidney, and brain were collected. After snap freezing in liquid nitrogen, the tissue samples were stored at -80°C for further determination of GABA concentration (Smits *et al.*, 2012).

Production performance variables

The number of dead ducks and air temperature were recorded daily during the trial. At day 19, 32, and 42, the rectal temperatures of 20 random individuals from each group were determined. At day 32 and 42, feed intake was calculated. Body weight at 42-days was averaged to per duck at the age of 42. At the end of the experiment, 20

Table 1: Composition and nutrient levels of the basal diet

| Ingredients | 0-19 days | 20-32 days | 33-42 days |
|--|-----------|------------|------------|
| Maize (%) | 65.87 | 67.32 | 69.82 |
| Wheat middling (%) | - | 3 | 6 |
| Distillers dried grains with soluble (%) | 2.86 | 4 | 4 |
| Vegetable oil (%) | - | 1.5 | 2.33 |
| Soybean meal (%) | 14.23 | 0.4 | - |
| Cottonseed meal (%) | 4 | 7 | 7 |
| Rapeseed dregs (%) | 3 | 4 | 0.6 |
| Peanut meal (%) | 5 | 5 | 4 |
| Maize protein meal (%) | 0.57 | 3.32 | 2.29 |
| Sodium chloride (%) | 0.3 | 0.36 | 0.36 |
| Limestone (%) | 1.35 | 1.29 | 1.22 |
| Di-calcium phosphate (%) | 0.99 | 0.75 | 0.51 |
| Choline (%) | 0.1 | 0.1 | 0.1 |
| Lysine (%) | 1.02 | 1.22 | 1.01 |
| Methionine (%) | 0.2 | 0.17 | 0.2 |
| Threonine (%) | 0.08 | 0.14 | 0.13 |
| Complex enzyme (%) | 0.02 | 0.02 | 0.02 |
| Mineral premix † (%) | 0.2 | 0.2 | 0.2 |
| Phytase (%) | 0.01 | 0.01 | 0.01 |
| Vitamin premix ‡ (%) | 0.2 | 0.2 | 0.2 |
| Calculated nutrient content | | | |
| Crude protein (%) | 19 | 17 | 15 |
| Metabolisable energy (kcal/kg) | 2900 | 3000 | 3100 |
| Calcium (%) | 0.9 | 0.8 | 0.7 |
| Total phosphorus (%) | 0.55 | 0.5 | 0.45 |
| Sodium chloride (%) | 0.3 | 0.35 | 0.35 |
| Digestible lysine (%) | 1.15 | 1 | 0.85 |
| Digestible methionine and cysteine (%) | 0.7 | 0.65 | 0.62 |
| Digestible threonine (%) | 0.6 | 0.56 | 0.5 |

† Mineral premix provided per kg of diet: manganese, 10 mg; zinc, 150 mg; iron, 250 mg; copper, 170 mg; selenium, 0.3 mg; iodine, 0.14 mg. ‡ Vitamin premix provided per kg of diet: vitamin A, 7000 IU; vitamin D3, 1500 IU; vitamin E, 13 IU; vitamin K3, 2 mg; vitamin B2, 3 mg; vitamin B6, 2 mg; vitamin B12, 0.006 mg; pantothenic acid, 0.1 mg; niacin 0.007 mg; pyridoxic acid, 0.005 mg

ducks in each group were randomly collected and slaughtered for carcass characterisation. The feathers, claws and inner organs, except for the lungs and windpipe of the ducks were removed, and the carcass was weighed with skin. The dressing percentage was designated as the ratio of carcass weight to live body weight.

Plasma metabolites and hormone levels

Plasma concentrations of glucose, uric acid (UA), triglyceride (TG), and free fatty acid (FFA) were measured spectrophotometrically with commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, P. R. China). Plasma T3 was measured by radioimmunoassay according to the method described by Darras *et al.* (1996). Briefly, T3 measurements were

performed using a commercial available T3 antiserum from Byk-Sangtec (Byc-Sangtec Diagnostica GmbH, Dietzenbach, Germany) combined with a specific tracer (Amersham International, Slough, England). The intra-assay coefficient of variation was 4.1%.

Determination of GABA content in the tissues

The determination of GABA was referred to Ishiwata *et al.* (2005) with minor modifications. Isocratic separation of GABA derivatives at 45°C was achieved using a reverse-phase high performance liquid chromatography (HPLC)-electrochemical detection system. This system comprises a 25 cm × 4.6 mm Spherisorb ODS2 5 µm column with a BAS detector containing a glassy carbon electrode set to +0.85 V (vs. Ag/AgCl) for quantification. The mobile

phase, with a flow rate of 1 ml/min, consisted of mobile phase A [acetonitrile /acetic acid buffer (70 mM, pH = 6.5)/EDTA (25/975/0.25 by vol)] and mobile phase B [acetonitrile water/methanol (45/40/15 by vol)].

All chemicals were of reagent grade and obtained from Sigma and Fisons (Ipswich, United Kingdom). Then, 54 mg o-phthalaldehyde was added to 1 ml ethanol, 1 ml sodium sulphite (1 M), and 18 ml disodium tetraborate (0.1 M, pH = 9.5). This reagent was stable at room temperature for more than several weeks. Refrigerated stock solutions of GABA were prepared by dissolution in water with the addition of disodium hydrogen phosphate (5 mM, pH = 7.4) where necessary. The derivatisation reaction proceeded at room temperature. Then, 10 µL of the derivatisation agent (phenylisothiocyanate: carbinol: sodium acetate: triethylamine: water, 1: 6: 1: 1: 1 by volume) was reacted with 1 ml of amino acid standard for 20 min in a polyethylene vial before injection into the column. For reaction with microdialysis samples (20 µL), the volume of derivatisation agent was reduced to 0.4 µL to prevent contamination of the chromatogram by excess reagent, which is electroactive.

Blood and tissue samples (80-100 mg) were homogenised in a semi-micro glass/glass homogeniser in 0.5 ml of 0.1 M perchloric acid containing 100 µM of ascorbic acid. The homogenate was centrifuged for 5 min at 12000 × g. An aliquot of supernate was collected, and a further aliquot was used for the concurrent measurement of monoamines and their metabolites on a different system. When required, dilution with four volumes of disodium hydrogen phosphate (5 mM, pH = 7.4) facilitated frozen storage at -20°C for

later analysis. Then, 20 µL of amino acid standard was added to 800 µL standard or sample supernatant, and then 100 µL of this mixture was reacted with 160 µL reagent for 20 min at room temperature. A 40 µL aliquot of the derivative was diluted with 20-fold excess water, and 20 µL was then injected onto the HPLC column. The amino acid concentrations in the samples were determined by the measurement of peak heights, calculation of the ratio of the peak height with the internal standard peak height, and comparison with the equivalent ratio from a standard solution.

Statistical analysis

Data are presented as means ± SEM. All data were subjected to paired t-test to determine the differences between means with no covariates. Significance was set at P<0.05. All analyses were performed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2008).

Results

The mean rectal body temperature of the ducks fed GABA-supplemented diet was not significantly different from that fed control diet in three different measuring intervals (Table 2). Gamma aminobutyric acid significantly decreased the ducks' feed intake (P<0.05) (Table 3). Gamma

Table 2: Effect of dietary γ-aminobutyric acid (GABA) on rectal temperature (n=20) in heat stressed ducks

| Age | Control (°C) | GABA (°C) | P-value |
|---------|--------------|------------|---------|
| 19 days | 42.18±0.14 | 42.24±0.09 | 0.7245 |
| 32 days | 42.08±0.07 | 41.92±0.09 | 0.1790 |
| 42 days | 41.84±0.05 | 41.68±0.07 | 0.1114 |

Table 3: Effect of dietary γ-aminobutyric acid (GABA) on feed intake (FI, n=5) survival rate (n=5), body weight (n=5), feed conversion ratio (FCR, n=5) and dressing percentage (n=20) in heat stressed ducks at 42 days

| Parameters | Control | GABA | P-value |
|---------------------------|----------------------------|----------------------------|---------|
| FI (20-32 days, g/bird/d) | 167.1 ± 3.84 ^a | 147.91 ± 2.56 ^b | 0.0032 |
| FI (32-42 days, g/bird/d) | 189.49 ± 1.66 ^a | 158.22 ± 4.75 ^b | 0.0003 |
| Survival rate (%) | 96.78 ± 0.34 | 97.00 ± 0.24 | 0.6085 |
| Initial body weight (kg) | 0.95 ± 0.02 | 0.98 ± 0.04 | 0.0824 |
| Final body weight (kg) | 2.60 ± 0.01 | 2.65 ± 0.02 | 0.0824 |
| FCR (kg/kg) | 2.15 ± 0.04 ^a | 1.96 ± 0.02 ^b | 0.0042 |
| Dressing percentage (%) | 71.60 ± 0.02 | 71.63 ± 0.03 | 0.3721 |

^{a, b} Means with different letters in a row differ significantly (P<0.05)

Table 4: Effect of dietary γ -aminobutyric acid (GABA) on plasma parameters (n=20) of heat stressed ducks at 42 days

| Parameters | Control | GABA | P-value |
|--------------------------------|----------------------------------|----------------------------------|---------|
| Uric acid (mg/l) | 452.92 \pm 66.78 | 353.17 \pm 75.39 | 0.3327 |
| Glucose (mmol/l) | 7.94 \pm 0.27 ^a | 6.89 \pm 0.37 ^b | 0.0290 |
| Triglyceride (μ mol/l) | 1.25 \pm 0.14 ^b | 1.99 \pm 0.30 ^a | 0.0400 |
| Free fatty acid (μ mol/l) | 1146.04 \pm 269.6 ^b | 2143.15 \pm 243.0 ^a | 0.0123 |
| T3 (μ mol/l) | 1.69 \pm 0.25 | 2.07 \pm 0.18 | 0.2309 |

^{a, b} Means with different letters differ significantly (P<0.05)

Table 5: Effect of dietary γ -aminobutyric acid (GABA) on GABA concentrations in blood and different tissues of heat stressed ducks (n=20) at 42 days

| Parameters | Control | GABA | P-value |
|----------------------|------------------------------|------------------------------|---------|
| Blood (nmol/ml) | 13.17 \pm 2.88 | 15.72 \pm 2.22 | 0.4981 |
| Heart (μ mol/g) | 0.27 \pm 0.02 | 0.32 \pm 0.03 | 0.1657 |
| Liver (μ mol/g) | 0.59 \pm 0.03 | 0.64 \pm 0.04 | 0.3365 |
| Kidney (nmol/g) | 18.80 \pm 1.21 | 20.23 \pm 1.75 | 0.5163 |
| Brain (μ mol/g) | 2.91 \pm 0.13 ^b | 3.51 \pm 0.05 ^a | 0.0030 |

^{a, b} Means with different letters differ significantly (P<0.05)

aminobutyric acid did not significantly affect survival rate, body weight, and dressing percentage of the ducks, however, GABA significantly decreased feed conversion ratio (P<0.05) (Table 3).

The plasma concentrations of UA and T3 were not significantly altered with GABA treatment; but the plasma glucose concentration was significantly decreased (P<0.05) and the plasma concentrations of TG and FFA were significantly increased (P<0.05) (Table 4).

The amount of GABA distribution in blood, heart, liver, and kidney was not significantly altered in ducks fed GABA diet compared with those fed basal diet, however, GABA treatment resulted in a higher amount of distribution in the brain tissue (P<0.01) (Table 5).

Discussion

A constant temperature of 29.4°C resulted in heat stress in ducks and elevated the relative weight of the adrenals, but the corticosterone concentration remained at control levels (Hester *et al.*, 1981). Unrestrained White Peking ducks expressed metabolic response to heat stress in ambient temperature of 30°C (Hagan and Heath, 1976). A similar temperature was applied in the current research to mimic the mild heat stress in Cherry Valley ducks. In the present

study, we found that 100 mg/kg dietary GABA reduced feed intake under heat stress and improved feed efficiency, which might be attributed to its contribution to regulate carbohydrate and lipid metabolism.

Gamma aminobutyric acid is one of the inhibitory neurotransmitters in the mammalian central nervous system that is involved in the control of feeding behaviour (Decavel and Van den Pol, 1990). Manipulation of GABA-sensitive cells in the nucleus accumbens shell could have a pronounced and specific effect on feeding behaviour in rats (Stratford and Kelley, 1997). Gamma aminobutyric acid and neuropeptide Y might crosstalk through distinct receptors and second messenger systems in the paraventricular nucleus (PVN) to augment feed intake (Pu *et al.*, 1999). Jonaidi and Noori (2012) suggested that GABA acts within the brain of broilers at a GABAA receptor to increase voluntary feed intake under normal temperatures. Olgiati *et al.* (1980) have shown that intracerebroventricular injection of GABA or inhibition of GABA-transaminase decreased feed intake in rats. Denbow (1991) reported that GABA acted within the brain of turkey to increase feed intake but not water intake. In the present study, GABA reduced the feed intake of heat-exposed meat ducks, which was consistent with a recent report in laying hens (Zhang *et al.*,

2011). Therefore, the effect of GABA on feed intake might possess species, dosage and temperature speciality.

The decreased feed intake by GABA in heat exposed ducks might attenuate the detrimental effect of high temperature. This conclusion was deduced by the tended reduction of rectal temperature in GABA treated ducks. In mice, γ -acetylenic GABA and γ -vinyl GABA, two catalytic irreversible inhibitors of GABA-transaminase, produced markedly sustained elevations in brain GABA concentrations and a reduction in the rectal temperature (Schechter and Tranier, 1977). Although GABA caused a decrease in feed intake, the ducks fed GABA reached a similar body weight compared with those fed basal diet. This might be related to the effect of GABA on carbohydrate and lipid metabolism. However, Zhang *et al.* (2011) found 50 mg/kg GABA tended to increase feed intake in heat stressed laying hens, while 100 mg/kg GABA showed a reversed tendency. Therefore, the effect of different dosage of GABA on duck's feed intake needs to be further addressed.

The unchanged plasma concentrations of UA and T3 suggested that GABA showed a minor effect on protein and whole energy metabolism in ducks. However, the decreased plasma glucose and increased plasma FFA and TG provided the evidences that GABA altered the carbohydrate and lipid metabolism in ducks. This might partly explain the improved feed efficiency in ducks fed GABA. The decreased plasma glucose indicated that GABA might stimulate the glucose utilization in ducks. The increased plasma FFA and TG revealed that GABA increased the fat mobilization. The altered nutrient flow from fat to protein could have resulted in the similar dress percentage between GABA and control ducks. However, the future research should better address plasma insulin and body fat deposition status to clarify the underlying mechanisms related to the change of glucose and lipid metabolism. The results of the current research were consistent with the findings in broilers and layers. In the most recent study, Dai *et al.* (2011) found that dietary GABA offered a potential nutritional strategy to prevent heat stress-related

depression in performance and carcass characteristics of broiler. Zhang *et al.* (2011) found that GABA improved laying performance and physical condition mainly by modulating hormone secretion, enhancing anti-oxidation and immune activity, and maintaining electrolyte balance in laying Roman hens.

Gamma aminobutyric acid is a signalling molecule that naturally exists in vertebrate's brain and plays important roles in neurotransmission. Gamma aminobutyric acid is also directly responsible for the regulation of muscle tone in humans (Watanabe *et al.*, 2002). Gamma aminobutyric acid is metabolised inside the mitochondria by GABA aminotransferase to generate alanine and succinate semialdehyde (SSA). Succinate semialdehyde is considered to be a reactive carbonyl and can lead to formation of free radicals and oxidative stress. Therefore, we determined the GABA distributions in the main internal organs of the ducks to see if there was more GABA reserved with dietary GABA supplementation. The similar amount of GABA in the blood, heart, liver and kidney was consistent with the findings in rats (Kuriyama and Sze, 1971). Furthermore, the relatively high amount of GABA in the brain was surprising at present. The blood-brain barrier in adult animals is impermeable to both blood-borne GABA and endogenous cerebral GABA (Kuriyama and Sze, 1971). Certain areas of the brain have no effective blood-brain barrier (Broadwell and Brightman, 1976). The periventricular nucleus can be reached by drugs such as extrinsic injected GABA (Müller *et al.*, 1999). More research is needed to investigate the way that GABA was enriched in the brain of the ducks.

In conclusion, our results suggested that 100 mg/kg dietary GABA decreased feed intake in heat stressed ducks but showed no detrimental effect on duck's performance, which might be derived from its regulation on carbohydrate and lipid metabolism. The supplementation of GABA in the diet might be beneficial for the heat exposed ducks.

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