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Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status

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To improve the quality of poultry meat and increase the health-promoting properties, poultry nutrition used additives such as phytobiotic substances and amino acids. The aim of this study was to analyze the possibility of improving production rates and meat quality by simultaneously supplementing broiler diets with garlic extract and β-alanine. A total of 1050 ROSS 308 broiler chickens were part of the experiment. The chickens were divided into several groups: the control group without additives (Control), groups with 0.5% garlic extract (G05) or 2% garlic extract (G2), groups with 0.5% added β-alanine (B0.5) or 2% added β-alanine (B2), and groups with both

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0.5% added garlic extract and 0.5% added β-alanine (BG0.5) or 2% garlic extract and 2% added β-alanine (BG2). Each group was further divided into six replicates, with each replicate consisting of 25 birds. After 35 days of rearing, the chickens were slaughtered, and analyses were conducted on breast and leg muscle chemistry, bioactive peptide content, and the oxidative status indicator dimalondialdehyde in muscles stored under refrigeration until day 10.The results showed significant improvements in certain aspects. The BG05 group exhibited an increase in final body weight (P<0.001) and improved feed utilization (P<0.001). The β-alanine-supplemented groups showed higher levels of protein (P<0.001), carnosine (P<0.001), and anserine (P<0.001) in both breast and leg muscles. Additionally, leg muscles showed increased levels of protein (P<0.001), carnosine (P<0.001),and anserine (P<0.001).Notably, the BG05 group contained lower levels of MDA in both breast and leg muscles (P<0.001).

KEY WORDS: broiler / meat / β-alanine / carnosine / garlic extract

Poultry meat is one of the most frequently chosen types of meat by consumers [Kralik *et al.* 2018]. Over the past 20 years, global meat production has increased by 47%, reaching 109 million tons, while poultry meat production has increased by 32% reaching 158 million tons [FAO, 2020]. This demand is expected to increase relatively with the growth of the human population. In 2018, global poultry meat consumption was 14.3 kg/person, and in Europe, it was 26.8 kg/person [Stanciu 2020]. It is estimated that poultry meat consumption could increase by 50% by 2050 [Henchion *et al.*2021]. The high consumption of poultry meat is influenced by its easy availability on the market, low price, easily digestible protein content, as well as the absence of cultural and religious contraindications [Kralik *et al.*2018, Zotte *et al.*2020].

Poultry meat is a valued animal product, not only for its nutritional value but also for its ease and speed of thermal processing [Juniper and Rymer 2018]. Beyond being a rich source of protein, fat, vitamins, and micro and macro elements, it contains biologically active peptides [Kralik *et al.* 2018, Petracci 2022]. These peptides are usually composed of a varying number of amino acids (from 2 to 20), which have many important functions in the human body. Notably, carnosine (β-alanyl-1-histidine) and anserine(β-alanyl-1-methyl-1-histidine) are significant biologically active dipeptides. Carnosine is composed of β-alanine and L-histidine, while anserine is a methyl derivative of carnosine. Carnosine is involved in the chelation of metal ions, pH buffering, complexing of dangerous carbonyl compounds, scavenging free radicals, exhibiting antiglycation and antioxidant activity, prolonging cell lifespan, and improving physical fitness [Xing *et al.* 2019, Lackner *et al.* 2021]. In addition, it shows protective properties for the body and inhibits the development of neurodegenerative diseases such as Alzheimer's and Parkinson's, often associated with aging. Additionally, carnosine reduces the tendency for tumorigenesis and reduces the risk of complications associated with type 2 diabetes [Lackner *et al.*2021; Suwanvichanee *et al.*2022].To increase the health-promoting qualities of chicken meat, it is possible to increase the content of the aforementioned peptides. The levels of carnosine can be achieved mainly by supplementing the chicken's ration with the substrate amino acids β-alanine and L-histidine [Qi *et al.*2018, Suwanvichanee *et al.* 2022]. Adding β-alanine not only increases the linear content of carnosine and β-alanine in chicken

muscles but also decreases the presence of 3,4-methylenedioxyamphetamine (MDA), one of the main markers of oxidative stress. At a 0.5% concentration, it also increases the proportion of breast and leg muscles [Qi *et al.* 2018].

Nowadays, consumers not only prefer meat enriched with health-promoting substances but also pay close attention to the welfare and feeding practices of the birds. There is a growing preference for animal-derived products obtained without the use of antibiotics and other pharmaceutical agents. To meet this growing demand and adhere to the ban on the preventive use of antibiotics, manufacturers are increasingly using herbal additives. These herbs contain active substances that show positive effects on animal health, production performance, and meat quality. Specifically, they serve as a source of phytobiotic substances with antioxidant properties and immune-boosting benefits [Przysiecki *et al*. 2010, Lukanov *et al.* 2015, Aarti and Khusro 2020, Santoso *et al.* 2020, Jakubowska and Karamucki 2021, Yeung *et al*. 2021abc, 2022, Abdel-Azeem and Abd El-Kader 2022].

Among the commonly used herbal additives, garlic, onion, thyme, and oregano stand out [Alfaig *et al.* 2014, Lukanov *et al.* 2015, Huminiecki *et al*. 2017, Yeung *et al*. 2021].The main constituent of garlic isallicin (diallylthiosulfinate), which is a non protein amino acid. Additionally, garlic is a rich source of various other substances such as alline (S-allylcysteinesulfoxide), allylmethanesulfinate, diallyldisulfide, diallyltrisulfide, allylmethyltrisulfide, S-allylmercaptocysteine, ajoene, and S-allylcysteine. These substances, owing to their sulfur atom binding, exhibit many properties, such as antioxidants, antimicrobial, antimutagenic, anticancer, anti-inflammatory, antiatherosclerotic, antidiabetic, and immune stimulating properties [Tadeusiewicz *et al.* 2014, Chen *et al.* 2021].

In a study conducted by Hossain *et al*. [2014], it was observed that the addition of 0.5%, 1%, and 2% garlic extract to the diet of broiler chickens resulted in an increase in body weight (BW) and a decrease in the feed conversion ratio (FCR) of the birds. Furthermore, Elmowalid *et al*. [2019] suggest that meat from chickens whose diets were supplemented with garlic extract may have a positive impact on consumer health. Such meat consumption might offer protection against antibiotic residues or toxic antibiotic metabolites and could reduce the risk of infection by bacterial pathogens [Elmowalid *et al.* 2019, Chen *et al.* 2021].

However, despite these findings, the synergistic effect of garlic extract and β-alanine on the production performance and quality of fresh and cold-stored poultry meat has not been thoroughly analyzed. Given the wide spectrum of actions exhibited by the biologically active compounds of garlic and the function of β-alanine in muscle tissue synthesis, it was hypothesized that their use as dietary supplements for broilers could have a positive effect on the growth, feed conversion, slaughter performance, and muscle quality of these birds.

The aim of the study was to analyze the possibility of improving production rates and meat quality in broiler chickens by simultaneously supplementing their diets with garlic extract and β-alanine.

Material and methods

Diets and experimental design

All experimental procedures involving broiler chickens were approved by the Third Local Ethics Committee on Animal Experimentation in Warsaw. The experiment involved 1050 one-day-old male ROSS 308 chicks and was conducted at the experimental farm of Warsaw University of Life Sciences (SGGW) – RZD Wilanów-Obory. The chicks' initial BWwas measured on day 0, and they were then divided into seven groups, each consisting of 150 chicks. These groups were further subdivided into six replicates, with each replicate comprising 25 birds. The chicks were fed a maize-wheat-soybean diet *ad libitum*, following a three-stage feeding system: starter from 0 to 16 days, grower from 16 to 28 days, and finisher from 28 to 35 days. The nutrient composition of the basic experimental diets for different growing phases is presented in Table 1. Chickens were divided into the following groups: without additives (control group – C), with the addition of 0.5% garlic extract (G05), 2% garlic extract (G2), 0.5% β-alanine supplement (B0.5), 2% β-alanine supplement (B2), 0.5% garlic extract and 0.5% β-alanine supplement (BG0.5), 2% garlic extract and 2% β-alanine supplement (BG2). The β-Ala was purchased from OstroVit sp. z.o.o. company. The garlic extract used in the experiment was purchased from BELLACO (Warsaw, Poland).

Tabele 1. Nutrient composition of basic experimental diets in different growing phases

Item		Starter	Grower	Finisher
Crude protein	(%)	22.1	22.1	18.7
Crude fiber	(%)	2.8	2.7	2.75
Crude fat	(%)	4.62	6.79	6.82
Crude ash	(%)	5.31	4.45	3.71
Lysine	(%)	1.35	1.21	1.1
Methionine	(%)	0.64	0.55	0.49
Calcium	(%)	0.78	0.6	0.43
Sodium	(%)	0.14	0.13	0.13
Phosphorus	(%)	0.55	0.46	0.37
Vitamin E (DL-alpha tocopherol)	Mg/kg	68.25	38.67	27.3
Vitamin A (retinol octane)	Jm.kg	1500	1000	1000
Vitamin D3	Jm.kg	5000	2800	2000
Iron (III) sulphate monohydrate (Fe)	mg/kg	60	50	48
Manganese sulphate (III) monohydrate (Mn)	mg/kg	100	80	72
Copper (III) sulphate pentahydrate (Cu)	mg/kg	20	18	6
Zinc sulphate monohydrate (Zn)	mg/kg	100	70	64
Granulated calcium iodate	mg/kg	1	1	0.8
Coated. granulated sodium selenite (Se)	mg/kg	0.35	0.35	0.28
Coccidiostat Salinomycin sodium salt	mg/kg	70	70	0

The chickens were kept according to the flock management manual for ROSS 308 [Aviagen 2019] on permeate straw pellets.

Growth performance

The body weight of individual chicks was determined individually (±1.0 g) at the time of insertion (0 D). Subsequently, the health status of the birds was checked daily. The feed consumption (FI) and mortality rates were continuously monitored and recorded.

The BW of the birds was measured at specific intervals, coinciding with feed ration changes, on days 16, 28, and 35. The data collected during the experiment, which included feed consumption and BW measurements of the birds, allowed the determination of the FCR, expressed as feed:gain, kg:kg. The FCR was calculated and corrected for mortality.

Slaughtering and sampling

On day 35 of the experiment, six birds were selected from each group, with an average BW representative of the respective group. These birds were chosen, one from each replication. After 8 h of starving, the chickens were slaughtered. The slaughter process involved electrical stunning followed by decapitation. Subsequently, the birds were plucked and eviscerated. The resulting carcasses were then subjected to the oviposition cooling method for 24 h at a temperature of 4°C.

Dissection of the carcasses was carried out according to the methodology described by Ziołecki and Doruchowski [1989]. The slaughter yield, which included the muscle content and giblet content (stomach, liver, and heart) relative to the carcass weight, was calculated.

Individual breast and leg muscles were weighed, labeled, preserved, and stored under refrigeration for further analysis. The collected muscles were homogenized, i.e., they were minced twice in a meat grinder with 3 mm holes and thoroughly mixed to ensure a consistent sample. The pH of the prepared sample was measured, and a chemical composition analysis was performed.

Subsequently, 20-g samples were placed in polyethylene (PE) film string bags measuring 100×150 mm and tightly closed. These samples were then stored under refrigeration at a temperature of 2.2 ± 0.3°C. On days 1, 3, 5, 7, and 10 of refrigerated storage, analyses were conducted to determine the levels of MDA and dipeptides in the samples.

pH

The pH value of the meat samples was determined according to PN-ISO 2917:2001 standard, using a CP-411 pH meter (Elmetron, Zabrze, Poland) using a glass-calomel electrode. Before measurement, the electrode was calibrated using pH 4.0 and 7.0 buffers. To ensure consistent and reliable results, three pH measurements were conducted for each meat sample, and the average of these three measurements was then calculated.

Chemical composition

The basic chemical composition of samples from breast and leg meat was determined with a Food Scan™ analyzer [Foss Electric, Hillerød, Denmark].

Indicator of redox state (MDA)

Ten serum samples, each measuring 250 µl, were utilized to determine the malondialdehyde (MDA) level according to the method proposed by Kapusta *et al*.

[2018]. In each sample, 25 µl of 0.2% 2,6-bis(1,1-dimethyl)-4-methylphenol (BHT) in ethanol and 1 ml of 5% trichloroacetic acid (aqueous, TCA, Merck, Warsaw, Poland) were added and mixed by vortexing. Subsequently, the samples were centrifuged at 14,000×g for 10 min, and 750 µl of the supernatant was transferred to a glass tube. To this, 500 µl of 0.6% aqueous thiobarbituric acid (Merck) was added, mixed, and incubated in a water bath at 90°C for 45 min. The resulting supernatants were then stored under cool conditions and centrifuged again at 4000×g for 5 min. Next, 100 µl of the clear supernatant was transferred into a microplate, and MDA concentration was determined at a wavelength of 532 nm using Tecan's NanoQuant Infinite M200 PRO analyzer (Tecan Austria GmBH, Grödig, Austria). Each sample was analyzed three times on days 1, 3, 5, 7, and 10 during the refrigerated storage of the muscle samples. The results were expressed as mM MDA/g of meat.

Dipeptide content

The levels of bioactive peptides, namely carnosine and anserine [Łukasiewicz *et al.* 2015], as well as Q10 and taurine [Purchas *et al.*2004] in the muscles, were determined using reverse-phase high-performance liquid chromatography (RP-HPLC) with an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) and a Jupiter C18 300A column (Phenomenex, Torrance, CA, USA).

The mobile phase A comprised acetonitrile and water (30:70) with 0.1% TFA acid (both reagents by Merck), while phase B comprised a mixture of acetonitrile and water (70:30) with 0.1% TFA. The flow rate through the column was set at 1.4 ml/min., and detection was performed at a wavelength of 214 nm. The injection volume of the final solution was 25 µl, and all samples were analyzed in duplicate. To confirm the identification of peaks, standards (Sigma-Aldrich, St. Louis, MO, USA) were performed.

The analyses were conducted on days 1, 3, 5, 7, and 10 during the refrigerated storage of the muscle samples.

Statistical analysis

The effect of the studied factors was evaluated using analysis of variance (ANOVA) unless the variable did not follow a normal distribution, in which case the Kruskal-Wallis test was used. The normality of the data was determined through theShapiro-Wilk test.

For multiple comparisons between treatment groups or between different storage periods, one-way ANOVA was applied based on the following models:

$$Y_{ij} = \mu + A_j + e_{ij} \text{ or } Y_{jk} = \mu + B_k + e_{ik}$$

Two-way ANOVA was applied for the evaluation of the effect of treatment and storage period as well as its interaction according to the following model:

$$Y_{ijk} = \mu + A_j \text{ or } B_k + [AB]_{jk} + e_{ijk}$$

where Y is a dependent variable; µ is the general mean; A_j is the effect of the treatment;

B_k is the effect of the storage period. The results of two-way ANOVA were presented as *P*-values.

To compare the means, Duncan's multiple range test was employed, and groups of means with no significant differences were identified using successive letters of the alphabet. Standard errors of the means [SEM] were presented as measures of variability.

The statistical analyses were conducted using Statistica 13 [TIBCO, 2017] software, and the significance level for all analyses was set at 0.05.

Results and discussion

Effect of diet

Table 2 shows the impact of the dietary inclusion of garlic extract and β-alanine on the growth performance, feed intake, and feed conversion ratio of broiler chickens during the experiment.The applied supplementation significantly affected BW (P<0.001), BWG (P<0.001), FI (P<0.001), and FCR (P<0.001). Specifically, the supplementation of 2%

Table 2. Effects (means±pooled SEM) of chicken dietary inclusion of garlic extract and β-alanine on growth performance, feed intake and feed conversion ratio

Parameter		Treatments							Pooled SEM	*P*-value
		Control	G05	B05	BG05	G2	B2	BG2		
					Starter (day 1-16)					
IBW[1]	kg	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.001	0.774
BWG[2](1)	kg	0.636^{a}	0.618^{ab}	0.632^{b}	0.605^{a}	0.601^{a}	0.597^{a}	0.594^{a}	0.025	0.012
FI[3](1)	kg	0.754^{e}	0.673^{a}	0.725^{cd}	0.708^{bc}	0.701^{b}	0.741^{de}	0.696^{b}	0.072	<0.001
FCR(1)	kg/kg	1.19^{c}	1.12^{a}	1.15^{ab}	1.18^{b}	1.17^{b}	1.25^{c}	1.18^{b}	0.009	<0.001
Mortality(1)	%	4.0	6.0	4.0	4.0	4.7	4.7	4.0	0.183	0.320
					Grower (day 17-28)					
BWG(2)	kg	1.17^{cd}	1.22^{d}	1.21^{d}	1.14^{c}	1.20^{d}	1.03^{a}	1.08^{b}	0.026	<0.001
FI(2)	kg	1.71^{c}	1.76^{c}	1.72^{c}	1.63^{ab}	1.68^{b}	1.51^{a}	1.55^{a}	0.064	<0.001
FCR[4](2)	kg/kg	1.45^{b}	1.44^{ab}	1.43^{a}	1.43^{a}	1.40^{a}	1.46^{b}	1.44^{ab}	0.011	<0.001
Mortality(2)	%	0	0	0	0	0.7	0.7	0.7	0.100	0.423
					Finisher (day 29-35)					
BWG(3)	kg	0.899^{a}	0.956^{b}	0.989^{b}	1.01^{c}	1.05^{c}	1.04^{c}	0.967^{b}	0.029	0.006
FI(3)	kg	1.60^{a}	1.70^{b}	1.74^{bc}	1.72^{b}	1.78^{c}	1.75^{c}	1.70^{b}	0.021	0.380
FCR(3)	kg/kg	1.78^{b}	1.78^{b}	1.76^{b}	1.70^{a}	1.70^{a}	1.68^{a}	1.76^{b}	0.020	<0.001
Mortality(3)	%	1.4	1.4	1.4	1.4	0.7	1.4	2.1	0.314	0.996
					Overall (day 1-35)					
BW[5](4)	kg	2.78^{ab}	2.84^{bc}	2.90^{d}	2.85^{cd}	2.90^{d}	2.74^{a}	2.72^{a}	0.033	<0.001
BWG(4)	kg	2.74^{ab}	2.80^{bc}	2.86^{d}	2.81^{cd}	2.86^{d}	2.70^{a}	2.68^{a}	0.053	<0.001
FI(4)	kg	4.05^{b}	4.13^{c}	4.19^{c}	4.06^{b}	4.16^{c}	4.01^{b}	3.95^{a}	0.058	<0.001
FCR*(4)	kg/kg	1.48^{b}	1.48^{b}	1.47^{ab}	1.44^{a}	1.45^{a}	1.48^{b}	1.47^{ab}	0.008	<0.001
Mortality[6](4)	%	5.3	6.7	5.3	5.3	6.7	6	6	0.387	0.731

[a-e]Means within a row with different letter in superscript differs significantly at P<0.05, data represented mean values of 6 replication per treatment.

Control, commercial basal feed; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

[1]IBW, initial/hatching body weight; [2]BWG, body weight gain; [3]FI, feed intake; [4]FCR, feed conversion ratio (kg diet/kg BW) were *Cumulative FCR for 35days; [5]BW, final (35days old) body weight; [6]cumulative mortality for 35 days.

Table 3. Mean values and pooled SEM for the calculated slaughter traits of the chicken fed with garlic extract and β-alanine

Parameter		Treatments							*P*-value	Pooled SEM
		Control	G05	B05	BG05	G2	B2	BG2		
BW	G	2796.5^{abc}	2850.0^{cd}	2936.5^{e}	2825.5^{bcd}	2899.7^{de}	2715.3^{a}	2756.2^{ab}	<0.001	14.78
Carcass	g	1987.7^{ab}	2049.5^{bc}	2075.2^{bc}	2035.3^{bc}	2093.2^{c}	1908.0^{a}	1940.7^{a}	<0.001	14.29
Carcass yield	g/100g BW	71.09	71.91	70.68	72.02	72.19	70.27	70.40	0.345	0.29
Breast		32.09	32.05	33.20	32.40	32.09	32.87	32.05	0.856	0.26
Legs		19.81^{ab}	21.14^{bc}	21.10^{bc}	21.44^{c}	20.66^{bc}	20.65^{bc}	19.20^{a}	0.010	0.19
Gizzard		0.69^{bc}	0.60^{ab}	0.75^{c}	0.65^{ab}	0.60^{ab}	0.64^{ab}	0.58^{a}	0.009	0.014
Heart		0.42	0.54	0.48	0.53	0.46	0.52	0.48	0.244	0.014
Liver		2.12	2.20	2.42	2.33	2.32	2.11	2.27	0.412	0.042
Fat		0.87	1.14	0.77	0.79	0.99	0.84	0.85	0.279	0.044
Offal Total		56.09^{ab}	57.67^{bc}	58.71^{c}	58.14^{bc}	57.12^{abc}	57.62^{bc}	55.43^{a}	0.020	0.29

$^{a-c}$ Different letters in the column indicate differences between treatment groups in a particular storage period, P ≤ 0.05 (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

Table 4. Chemical composition of chicken breast meat

Muscle	Parameter	Group							P-value	SEM
		Control	G05	B05	BG05	G2	B2	BG2		
Brest	protein	22.20^{a}	22.34^{ab}	22.42^{b}	22.52^{b}	22.51^{b}	22.83^{c}	22.80^{c}	<0.001	0.040
	moisture	74.46	73.85	74.08	73.91	73.87	74.21	74.15	0.621	0.096
	lipids	2.67	2.98	2.88	2.46	2.88	2.36	2.54	0.172	0.074
	collagen	0.93	1.10	0.81	1.02	0.98	0.75	1.00	0.094	0.035
Legs	protein	19.96^{a}	20.23^{abc}	20.33^{abc}	20.42^{c}	20.03^{ab}	20.50^{c}	20.37^{bc}	0.025	0.052
	moisture	71.09^{ab}	70.94^{a}	71.89^{bc}	71.70^{abc}	71.22^{ab}	71.37^{abc}	72.10^{c}	0.020	0.108
	lipids	7.86^{c}	7.72^{bc}	7.00^{a}	6.94^{a}	8.04^{c}	7.10^{ab}	6.79^{a}	0.001	0.107
	collagen	1.27	1.27	1.33	1.19	1.18	1.18	1.08	0.107	0.024

$^{a-c}$ Different letters in the column indicate differences between treatment groups in a particular storage period, P ≤ 0.05 (one-way ANOVA, Duncan test); Contol, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

garlic extract (G2) and 0.5% β-alanine (B05) influenced the highest BW compared to the control group (P<0.001).Throughout the experiment, chickens in groups B05, BG05, and G2 achieved the highest BWG (*P*<0.05). The BG05 group had the lowest FCR (*P*<0.05]. However, there was no significant effect of supplementation on chick mortality during the whole experimental period (1-35 days) (*P*=0.731).

Table 3 shows the slaughter traits of chickens fed with garlic extract and β-alanine. The applied supplementation had a significant effect on BW (P<0.001), carcass (P<0.001), leg muscle content (*P*=0.010), gizzard (*P*=0.009), and Offal Total (*P*=0.020). However, no statistically significant differences were observed in

carcass yield (*P*=0.345) and the proportion of breast muscle (*P*=0.856), heart content (*P*=0.244), liver content (*P*=0.412), and fat (*P*=0.279).

Table 4 shows the chemical composition of the breast and leg muscles. The diet used had a significant effect on the protein content of both the breast (P<0.001) and leg muscles (*P*=0.025). The highest protein levels in chicken breast muscles were found in groups B2 and BG2 (P<0.001), while in leg muscles, the highest protein levels were found in groups BG05 and B2 (*P*=0.025). However, there was no significant effect of garlic extract and β-alanine supplementation on water content (*P*=0.621), fat (*P*=0.172), and collagen (*P*=0.094) in breast muscles. In leg muscles, protein levels were highest in the B2 and BG05 groups (*P*=0.025).

Table 5 shows the pH values 24 h after slaughter in breast and leg muscles. The applied diet had a significant effect on the pH value in both breast (P<0.001) and leg muscles (*P*=0.004). In the breast muscles, the level of supplements used in the B2 and BG2 groups affected the pH value (P<0.001).

Tables 6 and 7 show the content of bioactive peptides (g/100 g of meat) and MDA levels (mM/g of meat) in broiler breast meat (Tab. 6) and leg meat (Tab. 7) concerning the storage time and the diet supplemented with garlic extract and β-alanine. The applied diet significantly affected the content of carnosine (P<0.001), anserine (P<0.001), taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001) in both muscle types (Tab. 6 and 7).

On the first day of storage, the breast muscles in the BG2 group showed the highest level of carnosine, which differed from the B2, BG05, and B05 groups (P<0.05). Additionally, MDA levels were higher in fresh chicken breast muscles from groups BG2 and B2 compared to groups G05 and B05 (P<0.05).

Table 5. Changes in broiler legs and breast meat pH in relation to diet supplemented with garlic extract and β-alanine

Parameter	Muscle	Group							P-value	SEM
		Control	G05	B05	BG05	G2	B2	BG2		
Legs	B	5.807^{bc}	5.832^{cd}	5.811^{bc}	5.899^{d}	5.808^{bc}	5.724^{ab}	5.706^{a}	<0.001	0.014
	L	6.022^{abc}	6.137^{c}	5.931^{a}	6.112^{bc}	6.106^{bc}	6.016^{ab}	6.024^{abc}	0.004	0.016

[a-d] Different letters in the column indicate differences between treatment groups in a particular storage period, P≤0.05 (one-way ANOVA, Duncan test); L – leg muscle, B – breast muscle, Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

Effect of storage time

The analysis revealed that the storage time had a significant effect on the content of anserine (*P*=0.008), taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001) in breast muscles. However, no significant effect of storage time was found for carnosine content in breast muscles (*P*=0.537).

In leg muscles, the storage time had a significant effect on the content of taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001). However, no significant effect of

Table 6. Bioactive peptides (g/100 g of meat) and oxidative status (mM/1 g of meat) in broiler breast meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine

Storage time (day)	Treatment	Item				
		Carnosine	Anserine	Taurine	Q10	MDA
	Control	64.7^{aA}	110.0	200.0	3.24	2.22^{abA}
	G05	71.0^{bB}	109.8^{B}	198.3^{B}	3.22^{B}	2.10^{aA}
	B05	73.8^{bc}	112.1^{A}	200.4^{B}	3.25^{B}	2.11^{aA}
1	BG05	74.8^{bc}	111.7	200.1^{B}	3.24^{B}	2.14^{abA}
	G2	69.9^{ab}	109.5^{AB}	197.9	3.21	2.17^{abA}
	B2	77.9^{c}	112.9	201.2^{BC}	3.26^{BC}	2.28^{bA}
	BG2	85.3^{dB}	112.2	200.5^{B}	3.25^{B}	2.30^{bA}
	Control	66.6^{aAB}	110.3	200.3	3.25	2.47^{cB}
	G05	68.5^{aAB}	109.7^{B}	198.2^{B}	3.22^{B}	2.26^{aB}
	B05	73.9^{bc}	112.2^{A}	200.5^{B}	3.25^{B}	2.28^{abB}
3	BG05	74.0^{bc}	111.5	199.9^{B}	3.24^{B}	2.29^{abB}
	G2	70.0^{ab}	109.6^{AB}	198.0	3.22	2.27^{aA}
	B2	77.8^{c}	112.5	200.8^{BC}	3.25^{BC}	2.42^{cB}
	BG2	82.9^{dAB}	112.2	200.5^{B}	3.25^{B}	2.41^{bcAB}
	Control	69.1^{abAB}	109.3^{ab}	202.5^{b}	3.24^{b}	2.55^{bBC}
	G05	67.8^{aAB}	107.8^{abAB}	196.3^{aAB}	3.19^{abAB}	2.30^{aB}
	B05	74.1^{bc}	110.9^{abA}	196.1^{aA}	3.19^{abA}	2.49^{bC}
5	BG05	74.1^{bc}	108.9^{ab}	197.4^{abAB}	3.21^{abAB}	2.42^{abC}
	G2	66.8^{a}	106.1^{aA}	194.6^{a}	3.17^{a}	2.33^{aA}
	B2	79.2^{c}	111.0^{ab}	196.1^{aA}	3.19^{abA}	2.47^{bB}
	BG2	79.0^{cA}	114.3^{b}	194.5^{aA}	3.17^{aA}	2.49^{bAB}
	Control	69.5^{aB}	111.6^{ab}	199.9^{ab}	3.24^{a}	2.64^{bC}
	G05	69.4^{aAB}	108.6^{aB}	198.0^{aB}	3.23^{aB}	2.64^{bC}
	B05	75.8^{b}	115.9^{bB}	205.8^{cC}	3.32^{bC}	2.57^{bC}
7	BG05	76.5^{b}	113.0^{ab}	200.5^{abB}	3.25^{aB}	2.61^{bD}
	G2	69.2^{a}	112.0^{abB}	198.8^{ab}	3.23^{a}	2.35^{aA}
	B2	77.4^{bc}	114.4^{b}	202.7^{bcC}	3.28^{abC}	2.70^{bC}
	BG2	81.5^{cAB}	115.5^{b}	206.7^{cC}	3.33^{bC}	2.56^{bB}
	Control	69.7^{bB}	109.4^{b}	197.8^{c}	3.21^{c}	2.90^{abcD}
	G05	67.7^{aA}	102.6^{aA}	191.3^{aA}	3.12^{aA}	2.83^{abD}
	B05	75.9^{c}	114.2^{bAB}	202.5^{dB}	3.28^{dB}	2.98^{bcdD}
10	BG05	76.2^{c}	110.9^{b}	196.1^{cbA}	3.19^{cbA}	2.75^{aE}
	G2	69.9^{b}	110.1^{bAB}	198.6^{c}	3.22^{c}	2.76^{aB}
	B2	78.4^{c}	112.5^{b}	197.6^{cAB}	3.21^{cAB}	3.06^{cdD}
	BG2	79.1^{cA}	112.5^{b}	194.3^{bA}	3.16^{bA}	3.14^{dC}
Pooled SEM		0.43	0.32	0.32	0.004	0.020
				P-value		
Effects of treatment		<0.001	<0.001	<0.001	<0.001	<0.001
Effects of storage time		0.537	0.008	<0.001	<0.001	<0.001
Effects of storage time× treatment		0.044	0.778	0.002	0.007	<0.001

[a-d]Different letters in the columnin dicate differences between treatment groups in a particular storage period, $P \leq 0.05$; [A,B,C,D,E]- capital letters indicate significant differences between storage times within the same treatment, $P \leq 0.05$ (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

storage time was found for the content of anserine (P=0.918) and taurine (P=0.119) in leg muscles.

Table 7. Bioactive peptides and oxidative status (g/100 g of meat) in broiler legs meat in relation to the time of storage and diet supplemeted with garlic extract and β-alanine

Storage time (day)	Treatment	Item				
		Carnosine	Anserine	Taurine	Q10	MDA
	Control	28.8^{ab}	68.4^{a}	143.2^{aA}	2.34^{aAB}	2.10^{abA}
	G05	28.5^{ab}	70.9^{abAB}	147.8^{bB}	2.40^{bB}	2.02^{aA}
	B05	31.8^{abc}	76.7^{cdB}	149.1^{bAB}	2.42^{bAB}	2.20^{abA}
1	BG05	31.4^{abc}	75.7^{bcd}	147.2^{bAB}	2.39^{bAB}	2.08^{abA}
	G2	25.8^{a}	71.9^{abc}	145.7^{abA}	2.37^{abA}	2.15^{abA}
	B2	34.3^{bc}	77.6^{d}	146.2^{abB}	2.38^{abB}	2.28^{abA}
	BG2	36.7^{c}	78.8^{d}	148.3^{b}	2.41^{b}	2.36^{bA}
	Control	26.8^{a}	69.1^{a}	143.7^{aA}	2.34^{aAB}	2.31^{AB}
	G05	28.7^{a}	71.7^{abB}	148.6^{bcB}	2.41^{bcB}	2.17^{AB}
	B05	32.2^{ab}	77.5^{cB}	149.7^{cB}	2.43^{cB}	2.38^{AB}
3	BG05	31.8^{ab}	75.5^{bc}	148.3^{bcAB}	2.41^{bcAB}	2.21^{AB}
	G2	27.0^{a}	72.0^{ab}	146.9^{bcA}	2.39^{bcA}	2.25^{A}
	B2	32.7^{ab}	77.6^{c}	146.2^{abB}	2.38^{abB}	2.41^{AB}
	BG2	36.4^{b}	79.1^{c}	148.5^{bc}	2.41^{bc}	2.46^{AB}
	Control	26.7^{a}	71.0^{a}	149.8^{aB}	2.39^{aB}	2.42^{BC}
	G05	27.9^{ab}	70.3^{aAB}	150.3^{abBC}	2.43^{abBC}	2.37^{BC}
	B05	32.3^{ab}	77.1^{bB}	154.2^{bC}	2.49^{bC}	2.46^{B}
5	BG05	32.8^{ab}	79.0^{b}	154.4^{bC}	2.49^{bC}	2.36^{AB}
	G2	28.4^{ab}	70.8^{a}	153.3^{abC}	2.47^{bC}	2.42^{B}
	B2	33.4^{ab}	81.5^{b}	152.6^{abC}	2.46^{bC}	2.56^{B}
	BG2	34.5^{b}	80.2^{b}	151.7^{ab}	2.45^{b}	2.59^{BC}
	Control	28.6^{ab}	65.9^{a}	141.4^{aA}	2.31^{aA}	2.60^{CD}
	G05	29.9^{ab}	69.1^{abAB}	152.8^{cC}	2.47^{cC}	2.56^{CD}
	B05	31.9^{ab}	77.8^{cB}	149.9^{bcB}	2.43^{bcB}	2.62^{B}
7	BG05	30.4^{ab}	78.8^{c}	150.7^{bcB}	2.44^{bcB}	2.44^{AB}
	G2	27.2^{a}	73.4^{bc}	150.3^{bcB}	2.43^{bcB}	2.57^{B}
	B2	33.8^{ab}	77.3^{c}	139.9^{aA}	2.29^{aA}	2.61^{B}
	BG2	35.5^{b}	78.4^{c}	146.8^{b}	2.39^{b}	2.64^{C}
	Control	27.6^{abc}	70.9^{ab}	145.0^{abA}	2.36^{abAB}	2.79^{abD}
	G05	26.0^{a}	66.9^{aA}	142.1^{aA}	2.32^{aA}	2.71^{abD}
	B05	32.8^{bc}	71.5^{abA}	145.4^{abA}	2.37^{abA}	2.91^{bC}
10	BG05	29.6^{abc}	74.8^{bc}	146.5^{bA}	2.38^{bA}	2.61^{aB}
	G2	26.4^{ab}	71.7^{ab}	145.5^{abA}	2.37^{abA}	2.78^{abC}
	B2	33.8^{c}	77.9^{c}	148.8^{bB}	2.41^{bB}	2.87^{bC}
	BG2	34.3^{c}	78.6^{c}	148.1^{b}	2.40^{b}	2.87^{bD}
Pooled SEM		0.41	0.39	0.31	0.004	0.021
				P-value		
Effects of treatment		<0.001	<0.001	<0.001	<0.001	<0.001
Effects of storage time		0.918	0.119	<0.001	<0.001	<0.001
Effects of storage time× treatment		0.999	0.548	<0.001	<0.001	0.999

$^{a-d}$-Different letters in the columnin dicate differences between treatment groups in a particular storage period, $P \leq 0.05$; ^{A,B,C,D}- capital letters indicate significant differences between storage times within the same treatment, $P \leq 0.05$ (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

It was observed that MDA levels in both breast and leg muscles increased with storage time. Among the breast muscles, the lowest increase in MDA levels was found in the muscles of BG05 chickens, with an increase of 22.18% between 1 and 10 days

of storage (P<0.05) (Tab. 6). A similar relationship was found in the leg muscles of BG05 chickens, with an increase in MDA levels of 20.3% (P<0.05) - Table 7.

Interaction diet ×storage time

The results revealed an interaction effect between storage time and the diet used on the content of carnosine (*P*=0.044), taurine (*P*=0.002), Q10 (*P*=0.007), and MDA (P<0.001) in breast muscles. However, the interaction effect on anserine content was not significant (*P*=0.778). In leg muscles, there was an interaction effect of storage time and diet used for taurine (P<0.001) and Q10 (P<0.001) content. However, there was no interaction effect of storage time and diet used on the content of carnosine (*P*=0.999), anserine (*P*=0.548), and MDA (*P*=0.999) in leg muscles.

In the breast muscles, carnosine levels decreased during storage in the BG2 group (P<0.05). On day 10 of storage, the carnosine level was 6.2g lower compared to the level in fresh breast muscles, and this difference was significant compared to the groups without β-alanine supplementation (P<0.05) - Table 6. However, for leg muscles, no significant interaction between the factors used was observed (*P*=0.999) - Table 7.

Principal component analysis

In PCA (Fig. 1), the results for the content of each bioactive peptide and MDA, as well as the values of individual production performance parameters such as FI, FCR, BWG, and slaughter analysis results, were represented by two new uncorrelated variables known as "principal components" (PC1 and PC2). The relationships between these parameters and the PC were interpreted based on their correlations.

The results indicated a negative correlation between the fat content of breast and leg muscles and the protein content of these muscles, as well as the peptides carnosine, anserine, Q10, and taurine.

In Figure 1, the two components, PC1 and PC2, accounted for 64.41% of the variation in the analyzed values, leaving a loss of 35.59% of the information.PC1 showed positive correlations with collagen content in breast and leg muscles, as well as the pH of these muscles, the fat content of these muscles, and various measures of BWG such as BWG-st, BWG-gr, BWG-fin, BW-tot, and BWG-tot. These values were mainly described in the G2, G05, and C groups. However, PC1 showed negative correlations with the protein content in breast and leg muscles, as well as the content of peptides such as carnosine, anserine, Q10, taurine, and changes in MDA. These values were mainly attributed to the B2 and BG2 groups.

On the other hand, PC2 showed negative correlations with FCR during different stages, including FCR-st, FCR-gr, FCR-fin, and FCR-tot as well as moisture content in breast muscles. These values were primarily linked to the Control group. PC2 also showed positive correlations with parameters like BWG-fin and BWG-st, the percentage of breast and leg muscles, and the protein and peptide content in breast muscles. These parameters were mainly described in the B05 and BG05 groups.

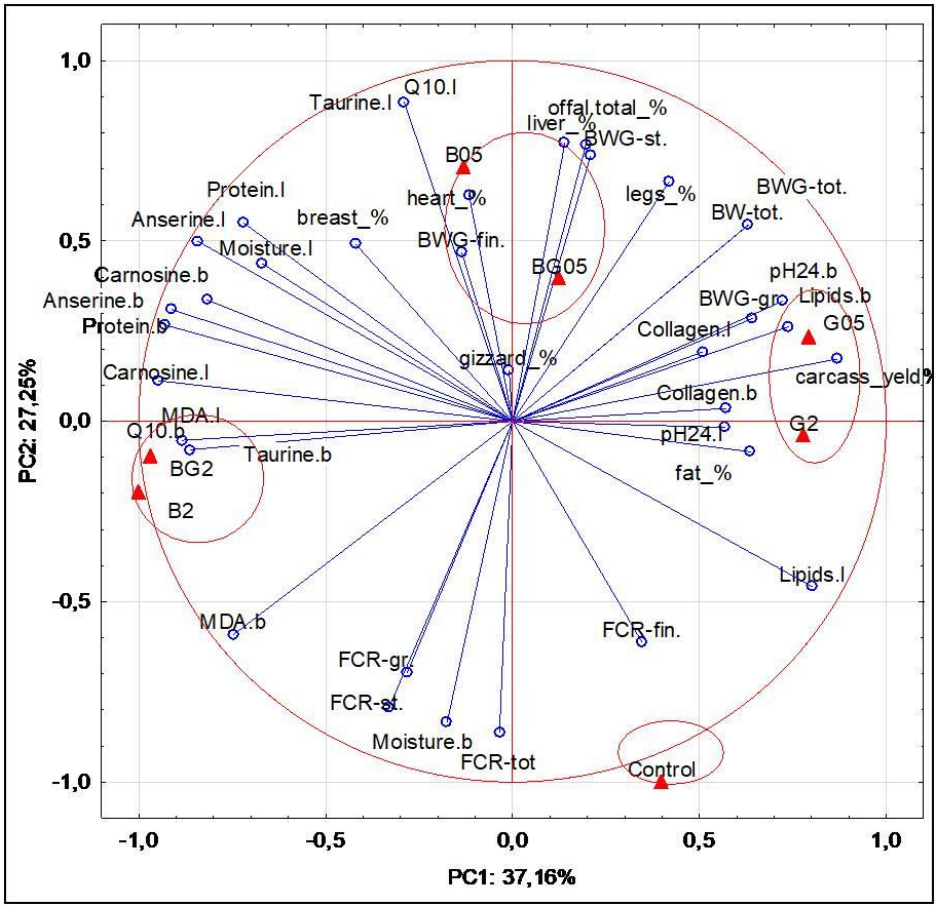

Fig. 1. First two components of PCA for slaughter analyses, performance and content of bioactive peptides and MDA during storage. D – day of storage; l, leg meat; b – breast meat; Control – commercial basal diet; G05 – diet supplemented with 0.5% of garlic extract; BO5 – diet supplemented with 0.5% of β-alanine; BG05 – diet supplemented with 0.5% each garlic extract and β-alanine; G2 – diet supplemented with 2% of garlic extract; B2 – diet supplemented with 2% of β-alanine; BG2 – diet supplemented with 2% each garlic extract and β-alanine; BW – body weight; BWG, body weight gain; FCR – feed conversion ratio (kg diet/kg BW) were: -st. for starter; -gr, for grower, -fin, for finisher and -tot, cumulative for 35days.

The dietary supplementation of broilers had a effect on the levels of carnosine, anserine, taurine, and Q10 in both breast and leg muscles. The use of β-alanine supplementation primarily increased the levels of carnosine and anserine in the muscles. Our results indicated that the groups with β-alanine supplementation contained significantly higher levels of these peptides compared to the groups without supplementation or those with garlic extract supplementation alone. This finding is consistent with previous studies conducted by Kralik *et al*. [2014], Łukasiewicz

et al. [2015], Qi *et al*. [2018], Lackner *et al*. [2021], and Suwanvichanee *et al*. [2022], which also reported an increase in carnosine and anserine content due to β-alanine supplementation. In our results (Tab. 5 and 6), the groups with β-alanine supplementation contained significantly higher levels of these peptides than the groups without supplementation or with garlic extract supplementation alone. An increase in carnosine and anserine content as a result of β-alanine supplementation was also found by other authors [Kralik *et al*. 2014, Łukasiewicz *et al*. 2015, Qi *et al*. 2018, Lackner *et al*. 2021, Suwanvichanee *et al*. 2022].

Kralik *et al*. [2014] found lower fat content in groups with β-alanine supplementation, which aligns with our experiment, where groups with garlic extract supplementation at 0.5% and 2% showed higher fat levels in both muscle types compared to the groups with β-alanine supplementation, which had lower fat levels in breast and leg muscles. This difference may be attributed to the health-promoting properties of increased carnosine content in the muscles and the greater activity of chickens fed the β-alanine-supplemented diet, leading to a higher protein content in the muscles of both types in the β-alanine-supplemented groups. Kralik *et al*. [2014] also observed an increase in protein content in leg and breast muscles as a result of supplementation. The addition of 0.5% β-alanine resulted in an increase of protein levels in breast muscles by 0.36g and in leg muscles by 0.89 g.

The activity of β-alanine and allicin in garlic extract has also affected the MDA content in both breast and leg muscles in chicken feed suplemented this aditions. In a study by Qi *et al*. [2018], a linear decrease in MDA was found in the β-alanine supplemented groups. Suwanvichenee *et al*. [2022] found lower muscle MDA levels following β-alanine supplementation at a level of 1%. In the case of pH, our study found lower values in groups supplemented with 2% β-alanine and 2% each of β-alanine and garlic extract. These values may have influenced the reduced muscle quality in the chickens of these groups. In the breast muscles, the levels of 0.5% β-alanine and 0.5% garlic extract influenced the reduction of MDA levels in fresh breast muscles. During storage, a linear increase in MDA levels was observed, and on day 10 of storage, breast muscles in the BG05 and B05 groups had the lowest levels of MDA. This may be due to the positive effect of both β-alanine and the synergistic effect of β-alanine and garlic extract. Similar results of MDA levels were shown in leg muscles.

The influence of garlic on rearing performance has been previously analyzed in the literature. However, its potential interaction with β-alanine supplementation remains unexplored. Among the groups studied, those receiving both 0.5% garlic extract and β-alanine supplementation exhibited the most favorable results, achieving the lowest FCR and a total gain of 2.81 kg throughout the rearing period. This gain was 2.5% higher than the Cgroup.

Lackner *et al*. [2021] reported better rearing results for mixed-sex ROSS 308 chicks. In the work of Lukanov *et al*. [2015], the addition of 0.8% garlic powder influenced the achievement of higher BW in ROSS 308 cockerels on day 35 of rearing.

The birds reached a BW of 2845 g, representing a 22.8% increase. Furthermore, Lukanov *et al*. [2015] found that birds supplemented with garlic powder exhibited significantly higher feed intake and higher FCR. The addition of 0.1, 0.2, and 0.3% essential garlic powder increased body weight in unsexed ROSS 308 chicken by 4%, 4.6%, and 5.1%, respectively. However, in the work of Pourali *et al*. [2010], the addition of 0.2% garlic powder increased the BW of ROSS 308 cockerels by 13.7%.Surprisingly, the addition of garlic powder at 1% had a negative effect on BW increase, resulting in chickens reaching a final BW 5% lower than those with the basal diet. Similarly, in the results obtained from the BG2 group, a lower BW of 2.2% was observed. The addition of β-alanine at 0.1% and 0.2% increased the BW of meat chickens by 2.9% and 6.3%, respectively.

In our results, the addition of 0.5% β-alanine increased BW by 4.1%, and when combined with garlic extract, it increased BW by 2.5%. However, a higher 2% addition of β-alanine negatively affected BW gains, resulting in a lower final weight of 1.5% and a lower final weight of 2.2% in the BG2 group (Tab. 2). In a study conducted by Lackner *et al*. [2021], the addition of 0.5% β-alanine did not lead to an increase in the BW of ROSS 308 broiler chickens. On day 33 of rearing, the chickens supplemented with 0.5% β-alanine showed a slightly lower proportion of breast muscle (0.5% decrease) and a slightly higher proportion of leg muscle (0.3% increase). However, these differences were not statistically significant.Similar results were obtained in the study by Qi *et al*. [2018], where the addition of 0.2% β-alanine resulted in a modest increase of 0.3% in breast yield but a reduction of 0.2% in leg muscle yield.

Likewise, Zhang *et al*. [2021] obtained comparable outcomes. The addition of 0.4% carnosine did not lead to a significant increase in the proportion of breast and leg muscles in broiler chickens. They also obtained a slightly lower carcass yield (69.6/100 g in the group with the addition of 0.4% carnosine and 69.8 in the group without the addition).

Pourali *et al*. [2010], using 0.2% and 1% garlic powder supplementation, did not find significant differences in the proportion of breast and leg muscles in ROSS 308 cockerels. At the 0.2% garlic powder level, both breast muscle (32.9% vs. 33.9% in the control group) and leg muscle (26.9% vs. 27.1% in the C group) proportions were slightly lower than the control group. On the other hand, the addition of 1% garlic powder resulted in a 0.2% increase in breast muscle proportion and a 1.4% increase in leg muscle proportion compared to the control group, but these differences were not significant. Amouzmehr *et al*. [2012] also reported lower proportions of breast and leg muscles when using 0.3% and 0.6% garlic extract supplementation. In the study by Lukanov *et al*. [2015], the addition of 0.8% garlic powder did not yield differences in carcass yield or the proportion of breast, gizzard, liver, and heart muscles.

Conclusion

The present study confirmed that dietary supplementation of broiler chickens with β-alanine and garlic extract together at a level of 0.5% has a beneficial effect

on chicken production performance (BW, FCR) and improves the oxidative status of meat. The observed lower levels of MDA in the breasts and legs of BG05 chickens suggest that the combination of both supplements in the diet can be successfully used in those processing branches where meat preservation is necessary. Furthermore, supplementing chicken diets with β-alanine alone can increase the levels of dietarily important peptides such as carnosine and anserine. Future research is needed to clarify the detailed mechanisms responsible for the synergistic effects of β-alanine and garlic extract.

Disclosures

The authors declare no conflicts of interest.

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