Impact of a phytogenic composition on feed conversion and certain digestive mechanisms in broiler chickens

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Abstract. Currently, a relevant direction in broiler chicken nutrition research is the search for and development of biologically active alternatives to antibiotic growth promoters, among which phytobiotics are included. The aim of this study was a comparative analysis of the effects of two developed phytogenic compositions on feed conversion and certain digestive mechanisms of Smena 9 broiler chickens, based on the study of gastrointestinal tract development and expression of the *SLC15A1* gene in the jejunum. Three groups were formed, each consisting of 43 birds. The first group (control) received complete compound feeds, while the second and third groups (experimental) were fed complete compound feeds supplemented with phytogenic compositions containing extracts of common chicory roots, maral root, creeping thyme shoots, and St. John's wort in various proportions. During different growth periods, live weight gain and feed intake were recorded, and feed conversion ratios were calculated. At 22 days, jejunal samples were collected from three roosters per group to analyze peptide transporter 1 gene expression, and at the end of the experiment, gastrointestinal tract development was assessed anatomically in three roosters per group. The use of the phytogenic composition in the ratio 0.4 : 0.35 : 0.15 : 0.1 favorably influenced nutrient assimilation processes, reflected in better gastrointestinal tract development, a 2.5% reduction in feed costs per 1 kg of weight gain compared to control and the Smena 9 standard, and a 6.5-fold increase in *SLC15A1* gene expression (p = 0.030). This phytogenic composition can be considered a feed additive for industrial poultry production aimed at improving the economic efficiency of poultry meat production.

1 Introduction

The development of microbial antibiotic resistance, partly caused by the irrational use of feed antibiotics in animal husbandry, has led to a complete or partial ban on antimicrobial agents in animal feeding. Consequently, there is an increased need to find accessible alternatives, among which phytogenic feed additives (phytobiotics) stand out. These

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biologically active products derived from plants exhibit a wide range of beneficial properties, including growth-promoting, antimicrobial, anti-inflammatory, and antioxidant effects [1].

One positive aspect of phytobiotics in livestock nutrition is their beneficial impact on digestive processes. They enhance digestion and nutrient absorption by stimulating the secretion of digestive juices, bile, saliva, and mucus; demonstrating a prebiotic effect by promoting the growth of beneficial gut microbiota; increasing digestive enzyme activities such as amylase and trypsin; prolonging the retention time of chyme in the digestive tract; and supporting the growth of intestinal villi [2]. Thus, the comprehensive effect of phytobiotics on gastrointestinal function creates favorable conditions for more efficient nutrient utilization, improving feed conversion ratios and, consequently, increasing meat production efficiency, including that of poultry [3].

Currently, various plant materials are actively being tested, including analyses of how different plants and their components influence digestion, highlighting the relevance of developing effective phytogenics. Recent studies have shown positive effects on feed conversion and digestive efficiency from plants such as *Callicarpa nudiflora* [4], *Terminalia chebula* [5], *Glycyrrhiza glabra* [6], *Zingiber officinale* [7], *Capsicum spp*. [8], among other phytogenic sources.

A key biomarker of physiological activity in response to various environmental factors is the relative level of gene expression. Genes associated with nutrient absorption processes are especially important for digestive organ activity. One such gene is the intestinal peptide transporter 1 (*PepT1*, *SLC15A1*), which plays a crucial role in protein nutrition by mediating di- and tripeptide transport from the intestinal lumen into enterocytes [9].

Based on these insights, the aim of the study was a comparative analysis of two developed phytogenic compositions on feed conversion and certain digestive mechanisms in broiler chickens, focusing on gastrointestinal tract development and *SLC15A1* gene expression in the jejunum.

2 Material and Methods

The study was reviewed and approved by the Bioethics Committee of the FSBEI of HE "Russian State Agrarian University – Moscow Timiryazev Agricultural Academy" (protocol № 43 dated April 26, 2025).

2.1 Design, Object, and Conditions of the Experiment

To achieve the research objective, a zootechnical trial lasting 35 days was conducted on broiler chickens of the Russian cross Smena 9 at the Preclinical and Clinical Testing Center for Veterinary Drugs of the Timiryazev Agricultural Academy from June to July 2025. The experiment was organized following the methodology for scientific and industrial research on feeding poultry, developed by the All-Russian Scientific Research and Technology Institute of Poultry.

Chickens were housed in vertical three-tier cage systems with an even distribution of birds across levels. Feeding and watering were provided ad libitum using vacuum/nipple drinkers and hopper/trough feeders at different stages of the broilers' ontogeny. Microclimate parameters (temperature, humidity), stocking density, nutrient content, and chemical composition of compound feeds (listed in Table 1) complied with the recommendations for the Smena 9 cross management.

Table 1. Nutritional value and chemical composition of the used compound feeds.

Indicators	Starter (0-10 days)	Grower (11-22 days)	Finisher (23-35 days)
Metabolic energy (kcal/100 g)	300.00	310.00	315.00
Crude protein (%)	22.80	20.80	19.50
Essential extract (%)	4.00	3.78	3.85
Crude fiber (%)	3.55	3.98	3.65
Total lysine (%)	1.33	1.17	1.10
Total methionine (%)	0.62	0.58	0.58
Total methionine + cystine (%)	0.97	0.91	0.89
Total threonine (%)	0.89	0.81	0.80
Calcium (%)	1.05	0.95	0.93
Available phosphorus (%)	0.77	0.67	0.71
Sodium (%)	0.21	0.17	0.16
Chlorine (%)	0.21	0.19	0.19

Using the balanced groups of analogues method, a control group and two experimental groups were formed, each consisting of 43 heads. The diet of the control group chicks included full-ration phase compound feeds: Starter (0-10 days), Grower (11-22 days), and Finisher (23-35 days). For the chicks in the experimental groups, two variants of a developed phytogenic composition were additionally introduced into the diet, containing: extract of common chicory roots (*Cichorium intubus* L.), standardized to 100% inulin; extract of maral root roots (*Rhaponticum carthamoides* Willd.), standardized to 0.505% phytoecdysteroid ecdysten; extract of perforate St John's wort shoots (*Hypericum perforatum* L.), standardized to 7.3% flavonoids expressed as rutin; extract of creeping thyme shoots (*Thymus serpyllum* L.), standardized to 18.4% tannins and 3.6% flavonoids expressed as rutin. The phytogenic compositions consisted of mixtures of evenly blended fine powders and were introduced into the compound feeds by stepwise mixing at a rate of 300 g/ton. The proportions of the extracts in the developed phytogenic compositions are presented in the experimental design in Table 2.

Table 2. Design of the experiment.

Indicators	Groups		
	1 Control	2 Experimental	3 Experimental
Level of phyto-combination introduction into the diet (g/t)	0	300	300
Proportion of common chicory extract in the combination (%)	-	40	35
Proportion of perforate St John's wort extract in the combination (%)	-	10	5
Proportion of maral root extract in the combination (%)	-	35	40
Proportion of creeping thyme extract in the combination (%)	-	15	20

The developed phytocompositions possess a range of biologically active properties attributed to the effects of specific components identified by us earlier [10]. For instance, chicory extract contributes to stabilizing the intestinal microbiome and promoting the growth of probiotic cultures, thereby supporting gut health. Maral root extract activates protein biosynthesis and skeletal muscle growth. St. John's wort extract exhibits a vitagene-stimulating effect in the liver with activation of its antioxidant functions. Thyme extract positively influences the meat quality of poultry.

2.2 Feed Conversion Accounting of Broiler Chickens

During the zootechnical trial, weekly individual weighings (n = 43) were conducted to analyze the effect of the developed phytogenic composition on the growth of broiler chickens. Electronic laboratory scales Merkuriy 122ACFJR-600.01 (Mertech, Russia) with an accuracy of 0.01 g and electronic scales M-ER 223 AC-15.2 Mary LCD (Mertech, Russia) with an accuracy of 2 g were used depending on the age of the birds. Broiler survival and total feed consumption were recorded. Individual feed consumption was calculated by dividing the total feed intake by the number of birds in the group, considering survival dynamics throughout the experiment. Feed cost per 1 kg of weight gain was determined by dividing the individual feed consumption over the entire rearing period by the average absolute weight gain of the chickens to assess feed conversion efficiency.

2.3 Analysis of the Development of Digestive Organs in Broiler Chickens

To identify the biological mechanisms underlying changes in feed conversion in broiler chickens, the expression of the *SLC15A1* gene (peptide transporter 1) was studied in cells of the jejunum tissue. For this purpose, at 22 days of age, 3 cockerels with average live weight were selected from each group and euthanized following the Recommendation No. 33 of the Eurasian Economic Commission Board dated November 14, 2023, "On Guidelines for Working with Laboratory (Experimental) Animals in Preclinical (Nonclinical) Studies." Subsequently, the abdominal cavity was opened, and fragments of the jejunum (central part of the organ) were collected and placed in the IntactRNA fixer (Evrogen, Russia). Samples were stored at −20°C.

The determination of the relative transcriptional activity of the gene was carried out at the educational and scientific collective-use center – "Service Laboratory of Comprehensive Chemical Compound Analysis" of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (Moscow, Russia). Total RNA was extracted from the samples using the RNA Solo kit (Evrogen, Russia), applying the TissueLyser II device (Qiagen, Netherlands) for tissue homogenization and subsequent cell lysis. Based on the isolated RNA, cDNA was synthesized using reverse transcriptase included in the Magnus kit (Evrogen, Russia) on the C1000 Touch instrument (Bio-Rad, USA). The quality and quantity of RNA and synthesized cDNA were analyzed on the Fluo-200 device (Allsheng, China) using the QuDu ssDNA kit (Thermo Fisher Scientific, USA). All RNA extraction and cDNA synthesis procedures were performed according to the manufacturers' instructions. The resulting cDNA was stored at −20°C.

For analysis of the relative expression of the chosen gene, gene-specific primer sequences were used, presented in Table 3, selected based on a review of international scientific literature and verified using the Ensembl genome browser.

Table 3. Nucleotide Sequence of Primers.

Gene	Primers	Author
ACTB (β-actin)	F: CTGTGCCCATCTATGAAGGCTA R: ATTTCTCTCTCGGCTGTGGTG	Laptev G.Yu. et al., 2023 [11]
SLC15A1 (solute carrier family 15 member 1)	F: AATTGGGCAGGCAGTCATGG R: AGCGCGATGAGAATCAAGCC	Park J.H. et al., 2020 [12]

The PCR-RV reaction was performed using the CFX96 Touch amplifier (Bio-Rad, USA) and the 5X qPCRmix-HS SYBR kit ("Eurogen," Russia). The housekeeping gene used as a control was the β-actin *ACTB* gene. The amplification protocol was as follows: 3 minutes at

95°C (initial denaturation); 30 seconds at 95°C (denaturation), 30 seconds at 60°C (primer annealing), 30 seconds at 72°C (elongation). The total reaction duration was 40 cycles. Relative expression levels were assessed using the $2^{-\Delta\Delta CT}$ method [13].

At the end of the growing period, at 35 days of age, after a 10-hour fasting, 3 roosters with average live weight per group and sex were selected from each experimental group. The selected chicks were euthanized for anatomical dissection following the methodology of the All-Russian Scientific Research and Technological Institute of Poultry in a specially equipped room using surgical instruments, with results recorded in protocols. Gastrointestinal organs were weighed using Mercury 122ACFJR-600.01 laboratory scales (Mertech, Russia) with a precision of 0.01 g.

2.4 Statistical Data Analysis

Statistical analysis of the obtained data was performed using the IBM SPSS Statistics 23 software package (SPSS Inc., Chicago, IL, USA, 2023) by the one-way analysis of variance (ANOVA) method with multiple comparisons using Tukey's test. Data normality was preliminarily checked using the Shapiro-Wilk test. Differences were considered statistically significant at $p \leq 0.05$.

3 Results and Discussion

3.1 Feed Conversion in Broiler Chickens

The analysis of the experimental results showed that the effectiveness of one of the developed phytogenic compositions was manifested in improved feed utilization by broiler chickens. At the same growth rate, broilers in the 2nd experimental group consumed less compound feed starting from the initial growing phase, as shown in Table 4.

Table 4. Dynamics of feed consumption and feed costs per 1 kg of broiler weight gain, $n = 43$.

Indicators	Groups		
	1 Control	**2 Experimental**	**3 Experimental**
Cumulative feed consumption (g/head) at age			
7 days	109.3	108.0	113.4
as % of control	100.00	98.8	103.8
14 days	498.6	473.1	482.4
as % of control	100.00	94.9	96.8
21 days	1237.8	1176.2	1195.7
as % of control	100.00	95.0	96.6
28 days	2288.9	2211.7	2186.3
as % of control	100.00	96.6	95.5
35 days	3454.6	3355.1	3310.9
as % of control	100.00	97.1	95.8
Feed conversion ratio at age (kg)			
7 days	0.712	0.684	0.727
as % of control	100.00	96.1	102.1
14 days	0.997	0.944	0.975
as % of control	100.00	94.7	97.8
21 days	1.186	1.139	1.164
as % of control	100.00	96.0	98.1
28 days	1.420	1.388	1.404
as % of control	100.00	97.7	98.9
35 days	1.660	1.620	1.662

as % of control	100.00	97.6	100.1

This led to a reduction in feed costs per 1 kg of weight gain. Over the entire 35-day growing period, feed costs per 1 kg of gain in the control group amounted to 1.66 kg/kg, which corresponds to the standard for the Smena 9 cross. In the 2nd experimental group, this indicator was 1.62 kg/kg, lower by 2.5%. Improvement of feed conversion in broilers under the influence of phytogenic additives based on plant extracts has been demonstrated in many studies reviewed by W. Windisch et al. [14]. This pattern was also confirmed in the present study.

Thus, the proportions of raw materials in the composition of the finished phytogenic mixtures are significant in terms of their impact on bird productivity, which is likely related to mechanisms of synergy and antagonism of biologically active compounds of the original plant extracts.

3.2 Development of the Digestive Organs of Broiler Chickens

The pattern of improved feed conversion in broiler chickens was partially explained by the results of anatomical studies (Table 5).

Despite the absence of statistically significant differences ($p > 0.05$), the assessment of the gastrointestinal tract condition in broiler chickens indicated better development of most digestive system organs in the experimental groups. This likely points to greater functional activity of the digestive organs, and consequently, to the activation of digestion and nutrient absorption processes under the influence of biologically active substances from phytochemical combinations.

Table 5. Development of the gastrointestinal tract organs of broiler chickens, $n = 3$, roosters.

Indicators	Groups			*p*-Value
	1 Control	**2 Experimental**	**3 Experimental**	
Mass of esophagus and crop, g	11.4±0.80	17.3±0.82	25.3±7.5	0.158
Mass of esophagus and crop relative to live weight, %	0.6±0.04	0.9±0.04	1.3±0.38	0.150
Mass of glandular stomach, g	8.9±0.43	10.6±0.94	11.3±1.44	0.314
Mass of glandular stomach relative to live weight, %	0.4±0.02	0.5±0.05	0.6±0.07	0.248
Mass of muscular stomach, g	15.9±1.33	24.2±2.57	21.3±3.88	0.183
Mass of muscular stomach relative to live weight, %	0.8±0.07	1.2±0.12	1.1±0.19	0.155
Mass of liver, g	53.6±4.02	57.5±8.63	55.2±4.77	0.904
Mass of liver relative to live weight, %	2.6±0.19	2.8±0.44	2.8±0.23	0.857
Mass of pancreas, g	4.0±0.54	3.7±0.83	3.7±0.24	0.902
Mass of pancreas relative to live weight, %	0.2±0.03	0.2±0.04	0.2±0.01	0.955
Mass of intestine, g	104.2±9.32	95.2±13.40	115.3±19.04	0.638
Mass of intestine relative to live weight, %	5.1±0.44	4.7±0.69	5.8±1.00	0.589

3.3 Expression of the *SLC15A1* Gene in the Jejunum of Broiler Chickens

According to the PCR-RV results shown in Figure 1, it was established that in the jejunum tissues of chickens from the 2nd experimental group, the expression of the *SLC15A1* gene was more than 6.5 times higher, with a statistically significant difference ($p = 0.030$). Thus, changes in the expression of this gene, as one of the key nutrient transporter genes, likely indicate more efficient digestion and nutrient absorption processes in the bird's intestine. The experimental data overall align with the findings of Al-Khalaifah H.S. et al., whose study showed that adding dried brewer's grains fermented by *Bacillus subtilis*, *Lactobacillus rhamnosus*, and *Saccharomyces cerevisiae* to the main diet at various inclusion levels increased the expression of the transporter gene *SLC2A1* in the duodenum of Ross 308 broilers, lowering feed conversion ratio and promoting better development of the muscular stomach [15]. A similar set of changes was observed in the present study when the corresponding phytogenic composition variant was included in the diet of the 2nd experimental group.

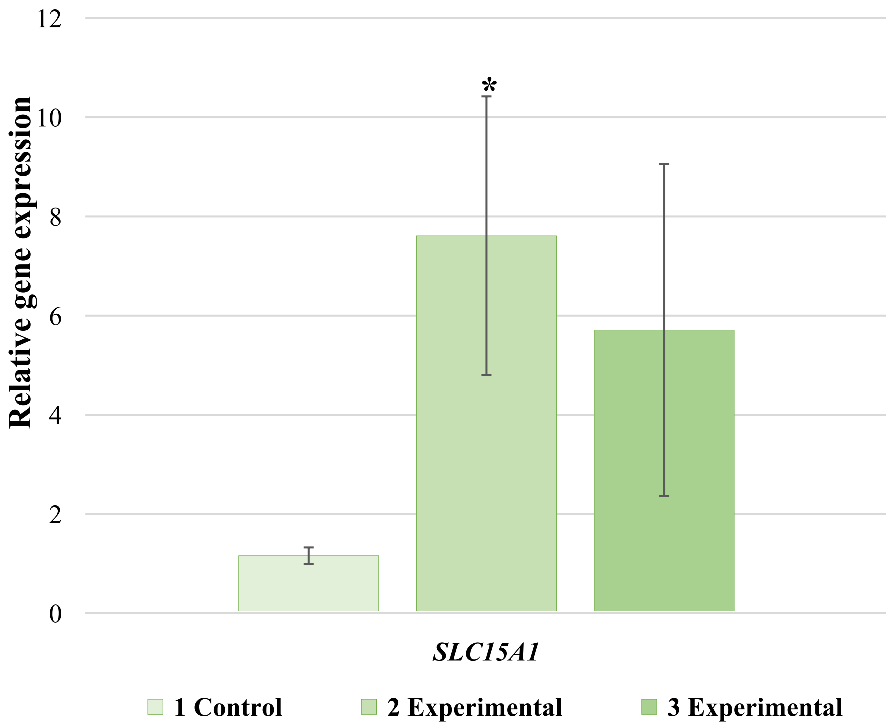

Fig. 1. The expression level of the *SLC15A1* gene in the tissues of the jejunum of broiler chickens (* – the difference in ΔCt values is statistically significant compared to the control group at $p \leq 0.05$; results are presented as mean ± standard error of the mean (M±m) for mRNA expression, $n = 3$, roosters).

4 Conclusion

The inclusion of a multicomponent phytogenic composition in the diet of broiler chickens, consisting of extracts of chicory, maral root, thyme, and St. John's wort in the ratio 0.4 : 0.35 : 0.15 : 0.1, positively influences the nutrient absorption processes. This is expressed by improved development of the gastrointestinal tract, a 2.5% reduction in feed consumption per 1 kg of weight gain compared to both the control and the Smena 9 cross standard, and a 6.5-fold increase in the expression of the peptide transporter gene *SLC15A1*. These results support considering the developed phytogenic composition as a feed additive in industrial poultry farming to enhance the economic efficiency of poultry meat production.

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