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THE EFFECT OF PHYTOGENIC ADDITIVES ON BIOCHEMICAL PARAMETERS OF BROILER CHICKEN TISSUES

Marina Ya. Kurilkina*, Tatyana A. Klimova, Shamil G. Rakhmatullin, Dmitry G. Deryabin, Kseniya N. Atlanderova, Galimzhan K. Duskaev
Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia

Keywords: *broiler chickens, quercetin, cinnamaldehyde, 7-hydroxycoumarin, chemical composition, mineral composition*

Abstract
The aim of this study was to evaluate the effect of combinations of bioactive compounds of plant origin (cinnamaldehyde, quercetin and 7-hydroxycoumarin) on productivity, meat quality and mineral metabolism in broiler chickens. During a 35-day experiment, 180 broiler chickens of the Arbor Acres cross were divided into 4 groups (n = 45): control (basal diet — BD) and three experimental treatments (BD with additives): I (cinnamaldehyde 30 mg/kg feed + quercetin 2.5 mg/kg feed), II (cinnamaldehyde 30 mg/kg feed + 7-hydroxycoumarin 0.3 mg/kg feed), III (combination of all three substances). Zootechnical parameters, chemical, amino acid and elemental composition of meat and liver from the experimental animals were assessed. Statistical significance was determined using the Mann-Whitney U-test ($p \le 0.05$). The greatest synergistic effect was demonstrated by the combination in the experimental group III. Compared to the control, in this group, the absolute live weight gain significantly increased by 880.7 g (by 51.4 %; $p \le 0.05$), and the average daily gain increased by 18.23 g (by 34.5 %; $p \le 0.05$). Feed conversion improved by 9.4 % (from 2.02 to 1.83 kg feed/kg weight gain), and the productivity efficiency index (EPEF) more than doubled by 257.04 points (from 209.37 to 466.41; $p \le 0.05$). The muscle tissue weight of carcasses in experimental group III was 246.5 g higher than the control value (31.9 %; $p \le 0.05$). A significant increase in the fat mass fraction was observed in the breast muscles of all experimental groups, e. g. in group I by 0.9 % ($p \le 0.001$). And in group II, an increase in the protein fraction by 2.2 % ($p \le 0.05$) was observed. The additives had a modulating effect on the mineral composition of tissues, causing, in particular, a decrease in the concentration of iron (Fe) in the breast muscles of groups I and III by 7.21 mg/kg (22.3 %; $p \le 0.001$) and 5.70 mg/kg (17.6 %; $p \le 0.001$), as well as an increase in the content of zinc (Zn) in the thigh muscles of groups I and II by 7.61 mg/kg (16.3 %; $p \le 0.05$) and 9.01 mg/kg (19.3 %; $p \le 0.01$), respectively. Thus, the combined use of cinnamaldehyde, quercetin and 7-hydroxycoumarin demonstrated a statistically significant positive effect on growth, feed efficiency and meat productivity of broilers, and also changed the biochemical profile of muscle tissue, which confirms the potential of this composition as an alternative to antibiotic growth promoters.

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Introduction

The intensification of poultry production to meet the growing demand for meat products faces significant challenges, including an increased risk of disease transmission and the need to maintain high rates of livestock growth. The established practice of addressing these challenges has been the use of antibiotics, not only for treatment but also as growth promoters [1,2,3]. However, the global problem of antibiotic resistance and consumer concerns about product safety have led to a ban on the use of antibiotic growth promoters and stimulated the search for effective and safe alternatives [4,5].

Plant-based alternatives to antibiotics represent a large and diverse group of phytobiotics, i. e. complexes of biologically active substances of plant origin. These include essential oils, tannins, saponins, flavonoids, alkaloids, and resin acids, which exhibit a broad spectrum of activity, including antimicrobial, antiviral, antioxidant, and anti-inflammatory effects [6]. However, phytobiotics are not the only possible alternative. Current research also focuses on probiotics, prebiotics, organic acids, enzymes, and bacteriophages. Each of these approaches has its own mechanism of action, and their effectiveness often depends on the synergistic effect of combined use. Therefore, the search for an optimal alternative lies not in opposing phytobiotics against antibiotics, but in a comprehensive assessment of the effectiveness of specific compounds or their combinations for modulating intestinal health, immune status, and poultry productivity.

This study aimed to investigate the effect of a specific composition of plant-based bioactive compounds on the performance and meat quality of broiler chickens. The selection of specific components — cinnamaldehyde, quercetin, and 7-hydroxycoumarin — was based on their

unique and complementary properties, addressing key performance and health issues in poultry production [7].

Cinnamaldehyde, the main component of cinnamon essential oil, was included due to its potent antimicrobial properties against a wide range of pathogenic and opportunistic microorganisms [8]. Hypothetically, it should improve the microbiological status of the gastrointestinal tract, reducing microbial load and competitive nutrient uptake, which, in turn, could increase feed digestibility and the efficiency of energy use for growth.

Quercetin, one of the most common flavonoids, was selected for its pronounced antioxidant and anti-inflammatory activity [9–11]. According to the literature, quercetin's ability to inhibit proinflammatory signaling pathways (e. g., mTOR) and modulate the immune response should reduce the level of systemic inflammation often associated with intensive farming practices [12,13]. This, in turn, may redirect energy resources from immune defense to muscle growth and synthesis processes, as well as improve meat quality by reducing oxidative stress.

7-hydroxycoumarin was selected as a promising modulator of pathogenic microflora virulence. Its ability to inhibit quorum sensing and biofilm formation [14–19] potentially reduces bacterial pathogenicity without direct bactericidal pressure on them, minimizing the risk of resistance development [20–23]. This is expected to contribute to maintaining intestinal health and resistance to infections.

Although the effects of some individual phytogenic additives have been studied, the potential synergistic effect of this particular combination on product quality parameters (chemical, amino acid, and mineral composition of meat) remains unclear. We hypothesized that this combination would have a comprehensive positive effect on meat productivity and meat quality in broilers through simultaneous effects on the microbiota, antioxidant status, and inflammatory processes.

Thus, the scientific novelty of this study lies in its comprehensive assessment of the effects of a synergistic composition of bioactive compounds (cinnamaldehyde, quercetin, and 7-hydroxycoumarin) on productivity, meat quality parameters, and mineral metabolism in Arbor Acres broiler chickens.

Objects and methods

The study was conducted in the vivarium of the Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (Orenburg) from February to August 2022. The objects of the study were Arbor Acres broiler chickens and bioactive compounds of plant origin (cinnamaldehyde, quercetin, 7-hydroxycoumarin).

Experimental design

180 7-day-old broiler chickens were divided into 4 groups using the analog method ($n = 45$): Control (C): Basal diet (BD); Experimental group I (EXP-I): BD + cinnamaldehyde (30 mg/kg feed) + quercetin (2.5 mg/kg feed); Experimental group II (EXP-II): BD + cinnamaldehyde (30 mg/kg feed) + 7-hydroxycoumarin (0.3 mg/kg feed); Experimental group III (EXP-III): BD + cinnamaldehyde (30 mg/kg feed) + quercetin (2.5 mg/kg feed) + 7-hydroxycoumarin (0.3 mg/kg feed) (Table 1).

Table 1. Experimental design

Objects of the study	Group	Experimental period	
		Preparation	Experiment
		Days	
		7	35
Arbor Acres broiler chickens	Control ($n = 45$)	BD	BD
	Experimental I ($n = 45$)		EXP-I
	Experimental II ($n = 45$)		EXP-II
	Experimental III ($n = 45$)		EXP-III

Husbandry and feeding

Husbandry conditions were identical for all groups. Diets were formulated according to the recommendations by the All-Russian Poultry Research and Technological Institute [24]. Feeding was twice daily, and intake was monitored daily.

Sampling

On the 42nd day, the test animals were slaughtered by the internal method. Samples of the breast and thigh muscles, as well as the liver, were collected immediately after slaughter and frozen (–18 °C).

Biosubstrate analysis

The samples were analyzed for their chemical composition: content of moisture (dried at 150 ± 2 °C), crude fat (Soxhlet extraction), crude protein (Kjeldahl method), and ash. Amino acid composition was determined by capillary electrophoresis on Kapel-105M system after acid hydrolysis of the samples and the production of FTC derivatives. Elemental composition: ICP-MS on Agilent 7900 ICP-MS mass spectrometer.

Chemical analysis of biosubstrates was performed using standardized methods at the testing center of the Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

Carcass morphometry

Eviscerated carcasses (standard definition: internal organs removed, head, skinless neck, feet up to the tarsal joint ± 20 mm) were weighed (Mercury 327 ACP LCD electronic scales, Russia, with an accuracy of ± 2 g). Cutting was performed according to the recommendations by the All-Russian Poultry Research and Technological Institute [24], with the main parts (breast, drumstick and thigh, wings, forequarters, and dorsal-scapular portion) isolated. The parts were anatomically deboned, and the isolated tissues (muscle, skin, veins, fat, and bones) were weighed (MW-II laboratory scales, China, with an accuracy of ± 0.01 g) to calculate the yield.

Determining the moisture content

To determine the moisture content of the test sample, 0.2 kg samples were taken. The biosubstrate samples were freed from their coverings (including the skin), then homogenized (repeatedly minced using a meat grinder), and then thoroughly mixed (the temperature of the resulting test sample was approximately 25 °C). Next, 0.01 kg ± 0.001 kg of purified sand and a glass rod for mixing the sand with the sample were transferred to a weighing cup, after which they were dried in a drying oven (SHC-80–01, Russia) at a temperature of 150 °C for 0.5 h. Then, the dried cups were closed with lids and placed in a desiccator, where they cooled to 20–22 °C, after which they were weighed to the third decimal place on electronic scales (VM-153, Russia). Prepared samples of the tested biosubstrates (3000 mg) were placed in the cups, after which the total mass of the cup with the contents was weighed. The samples with sand were thoroughly mixed using a glass rod and transferred to a drying oven at a temperature of 150 °C for 60 min for drying. After drying, the cups were covered with lids and transferred to a desiccator to cool to 20–22 °C. The cups were then weighed to the third decimal place. To calculate the moisture content in%, the following formula was used:

$$A = ((a - b)/(a - c)) \times 100\,\%, \quad (1)$$

where:

A is the moisture mass fraction;

a is the weight of the cup containing biosubstrate sample, glass rod, and sand, g;

b is the weight of the cup containing biosubstrate sample, glass rod, and sand after drying, g;

c is the weight of the cup containing the glass rod and sand, g.

Determining the ash content

To determine the ash content, a weighed portion of the air-dried sample (1–2 g ± 0.0001 g) and 3 ml of ashing agent (magnesium acetate in ethyl alcohol with added iodine) were placed into calibrated crucibles. The ashing agent was ignited, and after it burned out, the crucibles were placed in a furnace (Natbertherm GmbH, Germany) at 500–550 °C until a grayish-white ash was obtained. The crucibles were then annealed to a constant weight. To calculate the ash content in%, the following formula was used:

$$A = (a - b) \times 100\,\%/c, \quad (2)$$

where:

A is the ash mass fraction;

a is the weight of the ash in the biosubstrate sample, g;

b is the weight of the ash from the ashing agent, g;

c is the weight of the initial portion of the biosubstrate, g.

Determining the fat content

To determine the fat content in the experimental biosubstrates, we used a method of repeated extraction of fat from a dried sample using a solvent in a Soxhlet extraction apparatus. The solvent was then removed from the samples, and the resulting fat was dried to a constant weight. A 5000 mg portion of the prepared sample was placed on a watch glass and dried in a drying oven for 60 minutes at 105 °C (SHC-80-01, Russia). The dried test sample was then placed in a specially prepared filter paper sleeve with a cotton wool base. Next, the watch glass was soaked in diethyl ether and transferred to a prepared sleeve. The sleeve was then sealed and transferred to a special desiccator in a Soxhlet apparatus. The extraction process was carried out for approximately 6 hours (the extract was poured off at an average rate of 7 times per hour) using an extraction flask and a water bath. The process was considered completed when no greasy stain from the extract remained on the filter paper. Upon completion of the extraction, the solvent was distilled from the extraction flask. The extraction flask, containing the fat after extraction, was then dried in a drying oven at 105 °C to a constant weight. To calculate the fat content in%, the following formula was used:

$$A = ((a - b) \times 100\,\%)/c, \quad (3)$$

where:

A is the fat mass fraction;

a is the weight of the extraction flask containing the fat, g;

b is the weight of the empty extraction flask, g;

c is the weight of the biosubstrate sample, g.

Determining the protein content

This method allows for the determination of the total nitrogen content in a sample, including nitrogen in various forms, such as ammonia. For this purpose, a weighed portion of the sample (2 g ± 0.001 g) was transferred to a Kjeldahl flask, anhydrous potassium sulfate (15 g) and concentrated sulfuric acid (25 ml) were added, everything was mixed, and the flask was placed on a heating device to dissolve the sample. After the sample was completely dissolved, the mineralization process was continued at boiling until the contents of the flask became transparent and then turned a pale green-blue color. After the flask contents became completely colorless, it was boiled for another 1.5 hours, with the total mineralization time being approximately 2 hours. The Kjeldahl flask containing the sample was then cooled (40 °C) and distilled water (50 ml) was added. The flask was then stirred and cooled to a temperature of 20–22 °C. To calculate the protein content in%, the following formula was used:

$$A = (0.0014 \times (a - b) \times c \times 100)/d \times 6.25, \quad (4)$$

where:

A is the protein mass fraction;

a is the amount of hydrochloric acid solution (0.1 mol/dm^3) used to titrate the test sample, cm^3;

b is the amount of hydrochloric acid solution (0.1 mol/dm^3) used to titrate the control sample, cm^3;

c is the correction factor to the nominal concentration of the hydrochloric acid solution;

0.0014 is the amount of nitrogen equivalent to 1 cm^3 of hydrochloric acid solution (0.1 mol/dm^3), g;

100 is the conversion factor for%;

d is the weight of the test sample, g;

6.25 is the conversion factor for protein.

Determining the amino acid composition

The analysis was performed using capillary electrophoresis on Kapel-105M device manufactured by Lumex-Marketing LLC (Russia) according to the standard procedure (M 04-38-2009). The amino acid composition was determined using the following mass fractions: arginine, lysine, tyrosine, phenylalanine, histidine, leucine and isoleucine (total), methionine, valine, proline, threonine, serine, alanine, and glycine in the form of phenylisothiocarbamyl derivatives. Samples (0.1 g) were placed in vials with heat-resistant screw caps and fluoroplastic liners for hydrolysis. 10 ml of HCL (1:1) was added and mixed. Acid hydrolysis was performed in a drying oven (SHC-80-01, Russia) for 15 ± 2 h, at a temperature of 110 °C. After that, the vials were removed from the cabinet and cooled to a temperature of 20–22 °C, then the contents were filtered (using a blue-ribbon filter). The resulting hydrolysate (50 mm^3) was transferred to glass weighing bottles and evaporated to dryness in a stream of warm air. Then the FTC derivative was obtained by adding 150 mm^3 of sodium carbonate solution and 300 mm^3 of phenyl isothiocyanate solution in isopropyl alcohol to the dry residue, stirring until the precipitate dissolved, closing the lid and leaving for 35 min at room temperature. Then the solution was evaporated to dryness in a stream of warm air. The dry residue was dissolved in 500 mm^3 of distilled water. The resulting solution for analysis was transferred to an Eppendorf tube and centrifuged (5 min, 5000 rpm) in a centrifuge (Eppendorf AG MiniSpin, Germany). The following formula was used to calculate the mass fraction of each amino acid in the sample (%):

$$A = 10 \times C/m, \quad (5)$$

where:

A is the mass fraction of amino acids, %;

10 is the volume conversion factor;

C is the measured mass concentration of the amino acid in the solution;

m is the weight of the test sample.

Determining the elemental composition

Pre-homogenized sample in its dried or native form (weighing from 0.1 to 0.3 g) was placed in TFM container and weighed to an accuracy of 0.0001 g (Pioneer PX 224 laboratory scales, USA). Nitric acid, hydrogen peroxide, and, if necessary, hydrofluoric or hydrochloric acid were added. Degradation of the organic matrix was performed in TOPEX+ microwave sample preparation system (PREEKEM, China) at the appropriate temperature and pressure (the degradation program is selected depending on the sample type). Elemental analysis was performed on Agilent 7900 ICP-MS single-quadrupole inductively coupled plasma mass spectrometer (Agilent, USA). Fe and Zn were analyzed in helium mode using a collision cell. Standard solutions were prepared from a multicomponent mixture of 23 elements from Merck (Germany) with the addition of additional reference samples. Statistical data were accumulated through a series of measurements across the entire mass range in duplicate. The analysis result is the concentration of the target elements, expressed as mg of element per kg of sample, taking into account the sum of instrumental and methodological errors.

Ethics statement

The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[1], the Declaration of Helsinki[2], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[3].

Statistical analysis

The significance of differences was assessed using the Mann-Whitney U-test (Statistica 10.0 package). The significance level was $p \leq 0.05$.

Results and discussion

The obtained research results revealed that the experimental groups of broiler chickens showed a trend toward an increase in average daily weight gain by 4.5–34.5 % compared to the control group. The greatest effect was observed in experimental group III ($p \leq 0.05$). This indicates a synergistic effect from the combined use of all three studied additives. In terms of absolute weight gain, the pattern completely replicates the dynamics of average daily weight gain, as these indicators are directly related. Experimental groups I and III demonstrated significantly higher absolute weight gain (by 34.4 % and 51.4 %, respectively) compared to the control group (Table 2).

The feed intake is crucial for interpreting the results. For example, in experimental group I, feed intake was 3,171.3 g (a decrease of 8.5 % relative to the control), in experimental group II it was 3,468.4 g (similar to the control), and in experimental group III it was 3,959.0 g (an increase of 14.2 % relative to the control). Thus, the improved gains in the experimental groups are not due to a simple increase in feed intake. Moreover, experimental group I demonstrated better gains while consuming less feed. This clearly indicates that the primary mechanism of action of phytogenic additives is improved nutrient digestibility, not appetite stimulation. Feed consumption per 1 kg of gain (feed conversion ratio, kg) is an integral indicator of feed efficiency. In the control group, this parameter was 2.02 kg of feed/kg of gain, which was higher than in the experimental groups by 6.9 % in group I, 6.4 % in group II, and 9.4 % in group III, respectively. All experimental diets reduced feed consumption per unit of gain. The best feed conversion was

[1] Federal Law of the Russian Federation dated December 27, 2018 No. 498-FZ "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation." Retrieved from https://docs.cntd.ru/document/552045936. Accessed May 17, 2025 (In Russian)

[2] WMA Declaration of Helsinki — ethical principles for medical research involving human subjects Retrieved from https://www.wma.net/policiespost/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed May 17, 2025

[3] ETS No. 123, Strasbourg, 1986) (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Retrieved from https://rm.coe.int/168007a67b. Accessed May 17, 2025

Table 2. Growing efficiency of test animals

Parameter	Group			
	Control	I	II	III
Average daily gain over 5 weeks, g	52.91 ± 10.6	60.90 ± 0.6	55.27 ± 3.7	71.14 ± 4.9[a]
Absolute gain, g	1714.8 ± 311.6	2304.8 ± 65.9[a]	1934.5 ± 127.8	2595.5 ± 166.1[a]
Feed intake, g	3467.0 ± 630.0	3171.3 ± 90.7	3468.4 ± 229.2	3959.0 ± 253.3
Feed consumption per 1 kg of live weight gain, g	2.02	1.88	1.89	1.83
Livability, %	96	98	98	98
EPEF	209.37 ± 66.32	442.57 ± 4.45[a]	308.28 ± 20.37[a]	466.41 ± 32.14[a]

Note: [a] — $P \le 0.05$ when compared to the control group.

achieved in group III, confirming the synergistic effect of the combination and its high cost-effectiveness.

Livability was very high in all groups (96–98 %), indicating good housing conditions and the absence of a negative impact of the additives on the animals' health. The Production Efficiency Factor (EPEF) is a complex parameter combining growth, livability, and feed conversion. In the control group, it was 209.37, while a significant increase was observed in all experimental groups ($p \le 0.05$).

Thus, the use of experimental phytogenic additives, particularly the combination of cinnamaldehyde, quercetin, and 7-hydroxycoumarin (group III), significantly improved all key livestock performance indicators: increased the rate and absolute live weight gain, significantly improved feed conversion and overall production efficiency (EPEF). Importantly, this positive effect was achieved primarily by increasing feed digestibility, not by increasing feed intake.

A study of meat productivity indicators in test animals revealed a significant advantage of using phytogenic additives in their diets only for group III (Table 3).

The control slaughter revealed the following. High pre-slaughter live weights were observed in experimental groups I and III, exceeding those of the control group by 13.1 % and 30.8 % ($p \le 0.01$). Experimental group III significantly exceeded the control group ($p \le 0.05$) in terms of eviscerated carcass weight by 31.5 %, muscle tissue by 31.9 %, and edible portion by 33.4 %, respectively. Feeding phytogenic additives to chickens in experimental groups II and III also demonstrated a beneficial effect on slaughter yield, which increased by 0.8–0.9 % relative to the control value.

Similar results were obtained in other studies involving the introduction of phytobiotics in broiler chicken diets. Thus, it was established that with the addition of a plant-based additive from the extract of edible chestnut wood, a high pre-slaughter live weight was observed relative to the control group, and the weight of eviscerated chickens from the experimental groups with the highest level of chestnut extract exceeded similar groups with the lowest level of this substance in the diet by 6.07 % ($p \le 0.05$) and 9.48 % ($p \le 0.05$), respectively [25]. Similar results were obtained in broiler chickens for the weight of semi-eviscerated carcasses in the work by Gheisar et al. [26]. This was probably due to the greater digestibility of nutrients in the experimental groups following the inclusion of phytobiotics, which contributed to a more intensive increase in live weight. Younis and Abdel-Latif previously reported that the slaughter yield of broiler chickens was significantly ($p \le 0.05$) higher when using water supplemented with 1 % red hot pepper extract [27]. High slaughter yields were also observed in test animals supplemented with turmeric [28].

Analysis of the chemical composition of breast tissue in animals from the experimental groups revealed an increase in fat concentration by 0.2–0.9 % ($p \le 0.001$) relative to the control group. In experimental group II, protein and dry matter concentrations also increased relative to the control group by 2.2 % ($p \le 0.05$) and 2.4 % ($p \le 0.01$) (Table 4).

We hypothesize that the increased fat content in the breast muscles of the experimental groups observed in the study is a direct consequence of the combined effects of the phytogenic additives on the metabolism of the test animals. This is not a random result, but a predictable phenomenon, explainable in terms of their biological activity, as their primary mechanisms involve improving nutrient absorption and energy metabolism. The greatest increase in breast muscle fat was observed in group I (cinnamaldehyde + quercetin). This is an interesting observation, suggesting that this combination most effectively triggers

Table 3. Slaughter parameters of test animals at the end of the experiment

Parameter	Group			
	Control	I	II	III
Pre-slaughter live weight, g	2135.5 ± 168.23	2415.3 ± 28.20	2236.0 ± 118.53	2794.0 ± 176.59[b]
Eviscerated carcass, g	1568.8 ± 185.77	1733.3 ± 21.73	1647.5 ± 79.53	2062.7 ± 149.03[a]
Muscle tissue, g	772.4 ± 143.32	823.2 ± 13.32	810.9 ± 43.24	1018.9 ± 15.19[a]
Bone tissue, g	451.0 ± 84.16	511.5 ± 1.39	470.8 ± 22.29	591.4 ± 23.31
Edible portion, g	1370.2 ± 140.72	1546.3 ± 20.77	1439.7 ± 70.60	1827.9 ± 150.00[a]
Inedible portion, g	634.4 ± 113.28	716.2 ± 13.84	655.0 ± 42.26	837.3 ± 24.29
Edible portion / Inedible portion	2.16 ± 0.045	2.16 ± 0.016	2.20 ± 0.035	2.18 ± 0.115
Slaughter yield, %	72.9 ± 1.10	71.8 ± 0.50	73.8 ± 0.86	73.7 ± 0.71

Note: [a] — $P \le 0.05$; [c] — $P \le 0.01$ when compared to the control group.

Table 4. Analysis of the chemical composition of breast muscles in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	76.6 ± 2.40	75.1 ± 2.55	74.2 ± 2.47	74.8 ± 2.26
Dry matter, %	23.4 ± 0.69	24.9 ± 0.65	25.8 ± 0.55[b]	25.2 ± 0.88
Fat, %	0.7 ± 0.03	1.6 ± 0.06[c]	1.0 ± 0.04[c]	0.9 ± 0.05[c]
Ash, %	0.99 ± 0.05	0.98 ± 0.04	0.99 ± 0.03	0.99 ± 0.05
Protein, %	21.7 ± 0.51	22.3 ± 0.65	23.9 ± 0.88[a]	23.3 ± 0.80

Note: [a] — $P \le 0.05$; [b] — $P \le 0.01$; [c] — $P \le 0.001$ when compared to the control group.

lipogenesis mechanisms in this particular muscle. Cinnamaldehyde likely plays a key role in stimulating digestion and enzyme secretion, while quercetin ensures metabolic stability through its antioxidant action. The best results in protein concentration were shown by groups II and III contained 7-hydroxycoumarin, which may indicate its special role in optimizing protein metabolism.

Thus, an increase in the fat content of breast muscles is a marker of improved overall metabolic status in animals. The phytogenic additives studied optimize digestion and energy absorption. Excess absorbed energy is naturally deposited as fat in tissues, including muscles.

The results showed that introducing the experimental additives into the diet did not significantly affect the amino acid composition of breast muscles in broiler chickens (Table 5).

At the same time, in the breast muscles of animals from the experimental groups, a tendency towards increasing concentrations was observed: arginine by 0.1 % and 0.2 % (groups II and III), lysine by 0.4 % and 0.2 % (groups II and III), tyrosine by 0.2 % (II group), phenylalanine by 0.2 % (II group), histidine by 0.1 % (II group), leucine + isoleucine by 0.3 % (II group), methionine by 0.1 % (groups II and III), threonine by 0.1 % (groups II and III), serine by 0.1 % (groups II and III), alanine by 0.1 % (II group) and glycine by 0.1 % (groups II and III) in comparison with the control.

The obtained results are consistent with literature data demonstrating that flavonoids in animal diets improve meat quality by optimizing the amino acid profile [29]. A likely reason is the pronounced antioxidant activity of flavonoid-containing plants, which reduces the level of free radicals and thereby promotes the preservation of amino acids in muscle tissue [30]. Our data also correlate with the findings by Haščík et al. [31], who demonstrated an increase in the amino acid concentration in chicken meat with the addition of a probiotic with anthocyanins and propolis extract, and Omar et al. [32], who noted improved growth in broilers under the influence of phenol-containing onion extracts due to increased amino acid digestibility and improved intestinal health.

It has also been previously established that many compounds of natural origin, especially those obtained from natural essential oils, have a positive effect on both carcass and meat quality parameters. An example of this is 1,8-cineole (eucalyptol), the main component of rosemary and eucalyptus essential oils, which, when added to broiler feed, increases body weight and weight gain, improves feed conversion ratio, and has a beneficial effect on the quality characteristics of the resulting meat [33]. Phytoncides have also been shown to significantly increase the amino acid concentration in broiler meat, indicating their role in improving meat quality [34,35]. Furthermore, it has been demonstrated that dietary supplementation with natural antioxidants may improve meat quality by preventing lipid peroxidation and protein denaturation, ultimately improving the fatty acid and amino acid profile of meat [36].

Analysis of the mineral composition of the breast muscles of the animals studied revealed intergroup differences in the concentrations of certain chemical elements (Table 6).

Thus, a reliable decrease in Ca was established in all experimental groups by 20.0 % ($p \le 0.01$), 13.3 % ($p \le 0.05$),

Table 5. Amino acid content of breast muscles in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	5.3 ± 0.11	5.1 ± 0.16	5.4 ± 0.18	5.5 ± 0.14
Lysine, %	6.9 ± 0.15	6.8 ± 0.11	7.3 ± 0.20	7.1 ± 0.13
Tyrosine, %	4.1 ± 0.18	4.1 ± 0.16	4.3 ± 0.19	4.1 ± 0.22
Phenylalanine, %	3.0 ± 0.09	3.1 ± 0.11	3.2 ± 0.08	3.0 ± 0.12
Histidine, %	2.9 ± 0.07	2.7 ± 0.06[a]	3.0 ± 0.07	2.9 ± 0.04
Leucine+Isoleucine, %	10.2 ± 0.25	10.2 ± 0.28	10.5 ± 0.31	10.1 ± 0.33
Methionine, %	2.1 ± 0.07	2.1 ± 0.10	2.2 ± 0.11	2.2 ± 0.11
Valine, %	4.2 ± 0.15	4.0 ± 0.14	4.2 ± 0.15	4.1 ± 0.09
Proline, %	2.8 ± 0.12	2.8 ± 0.17	2.8 ± 0.11	2.8 ± 0.15
Threonine, %	3.1 ± 0.18	3.1 ± 0.14	3.2 ± 0.15	3.2 ± 0.07
Serine, %	2.8 ± 0.14	2.7 ± 0.12	2.9 ± 0.15	2.9 ± 0.12
Alanine, %	7.1 ± 0.25	6.8 ± 0.27	7.2 ± 0.19	7.1 ± 0.21
Glycine, %	3.4 ± 0.16	3.3 ± 0.16	3.5 ± 0.17	3.5 ± 0.11

Note: [a] — $P \le 0.05$ when compared to the control group.

Table 6. Elemental composition of breast muscles in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.30 ± 0.02	0.24 ± 0.01[b]	0.26 ± 0.01[a]	0.21 ± 0.01[c]
P, g/kg	9.00 ± 0.37	8.43 ± 0.38	8.51 ± 0.29	9.17 ± 0.38
Na, g/kg	1.98 ± 0.07	1.78 ± 0.07[a]	1.52 ± 0.06[c]	1.93 ± 0.08
K, g/kg	14.98 ± 0.78	14.26 ± 0.60	14.63 ± 0.61	15.34 ± 0.63
Mg, g/kg	1.12 ± 0.05	1.07 ± 0.04	1.11 ± 0.04	1.15 ± 0.04
B, mg/kg	1.85 ± 0.10	1.39 ± 0.06[c]	1.80 ± 0.14	1.49 ± 0.06[b]
Mn, mg/kg	0.67 ± 0.02	0.55 ± 0.02[c]	0.63 ± 0.03	0.62 ± 0.02
Co, mg/kg	0.02 ± 0.003	0.01 ± 0.001[b]	0.02 ± 0.001	0.02 ± 0.001
Fe, mg/kg	32.39 ± 1.10	25.18 ± 1.38[c]	32.08 ± 3.40	26.69 ± 1.20[c]
Zn, mg/kg	25.65 ± 0.85	26.65 ± 0.93	24.26 ± 1.04	25.22 ± 0.86
Se, mg/kg	0.69 ± 0.062	0.77 ± 0.091	0.73 ± 0.265	0.90 ± 0.356
Cu, mg/kg	1.12 ± 0.05	1.25 ± 0.04[a]	1.08 ± 0.03	1.07 ± 0.04

Note: [a] — $P \le 0.05$; [b] — $P \le 0.01$; [c] — $P \le 0.001$ when compared to the control group.

30.0 % ($p \le 0.001$) and Na by 10.1 % ($p \le 0.05$) in group I and 23.2 % ($p \le 0.001$) in the group II, relative to the control. Similarly, B decreased by 24.9 % ($p \le 0.001$) and 19.5 % ($p \le 0.01$) in experimental groups I and III, Mn and Co by 17.9 % ($p \le 0.001$) and 50.0 % ($p \le 0.01$) in group I, and Fe by 22.3 % ($p \le 0.001$) and 17.6 % ($p \le 0.001$) in groups I and III, relative to the control. It should be noted that in the breast muscles of the studied chickens, a reliable decrease in Ca concentration due to the introduction of the tested phytogenic additives may be associated with the formation of tannin-calcium complexes in the gastrointestinal tract of broilers [37].

The thigh muscles of experimental broiler chickens also showed a tendency toward changes in chemical composition (a significant increase in fat in group I, a decrease in protein in all groups). The dynamics in the thigh muscles differ from those in the breast muscles, which is physiologically justified, since these muscles have different fiber types (white/glycolytic in the breast muscles, red/oxidative in the thigh muscles) and different metabolism. A significant increase in fat by 0.4 % ($p \le 0.001$) in group I is consistent with the mechanisms described above for the breast muscles. Enhanced lipogenesis and lipid transport affect all muscles. The lack of significant changes in groups II and III may indicate that the additive combinations affect metabolism differently in different muscle types. Thigh muscles, which are more metabolically active due to their oxidative nature, may utilize incoming lipids differently. The decrease in the mass fraction of protein in all experimental groups (by 0.6 %-1.6 %) was not significant (Table 7).

Table 7. Analysis of the chemical composition of the thigh muscles in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	76.1 ± 2.41	76.8 ± 2.58	77.5 ± 2.38	76.6 ± 2.37
Dry matter, %	23.9 ± 0.57	23.2 ± 0.65	22.5 ± 0.48	23.4 ± 0.62
Fat, %	1.5 ± 0.06	1.9 ± 0.07[a]	1.6 ± 0.05	1.6 ± 0.06
Ash, %	0.99 ± 0.02	0.98 ± 0.01	0.98 ± 0.03	0.98 ± 0.01
Protein, %	21.5 ± 0.47	20.3 ± 0.65	19.9 ± 0.96	20.9 ± 0.51

Note: [a] — $P \le 0.001$ when compared to the control group.

As in the breast muscles, ash content remained stable and unchanged across all groups. Unlike the breast muscles, the thigh muscles showed less sensitivity to the additives. The only significant effect was an increase in fat concentration in the group receiving the combination of cinnamaldehyde and quercetin.

Thus, the phytogenic additives studied exert a complex effect on metabolism, enhancing feed digestibility, energy metabolism, and lipogenesis, which is reflected in changes in the chemical composition of both the breast and thigh muscles.

Relative to the control, the amino acid composition of the thigh muscles (Table 8) was distinguished by an increase in concentrations in the experimental group I: arginine by 0.4 %, lysine, tyrosine, phenylalanine, proline, serine by 0.1 %, leucine + isoleucine and alanine by 0.2 %, in the experimental group III: methionine by 0.1 % and arginine by 0.2 %.

Table 8. Amino acid content of thigh muscles in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	5.1 ± 0.10	5.5 ± 0.23	5.2 ± 0.06	5.3 ± 0.07
Lysine, %	7.1 ± 0.11	7.2 ± 0.17	6.8 ± 0.14	7.1 ± 0.21
Tyrosine, %	3.1 ± 0.11	3.2 ± 0.15	3.0 ± 0.17	3.0 ± 0.22
Phenylalanine, %	3.0 ± 0.06	3.1 ± 0.13	3.0 ± 0.10	3.0 ± 0.07
Histidine, %	2.4 ± 0.04	2.4 ± 0.09	2.3 ± 0.05	2.4 ± 0.06
Leucine + Isoleucine, %	10.0 ± 0.27	10.2 ± 0.24	9.7 ± 0.24	10.0 ± 0.25
Methionine, %	2.1 ± 0.09	2.1 ± 0.15	2.1 ± 0.08	2.2 ± 0.07
Valine, %	4.0 ± 0.11	4.0 ± 0.13	3.8 ± 0.12	3.9 ± 0.11
Proline, %	2.8 ± 0.08	2.9 ± 0.15	2.7 ± 0.17	2.8 ± 0.10
Threonine, %	3.4 ± 0.06	3.4 ± 0.09	3.2 ± 0.16	3.3 ± 0.12
Serine, %	3.0 ± 0.17	3.1 ± 0.08	2.9 ± 0.11	3.0 ± 0.12
Alanine, %	6.1 ± 0.16	6.3 ± 0.15	6.0 ± 0.19	6.1 ± 0.10
Glycine, %	3.6 ± 0.19	3.6 ± 0.23	3.5 ± 0.17	3.5 ± 0.11

In contrast, in the experimental group II, decreased levels of lysine, tyrosine, histidine, leucine + isoleucine, methionine, proline, threonine, serine, alanine, and glycine were observed. However, these changes were insignificant.

The overwhelming majority of previously cited studies have found positive dynamics in the accumulation of macro- and microelements in the muscle and liver tissues of farm animals as a result of the use of a mixture of phytogenic additives (a mixture of oregano, anise, and citrus essential oils) in their diets [38]. Similar results were obtained in our study, when analyzing the elemental composition of the thigh muscles of experimental broilers (Table 9).

As a result of a comparative analysis of the concentrations of chemical elements in the thigh muscles of experimental broilers, a reliable increase in Cu content was established in all experimental groups by 22.6 % ($p \leq 0.001$), 14.7 % ($p \leq 0.01$), 15.3 % ($p \leq 0.01$) relative to the control. In the experimental group I, an increase in the content of Mn, Fe and Zn was noted by 57.4 % ($p \leq 0.001$), 27.2 % ($p \leq 0.001$) and 16.3 % ($p \leq 0.05$) relative to the control. In experimental group II, the levels of Ca, Mn, Fe, and Zn increased by 13.8 % ($p \leq 0.01$), 22.1 % ($p \leq 0.001$), 18.1 % ($p \leq 0.01$), and 19.3 % ($p \leq 0.01$), respectively, relative to the control. In experimental group III, a 50.0 % decrease in Co content ($p \leq 0.001$) and a 17.2 % increase in Ca content ($p \leq 0.001$) relative to the control values were recorded.

Analysis of the liver chemical composition revealed a decrease in the moisture content in animals from all experimental groups compared to the control (Table 10).

All experimental groups showed increases in dry matter by 0.77 %, 2.91 % ($p \leq 0.05$), and 1.88 %. A statistically significant increase in fat content ($p \leq 0.05$) was also noted in all experimental groups by 0.39 %, 1.68 %, and 0.64 % relative to the control. The greatest effect was observed in group II, where fat content reached 4.31 % (versus 2.63 % in the control). Protein content also increased relative to the control value by 0.38 %–1.24 %. However, the data were not significant. Ash content remained unchanged.

All studied bioactive compound compositions had a significant effect on the liver, increasing fat and dry matter content. This may indicate activation of metabolic processes in the liver or changes in lipid metabolism. The most potent effect was demonstrated by the combination of cinnamaldehyde and 7-hydroxycoumarin (group II).

A comparative analysis of amino acid concentrations in the liver of test animals revealed no significant differences between groups (Table 11).

In all experimental groups, the dynamics of increasing content was recorded: lysine by 0.11 %–0.66 %, tyrosine by 0.05 %–0.18 %, phenylalanine by 0.02 %–0.27 %, histidine by 0.01 %–0.19 %, leucine + isoleucine by 0.06 %-0.66 %, valine by 0.03–0.32 %, proline by 0.03 %–0.19 %, threonine by 0.01 %-0.19 %, glycine by 0.08 %–0.3 %, respectively, relative to the control. In experimental group I, a slight increase in serine and alanine concentrations was observed by 0.01 % and 0.04 %, respectively. In experimental group III, arginine, serine, and alanine increased by 0.4 %, 0.17 %, and 0.4 %, respectively. Methionine levels remained virtually unchanged across all experimental groups.

It was found that the use of experimental additives in the diets also had a certain effect on the elemental composition in the liver of experimental broiler chickens (Table 12).

Table 9. Elemental composition of thigh muscles in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.29 ± 0.01	0.30 ± 0.01	0.33 ± 0.01[b]	0.34 ± 0.01[c]
P, g/kg	8.78 ± 0.30	8.67 ± 0.29	8.51 ± 0.28	8.26 ± 0.39
Na, g/kg	2.56 ± 0.14	2.68 ± 0.09	2.74 ± 0.10	2.70 ± 0.09
Mg, g/kg	1.09 ± 0.03	1.08 ± 0.05	1.05 ± 0.04	1.02 ± 0.04
K, g/kg	14.97 ± 0.61	14.65 ± 0.47	14.74 ± 0.52	13.71 ± 0.44
B, mg/kg	1.78 ± 0.10	1.69 ± 0.13	1.88 ± 0.11	1.76 ± 0.11
Mn, mg/kg	0.68 ± 0.02	1.07 ± 0.05[c]	0.83 ± 0.03[c]	0.65 ± 0.03
Co, mg/kg	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.01 ± 0.001[c]
Fe, mg/kg	35.79 ± 1.18	45.51 ± 1.96[c]	42.27 ± 1.86[b]	34.23 ± 2.67
Zn, mg/kg	46.76 ± 2.24	54.37 ± 2.17[a]	55.77 ± 2.18[b]	50.80 ± 1.68
Se, mg/kg	0.84 ± 0.367	0.76 ± 0.064	0.59 ± 0.065	0.77 ± 0.114
Cu, mg/kg	1.90 ± 0.06	2.33 ± 0.10[c]	2.18 ± 0.08[b]	2.19 ± 0.09[b]

Note: [a] — P ≤ 0.05; [b] — P ≤ 0.01; [c] — P ≤ 0.001 when compared to the control group.

Table 10. Analysis of the chemical composition of the liver in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	82.42 ± 2.32	81.65 ± 2.51	79.51 ± 2.49	80.54 ± 2.19
Dry matter, %	17.58 ± 0.71	18.35 ± 0.58	20.49 ± 0.86[a]	19.46 ± 0.76
Fat, %	2.63 ± 0.09	3.02 ± 0.06[a]	4.31 ± 0.36[a]	3.27 ± 0.16[a]
Ash, %	0.97 ± 0.006	0.97 ± 0.04	0.96 ± 0.06	0.97 ± 0.08
Protein, %	13.98 ± 0.46	14.36 ± 0.54	15.22 ± 0.68	15.22 ± 0.89

Note: [a] — P ≤ 0.05 when compared to the control group.

Table 11. Amino acid content of the liver in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	4.15 ± 0.18	4.03 ± 0.21	3.88 ± 0.19	4.55 ± 0.17
Lysine, %	4.12 ± 0.23	4.39 ± 0.31	4.23 ± 0.19	4.78 ± 0.28
Tyrosine, %	2.08 ± 0.13	2.19 ± 0.18	2.13 ± 0.14	2.26 ± 0.21
Phenylalanine, %	2.71 ± 0.09	2.76 ± 0.06	2.74 ± 0.09	2.98 ± 0.12
Histidine, %	1.59 ± 0.05	1.65 ± 0.07	1.6 ± 0.06	1.78 ± 0.07[a]
Leucine + Isoleucine, %	7.9 ± 0.32	8.07 ± 0.29	7.96 ± 0.33	8.56 ± 0.37
Methionine, %	1.57 ± 0.07	1.59 ± 0.06	1.57 ± 0.04	1.57 ± 0.05
Valine, %	3.53 ± 0.16	3.56 ± 0.19	3.56 ± 0.14	3.85 ± 0.15
Proline, %	2.64 ± 0.10	2.68 ± 0.12	2.67 ± 0.09	2.83 ± 0.12
Threonine, %	2.69 ± 0.12	2.7 ± 0.11	2.7 ± 0.16	2.88 ± 0.18
Serine, %	2.71 ± 0.15	2.72 ± 0.12	2.68 ± 0.16	2.88 ± 0.14
Alanine, %	4.37 ± 0.19	4.41 ± 0.22	4.37 ± 0.16	4.77 ± 0.19
Glycine, %	2.95 ± 0.24	3.03 ± 0.19	3.03 ± 0.21	3.25 ± 0.17

Note: [a] — $P \le 0.05$ when compared to the control group.

Table 12. Elemental composition of liver in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.36 ± 0.01	0.36 ± 0.02	0.39 ± 0.01[a]	0.41 ± 0.02[a]
P, g/kg	11.86 ± 0.55	12.18 ± 0.71	11.36 ± 0.37	11.71 ± 0.50
B, mg/kg	1.65 ± 0.07	1.58 ± 0.06	1.83 ± 0.12	1.61 ± 0.12
Na, g/kg	4.14 ± 0.18	4.77 ± 0.25[a]	4.06 ± 0.14	4.15 ± 0.14
K, g/kg	11.60 ± 0.65	11.91 ± 0.73	10.76 ± 0.43	11.18 ± 0.40
Mg, g/kg	0.79 ± 0.03	0.86 ± 0.05	0.78 ± 0.02	0.79 ± 0.03
Mn, mg/kg	8.19 ± 0.48	7.51 ± 0.43	8.60 ± 0.28	7.46 ± 0.27
Fe, mg/kg	342.79 ± 14.40	224.10 ± 7.17[c]	256.18 ± 9.22[c]	307.84 ± 13.24
Zn, mg/kg	107.78 ± 3.99	111.12 ± 4.67	88.00 ± 3.08[c]	126.02 ± 5.54[b]
Co, mg/kg	0.07 ± 0.003	0.07 ± 0.004	0.06 ± 0.003[a]	0.07 ± 0.004
Cu, mg/kg	14.94 ± 0.58	12.53 ± 0.43[b]	12.08 ± 0.39[c]	12.70 ± 0.46[b]
Se, mg/kg	2.52 ± 0.26	2.60 ± 0.21	2.40 ± 0.37	2.64 ± 0.43

Note: [a] — $P \le 0.05$; [b] — $P \le 0.01$; [c] — $P \le 0.001$ when compared to the control group.

Thus, a reliable decrease in Cu was noted in all experimental groups by 16.1 % ($p \le 0.01$), 19.1 % ($p \le 0.001$), 15.0 % ($p \le 0.01$) relative to the control values. In the experimental group I, an increase in Na content by 15.2 % ($p \le 0.05$) and a decrease in Fe by 34.6 % ($p \le 0.001$) were observed. In the experimental group II, Fe, Zn and Co contents decreased by 25.3 % ($p \le 0.001$), 18.4 % ($p \le 0.001$) and 14.3 % ($p \le 0.05$), and Ca content increased by 8.3 % ($p \le 0.05$). In experimental group III, Ca and Zn contents increased by 13.9 % ($p \le 0.05$) and 16.9 % ($p \le 0.01$) compared to the control group. These results are consistent with previous studies showing that the inclusion of coumarin and *Bacillus cereus* probiotic in animal diets promotes an increase in Ca and Zn levels in liver tissue [39].

Since coumarin is a component of many plants, our results on the effect of 7-hydroxycoumarin on mineral metabolism may be compared with studies of plant extracts. For example, the addition of *Boswellia serrata* essential extract to broiler diets resulted in increased Ca levels in breast and thigh muscles and liver, and Mg levels in thigh muscles and liver, while simultaneously reducing Cu content in these tissues [40]. In our study, the addition of 7-hydroxycoumarin caused a similar effect on Ca (in thigh muscles and liver) and Cu (in the liver). Data on the direct effect of the studied bioactive compounds (cinnamaldehyde, quercetin, 7-hydroxycoumarin) on the mineral composition of broiler tissues are limited. However, it is known that the concentration of elements in the liver of farm animals depends on feeding and the environment [41]. Overall, our results confirm the ability of phytogenic additives to specifically influence the accumulation of microelements in animal tissues, where a moderate correlation with the total polyphenol content was observed [42]. This effect may be due to the antagonism between the components of the extracts and metal ions, as well as their ability to compete in the formation of metal complexes or chelate the transition metal ions [13, 43, 44], which ultimately modulates the accumulation of the latter in muscles and liver.

Conclusion

The study demonstrated the high efficacy of a combination of bioactive compounds (cinnamaldehyde, quercetin, and 7-hydroxycoumarin) in broiler chicken diets. The complete combination (group III) demonstrated the greatest synergistic effect, as evidenced by a significant increase in absolute weight gain by 51.4 % ($p \le 0.05$) and a more than twofold increase in the EPEF.

The improved productivity of broiler chickens in the experimental groups was accompanied by positive changes in meat quality: a significant increase in breast muscle fat content by 0.2–0.9 % ($p \le 0.001$) and protein content by 2.2 % ($p \le 0.05$) in group II. The additives modulated mineral metabolism, causing statistically significant changes in Fe, Zn, and Cu concentrations in muscle tissue and liver ($p \le 0.05$ to $p \le 0.001$).

Thus, the obtained results demonstrate that the developed composition of bioactive substances, especially the complete composition (cinnamaldehyde, quercetin, and 7-hydroxycoumarin), is highly effective for stimulating growth, improving meat quality, and optimizing metabolic processes in broiler chickens. This combination is a promising alternative to antibiotic growth promoters in modern poultry farming.

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AUTHORS INFORMATION

Marina Ya. Kurilkina, Candidate of Biological Sciences, Senior Researcher, Testing Center, Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia.
E-mail: k_marina4@mail.ru
ORCID: https://orcid.org/0000-0003-0253-7867
* corresponding author

Tatyana A. Klimova, Candidate of Biological Sciences, Researcher, Testing Center, Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia. E-mail: klimovat91@mail.ru
ORCID: https://orcid.org/0000-0003-4298-1663

Shamil G. Rakhmatullin, Candidate of Biological Sciences, Senior Researcher, Head of Patent Department, Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia. E-mail: shahm2005@mail.ru
ORCID: https://orcid.org/0000-0003-0143-9499

Dmitry G. Deryabin, Doctor of Medical Sciences, Professor, Leading Researcher, Laboratory of Breeding and Genetic Research in Animal Husbandry, Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia. E-mail: dgderyabin@yandex.ru
ORCID: https://orcid.org/0000-0002-2495-6694

Kseniya N. Atlanderova, Candidate of Biological Sciences, Researcher, Testing Center, Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia. E-mail: atlander-kn@mail.ru
ORCID: https://orcid.org/0000-0003-3977-4831

Galimzhan K. Duskaev, Doctor of Biology Sciences, Professor of Russian Academy of Sciences, Leading Researcher, Department of Farm Animal Feeding and Feed Technology named after S. G. Leushin, Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences. 29, 9 January Str., 460000, Orenburg, Russia. E-mail: gduskaev@mail.ru
ORCID: https://orcid.org/0000-0002-9015-8367

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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