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Role of gamma-aminobutyric acid in regulating feed intake in commercial broilers reared under normal and heat stress conditions.

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ABSTRACT

This study was conducted to investigate the effects of dietary GABA supplementation on blood biochemical parameters, the overall growth performance, and the relative mRNA expression of some FI- regulating genes in broiler chickens. A total of 192, three-day old chicks of mixed sex from two commercial broiler strains (Ross 308 and Cobb 500) were distributed into 2 groups; a control group and GABA-supplemented group (100 mg/kg diet). When the chicks reached 21 days of age, each group of each strain was randomly subdivided into two subgroups: one was exposed to HS (33 ± 2 °C for 5 h/day for 2 weeks), while the other remained at thermoneutral temperature (24 °C). GABA significantly improved bird growth performance under normal and HS conditions, by increasing body weight (BW), weight gain (WG), and FI and significantly reduced the elevated body temperature of birds under HS. GABA supplementation increased FI by reducing the mRNA expression levels of FI-inhibiting neuropeptides, such as *POMC, leptin, Ghrelin*, and *CCK,* during HS and by increasing the expression of FI-stimulating neuropeptides such as *AgRP* and *NPY*. Moreover, GABA significantly altered *FAS* and *ACC* gene expression, resulting in significant increases in abdominal fat content in birds reared normally. In contrast, GABA lowered fat content in Cobb birds and increased it in Ross birds under HS. Therefore, GABA (100 mg/kg diet) is a strong FI-stimulating neurotransmitter and its regulatory effects depend on broiler strain and housing temperature.

1. Introduction

Feed intake (FI) is a key factor which affects broiler growth performance, nutrient utilization, health, and production efficiency. Therefore, maintaining birds maximum FI is considered one of the main goals of poultry breeders. FI in poultry is controlled both central and peripheral mechanisms. Central regulation plays a fundamental role in energy homeostasis and feeding responses and is mediated through a complex neuronal network within the hypothalamus called the hypothalamic melanocortin system (Cline et al., 2008; Nahon, 2006; Richards and Proszkowiec-Weglarz, 2007).

The hypothalamic melanocortin system is composed of two sets of neurons. The first set expresses neuropeptide Y (NPY) and agouti-related protein (AgRP), while the second expresses proopiomelanocortin (POMC), a precursor to α-melanocyte-stimulating hormone. Stimulation of NPY/AgRP-expressing neurons mediates an anabolic effect by increasing FI and energy storage, while stimulation of POMC-expressing neurons stimulates a catabolic response resulting in FI reduction. Therefore, balance in the activity of hypothalamic melanocortin neurons is very important to maintain whole-body energy balance and body weight (BW) (Richards et al., 2010). Peripheral regulation of FI involves several neuropeptides, such as cholecystokinin (CCK), ghrelin, leptin and peptide YY, that regulate appetite by controlling the physiological function of the digestive tract and sending response signals to the central nervous system (Arora and Anubhuti, 2006).

Abbreviations: GABA, Gamma-aminobutyric acid; NPY, Neuropeptide Y; AgRP, agouti-related protein; POMC, proopiomelanocortin; CCK, cholecystokinin; FI, Feed intake; HS, heat stress; BW, body weight; WG, weight gain; FCR, feed conversion ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, Aspartate Aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; IGF, Insulin like growth factor; GHR, growth hormone receptor; FAS, fatty acid synthetase; ACC, acetyl Co A carboxylase; AMPK, Adenosine monophosphate-activated protein kinase; mTOR, mammalian target of rapamycin

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Table 1
Experimental design.

Bird's strain	Group Name	Temp °C	Treatment
Cobb (C)	CN + C	24 ± 2	Normal (N) + No GABA supplementation
	CN + GABA	24 ± 2	Normal (N) + GABA (100 mg/kg diet)
	CH+ C	33 ± 2	Heat stress (H) + No GABA supplementation
	CH + GABA	33 ± 2	Heat stress (H) + GABA (100 mg/kg diet)
Ross (R)	RN + C	24 ± 2	Normal (N) + No GABA supplementation
	RN + GABA	24 ± 2	Normal (N) + GABA supplementation
	RH + C	33 ± 2	Heat stress (H) + No GABA supplementation
	RH + GABA	33 ± 2	Heat stress (H) + GABA supplementation

Stimulation of the central and peripheral nervous systems by different nutrients and environmental stimuli results in the production of a variety of signalling molecules, such as neuropeptides, hormones and metabolic products that can bind to specific receptors and initiate signalling cascades in particular neuron subgroups in the hypothalamus, resulting in the regulation of FI and energy utilization (Proszkowiec-Weglarz et al., 2006).

Ferket and Gernat (2006) reported several factors affecting FI of birds, including dietary factors (nutrient composition and feed pellet quality) and managemental factors (access to feed and water, climatic changes, stocking density, and disease control). Stressors such as heat stress (HS) have adverse effects on bird feed consumption, influencing growth rate. The absence of sweat glands and the presence of feathers covering the body make poultry highly sensitive and susceptible to HS; fast growing broilers especially susceptible due to their high metabolic activity (Deeb and Cahaner, 2002; Settar et al., 1999). Several earlier studies investigated the impacts of HS, which was found to be associated with impaired growth performance and lowered FI (Sohail et al., 2012), intestinal morphological changes and microflora imbalance (Li et al., 2015), reduced uptake and utilization of nutrients (Chen et al., 2015) and immunosuppressive effects (Bartlett and Smith, 2003). During HS exposure, birds change their performance and physiological homeostasis to reduce body temperature. They reduce FI to decrease heat production inside the body, which consequently reduces the nutrients needed for growth and decreases feed utilization efficiency.

Improving bird management during stress conditions and alleviating negative impacts are important for successful poultry production. Various strategies have been applied, such as ventilation, and shading. The nutritional strategies include administration of nutrient-dense diets; dietary supplementation with additives such as antioxidants, vitamins, minerals, probiotics, prebiotics and essential oils; and water supplementation with electrolytes.

Gamma-aminobutyric acid (GABA), a non-protein amino acid is commonly distributed in the tissues of animals and plants. In addition to its main role as an inhibitory neurotransmitter in the central nervous system, GABA participates in other physiological functions in the body, including thermoregulation (Jha et al., 2001). GABA is found at high concentrations in the hypothalamic nuclei and is released in large amounts to the extracellular tissues of the brain during HS conditions (Frosini et al., 2000). In addition, GABA reduces depression and anxiety, lowers blood pressure and improves the immune response during stress conditions (Hayakawa et al., 2004; Inoue et al., 2003). Another important role of GABA is in controlling and regulating feed consumption behaviour (Decavel and Van den Pol, 1990). Recently, GABA has been found to ameliorate the detrimental impacts of HS in laying hens (Zhang et al., 2012a) and broilers (Al Wakeel et al., 2017).

Therefore, we hypothesized that dietary inclusion of GABA as a feed additive could be a nutritional strategy to reduce the depressive effects of HS on FI and its consequences on growth performance. The objective of this study was to investigate the mechanistic role of GABA in regulating feed consumption in broiler chickens reared under normal temperature and exposed to HS through analyzing the effects of dietary GABA supplementation on blood biochemical parameters, overall growth performance and the expression levels of select FI-regulating genes in two commercial broiler strains, Ross 308 and Cobb 500. Additionally, to determine whether any increases in bird WG following GABA supplementation were due to increases in muscle or fat, we examined the expression levels of muscle growth and fat metabolism-related genes.

2. Materials and methods

The bird management followed the regulations of the Animal Care and Ethics Committee at the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

2.1. Bird management, experimental design and diet preparation

A total of 192, mixed sex, three-day old chicks from two commercial broiler strains (Ross 308 and Cobb 500) were used in this study to clarify the difference in their performance (El-Kassas et al., 2018). The chicks were received, kept together and fed a basal diet (BD) without GABA supplementation for 2 days allowing birds to recover from transportation stress. At day 3 of chicks' age, they were individually weighed (average initial BW 64.85 ± 0.95 g) then randomly distributed in environmentally controlled rooms (3 m width, 15 m length, 3 m height) at the research facility. For each strain, 96 chicks were allocated into two experimental groups, control and GABA supplemented (100 mg/kg diet) from day 3 until the end of the experiment. Each treatment was replicated three times (16 birds/replicate). From the first day, the brooding temperature was 34 °C which was decreased gradually to reach the normal rearing temperature (22–25 °C) at 21 days of age. At day 21 of age, the experimental groups were subdivided randomly into two subgroups: one exposed to HS for 2 weeks (33 ± 2 °C for 5 h from 10:00 a.m. to 15:00 h, and then 24 °C from 15:00 to 10:00) and the other housed under normal housing temperature (24 °C as thermoneutral group) as shown in Table 1. The temperature was elevated using electric heaters that kept a fixed temperature. This HS routine was done to simulate the most common heat wave conditions in the subtropical countries and based on earlier researches (Akbarian et al., 2016; Azad et al., 2013; El-Kassas et al., 2018; Sohail et al., 2011). Also, to guarantee the complete development of the bird thermoregulatory center that is not well-developed at earlier ages. Moreover, the HS is more severe at the mature age because the birds consume more feed and are covered with feathers and having no sweat glands are more susceptible to HS.

Through the study, birds were housed in floor pens (1 m × 1.5 m) at stocking density of eight birds/m^2. The roof of the house was insulated to prevent heating from outside. Temperature and relative humidity were checked three times a day using digital thermometers to guarantee the stability of temperature and ventilation. The thermometers reading accuracy was checked two times/day using a manual thermometer and a hygrometer, which were placed at the center of the house and nearby the digital thermometers to ensure similar readings. Basal diet (BD) was formulated to meet the nutrient requirements for poultry (NRC, 1994). Ingredients composition of the BD and its calculated analysis are

Table 2
Ingredients and calculated composition of the basal diet.

Ingredients (%)	Starter	Grower	Finisher
Yellow corn	56.60	62.51	66.62
Corn Gluten Meal	5.80	3.50	2.00
Soybean meal (47%)	31.50	27.61	25.21
Soybean Oil	2.30	2.50	2.50
DCP[a]	1.50	1.50	1.50
Limestone [b]	1.40	1.40	1.40
Mineral premix [c]	0.10	0.10	0.10
Vitamin premix [d]	0.10	0.10	0.10
Common salt	0.30	0.30	0.30
DL- methionine [e]	0.20	0.22	0.22
Lysine HCl [f]	0.15	0.21	0.21
Choline chloride	0.05	0.05	0.05
Calculated Composition [g]			
Crude protein (%)	22.82	20.02	18.19
Calcium (%)	0.92	0.91	0.90
Available Phosphorus (%)	0.47	0.46	0.46
Lysine (%)	1.20	1.15	1.07
Methionine (%)	0.58	0.55	0.52
ME (Kcal/kg)	3061.99	3110.00	3137.0
Calorie/protein ratio [h]	134.2	155.33	172.49

[a] DCP = Dicalcium phosphate (contain 18% Phosphorus and 21% Calcium).

[b] Limestone (contain 35% calcium).

[c] Mineral premix: produced by Nutristar international Co. and contained (each 1 kg) 60000 mg Mn (Mn oxide), 50000 mg Zn (Zn oxide), 5000 mg Cu (copper sulphate), 30000 mg Fe (Fe chloride), 500 mg I (calcium iodate), 100 mg Se (sodium selenate) and 100 mg Co (cobalt sulphate) and calcium carbonate as a carrier up to 1 kg.

[d] Vitamin premix produced by Nutristar international Co. and contained (per 1 kg) vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 1000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B1210 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, antioxidant-ethoxyquin 250 mg with calcium carbonate upto 1 kg as carrier.

[e] DL-Methionine (Produced by Evonik Co. and contain 99% methionine).

[f] Lysine = lysine hydrochloride (contain 98% Lysine).

[g] Calculated composition according to NRC (1994).

[h] Calorie/protein ratio = ME Kcal/CP%.

illustrated in Table 2. Starter diet was fed for the first 2 weeks, followed by grower diet for another two weeks (3rd and 4th week) and finally the finisher diet until the end of the experiment. GABA was purchased from (Sigma, St. Louis, MO, USA), and supplemented at 100 mg/kg diet. During this study, birds had free access to feed and water. Newcastle disease vaccination program was done on days 7, 18, and 28 of age. The infectious bursal disease (Gumboro) vaccination was done on day 12. The management in all pens, including floor space, light intensity, and food and water supply was similar. The relative humidity was kept at an average of 70% during the experiment for all groups. Bird growth performance was assessed weekly by measuring BW, FI, and feed conversion ratio (FCR) which was calculated based on the feed consumed and gain obtained.

2.2. Sample collection

At the end of the fifth week of experiment, all birds in each replicate were first weighed to assess the live BW followed by measuring rectal temperature using a digital thermometer inserted into cloaca to approximately 3 cm. A total of 6 birds from each treatment (two from each replicate) were randomly selected and used for sample collection immediately at the end of heat treatment. Blood samples were drawn from the jugular vein of birds using a non-heparinized syringes and collected in clean vials for serum separation by centrifugation at 3000 rpm for 10 min. The clear samples were kept at −20 °C for further analysis of serum biochemical parameters. After that, birds were killed by cervical dislocation under mild anesthesia. From each bird, hypothalamic and liver (from the left lobe) specimens were collected, rapidly frozen in liquid nitrogen and kept at −80 °C for gene expression of FI-regulating genes. Birds were then eviscerated, abdominal fat was collected and weighed.

2.3. Blood biochemical parameters

Serum samples were used for determination of the following blood biochemical constituents: total protein, albumin, glucose, cholesterol, triglyceride (TG), low and high-density lipoprotein (LDL and HDL), aspartate Aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). These parameters were measured by spectrophotometer using standard commercial kits (Biodiagnostic Co, Egypt) according to the manufactures instructions.

2.4. Real time PCR

The RNA was extracted using 30–50 mg of hypothalamic and liver tissues from six birds ($n = 6$) from each treatment (two birds/replicate) using TRI reagent (easy-RED™, iNtRON Biotechnology, South Korea), following the manufacturers' protocol. The integrity of RNA was verified using gel electrophoresis followed by visual assessment of rRNA bands (18S and 28S) in 2% agarose gel stained with ethidium bromide. The RNA concentration was determined using Nano drop (UV-Vis spectrophotometer Q5000, Quawell, USA). Nearly, 2 μg of RNA sample was reverse transcribed using the SensiFAST™ cDNA synthesis kit (Bioline, United Kingdom). The cDNA product was verified by conventional PCR using house-keeping gene primers and analyzed by agarose gel electrophoresis.

Specific primers (Table 3) were used to amplify FI-regulatory genes. In this regard, qPCR was done using the SensiFast™ SYBR Lo-Rox kit (Bioline, United Kingdom) and MxPro qPCR system (Agilent Technologies, USA). The qPCR mix consisted of 10 μl of mastermix, 0.5 μM of each primer and 2 μl of cDNA. The thermal cycling conditions were: initial denaturation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 15 s, annealing for 1 min at 60 °C for all genes. Dissociation curves were analyzed starting at 65 °C and ending at 95 °C, with increases of 0.5 °C every 5 s to confirm the specificity of the PCR products. Analysis of the dissociation curve showed only one peak at the specific melting temperature for all tested genes showing that the PCR products were specifically amplified. The genes were tested in duplicates for six samples from each treatment. CT values were determined for each sample and incorporated in "fold change" calculation according to the Livac method (Livak and Schmittgen, 2001). The mRNA expression for each sample was normalized against house-keeping gene (β actin and GAPDH) and its comparable values of the control group (no GABA supplementation at normal temperature).

2.5. Statistical analysis

The results were analyzed using a linear mixed-effects model (MIXED) procedure in the SPSS package (©IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM). The model included heat treatment, broiler strain, and GABA dietary supplementation as fixed factors and the animals within the different treatments as a random effect. Multiple mean comparisons were done using Tukey's multiple comparison test. The results were shown as the mean ± standard error of the mean (SEM) and the figures were created using GraphPad Prism 6 (©GraphPrism Software, La Jolla, CA, USA).

3. Results

3.1. Growth performance and body temperature

Table 4 shows the effects of HS, broiler strain, dietary GABA supplementation and interactions among factors on bird growth performance. These factors significantly modified the growth performance of

Table 3
Primer sequences (5'-3') used in real-time PCR.

Gene	Primer	Ref. Seq. Accession No.	Annealing
β –actin	F: ACCTGAGCGCAAGTACTCTGTCT R: CATCGTACTCCTGCTTGCTGAT	NM_205518.1 (Xie et al., 2014)	60
GAPDH[a]	F: GGGCACGCCATCACTATCTTC R: ACCTGCATCTGCCCATTTGAT	NM_204305 (Nerren et al., 2009)	60
AgRP [b]	F: GGAACCGCAGGCATTGTC R: GTAGCAGAAGGCGTTGAAGAA	NM_001031457 (Liu et al., 2014)	63
Ghrelin	F: CCTTGGGACAGAAACTGCTC R: CACCAATTTCAAAAGGAACG	AB075215 (Song et al., 2012)	63
CCK [c]	F: CAGCAGAGCCTGACAGAACC R: AGAGAACCTCCCAGTGGAACC	NM_001001741 (He and Lu, 2018)	58
Leptin	F: CGTCGGTATCCGCCAAGCAGAGGG R: CCAGGACGCCATCCAGGCTCTCTGGC	Ashwell et al. (1999)	58
POMC [d]	F: CGCTACGGCGGCTTCA R: TCTTGTAGGCGCTTTTGACGAT	NM_001031098 (Song et al., 2012)	63
FAS [e]	F: GGAAGATCTGGAGGCTCGTG R: AAAGGAAGCAGCAGCAAAGC	NM_205155.2(Elshobokshy, 2017)	63
ACC [f]	F: AATGGCAGCTTTGGAGGTGT R: TCTGTTTGGGTGGGAGGTG	NM_205505 (Liu et al., 2016)	63
GHR [g]	F: AACACAGATACCCAACAGCC R: AGAAGTCAGTGTTTGTCAGGG	(Kamel et al., 2016)	60
IGF [h]	F: CACCTAAATCTGCACGCT R: CTTGTGGATGGCATGATCT	(Kamel et al., 2016)	60
NPY [i]	F: GAGGCACTACATCAACCTCATCAC R: TGTTTTCTGTGCTTTCCCTCAA	Liu et al. (2014)	63

[a] *GAPDH* = glyceraldehyde 3-phosphate dehydrogenase.
[b] *AgRP* = agouti-related peptide.
[c] *CCK* = cholecystokinin.
[d] *POMC* = pro-opiomelanocortin.
[e] *FAS* = fatty acid synthetase.
[f] *ACC* =acetyl Co A carboxylase.
[g] *GHR* = growth hormone receptor.
[h] *IGF* = Insulin like growth factor.
[i] *NPY* = neuropeptide Y. Genes 5, 6, 7 and 8 were tested in the liver tissue while the rest in the hypothalamic tissue.

Ross and Cobb birds. At the end of week 5 and 6, HS ($P = 0.031$ and $P = 0.005$, respectively), GABA supplementation ($P = 0.021$ and $P = 0.045$, respectively) had significant effects on BW without any effects of broiler strain ($P = 0.061$) or interactions among factors ($P = 0.059$). On the other hand, WG and FCR were significantly affected by HS ($P = 0.027$ and $P = 0.001$, respectively) without any effect of GABA supplementation ($P = 0.131$), broiler strain ($P = 0.082$) or interaction among factors ($P = 0.075$). Under normal housing temperatures, BW was significantly higher in the 5th week in Ross birds receiving dietary GABA supplementation (RN + GABA) than in non-supplemented Ross birds (RN + C) ($P < 0.05$). However, no differences were observed during week 6 ($P > 0.05$). Additionally, there were no differences in WG or FCR for these same groups ($P > 0.05$), but an increase in total FI was found. Following HS exposure, growth performance was significantly better in Ross birds receiving GABA-supplemented diets (RH + GABA) than in those receiving the control diet (RH + C). Significant increases in BW and, WG, and improved FCRs were observed in weeks 5 and 6 ($P < 0.05$). In Cobb birds exposed to HS and supplemented with GABA (CH + GABA), BW was significantly higher than that of control birds in weeks 5 and 6 ($P < 0.05$), but there were no differences in total WG and FCR ($P > 0.05$). In addition, the FCR in Ross birds (RH+ GABA) was

Table 4
Effect of dietary supplementation of GABA on growth performance of broiler chickens.

Groups	Initial Wt. (g)	Wt. after HS (5th week Wt.) (g)	Final Wt. (6th week Wt.) (g)	Total gain (g)	Total FI (g)	Average final FCR [a]
Cobb						
CN+ C	65.60 ± 1.15	1698.43 ± 41.47^{a}	2061.00 ± 37.56^{a}	1995.4 ± 49.31^{a}	3134.80	1.57 ± 0.04^{aA}
CN+ GABA	63.27 ± 1.11	1750.14 ± 36.93^{a}	2145.33 ± 0.35^{a}	2082.06 ± 38.74^{a}	3314.86	1.59 ± 0.04^{aA}
CH + C	65.69 ± 1.11	1633.25 ± 53.98^{b}	1971.38 ± 64.93^{b}	1905.69 ± 110.07^{a}	4169.32	2.19 ± 0.09^{aA}
CH+ GABA	62.92 ± 1.67	1715.50 ± 12.49^{a}	2158.62 ± 76.94^{a}	2095.7 ± 76.48^{a}	4238.96	2.02 ± 0.08^{aA}
Ross						
RN+ C	65.72 ± 1.01	1611.68 ± 47.83^{b}	2167.00 ± 53.69^{a}	2101.28 ± 64.00^{a}	3051.96	1.50 ± 0.06^{aA}
RN+ GABA	63.46 ± 1.08	1746.95 ± 40.19^{a}	2178.56 ± 57.68^{a}	2115.1 ± 54.04^{a}	3393.78	1.60 ± 0.05^{aA}
RH+C	65.67 ± 1.18	1596.90 ± 47.51^{b}	1980.13 ± 67.73^{b}	1914.46 ± 65.02^{b}	3938.20	2.06 ± 0.06^{aA}
RH + GABA	66.50 ± 1.64	1714.38 ± 37.03^{a}	2183.75 ± 45.07^{a}	2117.25 ± 95.02^{a}	3752.97	1.77 ± 0.06^{bB}

Data is presented as means ± SEM (n=8 bird/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same strain. The uppercase letters represent the statistical significance ($P < 0.05$) between Ross and Cobb chickens.

CN + C: Cobb birds kept at normal temperature (N) without GABA supplementation; CN + GABA: Cobb birds kept at normal temperature (N) with GABA supplementation; CH+ C: Cobb birds subjected to heat stress (H) without GABA supplementation; CH + GABA: Cobb birds subjected to heat stress (H) with GABA supplementation; RN + C: Ross birds kept at normal temperature (N) without GABA supplementation; RN + GABA: Ross birds kept at normal temperature (N) with GABA supplementation; RH+ C: Ross birds subjected to heat stress (H) without GABA supplementation; RH + GABA: Ross birds subjected to heat stress (H) with GABA supplementation.

[a] FCR = total FI/Total gain.

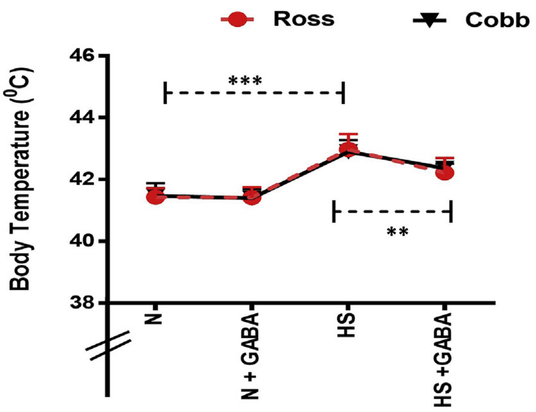

Fig. 1. Effect of dietary supplementation of GABA on body temperature normally and following HS in Ross and Cobb birds. Body temperature was reported in all chickens (n = 24, 8 birds/replicate) which were reared under normal temperature and supplemented with their GABA (100 mg/kg diet) (N and N + GABA, respectively). HS + GABA represent the body temperature for birds exposed to chronic HS and supplemented with GABA. The Results are expressed as mean ± SEM. ** and *** denote statistical significance at $P < 0.01$ and $P < 0.001$, respectively.

significantly better than that in Cobb birds (CH + GABA) ($P < 0.05$). Moreover, total FI was higher in GABA-supplemented heat-stressed Cobb birds and lower in GABA-supplemented heat-stressed Ross birds (RH + GABA) than in non-supplemented control birds reared under HS. The effect of GABA supplementation on bird growth performance was associated with significant modulation of bird body temperature. GABA supplementation ($P = 0.002$), HS ($P = 0.001$) significantly modulated body temperature of birds, with no effect of strain and interaction ($P = 0.114$). Exposure to cyclic chronic HS stimulated a marked rise in body temperature in the two broiler strains ($P < 0.05$) compared to the normally housed chickens) (Fig. 1). Interesting, dietary inclusion of GABA significantly attenuated the body temperature elevations in Ross and Cobb birds reared under HS ($P < 0.05$).

3.2. Serum biochemical parameters

The effects of different housing temperatures, broiler strains, GABA dietary supplementation and interactions among factors on serum biochemical parameters are illustrated in Table 5. Non-significant modulations in the serum concentrations of total protein, albumin, globulin and glucose in the two strains housed under normal temperature or exposed to HS were observed ($P > 0.05$). Moreover, AST and ALP concentrations were significantly modified by GABA supplementation ($P = 0.031$ and $P = 0.002$, respectively) and housing temperatures ($P = 0.006$ and $P = 0.012$, respectively) irrespective of broiler strains and without any interactions ($P = 0.082$ and $P = 0.127$, respectively).

Cobb birds fed a diet supplemented with GABA and housed under normal temperatures (CN + GABA) showed significantly higher AST and ALP concentrations than non-supplemented birds under normal temperatures (CN + N) ($P < 0.05$). GABA-supplemented Cobb birds under HS showed significantly higher ALP concentration increased ($P < 0.05$) than non-supplemented Cobb birds under HS (CH + GABA). In Ross birds, GABA supplementation under HS conditions (RH + GABA) significantly elevated ALP, AST and ALT concentrations ($P < 0.05$) compared with control group under HS conditions (RH + C group). In addition, Cobb birds exposed to HS without GABA supplementation (CH + C) demonstrated higher concentrations of AST than the corresponding Ross birds (RH + C) ($P < 0.05$).

3.3. Serum lipid profiles and abdominal fat content

Table 6 presents the effects of heat treatment, broiler strain, GABA supplementation and interaction among factors on serum lipid profile and abdominal fat content. Significant effects were observed for HS, broiler strain, GABA supplementation and interactions. LDL was significantly modified by strain ($P = 0.014$), HS ($P = 0.001$), GABA supplementation ($P = 0.001$) and interaction ($P = 0.047$); while, HDL was altered by HS, and GABA supplementation ($P < 0.001$) and interaction among factors ($P = 0.011$) without any effect of strain ($P = 0.157$). Regarding cholesterol and TGs concentrations; they were significantly modified by strain ($P = 0.044$ and $P = 0.009$, respectively), HS ($P = 0.035$ and $P = 0.015$, respectively), GABA supplementation ($P = 0.046$ and $P = 0.021$, respectively) without any effect of interaction among factors ($P = 0.09$). Additionally, abdominal fat contents were significantly altered by all factors: strain ($P = 0.039$), HS ($P = 0.008$), GABA supplementation ($P = 0.017$) and interactions ($P = 0.002$).

Under normal housing temperatures, dietary inclusion of GABA for Cobb birds (CN + GABA) significantly increased TG and HDL concentrations along with abdominal fat content ($P < 0.05$). LDL concentration were markedly lower in the CN + GABA group than in the CN + C ($P < 0.05$). Cobb birds under HS and receiving GABA supplementation (CH + GABA) exhibited distinct increases in cholesterol and LDL concentrations ($P < 0.05$) compared with Cobb birds under HS not receiving GABA (CH + C). In addition, significantly lower abdominal fat content was found in CH + GABA birds compared to

Table 5
Effect of dietary supplementation of GABA on some serum biochemical parameters of broiler chickens.

Group	Total protein g/dl	Albumin g/dl	Globulin [a] g/dl	AST [b] U/L	ALT [c] U/L	ALP[d] U/L	Glucose mg/dl
Cobb							
CN+ C	3.32 ± 0.04	1.64 ± 0.12	1.68 ± 0.15	163.00 ± 5.21^{bA}	6.00 ± 0.58^{a}	2403.00 ± 26.56^{b}	206.00 ± 5.13
CN+ GABA	3.36 ± 0.28	1.70 ± 0.03	1.65 ± 0.26	174.00 ± 5.24^{aA}	5.33 ± 0.19^{a}	3286.33 ± 69.07^{a}	205.00 ± 17.70
CH+ C	3.21 ± 0.06	1.66 ± 0.02	1.55 ± 0.08	183.67 ± 13.67^{aA}	5.67 ± 0.69^{a}	2927.00 ± 67.83^{b}	239.00 ± 0.67
CH+ GABA	3.11 ± 0.04	1.54 ± 0.01	1.58 ± 0.05	181.00 ± 3.67^{aA}	5.67 ± 0.19^{a}	4013.67 ± 33.54^{a}	247.33 ± 4.44
Ross							
RN+ C	3.65 ± 0.17	1.88 ± 0.01	1.77 ± 0.17	163.33 ± 2.50^{aA}	3.33 ± 0.19^{a}	2271.67 ± 58.50^{a}	173.33 ± 5.60
RN + GABA	3.22 ± 0.10	1.84 ± 0.04	1.38 ± 0.06	169.00 ± 3.84^{aA}	5.00 ± 0.33^{a}	2537.00 ± 58.14^{a}	197.67 ± 4.30
RH + C	3.61 ± 0.05	1.69 ± 0.03	1.92 ± 0.08	158.67 ± 5.97^{bB}	4.67 ± 0.38^{b}	3832.67 ± 98.98^{b}	241.67 ± 1.95
RH + GABA	3.13 ± 0.06	1.70 ± 0.07	1.43 ± 0.01	192.33 ± 0.51^{aA}	7.00 ± 1.20^{a}	4133.33 ± 67.29^{a}	266.33 ± 6.77

Data is presented as means ± SEM (n=6 bird, 2 bird/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same strain. The uppercase letters represent the statistical significance ($P < 0.05$) between Ross and Cobb chickens.

[a] Globulin: total protein – albumin.

[b] AST: Aspartate Aminotransferase.

[c] ALT: alanine aminotransferase.

[d] ALP: alkaline phosphatase.

Table 6

Effect of dietary supplementation of GABA on serum lipid profile and average abdominal fat content of broiler chickens.

Group	Cholesterol mg/dl	Triglyceride mg/dl	HDL[a] mg/dl	LDL[b] mg/dl	Average Abdominal Fat Content (g)
Cobb					
CN+ C	145.33 ± 0.69^{a}	190.00 ± 4.33^{bA}	77.03 ± 2.08^{b}	30.30 ± 2.25^{aA}	10.20 ± 2.10^{b}
CN+ GABA	146.33 ± 2.22^{a}	219.67 ± 12.19^{aA}	112.10 ± 1.54^{a}	14.03 ± 1.58^{bA}	16.40 ± 1.18^{a}
CH+C	149.67 ± 5.60^{b}	233.67 ± 7.13^{aA}	75.23 ± 1.56^{b}	27.70 ± 4.26^{bB}	15.10 ± 1.26^{a}
CH+ GABA	164.67 ± 3.67^{a}	230.00 ± 14.98^{aA}	73.00 ± 0.92^{b}	45.67 ± 0.98^{aA}	9.40 ± 1.24^{b}
Ross					
RN + C	157.33 ± 6.08^{a}	182.00 ± 14.43^{bA}	92.70 ± 6.33^{a}	28.23 ± 3.06^{aA}	9.00 ± 0.68^{b}
RN + GABA	154.33 ± 4.43^{a}	201.00 ± 2.03^{aA}	91.20 ± 3.16^{a}	22.60 ± 3.62^{aA}	13.40 ± 1.37^{a}
RH + C	163.00 ± 0.58^{a}	174.33 ± 1.95^{bB}	84.27 ± 1.56^{a}	43.87 ± 1.56^{aA}	6.30 ± 2.06^{b}
RH + GABA	165.33 ± 3.75^{a}	224.00 ± 7.53^{aA}	73.23 ± 0.55^{b}	47.30 ± 2.17^{aA}	13.30 ± 1.49^{a}

Data is presented as means ± SEM (n=6 bird, 2 bird/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same strain. The uppercase letters represent the statistical significance ($P < 0.05$) between Ross and Cobb chickens.

[a] HDL: high-density lipoprotein.

[b] LDL: low-density lipoprotein.

CH + C birds ($P < 0.05$).

GABA supplementation in normally housed Ross birds (RN + GABA) significantly increased TGs and abdominal fat content ($P < 0.05$). Under HS, the GABA-supplemented diet (RH + GABA) significantly elevated TGs ($P < 0.05$) when compared to the control diet (RH + C). Markedly reduced HDL concentration and increased abdominal fat content were found in the RH + GABA group compared to the RH + C group ($P < 0.05$). In addition, Ross birds exposed to HS (RH+C) showed significantly lower concentration of TGs than corresponding Cobb birds (CH + C) ($P < 0.05$).

3.4. Gene expression levels of feed intake regulatory genes

The gene expression levels of *POMC* was significantly modulated by HS and GABA supplementation ($P < 0.001$ for both), strain ($P = 0.022$) and interactions among factors ($P = 0.002$). HS exposure significantly upregulated *POMC* mRNA expression levels ($P < 0.05$) in Ross and Cobb birds compared with those under normal rearing temperatures (Fig. 2A). Dietary supplementation of GABA in Cobb birds exposed to HS markedly downregulated *POMC* expression levels ($P < 0.05$). *POMC* expression levels in Ross birds exposed to HS and supplemented with GABA were significantly higher than those in Cobb birds under the same conditions ($P < 0.05$).

AgRP expression was also significantly affected by HS ($P = 0.015$), broiler strain ($P = 0.023$), GABA supplementation ($P = 0.004$) and their interactions among factors ($P = 0.022$) (Fig. 2B). In Ross birds, *AgRP* mRNA expression was not different between the GABA-supplemented group reared under normal housing temperature (N + GABA) compared with its corresponding group without GABA (N) ($P > 0.05$). However, Ross birds supplemented with GABA and exposed to HS (HS + GABA) showed significantly higher *AgRP* expression than normally housed and heat-stressed birds without GABA ($P < 0.05$). In Cobb birds, *AgRP* expression was not different between the group given dietary GABA and reared under normal housing temperatures (N + GABA) and the N group ($P > 0.05$). On the other hand, significant *AgRP* upregulation was found Cobb birds exposed to HS without GABA supplementation ($P < 0.05$). However, GABA supplementation in Cobb birds exposed to HS (HS + GABA) didn't attenuate the increase in *AgRP* expression caused by HS ($P > 0.05$). The effect of HS exposure on *AgRP* expression was significantly greater in Cobb birds than in Ross birds ($P < 0.05$).

The relative gene expression of *NPY* (Fig. 2C) was significantly altered by GABA supplementation and HS ($P = 0.001$) with no effect of strain or interactions ($P = 0.064$ and $P = 0.247$, respectively). Ross birds exposed to HS and those supplemented with GABA either under normal housing temperatures or HS showed non-significant increases in *NPY* mRNA expression ($P > 0.05$). In Cobb birds housed under normal temperatures, GABA supplementation (N + GABA) did not significantly affect the relative gene expression of *NPY* ($P > 0.05$). HS exposure also, did not affect *NPY* expression levels ($P > 0.05$). However, exposure of GABA supplemented Cobb birds to HS (HS + GABA) significantly upregulated *NPY* mRNA expression ($P < 0.05$). There were no differences between the strains due to HS or GABA supplementation ($P > 0.05$).

The relative mRNA expression levels of *leptin* (Fig. 3A) were significantly modified by HS ($P = 0.001$), broiler strain ($P = 0.001$), GABA supplementation ($P = 0.001$), and interactions among factors ($P < 0.001$). Under normal housing temperatures, GABA supplementation to Ross birds (N+GABA) did not modify the gene expression levels of *leptin* ($P > 0.05$). Under HS conditions, *leptin* was significantly upregulated without or with GABA supplementation (HS or HS + GABA, respectively) ($P < 0.05$), although the degree of upregulation was significantly higher in birds reared without GABA supplementation ($P < 0.05$). In Cobb birds, neither HS nor GABA supplementation affected the expression of *leptin* ($P > 0.05$). Ross birds exhibited significantly higher expression of the *leptin* gene in the HS and HS + GABA groups compared to the corresponding Cobb groups ($P < 0.05$).

The mRNA expression of the *ghrelin* gene (Fig. 3B) was significantly altered by HS ($P = 0.001$) and GABA ($P = 0.0002$) irrespective of broiler strain ($P = 0.085$), and there were no interactions between different treatments ($P = 0.063$). The two studied broiler strains (Ross and Cobb) did not show any changes in relative *ghrelin* gene expression following GABA supplementation under normal temperatures or HS ($P > 0.05$). HS exposure in non-supplemented birds significantly upregulated *ghrelin* mRNA expression ($P < 0.05$) in Ross and Cobb. This effect was significantly attenuated by GABA supplementation in heat-stressed Ross birds ($P < 0.05$).

Relative *CCK* gene expression (Fig. 3C) was significantly modified by HS ($P = 0.005$), GABA supplementation ($P = 0.002$), broiler strain ($P = 0.033$) and interactions ($P = 0.017$). In Ross birds, HS induced distinct increase in *CCK* gene expression compared with normal temperatures ($P < 0.05$). In contrast, in Cobb birds, HS did not affect *CCK* gene expression and was significantly lower in these birds than in heat-stressed Ross birds ($P < 0.05$). GABA inclusion did not change the effects of HS on *CCK* expression in Ross birds (HS + GABA) ($P > 0.05$). In Cobb birds, GABA supplementation (N + GABA or HS + GABA) did not alter the expression levels of *CCK* ($P > 0.05$).

3.5. Gene expression levels of muscle growth and fat metabolism related genes

The aforementioned results showed that dietary GABA supplementation modified the expression levels of some FI-regulating genes,

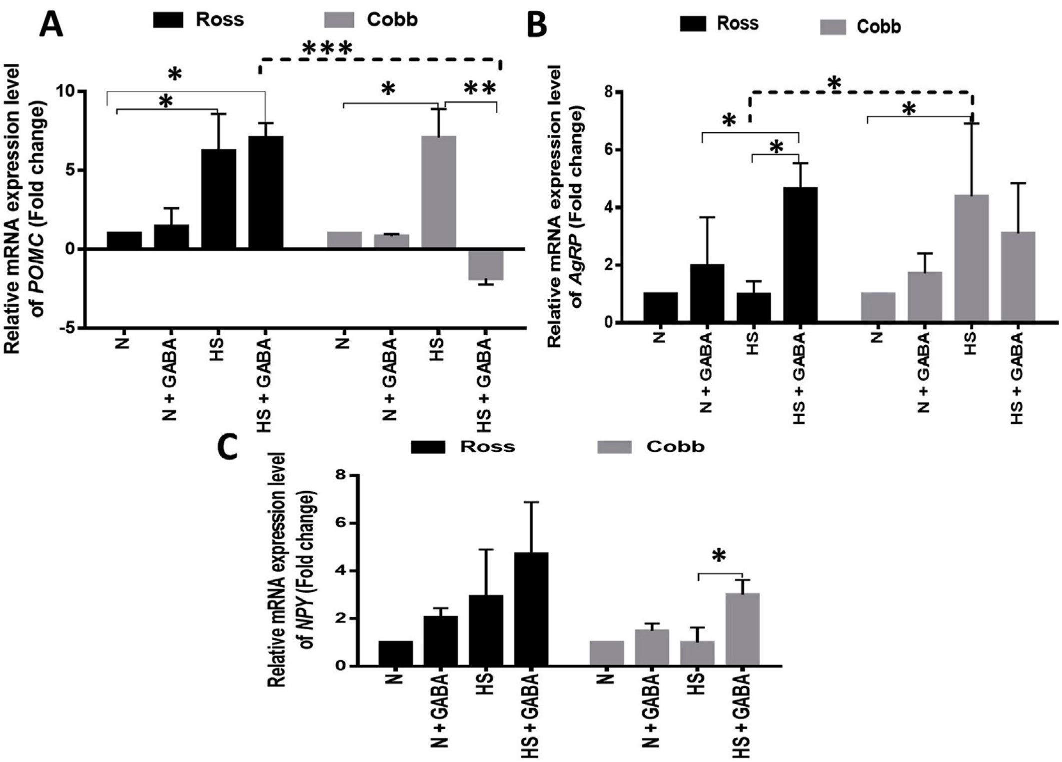

Fig. 2. The impact of GABA dietary supplementation on *POMC, AgRP* and *NPY* mRNA transcript levels in hypothalamus. mRNA transcript levels (fold changes) were assessed using qPCR for 6 birds (n = 6, 2 birds/replicate) from normally housed and HS exposed of Ross and Cobb chickens. The expression level of each gene was normalized against its control group and *β-actin* + *GAPDH*. A represents *POMC* mRNA copies, B shows *AgRP* fold change and C is for *NPY*. The (N and HS) means normally housed and heat-stressed birds, without GABA supplementation, respectively. (N + GABA and HS + GABA) refers to birds housed under normal temperature and exposed to HS, and fed diet supplemented with 100 mg GABA, respectively. Values are shown as mean ± SEM, *, ** and *** denote statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

such as *POMC, AgRP, NPY, leptin, ghrelin* and *CCK*. Moreover, GABA affected fat deposition; it resulted in significant increases in abdominal fat contents in birds under normal housing temperatures, while in those under HS, it lowered fat content in Cobb birds and increased it in Ross birds. Therefore, we investigated whether the changes in WG following GABA supplementation were due to changes in muscle or fat content by examining the expression levels of two muscle growth-regulating genes, insulin-like growth factor (*IGF*) and growth hormone receptor (*GHR*), and two fat metabolism-related genes, fatty acid synthetase (*FAS*) and acetyl-CoA carboxylase (*ACC*) (Fig. 4).

With regards to *GHR* (Fig. 4A), GABA supplementation and HS significantly modified *GHR* expression ($P < 0.001$) irrespective of broiler strain ($P = 0.077$) and without interactions ($P = 0.129$). Under normal temperatures, significant upregulation of *GHR* mRNA levels was found in Ross birds supplemented with GABA (N + GABA) ($P < 0.05$). Rearing Ross chickens under HS without GABA supplementation did not affect *GHR* expression levels ($P > 0.05$); as the expression was similar to that of normally housed chickens. Additionally, when Ross birds were supplemented with GABA and reared under HS (HS + GABA), they did not show changes in *GHR* expression levels ($P > 0.05$). Cobb birds reacted similarly, *GHR* expression was upregulated in normally housed chickens fed diets supplemented with GABA (N + GABA) ($P < 0.05$). *GHR* expression in birds subjected to HS without GABA was not different than in birds subjected to HS and GABA (HS + GABA) ($P > 0.05$). Regarding *IGF*, GABA supplementation ($P = 0.041$), HS ($P = 0.049$), broiler strain ($P < 0.001$) and interactions among factors ($P < 0.001$) significantly modified *IGF* levels (Fig. 4B). Ross birds reared under normal housing temperatures did not exhibit any changes in its levels ($P > 0.05$). However, when Ross birds were exposed to HS with or without GABA feeding (HS + GABA or HS respectively), significant upregulation was found ($P < 0.05$). On the other hand, in Cobb birds, GABA supplementation under normal temperatures and HS did not significantly change *IGF* gene expression levels ($P > 0.05$). The effects of HS alone and HS+GABA in Cobb chickens were clearly weaker than those in Ross chickens ($P < 0.05$).

The expression levels of fat metabolism related genes*, FAS* and *ACC* are presented in Fig. 4C and Fig. 4D. *FAS* was significantly modulated by HS ($P = 0.037$), GABA supplementation ($P = 0.013$), broiler strain ($P = 0.025$), and interactions ($P = 0.018$); while was ($P < 0.001$) for all these factors incase *ACC*). In Ross birds, only marked upregulation of *FAS* expression was induced by GABA supplementation under HS ($P < 0.05$). However, GABA supplementation in Cobb birds did not change *FAS* mRNA expression ($P > 0.05$). In addition, the effects of GABA supplementation on heat-stressed Ross birds were clearly higher than those in Cobb birds ($P < 0.05$). Regarding *ACC*, Ross birds exposed to HS showed distinct upregulation of *ACC* relative mRNA levels in hepatic tissue ($P < 0.05$). This effect was attenuated by GABA supplementation (HS + GABA) ($P < 0.05$). In Cobb birds, GABA supplementation under normal housing temperatures (N + GABA) stimulated significant increases in *ACC* mRNA expression ($P < 0.05$). The effects of HS alone and HS with GABA supplementation were significantly greater on Ross birds than on Cobb birds ($P = 0.003$ and $P < 0.001$, respectively).

4. Discussion

HS is one of the most challenging environmental stressors for poultry. It affects negatively the poultry welfare and production and can result in large economic losses. It impairs bird growth performance and induces immunosuppression (Attia et al., 2011; Niu et al., 2009; Sohail et al., 2012). The present study confirmed these findings; compared with birds reared under normal housing temperature, HS reduced

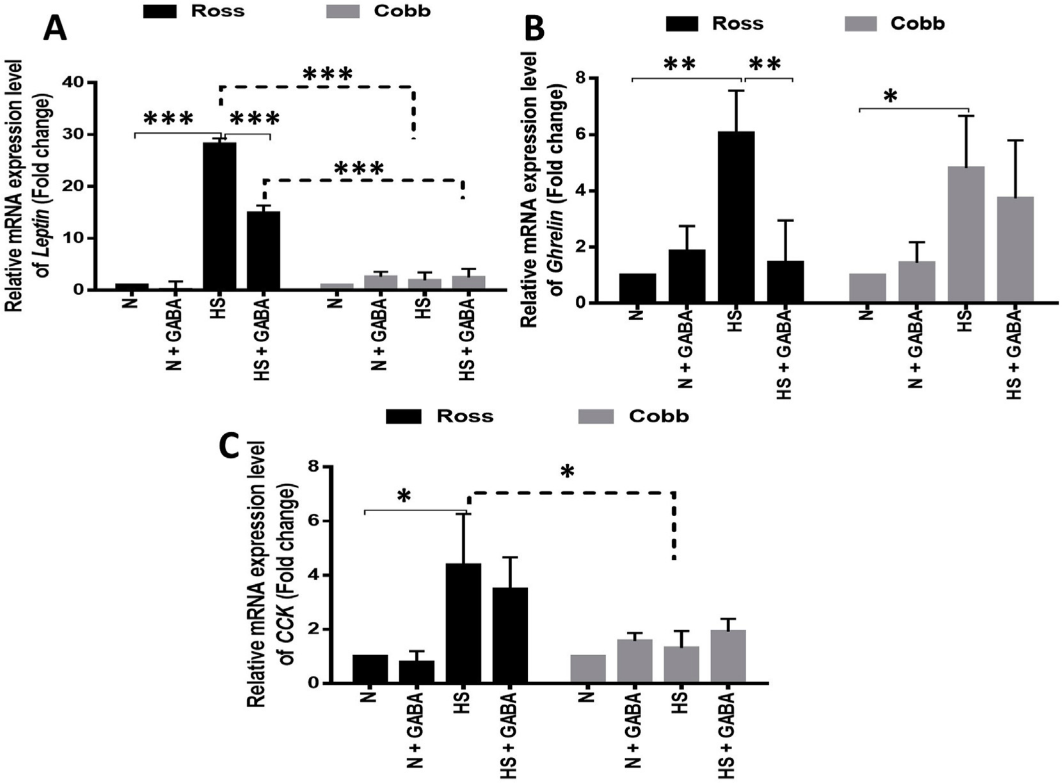

Fig. 3. Effect of dietary inclusion of GABA on relative mRNA expressions of *Leptin, Ghrelin,* and *CCK* in hepatic tissue. Expression levels were assessed using qPCR for 6 birds (n = 6, 2 birds/replicate) from normally housed and HS exposed Ross and Cobb chicken. The expression level of each gene was normalized against its control group and *β-actin* + *GAPDH*. A represents *Leptin* fold changes, B shows *Ghrelin* fold changes and C is for *CCK*. The (N and HS) means normally housed and heat-stressed birds, without GABA supplementation, respectively. (N + GABA and HS + GABA) refers to birds housed under normal temperature and exposed to HS, and fed diet supplemented with 100 mg GABA, respectively. Values are mean ± SEM, *, ** and *** denote statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

the BW of all birds and decreased FI, which consequently affected the FCR (especially in Ross birds). Under conditions of high environmental temperatures, birds attempt to achieve normothermic body temperatures by promoting heat loss to their surroundings and reducing metabolic heat production by reducing FI (Nilsson et al., 2016). Although, decreasing feed consumption alleviates the detrimental impacts of HS on bird performance (Lara and Rostagno, 2013), it also reduce bird nutrient requirements and affects its growth (Bonnet et al., 1997). In this study, dietary GABA supplementation improved the growth performance of birds reared under normal and HS conditions by increasing BW and WG, especially in Ross birds under HS. This improvement maybe attributable to the increased FI in the GABA-supplemented groups, an effect that has also been documented by others (Keun-Tae et al., 2016; Tajalli et al., 2006). As mentioned previously, dietary inclusion of GABA increased WG and FI; however, it did not noticeably affect the FCR (except in Ross birds under HS) and possibly because of the simultaneous increase in WG and FI, which occurred as a result of improved serum GABA content following GABA supplementation (Hu et al., 2016). GABA seems not to have a direct effect on the digestion and metabolism of nutrients in birds but improves performance by increasing FI (Dai et al., 2011; Hu et al., 2016). Unlike other groups supplemented with GABA, Ross birds subjected to HS and fed GABA-supplemented diet showed significant reduction in FI but, improvement in BW. This result is consistent with that of Zhigang et al. (2013), who reported improved BW despite reduced FI following GABA feeding in Cherry Valley ducks. This effect could help explain the significant improvement in FCR of this strain. The improved FCR following GABA feeding could be due to increases in jejunal villus length, crypt depth, and mucous membrane thickness, which in turn would improve nutrient absorption (Al Wakeel et al., 2017). Additionally, the higher BW in the Ross birds (RH + GABA) might be associated with the higher fat content resulting from GABA supplementation. The different responses between Cobb and Ross birds are apparently genetically-based (Abdo et al., 2017) and might be associated with individual variations in HS intensity and duration (Lara and Rostagno, 2013). Furthermore, the growth-promoting effects of GABA are significantly associated with reductions in body temperature, helping to ameliorate HS-associated effects (Dai et al., 2011; Hu et al., 2016; Zhang et al., 2012a). The reduction of bird temperature due to GABA supplementation might be associated with increases in serum GABA levels (Dai et al., 2011) which in turn reduce the production of prostaglandin (PG) especially PGE2, alleviating the HS-induced body temperature increases (Al Wakeel et al., 2017).

Serum biochemical constituents are important diagnostic tools especially for subjects under conditions of stress. Dietary GABA supplementation increased the serum concentrations of ALP, AST, ALT and glucose. These effects are similar to the results obtained by Hu et al. (2008) but are inconsistent with those of Hu et al. (2016), who detected reduced concentrations of AST, ALT and LDH and increases in the ALP enzyme activity upon GABA administration. Serum lipid profiles were also altered by GABA dietary supplementation depending on strain and temperature interactions. GABA increased cholesterol and TG and lowered LDL concentrations in birds reared under normal conditions. Similar increases in TG concentrations were shown by Zhigang et al. (2013) in Cherry Valley ducks supplemented with 100 mg GABA/kg diet. The increased TG concentrations may have been associated with the observed increases in abdominal fat content upon dietary GABA supplementation in normally housed birds. This finding is similar to that of (Dai et al., 2011) and could be explained by the increased serum TGs. Somewhat different effects were observed in birds exposed to HS

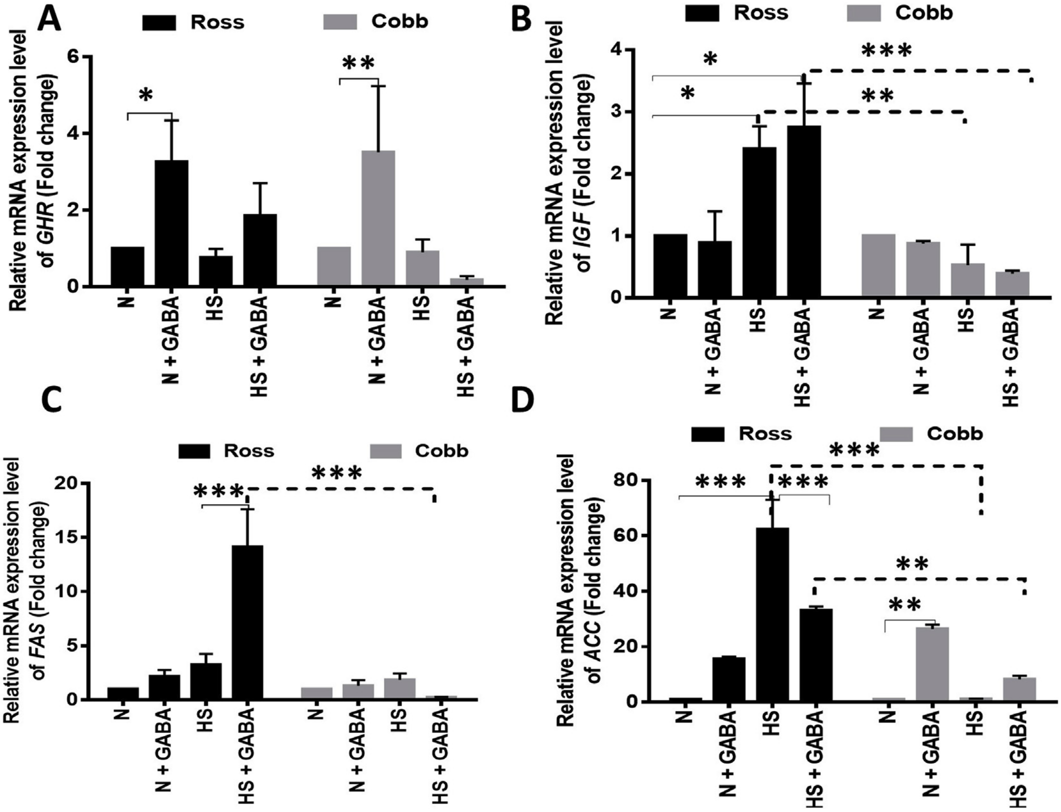

Fig. 4. Effect of dietary inclusion of GABA on relative mRNA expressions of *GHR, IGF, ACC and FAS* in the liver. Expression levels were assessed using qPCR for 6 birds (n = 6, 2 birds/replicate) from normally housed and HS exposed Ross and Cobb chicken. The expression level of each gene was normalized against its control group and *β-actin* + *GAPDH*. A represents *GHR* fold changes, B shows *IGF* fold changes, C is for *FAS* and D is for *ACC* fold changes. Values are mean ± SEM, *, ** and *** denote statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

without GABA supplementation, as cholesterol and TGs were reduced. However, Cobb birds reared under HS without GABA supplementation showed elevated abdominal fat content. This finding is consistent with that of (Zhang et al., 2012b), who found elevated fat deposition in the breast muscle of birds exposed to HS. This result might be attributable to reduced heat loss and increased energy production from ingested food, which resulted in deposition of body lipids (Geraert et al., 1996). Interestingly, GABA supplementation in heat-stressed Cobb birds reduced lipid deposition and decreased abdominal fat contents despite the increases in serum TGs. This effect may be due to increased serum levels of GABA (Hu et al., 2016), which can stimulate the turnover of fat and the release of free fat acids and glucose into serum to be available to all cells as energy sources (Dai et al., 2011). Ross birds responded differently than Cobb birds to GABA supplementation during HS, demonstrating increases in abdominal fat content. This different response is possibly due to the different genetic backgrounds between the Ross and Cobb strains (Abdo et al., 2017). In addition, it is perhaps due to the increased concentrations of TG in the GABA-supplemented groups. Based on these findings, GABA might have an important role in the metabolism of nutrients especially lipids.

To more thoroughly elucidate the mechanism by which GABA regulates FI in these birds, we assessed the effects of GABA on the gene expression levels of select satiety-associated molecules such as *NPY, AgRP, POMC*, *CCK*, *Ghrelin,* and *Leptin.* FI and energy utilization are regulated by a variety of signalling molecules produced in the central and peripheral tissues in response to nutritional changes (Wilding, 2002). In this study, the observed increases in FI in Cobb birds following GABA supplementation were associated with the regulatory effect of GABA on the gene expression levels of *POMC, AgRP* and *NPY*. Under normal temperatures, GABA did not affect the *POMC* expression. Ross birds reared under HS in the absence of GABA supplementation showed upregulation of *POMC* resulting in reduction in FI. Interestingly, in Cobb birds, GABA ameliorated the adverse effect of HS on FI and downregulated *POMC* gene expression levels. However, GABA supplementation to heat-stressed Ross birds markedly upregulated *POMC,* which could explain the reduced FI in this strain. This different response between Ross and Cobb birds is perhaps due to their genetic differences (Abdo et al., 2017). On the other hand, GABA supplementation upregulated *AgRP* mRNA levels in both strains especially in Ross birds under HS. However, the observed decreases and increases in *AgRP* levels in Ross birds and Cobb birds, respectively under HS without GABA supplementation; might explain the differences in FI responses of the two strains. Moreover, dietary inclusion of GABA stimulated higher expression levels of *NPY,* especially in Cobb birds reared under HS (CH + GABA). This finding is consistent with that of (Jonaidi and Noori, 2012) who concluded that *NPY* had a FI-stimulating effect through its action on $GABA_A$ receptors in the brain. Similarly, Wu et al. (2016) detected elevated *NPY* expression in the brain of grass carp supplemented with 50 or 100 mg GABA/kg diet. Furthermore, Li et al. (2015) suggested that increasing plasma *NPY* concentrations could be one way by which GABA enhanced the growth of weaned pigs. Furuse (2002) also, concluded that high expression of *NPY* in the brain could enhance FI in chicks. In summary, the stimulatory effect of GABA on FI is associated with increases in the expression of orexigenic *NPY/AgRP* and decreases in the expression of anorexigenic *POMC* that ultimately determine energy status and BW.

Peripherally, the *leptin* hormone regulates feed consumption by controlling the melanocortin system in the hypothalamus, stimulating melanocyte stimulating hormone (α-MSH) expression and inhibiting *AgRP* production (Dridi et al., 2005) to reduce chicken FI. While *ghrelin* inhibits FI by increasing plasma corticosterone (Saito et al., 2005; Yunianto et al., 2007). *CCK* is a polypeptide hormone secreted by the gastrointestinal mucosa that acts to reduce FI by binding to its receptors, CCKAR and CCKBR (El-Kassas et al., 2016), to activate POMC neurons and cause appetite suppression (Fan et al., 2004). In the present study, under normal temperatures, GABA failed to alter the expression levels of these three molecules. These findings are consistent with those of Wu et al. (2016), who reported that the expression levels of *CCK* and *leptin* in grass carp were not altered by dietary GABA. However, HS stimulated the expression of *leptin, ghrelin* and *CCK*, perhaps explaining the reduction in FI in HS exposed birds (especially in Cobb birds). Interestingly, GABA supplementation lowered the expression levels of these genes in heat-stressed birds (especially in Ross birds, RH + GABA). The responses of the studied genes are controlled by the intensity and duration of HS exposure; acute HS did not alter the central expression levels of *NPY, AgRP, POMC, CCK,* and *ghrelin* (Lei et al., 2013). On the other hand, the mRNA levels of *ghrelin* were significantly increased in some parts of the digestive system and those of *CCK* were increased in the duodenum, indicating that acute HS had no influence on the gene expression of central appetite-regulating peptides. The decreased FI in birds exposed to acute HS is attributable to an instant physiological response, namely, the flight or fight response; in which the birds spent more time fighting stress than feeding, resulting in decreased FI (Lei et al., 2013). On the other hand, under long-term exposure to chronic HS, reductions in FI are associated with modulation of FI regulating gene expression such as increased expression of *ghrelin* in laying hens exposed to 7 days of HS (Song et al. (2012). Therefore, the described results suggest that the role of GABA in regulating the bird FI is more prominent during HS exposure than under normal housing temperatures.

Since GABA promoted an increase in BW and modified abdominal fat content, the current study also aimed to investigate whether this increase was due to increases of muscle growth or fat deposition by examining the expression levels of select muscle-regulating and fat-metabolism associated genes. Growth hormone (GH) is the key regulator of growth rate and body composition, controlling the differentiation of muscle cells, adipocytes, and other cells required for development and growth (Kim, 2010). The activity of GH occurs through its binding to the GHR (Kita et al., 2005), which initiates hepatic synthesis and release of insulin-like growth factor-I (*IGF-I*) (Xu et al., 1995) into the circulation, stimulating cell proliferation, growth and metabolism in chickens (Scanes, 2009; Tanaka et al., 1996). Our findings showed that GABA increased *GHR* expression in Ross and Cobb birds reared under normal housing temperatures, suggesting that GABA stimulated the direct binding of GH activity to its receptor stimulating BW gain by increasing the synthesis and release of IGF. However, under HS conditions, reductions of GHR expression were observed. Interestingly, GABA supplementation during HS stimulated GHR expression in Ross birds but failed to stimulate it in Cobb birds. These results suggest that GABA inhibits the release of *IGF* in the liver of Cobb birds under HS due to inhibition of GH binding to its receptor (GHR) (Vanderpooten et al., 1991). It has been reported that circulating *IGF-1* concentrations are positively associated with BW in broiler chickens (Scanes, 2009). Moreover, Beccavin et al. (2001) reported that genotype significantly affects *IGF-I* expression, consequently influencing growth rate in broiler chickens.

Regarding the fat metabolism-related genes; *FAS* and *ACC*, dietary GABA supplementation strongly modulated their expression levels, and this effect was associated with decreased fat deposition in the abdomen of the birds. The enzymes ACC and FAS play key roles in lipogenesis (Huang et al., 2008). The enzyme ACC is involved in the conversion of acetyl-CoA into malonyl-CoA and then into palmitate; and this pathway is mediated by FAS and ends in TG formation (Richards, 2003). HS exposure activates two related energy utilization pathways: the adenosine monophosphate-activated protein kinase (AMPK) pathway and mammalian target of rapamycin (mTOR) pathway, (Richards et al., 2010). AMPK activation inhibits the action of *ACC*, preventing the production of malonyl-CoA and blocking the lipogenesis pathway as well as stimulating *Ag RP/NPY* neuropeptides resulting in increased FI (Richards et al., 2010). Unlike AMPK activation, mTOR activation induces protein synthesis, growth and proliferation and stimulates the activity of *POMC,* resulting in reduced FI and enhancing energy utilization. In the present study, GABA supplementation upregulated *ACC* in birds reared under normal temperatures (especially Cobb birds). However, Ross birds under HS only without GABA supplementation, showed elevated expression of *ACC,* and this effect was reversed in birds with GABA supplementation. The effect of GABA is probably associated with activation of AMPK, which induces *AgRP/NPY* neuropeptides, results in increased FI and reduced energy use, in turn inducing the action of the mTOR pathway. The mTOR pathway encourages synthesis of nutrients such as fatty acids, in a process mediated by *ACC* and *FAS*. *FAS* expression was also regulated by GABA supplementation. GABA upregulated *FAS* expression in Ross birds reared under HS, but, failing to induce the same effect in Cobb birds. Therefore, GABA seems to be involved in the regulation of fat metabolism.

5. Conclusion

In summary, GABA improved the growth performance of broiler chickens especially under HS by affecting BW, WG and FI through modulating of FI-regulating neuropeptides. It lowered the expression levels of FI-inhibiting neuropeptides, like *POMC*, *leptin*, *Ghrelin,* and *CCK,* during HS, whereas upregulated stimulatory ones like *AgRP* and *NPY* underneath each thermoneutral and HS conditions. The increased WG in this study could be related to the influence of GABA on lipid metabolism and might be derived from the deposition of more fat than protein. Future research addressing the nature of body composition in relation to the metabolism of different nutrients is needed.

Disclosure statement

No conflicts of interest, financial, or otherwise, are declared by the author (s).

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