

# Effects of gamma-aminobutyric acid alone and in combination with coated sodium butyrate on growth performance, meat quality and nutrient utilisation in heat-stressed broiler chickens

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**ABSTRACT.** This study investigated the effects of gamma-aminobutyric acid (GABA) and its combination with coated sodium butyrate (CSB) on growth performance, meat quality, and nutrient utilisation in heat-stressed broilers. In Trait 1, 90 newly hatched male Ross 308 broilers were randomly allocated to 3 dietary treatments for 42 days: a positive control diet (PC), PC + 0.05% (20% GABA), and PC + 0.03% (20% GABA) + 0.05% (50% CSB). In Trait 2, 120 birds were divided into 4 dietary treatment groups for 24–28 days: PC, a negative control (NC), formulated by reducing PC metabolizable energy by 100 kcal/kg, NC + 0.05% (20% GABA), and NC + 0.03% (20% GABA) + 0.05% (50% CSB). All groups were exposed to chronic heat stress in a closed house and acute heat stress during transportation. In Trait 1, GABA or its combination with CSB improved feed conversion ratio, European production efficiency index, post-transport body weight, heterophil-to-lymphocyte ratio, and thiobarbituric acid reactive substances ( $P < 0.05$ ). In Trait 2, GABA or its combination with CSB increased apparent metabolizable energy, and nitrogen-corrected metabolizable energy ( $P < 0.01$ ) compared to the NC. In conclusion, the GABA + CSB combination resulted in the highest improvement in growth performance and meat quality. GABA and its combination with CSB also increased the energy matrix value of broiler feed.

## Introduction

Stressor factors occur throughout the production cycle and include heat stress, adverse ambient conditions, overcrowding, vaccination programs, transport, noise, poor water quality, excess ammonia, and insufficient access to feed or water. When stress impairs gut function, overall performance and meat quality decline.

Gamma-aminobutyric acid (GABA) is an established safe feed additive with multiple benefits for animals. It is a four-carbon non-protein amino acid and the primary inhibitory neurotransmitter in the central nervous system. GABA is associated with the hypothalamic-pituitary-adrenal (HPA) axis of animals (Cullinan et al., 2008) and can reduce levels of epinephrine (EP), norepinephrine (NEP), and cortisol, thereby alleviating stress and

promoting relaxation. It also stimulates growth hormone and triiodothyronine (T3-H) secretion (Park and Kim, 2015; Jeong et al., 2020). Additionally, GABA supports immune function by modulating anti-inflammatory responses, antioxidant activity, and gut microbiota balance (Xie et al., 2012; Al Wakeel et al., 2017). Together, these effects contribute to improved productive performance and meat quality (Park and Kim, 2015; Jeong et al., 2020).

GABA is also present in the enteric nervous system, where it is thought to act on the peripheral nervous system via the gut-brain axis. Peripheral regulation of feed intake (FI) involves several neuropeptides, including cholecystokinin (CCK), ghrelin, leptin, and peptide YY, that control appetite through physiological function of the digestive tract and send response signals to the central nervous system. Li et al. (2023) reported that GABA can increase FI under heat stress by reducing mRNA expression levels of FI-inhibiting neuropeptides, such as pro-opiomelanocortin (POMC), leptin, ghrelin, and CCK, while enhancing the expression of FI-stimulating neuropeptides like agouti-related protein (AgRP) and neuropeptide Y (NPY).

Butyric acid, a short-chain fatty acid, serves as a primary energy source for body cells. When coated with sodium butyrate (CSB), it becomes relatively stable and is slowly released, ensuring targeted effect on the entire gut. Butyric acid supports colonic epithelial cell mucosa, which is the most important energy source, and increases the surface of nutrient absorption, including amino acids, glucose, fatty acids, calcium, and minerals (Zhao et al., 2021). Butyrate also selectively promotes the growth of beneficial microbiota and helps maintain microbial balance in the gastrointestinal tract through its antibacterial properties, while also partially attenuating inflammatory responses (Jiang et al., 2015). Previous research has confirmed that supplementing poultry feed with butyric acid improves gut health, nutrient digestibility, and overall growth performance in birds (Mazur-Kuśnerek et al., 2024).

In our previous studies, we found that adding GABA to the diet of commercial broiler chickens along with CSB resulted in a significant improvement in growth performance. Based on these findings, we conducted a novel study to evaluate the effects of dietary supplementation with GABA and its combination on meat quality and nutrient utilisation in heat-stressed broiler chickens.

## Material and methods

This experiment, including all protocols (U1-02538-2559) involving animals were approved and reviewed by the Animal Care & Use Committee of the Faculty of Agriculture Innovation and Technology Ethics Committee, Rajamangala University of Technology Isan, Thailand.

### Birds, diets and housing

In Trait 1, 90 newly hatched male Ross 308 broiler chicks were allocated to three treatments, each consisting of six pens with five birds per pen (group weighing). A practical maize-soy bean meal (SBM)-based diet positive control (PC) was formulated as the basal diet for all growing phases, starter (days 0–10), grower (days 10–24), and finisher (days 24–42). The test products, 20% GABA (SynRelax product by Zhumadian Huazhong Chia Tai Co., Ltd, Zhumadian, HA, China) and 50% SB (InduceAcid-buty product by Zhumadian Huazhong Chia Tai Co., Ltd), were added to the basal diets according to the treatment design. The three treatments were as follows: T1: control diet (CON, practical maize-SBM diet), T2: CON + 0.05% (20% GABA), and T3: CON + 0.03% (20% GABA) + 0.05% (50% CSB). The basal diet was formulated based on Ross (2019) nutrient recommendations. Test products were added to the diet during mash mixing at the specified inclusion levels (Table 1). All feeds were pelleted at a conditioning temperature of 82 °C (range 80–84 °C), with a pellet diameter of 3 mm. Crumbled pellets were administered during the first 10 days.

Each pen was equipped with a tubular feeder and two nipple water drinkers, allowing *ad libitum* access to feed and water. Lighting and management programs followed the Ross 308 broiler management manual. All birds were vaccinated for Newcastle and infectious Bronchitis diseases at 7 days and Gumboro disease at 14 days of age. Other management practices adhered to the Ross 308 manual.

The experiments were conducted in a closed-sided house with concrete floor pens and rice hull bedding. House temperature was maintained according to bird age: 20–30 °C with 60% relative humidity (RH) from day 0 to 28, and 23–25 °C with 60–70% RH from day 28 to 42 under normal ambient conditions. Chronic heat stress was applied from day 28 to 42 by exposing birds to 33 ± 1 °C and 60% RH for 3 h daily (12:00–15:00).

Trait 2, a separate group of birds was reared in floor pens and fed practical diets from hatch until 21 days of age. On day 21, 120 birds with uniform body weight were selected for a digestibility trial and allocated to 24 metabolic cages, 5 birds per cage. Four diets were prepared with GABA and CSB supplementation according to the treatment design: T1: positive control (PC, practical maize-SBM diet); T2: negative control (NC, 100 kcal/kg lower ME than T1); T3: NC + 0.05% (20% GABA) and T4: NC + 0.03% (20% GABA) + 0.05% (50% CSB) (Table 2). All diets were prepared in a pelleted form at 80–84 °C, with a pellet diameter of 3 mm. Birds were weighed on day 24 and exposed to chronic heat stress at 33 ± 1 °C and 60% RH for 3 h daily (12:00 to 15:00) in the metabolic cages for five consecutive days (days 24–28).

### Growth performance and meat quality (Trait 1)

Body weight and feed consumption were recorded on a pen basis at the beginning and end of each feeding phase. Dead or culled birds were weighed, and the cause of death or culling was documented. Final body weight (FBW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and livability were calculated for 0–10, 10–24, 24–42, and 0–42 days of age periods. The European production efficiency index (EPEI) was calculated using the following equation (Euribrid, 1994):

$$\text{EPEI (\%)} = \frac{\text{body weight (kg)} \times \text{livability (\%)}}{\text{age (days)} \times \text{feed conversion ratio}} \times 100.$$

On day 42, all birds were fasted for 6 h before body weight measurement. From each treatment group, 15 birds (45 birds total) were randomly selected and weighed individually. Birds from each pen were placed in cages (53 × 72 × 31 cm), five birds per cage, and nine cages were assigned to each treatment. All cages were transported by truck for 1 h (approximately 50 km). During transport, the temperature in the vehicle was 37–38 °C, and the outside temperature was 36–37 °C, exposing the birds to acute heat stress. After transport, birds were individually re-weighed by treatment group, and then selected for carcass evaluation. Before slaughter, 10 birds from each treatment were selected for blood sample collection from the jugular veins. A complete blood count (CBC) was performed, including red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and haematocrit (Hct). Differential WBC composition (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were also determined. All parameters were mea-

sured using an MS9-5V automatic blood cell counter (Melet Schloesing Laboratoires, Osny, France). After blood sample collection, birds were slaughtered for carcass trait evaluation (dressing carcass, breast meat, thigh, drumstick, wing, and abdominal fat). Breast and thigh samples were collected from 15 birds per treatment. Breast meat samples were analysed for meat pH using a calibrated, WTW pH340-A glass-electrode pH meter (WTH Measurement Systems Inc., Fort Myers, FL, USA). Drip loss was measured on about 2 g of breast sample using the plastic bag method described by Honikel (1998). Thigh meat samples were analysed for fat oxidation using the thiobarbituric acid reactive substances (TBARS) method, as described by Witte et al. (1970). TBARS values were expressed as mg of malondialdehyde (MDA) per kg of muscle, with extraction performed using a 20% (w/v) trichloroacetic acid solution.

### AME and nutrient retention (Trait 2)

The four treatment diets were randomly assigned to 24 cages. Following a three-day adjustment period to the cage and feed, all feeders were removed and excreta trays were cleaned. The remaining feed in each feeder was weighed to mark the start of a 96-h excreta collection period (days 25–28).

During this collection period, BWG and total FI for birds in each cage were recorded. All wet excreta from each cage were collected daily and immediately stored at –20 °C. After the four-day period, the excreta samples from each cage were pooled, dried at 80 °C for 24 h, and subsequently weighed and ground. The test diets and dried excreta samples were analysed for dry matter (DM) content (105 °C, 4 h), gross energy using an isoperibol bomb calorimeter (Leco model AC-350), and crude protein via the Kjeldahl method, following AOAC International (2000) procedures. BWG, FI, and mortality over the 4-day collection period were also recorded. The apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) of the test diets were calculated using the equations provided by Abdollahi et al. (2021):

$$\begin{aligned} \text{AME (kcal/kg)} &= \cdot \\ &= \frac{(\text{GE feed} \times \text{g feed consumed}) - (\text{GE excreta} \times \text{g excreta})}{(\text{g feed consumed})} \end{aligned}$$

where: GE – gross energy, NR represents nitrogen retention, assumed as 20% of body weight gain or loss divided by 6.25, and K is the constant equal to 8.21 kcal/g nitrogen retention:

$$\text{AMEn (kcal/kg)} = \frac{((\text{GE feed} \times \text{feed consumed}) - (\text{GE excreta} \times \text{excreta}) - (\text{NR} \times \text{K}))}{(\text{feed consumed})}$$

where: GE – gross energy, NR represents nitrogen retention, assumed as 20% of body weight gain or loss divided by 6.25, and K is the constant equal to 8.21 kcal/g nitrogen retention.

### Statistical analysis

The data were analysed using analysis of variance (ANOVA) in the SAS software (SAS Institute Inc., Cary, NC, USA). Trait 1 was arranged in a completely randomised design (CRD), and Trait 2 was arranged in a randomised complete block design (RCBD). Significant differences between treatment means were separated using Duncan's multiple range test at a 5% significance level.

## Results

### Growth performance, carcass and meat quality (Trait 1)

The performance of birds fed the three experimental diets over the 42-day period remained within the expected range for birds receiving practical maize-SBM diets (Table 1). Nutritional analysis of the feed mixtures using AOAC methods did not show

any significant differences compared to the tabulated values (data not shown). As shown in Table 3, during phases I (days 0–10), II (days 10–24), and III (days 24–42), broilers fed GABA or GABA + CSB diets had higher BWG, lower FI (phase III), and significantly improved FCR compared to the control group ( $P < 0.05$ ). Overall, GABA supplementation alone or combined with CSB increased BWG and significantly improved post-transport BW, FCR, and EPEI relative to the control ( $P < 0.05$ ). The effect in the GABA + CSB group was slightly stronger than in the group receiving GABA alone. Although not statistically significant, livability was slightly higher in the GABA and GABA + CSB groups, with mortality of 7% (two birds) in the control group. Body weight loss after 1-h transport from farm to slaughterhouse tended to be lower in birds receiving feed supplemented with GABA or the combination with CSB compared to the control group (Table 4).

No significant differences were observed in most blood parameters studied between the groups receiving GABA or its combination with CSB. Interestingly, GABA supplementation reduced heterophil (H) counts and increased lymphocyte (L) counts, resulting in a significantly lower H/L ratio compared to the control group ( $P < 0.05$ ; Table 5).

**Table 1.** Composition and calculated nutrient content of basal diets (Trait1)

Ingredient	Starter (0–10 days)			Grower (10–24 days)			Finisher (24–42 days)		
	CON	GABA	combination	CON	GABA	combination	CON	GABA	combination
Corn 8%	53.814	53.764	53.734	52.399	52.349	52.319	57.549	57.499	57.469
SBM (dehulled) 48%	33.225	33.225	33.225	32.083	32.083	32.083	24.997	24.997	24.997
Full fat soybean	6	6	6	8	8	8	10.000	10.000	10.000
Soybean oil	1.493	1.493	1.493	2.612	2.612	2.612	2.943	2.943	2.943
MDCP	2.173	2.173	2.173	1.915	1.915	1.915	1.685	1.685	1.685
Limestone	1.207	1.207	1.207	1.077	1.077	1.077	0.983	0.983	0.983
Salt	0.285	0.285	0.285	0.289	0.289	0.289	0.294	0.294	0.294
Broiler vitamin/mineral premix	0.2	0.2	0.2	0.2	0.2	0.2	0.200	0.200	0.200
DL-methionine	0.346	0.346	0.346	0.306	0.306	0.306	0.277	0.277	0.277
L-lysine HCl	0.243	0.243	0.243	0.196	0.196	0.196	0.185	0.185	0.185
L-threonine	0.143	0.143	0.143	0.092	0.092	0.092	0.065	0.065	0.065
L-valine	0.041	0.041	0.041	0.015	0.015	0.015	0.010	0.010	0.010
L-arginine	0.005	0.005	0.005						
L-isoleucine	0.014	0.014	0.014	0.042	0.042	0.042	0.037	0.037	0.037
Sodium bicarbonate	0.165	0.165	0.165	0.168	0.168	0.168	0.161	0.161	0.161
Choline chloride 60%	0.096	0.096	0.096	0.055	0.055	0.055	0.061	0.061	0.061
Pellet binder	0.05	0.05	0.05	0.3	0.3	0.3	0.300	0.300	0.300
Antimold	0.3	0.3	0.3	0.2	0.2	0.2	0.200	0.200	0.200
Coccidiostat* (12% Salinomycin)	0.2	0.2	0.2	0.05	0.05	0.05	0.05	0.05	0.05
GABA (20%)		0.05	0.03		0.05	0.03		0.05	0.03
CSB (50%)			0.05			0.05			0.05
Total	100	100	100	100	100	100	100	100	100

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Table 1. continued

Ingredient	Starter (0–10 days)			Grower (10–24 days)			Finisher (24–42 days)		
	CON	GABA	combination	CON	GABA	combination	CON	GABA	combination
Nutrient composition, %									
weight	1	1	1	1	1	1	1	1	1
dry matter	88.8	88.8	88.8	88.79	88.79	88.79	88.79	88.79	88.79
ME for poultry	3000	3000	3000	3100	3100	3100	3100	3 100	3 100
crude protein	23	23	23	21.5	21.5	21.5	21.5	21.5	21.5
crude fat	5.06	5.06	5.06	6.56	6.56	6.56	6.56	6.56	6.56
linoleic acid	2.54	2.54	2.54	3.27	3.27	3.27	3.27	3.27	3.27
crude fiber	3.42	3.42	3.42	3.53	3.53	3.53	3.53	3.53	3.53
ash	6.36	6.36	6.36	6.13	6.13	6.13	6.13	6.13	6.13
digestible lysine (poultry)	1.28	1.28	1.28	1.15	1.15	1.15	1.15	1.15	1.15
digestible methionine (poultry)	0.655	0.655	0.655	0.593	0.593	0.593	0.593	0.593	0.593
digestible cysteine (poultry)	0.295	0.295	0.295	0.277	0.277	0.277	0.277	0.277	0.277
digestible methionine + cysteine (poultry)	0.95	0.95	0.95	0.87	0.87	0.87	0.87	0.87	0.87
digestible threonine (poultry)	0.86	0.86	0.86	0.77	0.77	0.77	0.77	0.77	0.77
digestible tryptophane (poultry)	0.245	0.245	0.245	0.211	0.211	0.211	0.211	0.211	0.211
digestible arginine (poultry)	1.37	1.37	1.37	1.276	1.276	1.276	1.276	1.276	1.276
digestible valine (poultry)	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.87
digestible isoleucine (poultry)	0.86	0.86	0.86	0.78	0.78	0.78	0.78	0.78	0.78
digestible leucine (poultry)	1.675	1.675	1.675	1.549	1.549	1.549	1.549	1.549	1.549
lysine	1.45	1.45	1.45	1.326	1.326	1.326	1.326	1.326	1.326
arginine	1.537	1.537	1.537	1.434	1.434	1.434	1.434	1.434	1.434
methionine	0.692	0.692	0.692	0.632	0.632	0.632	0.632	0.632	0.632
methionine + cysteine	1.08	1.08	1.08	0.991	0.991	0.991	0.991	0.991	0.991
cystine	0.387	0.387	0.387	0.359	0.359	0.359	0.359	0.359	0.359
threonine	1.006	1.006	1.006	0.909	0.909	0.909	0.909	0.909	0.909
tryptophan	0.279	0.279	0.279	0.256	0.256	0.256	0.256	0.256	0.256
glycine + serine	1.938	1.938	1.938	1.886	1.886	1.886	1.886	1.886	1.886
histidine	0.614	0.614	0.614	0.577	0.577	0.577	0.577	0.577	0.577
isoleucine	0.992	0.992	0.992	0.96	0.96	0.96	0.96	0.96	0.96
leucine	1.903	1.903	1.903	1.786	1.786	1.786	1.786	1.786	1.786
valine	1.119	1.119	1.119	1.027	1.027	1.027	1.027	1.027	1.027
phenylalanine	1.083	1.083	1.083	1.042	1.042	1.042	1.042	1.042	1.042
calcium	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.87
phosphorus-total	0.826	0.826	0.826	0.771	0.771	0.771	0.771	0.771	0.771
phosphorus-available	0.48	0.48	0.48	0.435	0.435	0.435	0.435	0.435	0.435
potassium	0.97	0.97	0.97	0.9	0.9	0.9	0.9	0.9	0.9
choline	1700	1700	1700	1600	1600	1600	1600	1600	1600
sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
chloride	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
DEB	253	253	253	235	235	235	235	235	235

SBM – soybean meal, MDPCP – monocalcium phosphate, DEB – dietary electrolyte balance; CON – control, GABA – gamma-aminobutyric acid, CSB – coated sodium butyrate, combination – GABA + CSB; nutrition specifications calculate from Ross (2019); raw materials profile: INRA – international research associates (2023) and NRC – nutrient requirements of poultry (1994) for metabolizable energy (ME); \* withdrawal during 35–42 days

Carcass traits were not significantly affected by GABA or GABA + CSB supplementation, although trends toward higher dressing percentage, breast, and thigh yield were observed (Table 6). Supplementation with GABA or GABA + CSB tended to reduce breast meat drip loss after 1 and 7 days of storage at 4 °C, and significantly decreased fat

oxidation in the thigh, as measured by the TBARS method ( $P < 0.05$ ) (Table 7).

### AME and nutrient retention (Trait 2)

Performances of birds fed the four experimental diets over the 4-day collection period were within the normal ranges for birds fed practical diets

**Table 2.** Composition and calculated nutrient content of experimental diets (Trait 2)

Ingredient	Experimental diets (Grower, 21–28 days)			
	PC	NC	NC + GABA	NC + combination
Corn 8%	52.399	54.323	54.273	54.243
SBM (dehulled) 48%	32.083	31.719	31.719	31.719
Full fat soybean	8	8	8	8
Soybean oil	2.612	1.047	1.047	1.047
MDCP	1.915	1.913	1.913	1.913
Limestone	1.077	1.08	1.08	1.08
Salt	0.289	0.287	0.287	0.287
Broiler vitamin/mineral premix	0.2	0.2	0.2	0.2
DL-methionine	0.306	0.303	0.303	0.303
L-lysine HCl	0.196	0.202	0.202	0.202
L-threonine	0.092	0.092	0.092	0.092
L-valine	0.015	0.015	0.015	0.015
L-arginine				
L-isoleucine	0.042	0.043	0.043	0.043
Sodium bicarbonate	0.168	0.17	0.17	0.17
Choline chloride 60%	0.055	0.055	0.055	0.055
Pellet binder	0.3	0.3	0.3	0.3
Antimold	0.2	0.2	0.2	0.2
Coccidiostat* (Salinomycin 12%)	0.05	0.05	0.05	0.05
GABA (20%)			0.05	–
CSB (50%)			0.03	0.05
Total	100	100	100	100
Nutrient composition, %				
weight	1	1	1	1
dry matter	88.79	88.61	88.61	88.61
ME for poultry	3100	3000	3000	3000
crude protein	21.5	21.5	21.5	21.5
crude fat	6.56	5.06	5.06	5.06
linoleic acid	3.27	2.51	2.51	2.51
crude fiber	3.53	3.55	3.55	3.55
ash	6.13	6.13	6.13	6.13
digestible lysine (poultry)	1.15	1.15	1.15	1.15
digestible methionine (poultry)	0.593	0.591	0.591	0.591
digestible cysteine (poultry)	0.277	0.279	0.279	0.279
digestible methionine + cysteine (poultry)	0.87	0.87	0.87	0.87
digestible threonine (poultry)	0.77	0.77	0.77	0.77
digestible tryptophane (poultry)	0.211	0.21	0.21	0.21
digestible arginine (poultry)	1.276	1.272	1.272	1.272
digestible valine (poultry)	0.87	0.87	0.87	0.87
digestible isoleucine (poultry)	0.78	0.78	0.78	0.78
digestible leucine (poultry)	1.549	1.556	1.556	1.556
lysine	1.326	1.325	1.325	1.325
arginine	1.434	1.43	1.43	1.43
methionine	0.632	0.631	0.631	0.631
methionine + cysteine	0.991	0.991	0.991	0.991
cystine	0.359	0.36	0.36	0.36
threonine	0.909	0.908	0.908	0.908
tryptophan	0.256	0.255	0.255	0.255

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Table 2 continued)

Ingredient	Experimental diets (Grower, 21–28 days)			
	PC	NC	NC + GABA	NC + combination
glycine + serine	1.886	1.885	1.885	1.885
histidine	0.577	0.577	0.577	0.577
isoleucine	0.96	0.958	0.958	0.958
leucine	1.786	1.793	1.793	1.793
valine	1.027	1.026	1.026	1.026
calcium	0.87	0.87	0.87	0.87
phosphorus-total	0.771	0.773	0.773	0.773
phosphorus-available	0.435	0.435	0.435	0.435
potassium	0.9	0.9	0.9	0.9
choline	1600	1600	1600	1600
sodium	0.16	0.16	0.16	0.16
chloride	0.23	0.23	0.23	0.23
DEB	235	235	235	235

SBM – soybean meal, MDCP – monocalcium phosphate, DEB – dietary electrolyte balance; CON – control, GABA – gamma-aminobutyric acid, CSB – coated sodium butyrate, combination – GABA + CSB; nutrition specifications calculated from Ross (2019); raw materials profile: INRA – international research associates (2023) and NRC – nutrient requirements of poultry (1994) for metabolizable energy (ME); \* withdrawal during 35–42 days

Table 3. Effects of GABA and combination on growth performance in broiler chicken

Item	CON	GABA	combination	SEM	<i>P</i> -value
Body weight gain, g					
day 0–10 (Phase I)	196 <sup>b</sup>	216 <sup>a</sup>	216 <sup>a</sup>	10.96	0.04
10–24 (Phase II)	883 <sup>b</sup>	978 <sup>a</sup>	977 <sup>a</sup>	15.34	0.02
24–42 (Phase III)	1967	1972	1989	20.04	0.78
0–42 (Overall)	3046	3166	3182	33.24	0.22
Feed intake, g					
day 0–10 (Phase I)	212 <sup>c</sup>	232 <sup>a</sup>	229 <sup>b</sup>	10.56	0.02
10–24 (Phase II)	1062 <sup>b</sup>	1182 <sup>a</sup>	1173 <sup>a</sup>	18.71	0.02
24–42 (Phase III)	3293 <sup>a</sup>	3176 <sup>b</sup>	3165 <sup>b</sup>	30.72	0.03
0–42 (Overall)	4567	4590	4567	40.42	0.97
FCR					
day 0–10 (Phase I)	1.079 <sup>a</sup>	1.073 <sup>a</sup>	1.058 <sup>b</sup>	0.00	0.02
10–24 (Phase II)	1.202 <sup>ab</sup>	1.208 <sup>a</sup>	1.200 <sup>b</sup>	0.00	0.02
24–42 (Phase III)	1.674 <sup>a</sup>	1.610 <sup>b</sup>	1.591 <sup>b</sup>	0.02	0.02
0–42 (Overall)	1.499 <sup>a</sup>	1.450 <sup>b</sup>	1.435 <sup>b</sup>	0.01	0.03
Livability, %					
day 0–10 (Phase I)	100	100	100	0.00	
10–24 (Phase II)	100	100	100	0.00	
24–42 (Phase III)	93	100	100	2.24	0.10
0–42 (Overall)	93	100	100	2.24	0.10
EPEI					
day 0–42 d (Overall)	465 <sup>b</sup>	526 <sup>a</sup>	534 <sup>a</sup>	17.42	0.04

FCR – feed conversion ratio, EPEI – European production efficiency index, CON – control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; data are presented as means ± SEM (standard error of the mean) (n = 6); <sup>ab</sup> – means with different superscripts are significantly different at *P* < 0.05

with the formulated dietary energy levels (Table 2). Analysis of the feed mixtures by AOAC methods showed no significant deviation from the tabulated nutrient values (data not shown). The effects of GABA and its combination with CSB on AME and protein retention (PR) are shown in Table 8. The AME (on DM basis) for the PC diet, NC diet, NC + GABA diet, and NC + GABA + CSB were 3191, 3071, 3164, and

3152 kcal/kg (*P* < 0.05), respectively. Supplementation with GABA or GABA + CSB significantly improved the ayAMEn of the low-energy NC diet compared with NC alone, without affecting growth performance from days 24 to 28 compared to PC (*P* > 0.05; Table 9). The improvements were estimated at 93 for GABA and 81 kcal/kg for GABA + CSB. PR was minimally affected by supplementation.

**Table 4.** GABA and combination on body weight change (loss) after transportation in broiler chickens day 42

Item	CON	GABA	combination	SEM	P-value
Body weight (before), g	3066 <sup>b</sup>	3187 <sup>a</sup>	3214 <sup>a</sup>	17.61	0.03
Body weight (after), g	2993 <sup>b</sup>	3135 <sup>a</sup>	3166 <sup>a</sup>	18.92	0.01
Body weight loss, g	73	52	48	5.86	0.17
Body weight loss, %	2.42	1.62	1.48	0.68	0.11

CON – control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; birds were placed in cages, 5 birds/cage, and transported by a truck for 1 h (about 50 km); data are presented as means  $\pm$  SEM (standard error of the mean) (n = 15); <sup>ab</sup> – means with different superscripts are significantly different at  $P < 0.05$

**Table 5.** Effect of GABA and combination supplementation on blood parameters in broiler chickens day 42

Item	CON	GABA	Combination	SEM	P-value
RBC $\times 10^6$ cells/ $\mu$ l	2.43	2.47	2.43	0.03	0.78
Haemoglobin, g/dl	14.75	15.03	15.23	0.20	0.72
Haematocrit, %	31.50	32.90	31.40	0.40	0.23
WBC, $\times 10^3$ cells/mm <sup>3</sup>	11.64	12.44	12.29	0.55	0.33
H $\times 10^3$ cells/mm <sup>3</sup>	4.37	4.05	4.13	0.18	0.10
Basophil, $\times 10^3$ cells/mm <sup>3</sup>	0.19	0.20	0.19	0.03	0.76
Eosinophil, $\times 10^3$ cells/mm <sup>3</sup>	0.12	0.15	0.05	0.04	0.12
L $\times 10^3$ cells/mm <sup>3</sup>	6.80	7.85	7.65	0.13	0.09
Monocyte, $\times 10^3$ cells/mm <sup>3</sup>	0.16	0.19	0.27	0.02	0.14
H/L ratio	0.64 <sup>a</sup>	0.52 <sup>b</sup>	0.54 <sup>b</sup>	0.02	0.05

RBC – red blood cell, WBC – white blood cell, H/L – heterophil/lymphocyte, CON – control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; data are presented as means  $\pm$  SEM (standard error of the mean) (n = 10); <sup>ab</sup> – means with different superscripts are significantly different at  $P < 0.05$

**Table 6.** Effect of GABA and combination supplementation on carcass quality in broiler chickens day 42

Item*	CON	GABA	Combination	SEM	P-value
Dressing, %	4.37	4.25	4.93	0.18	0.25
Breast meat, %	0.19	0.18	0.19	0.03	0.76
Thigh, %	0.12	0.15	0.05	0.28	0.12
Drum stick, %	6.80	6.85	7.65	0.28	0.39
Wing, %	0.16	0.19	0.27	0.02	0.14
Abdominal fat	64.3	62.08	64.58	1.86	0.12

CON – control, GABA – gamma-aminobutyric acid, Combination – GABA + coated sodium butyrate, \* carcass traits as % of carcass weight; data are presented as means  $\pm$  SEM (standard error of the mean) (n = 15);  $P > 0.05$  – not statistically significant

**Table 7.** Effect of GABA and combination supplementation on meat quality in broiler chickens day 42

Item	CON	GABA	combination	SEM	P-value
Meat pH	6.3	6.28	6.34	0.03	0.69
Drip loss					
1 d, %	3.25	2.92	2.59	0.15	0.21
7 d, %	6.58	6.25	5.81	0.24	0.43
TBARS, mg/kg	2.25 <sup>a</sup>	1.96 <sup>ab</sup>	1.66 <sup>b</sup>	0.08	0.01

TBARS – thiobarbituric acid reactive substances; CON – control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; data are presented as means  $\pm$  SEM (standard error of the mean) (n = 15); <sup>ab</sup> – means with different superscripts are significantly different at  $P < 0.05$

**Table 8.** Effect of GABA and combination supplementation on performance in broiler chickens days 24–28

Item	PC	NC	NC + GABA	NC + combination	SEM	P-value
Initial body weight, g	1052	1084	1075	1080	4.82	0.07
Final body weight, g	1594	1617	1628	1644	8.03	2.30
Body weight gain, g	542	533	553	563	5.87	0.17
Feed intake, g	609	620	627	643	5.48	4.07
FCR	1.12	1.16	1.14	1.14	0.01	0.17
Livability, %	100	100	100	100		

FCR – feed conversion ratio; PC – positive control, NC – negative control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; data are presented as means  $\pm$  SEM (standard error of the mean) (n = 6);  $P > 0.05$  – not statistically significant

**Table 9.** Effect of GABA and combination supplementation on dietary apparent metabolizable energy and protein retention in broiler chickens days 24–28

Item	PC	NC	NC + GABA	NC + combination	SEM	P-value
AME as fed, kcal/kg	3040 <sup>a</sup>	2919 <sup>b</sup>	3020 <sup>a</sup>	3037 <sup>a</sup>	12.96	0.00
AME as dry, kcal/kg	3457 <sup>a</sup>	3329 <sup>b</sup>	3427 <sup>a</sup>	3411 <sup>a</sup>	13.25	0.00
AMEn as fed, kcal/kg	2806 <sup>a</sup>	2693 <sup>b</sup>	2789 <sup>a</sup>	2806 <sup>a</sup>	12.59	0.00
AMEn as dry, kcal/kg	3191 <sup>a</sup>	3071 <sup>b</sup>	3164 <sup>a</sup>	3152 <sup>a</sup>	12.91	0.00
PR as fed, %	54.19	54.40	55.78	55.32	0.50	0.66
PR as dry, %	61.62	62.04	63.29	62.14	0.56	0.78

AME – apparent metabolizable energy, AMEn – apparent metabolizable energy, nitrogen-corrected, PR – protein retention, PC – positive control, NC – negative control, GABA – gamma-aminobutyric acid, combination – GABA + coated sodium butyrate; data are presented as means  $\pm$  SEM (standard error of the mean) (n = 6); <sup>ab</sup> – means with different superscripts are significantly different at  $P < 0.05$

## Discussion

Based on the findings of the current study (Trait 1), the improved performance observed with dietary GABA may be due to its previously reported effects on neuroendocrine-mediated nutrient metabolism (Zhang et al., 2012). GABA supplementation in the diets of broiler hens has been shown to reduce stress, regulate appetite, and improve nutrient utilisation (Chen et al., 2014). In the present study, GABA or GABA combined with CSB significantly improved growth performance and reduced TBARS in broiler meat under heat-stress conditions, indicating enhanced oxidative stability. Lipid oxidation is the main process leading to meat quality deterioration (Smet et al., 2008). Oxidative stress is caused by an imbalance between the production of reactive species and the natural antioxidant capacity of cells to eliminate them (Scandalios, 2005). The present findings support the protective role of GABA in limiting heat-stress-related damage, likely through increased antioxidant enzyme activity. Higher antioxidant enzyme activity reduces lipid peroxidation, which ultimately lowers MDA content (Zhu et al., 2015). We also examined the effect of heat stress (HS) on blood physiological parameters. Chronic HS exposure decreased haemoglobin concentration and increased the H/L ratio, well-recognised stress indicators in poultry. Under stress, the number of H increases and L decreases, resulting in a higher H/L ratio. This effect may be attributed to GABA-mediated reduction in glucocorticoid levels, which promotes rapid release of heterophils from bone marrow into circulation (Fathi et al., 2023). This mechanism likely contributed to the lower H/L ratio observed during transport stress in this study.

Supplementing the GABA with CSB in the diet improved BWG by 16 g/bird, FCR by 2 points, and EPEI by 8 points compared to GABA alone. Several studies have shown that butyric acid does not

significantly affect feed consumption, while significant improvements in FCR and BWG were noted in birds receiving organic acids, indicating better absorption and nutrient utilisation than in birds on the control diet (Kaczmarek et al., 2016). CSB also increased energy digestibility because of a larger absorptive surface area related to better gastrointestinal epithelium development and activation of protein receptors that stimulate transporter expression within enterocytes, ultimately affecting FCR (Liu et al., 2019). This support of intestinal health has been shown to reduce pathogenic bacteria and improve villus morphology, which benefits gut function, digestion, and growth performance in broilers (Lum et al., 2018; Deepa et al., 2020). Moreover, our research has shown that combining GABA with CSB tended to reduce body weight loss after transportation by 7.6%, with a subsequent reduction in drip loss by 11% (1 day) and 7% (7 days), and TBARS by 15% compared to GABA alone. The addition of CSB to GABA may improve antioxidant status, as butyrate shows strong antioxidant activity both *in vivo* and *in vitro* (Russo et al., 2012). We assessed intestinal antioxidant activity by monitoring several antioxidant-related enzymes. Superoxide dismutase (SOD) and catalase (CAT) activities are considered the first line of defence in scavenging free radicals to protect cells from oxidative damage caused by stress. Miao et al. (2022) reported that short-chain fatty acids (SCFAs) including CSB, positively affected intestinal antioxidant capacity, as measured by SOD, CAT, total antioxidant capacity (T-AOC), and interleukin-10 (IL-10), and were inversely associated with pro-inflammatory cytokines. Therefore, the observed changes in antioxidant indices and cytokines can be attributed to higher SCFA production, which would support gut immunity and function. This mechanism explains the improved growth performance and meat quality observed in the current study.

In Trait 2, the data indicated that diets containing GABA or its combination with CSB improved nutrient utilisation in broilers under heat-stress conditions. GABA intake may reduce stress by lowering the levels of cortisol and certain neurotransmitters, thus limiting cell damage in the digestive tract, and supporting healthy gut microflora and immune function (Wen et al., 2021). Previous studies have demonstrated that heat stress can inhibit digestive enzyme activity, reduce nutrient absorption and mucosal immune function. GABA can mitigate these effects and protect the intestinal mucosa, thereby improving nutrient absorption (Chen et al., 2014; 2015). Other studies have demonstrated that GABA supplementation can also help promote the secretion of growth hormones and regulate thyroid-stimulating hormone, which in turn may influence appetite and improve nutrient utilisation (Fan et al., 2007; Dai et al., 2011; Zhang et al., 2012). Additionally, GABA have been shown to exert antioxidant effects by reducing oxygen-free radical levels (Chen et al., 2014). These antistress and antioxidant effects likely contributed to the improvement in AMEn without any loss of broiler performance. However, protein retention did not differ from the control and GABA groups. The observed effect could be attributed to elevated serum GABA levels, which may increase the breakdown of fat and carbohydrates by digestive enzymes and the release of free fatty acids and glucose into the bloodstream, making them available as energy substrates (Dai et al., 2011; Chen et al., 2015). These findings suggest that GABA may have an important role in nutrient metabolism, especially energy.

In this study, supplementing GABA or its combination with CSB did not affect energy use (AMEn). It is possible that adding GABA at 100 ppm (0.05% of a 20% GABA diet) or 60 ppm (0.03% of a 20% GABA diet) together with 250 ppm (0.05% of a 50% CSB diet) provides similar energy utilisation efficiency. CSB may also support colonocyte energy supply, stimulate villus development, support antioxidant activity, and maintain gut health thereby helping sustain a balanced microflora and immune function (Ahsan et al., 2016; Wu et al., 2018; Deepa et al., 2020). These effects may have contributed to improved growth performance and meat quality in broilers exposed to chronic and acute heat stress in Trait 1.

## Conclusions

The study concluded that supplementing diets with gamma-aminobutyric acid (GABA) or its combination with coated sodium butyrate in

heat-stressed broiler chickens improved growth performance and nitrogen-corrected apparent metabolizable energy. The combination of these supplements provided better performance and meat quality compared to GABA alone. Implementing this approach may offer economic benefits for the animal nutrition sector, including feed mills, farms, and broiler slaughterhouses.

## Conflict of interest

The Authors declare that there is no conflict of interest.

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