

# Journal of Poultry Sciences and Avian Diseases

Journal homepage: [www.jpsad.com](http://www.jpsad.com)



## Performance, Intestinal Health, Cecal Microbiota, and Nutrient Transporter Gene Expression between Arian and Ross Broiler Chickens under Wheat-Based Diets



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### Article Info

#### Article type:

Original Research

#### How to cite this article:

Oudi Zare, S., Hosseini Moghaddam, S. H., Mohiti-Asli, M., Ghalyanchi Langeroudi, A., & Ghavi Hossein-Zadeh, N. (2026). Performance, Intestinal Health, Cecal Microbiota, and Nutrient Transporter Gene Expression between Arian and Ross Broiler Chickens under Wheat-Based Diets. *Journal of Poultry Sciences and Avian Diseases*, 4(3), 1-12.

<http://dx.doi.org/10.61838/kman.jpsad.154>



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### ABSTRACT

This experiment examined the responses of Arian and Ross broiler strains to wheat-based diets formulated with elevated levels of meat meal, intended to disrupt gut health and induce dysbiosis. The objective was to evaluate strain-dependent resilience and determine the extent to which gut health-promoting feed additives could mitigate potential genetic predispositions to intestinal dysfunction. Six dietary treatments were administered: the basal diet without additives (control) and the basal diet supplemented with either an antibiotic, probiotic, prebiotic, organic acid, or phytobiotic. Evaluated parameters included growth performance traits, carcass weight, organ weights, intestinal length, clinical and histological indicators of intestinal health, and gut microbial populations. Additionally, the expression of key nutrient transporter-related genes (*SLC7A5*, *SLC7A6*, *SLC7A9*, *SLC6A19*, *GLUT2*, and *SI*) was quantified. The Ross strain exhibited significantly ( $p < 0.05$ ) higher feed intake than the Arian strain (4543 g vs. 3977 g), a superior feed conversion ratio (1.66 vs. 1.76), higher live weight (2713.2 g vs. 2232.3 g), carcass weight (2030 g vs. 1637 g), and also intestinal length (226.1 mm vs. 202.9 mm). Despite the potentially challenging nature of the basal diet, no significant differences in dysbacteriosis or necrosis scores were observed between the two strains ( $p > 0.05$ ), indicating effective adaptation in both strains. The microbial populations of *Escherichia coli*, *Lactobacillus*, and *Clostridium perfringens* were significantly higher in Ross birds than in Arian ( $p < 0.05$ ). Among diets, the phytobiotic-supplemented diet significantly upregulated the expression of *SLC6A19*, *SLC7A5*, and *SLC7A6* genes in the Ross strain ( $p < 0.05$ ). While organic acids significantly enhanced *SLC7A6* expression in both strains, antibiotics significantly upregulated *SLC7A9* in the Ross strain. The expression of *GLUT2* was not significantly affected by genetic strain ( $p > 0.05$ ). These findings suggest that the improved performance of Ross 308 broilers, particularly under phytobiotic supplementation, may be partially attributed to enhanced expression of nutrient transporter genes in response to intestinal challenge.

**Keywords:** Arian Broiler, Gene Expression, Health-Promoting Additives, Nutrient Transporters, Ross 308, Wheat-Based Diet.

#### Article history:

Received 24 August 2025

Revised 11 November 2025

Accepted 22 February 2026

Published online 01 January 2026

Final Published 01 July 2026

## 1 Introduction

The poultry industry has undergone remarkable development over recent decades, which has been largely driven by advancements in genetics and breeding. These innovations have resulted in the emergence of various commercial broiler strains optimized for growth performance, feed efficiency, and carcass yield (Al-Marzooqi et al., 2019; Ikusika et al., 2020). Among these, the Ross 308 is widely used worldwide, while the Arian strain has been selectively bred and adopted in Iran. These strains differ in size, physiology, and possibly in their responses to nutritional and environmental stressors.

The Arian broiler produced in Iran is derived from Arian commercial broiler lines, which were established in 1991 from the Hybro N pedigreed broiler lines imported from the Netherlands. Now the elite birds of these pedigreed-pure lines are reared with the low-density diet to reduce susceptibility to feed quality variation. Furthermore, they are genetically selected for at least eight economically significant traits.

One well-documented issue associated with the Arian strain is its increased susceptibility to ascites, particularly when raised at high altitudes where oxygen availability is limited (Azizian et al., 2013). Since the gastrointestinal tract accounts for more than 20% of the body's total oxygen consumption, it has been hypothesized that improving gut health could reduce oxygen demand, thereby enhancing overall physiological resilience.

Wheat-based diets are known to pose specific challenges to gut health in broilers due to their high content of soluble non-starch polysaccharides (NSPs), particularly pentosans, which increase intestinal viscosity, impair nutrient digestibility, and can contribute to dysbiosis and enteritis. The inclusion of meat meal may further complicate gut integrity by altering microbial populations and promoting digestive stress. Together, these ingredients were intentionally used to provoke subclinical gut disturbances, thereby allowing for the evaluation of strain-specific responses and the potential ameliorative effects of feed additives (Bautil et al., 2023; Vasanthakumari et al., 2023). Feed additives such as probiotics, prebiotics, phytobiotics, and organic acids are commonly used to modulate gut microbiota and the immune response in modern broilers, offering a wide range of health benefits (Krysiak et al., 2021).

Nutrient absorption is regulated by specific transporters across the plasma membrane in the small intestine, and

alterations in these transporters may contribute to reduced body weight and feed efficiency. The SLC gene family includes several key nutrient transporters, such as SGLT1 (*SLC5A1*), SGLT4 (*SLC5A9*), GLUT5 (*SLC2A5*), GLUT2 (*SLC2A2*), PepT1 (*SLC15A1*), EAAT3 (*SLC1A1*), ASCT1 (*SLC1A4*), LAT1 (*SLC7A5*), and y+LAT1 (*SLC7A7*) (Su et al., 2014). The SI gene is typically expressed on the surface of intestinal epithelial cells, where it facilitates the production of the enzyme sucrase-isomaltase, which is essential for starch and sugar digestion. This enzyme breaks down sucrose and maltose into simple sugars, allowing their absorption by intestinal epithelial cells. GLUT2 is present on both the basolateral and apical membranes of the intestine, where it plays a crucial role in glucose absorption and the transport of monosaccharides from enterocytes into the bloodstream (Kellett et al., 2008).

The current experiment was designed to compare the Arian and Ross 308 broiler strains under a dietary challenge involving wheat- and meat-based diets, both with and without feed additives, to assess differences in growth performance at various ages including feed intake (FI), weight gain (WG) and feed conversion ratio (FCR), gut health, intestinal morphology, microbial composition, and nutrient transporter gene expression. Although conducted at low altitude without the aim of directly evaluating ascites, the experiment aimed to induce intestinal stress through diet to reveal inherent differences in gut function and adaptability between the two strains, thereby identifying potential genetic and nutritional strategies to enhance physiological resilience in broiler production.

## 2 Material and Methods

### 2.1 Experimental treatments and diets

A total of 960 one-day-old male broiler chicks (480 Ross 308 strain and 480 Arian strain) were reared in a completely randomized design with six dietary treatments per strain, five replications per treatment, and 16 chicks per replication for six weeks. To evaluate the effects of various feed additives on broiler gut health under dietary stress, birds were assigned to the following dietary treatments: 1) basal diet containing wheat and meat meal without any feed additives (control), 2) the basal diet + antibiotic (avilamycin at 100 g/ton of feed), 3) the basal diet + probiotic (200 g/ton of feed; Bioguil, Zist Yar Varena, Iran), 4) the basal diet + prebiotic (500 g/ton of feed; KimiaMOS, Kimiazym, Iran), 5) the basal diet + organic acid (0.2% of the diet; Acidifier4+, Bonda Faravar, Iran), and 6) the basal diet +

phytobiotic (0.2% of the diet; O.X.Plant, Fanavari Novin Akam, Iran). The wheat-based diet had 45-55% wheat and 9-10% meat meal. The components and chemical

compositions of the diets (Table 1) were prepared based on the strain's standard nutritional requirements tables.

**Table 1.** Ingredients and composition of starter, grower, and finisher diets for Ross and Arian broiler chickens

Ingredient	Arian Strain			Ross Strain		
	Starter (d 0–14)	Grower (d 15–24)	Finisher (d 25–42)	Starter (d 0–10)	Grower (d 11–24)	Finisher (d 25–42)
Soybean meal (44% Crude protein)	26.18	20.68	16.17	25.75	21.24	16.17
Corn	17.38	21.46	15.06	17.68	20.77	15.07
Wheat	45	45	55	45	45	55
Soybean oil	0.46	0.91	1.66	0.42	1.00	1.66
Meat meal	9	10	10	9	10	10
Calcium carbonate	0.54	0.52	0.57	0.53	0.52	0.57
Common salt	0.14	0.02	0.01	0.12	0.03	0.02
Sodium bicarbonate	0.06	0.21	0.35	0.09	0.20	0.35
L-Threonine (99%)	0.14	0.14	0.13	0.19	0.14	0.12
dl-Methionine (98%)	0.31	0.28	0.25	0.35	0.29	0.25
L-Lysine HCl (78%)	0.30	0.30	0.31	0.37	0.32	0.30
Vitamin premix1	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated composition						
Metabolizable energy (kcal/kg)	2882	2950	3025	2882	2950	3025
Crude protein (g/kg)	231.9	215.4	202.1	231.5	217.7	202.1
Digestible lysine (g/kg)	11.80	10.70	9.80	11.22	10.90	9.70
Digestible methionine (g/kg)	6.00	5.50	5.00	6.30	5.70	5.10
Digestible methionine + cystine (g/kg)	8.80	8.10	7.50	9.10	8.30	7.60
Digestible threonine (g/kg)	7.80	7.20	6.60	8.20	7.30	6.50
Digestible arginine (g/kg)	13.10	11.9	10.8	13.0	12.0	10.8
Digestible valine (g/kg)	8.90	8.20	7.60	8.80	8.30	7.60
Digestible isoleucine (g/kg)	7.70	7.0	6.40	7.60	7.10	6.40
Calcium (g/kg)	10.00	8.60	8.20	10.00	8.60	8.20
Available phosphorus (g/kg)	4.70	4.30	4.00	4.70	4.30	4.00
Total phosphorus (g/kg)	7.50	7.00	6.70	7.50	7.00	6.70
Sodium (g/kg)	1.60	1.60	1.60	1.60	1.60	1.60
Potassium (g/kg)	9.10	8.50	7.90	9.10	8.50	7.90
Chloride (g/kg)	2.90	2.30	2.30	2.90	2.30	2.30
DEB (mEq/kg)	221	222	207	221	222	207

<sup>1</sup>Vitamin premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; nicotinic acid, 30 mg; pantothenic acid, 10 mg; vitamin B6, 3 mg; folic acid, 1 mg; vitamin B12, 0.15 mg and choline, 500 mg.

<sup>2</sup>Mineral premix provided the following per kilogram of diet: iron, 50 mg; iodine, 1 mg; manganese, 100 mg; zinc, 85 mg; copper, 10 mg; selenium, 0.2 mg.

Bioguil probiotic containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus delbrueckii*, and *Bacillus coagulans* with a concentration of 10<sup>10</sup> CFU/g. Organic acids with the commercial brand of Acidifier4+ included: formic acid, propionic acid, lactic acid, citric acid, and the salts: copper chloride, zinc, and sodium. The commercial prebiotic product of KimiaMOS is rich in mannan-oligosaccharides (MOS) and beta-glucans. O.X.Plant is a phyto-genetic product which contains a microencapsulated complex of natural essential oils of oregano and thyme, garlic, and capsicum oleoresin. The main bioactive ingredients in O.X.Plant are various terpenoids, aromatic hydrocarbons, and aromatic aldehydes, including carvacrol, thymol, allicin, and capsaicin, which

come from natural essential oils of savory, thyme, garlic, and red chili pepper.

The experiment was conducted at the University of Guilan's Poultry Research Facility, located at a very low altitude from sea level, ensuring that altitude-related effects such as hypoxia or ascites were not contributing factors.

## 2.2 Performance measurements and carcass characteristics

The chick weight and feed consumption were measured weekly to calculate daily weight gain (DWG), average daily feed intake (DFI), and FCR. The carcass weight and 10 various internal organs of two chickens/replicate were recorded at 42 days. Additionally, the weight and length of different segments of the intestine, the jejunum, the ileum, and the duodenum, were recorded. The mucosal tissue of the three intestinal segments was examined to check for necrosis

and dysbiosis. The necrosis scoring method (Prescott et al., 1978). was evaluated based on four-point observations: 0: No macroscopic lesions; 1: Thin and fragile intestinal wall, discolored and aged appearance; 2: The presence of necrotic ulcers and foci, pale and aged appearance, small amounts of gas production; 3: Large necrotic areas, gas-filled bowel, small blood spots; 4: Severe and extensive necrosis, pronounced bleeding, and large amounts of gas accumulation in the intestine. The dysbacteriosis scoring method (Teirlynck et al., 2011). Evaluated six parameters: intestinal ballooning, intestinal inflammation, free and reduced gut contents, intestinal thickness and frangibility, and undigested food.

### 2.3 Gut microbiota and intestinal histomorphology

Approximately one gram of caecum digesta was immediately collected to enumerate four bacterial species: coliforms, *Escherichia coli*, *Lactobacillus*, and *Clostridium perfringens*. The culture media for coliforms (MacConkey Agar, MC) and *Escherichia coli* (Eosin Methylene Blue Agar, EMB) were incubated at 37°C for 48h aerobically. Meanwhile, the culture media for *Lactobacillus* (De Man, Rogosa, and Sharpe Agar, MRS) and *Clostridium perfringens* (Tryptose Sulfite Cycloserine Agar, TSC) were placed in an anaerobic jar and incubated at 37°C for 72h under anaerobic conditions. Following incubation, bacterial colonies were enumerated. The most appropriate dilution factors used to determine colony-forming units (CFU) were 10<sup>-5</sup> for *Clostridium perfringens*, and 10<sup>-6</sup> for *Lactobacillus*, coliforms, and *Escherichia coli*. Colony counts were expressed as Log<sub>10</sub> CFU/g of digesta.

A tissue sample was obtained from the middle portion of the jejunum to examine the histology of the small intestine.

The samples were prepared according to the usual histological method. Paraffin tissue sections were put on the glass slides, deparaffinized, and stained with xylene 1 and 2 (each container for 15 min), dehydrated with serial dilutions of ethanol, stained with hematoxylin and eosin, and counterstained with serial dilutions of ethanol. Stained samples were observed using a stereomicroscope. Periodic acid-Schiff PAS staining was used to count the number of goblet cells that appeared red or purple after staining. The six morphometric measures were villus height (VH), villus width (VW), crypt depth (CD), goblet cells (GC), muscle thickness (MT), and lamina propria (LP).

### 2.4 RNA extraction, cDNA synthesis, and gene expression measurement

RNA extraction from homogenized jejunum tissue (30 mg) was performed using the Samzol™ Reagent Trizol-based extraction kit (Sambio, South Korea, Cat. #Sam015). RNA integrity was confirmed via agarose gel electrophoresis. cDNA synthesis was performed using the SinaClon First Strand cDNA Synthesis Kit according to the manufacturer’s protocol with a Thermal Cycler (Biometra TAdvanced PCR, Iran). The thermal cycling conditions were: 25°C for 5 min, 45°C for 60 min, and 70°C for 5 min to inactivate the reverse transcriptase enzyme.

The β-actin and *GAPDH* reference genes were evaluated using GeNorm. Primer design for reference and target genes was done using Primer (Version 5) software (Table 2). Quantitative PCR (qPCR) was conducted in triplicate using the HS-qPCRMix X2 Master Mix (SinaClone, Tehran, Iran, #MM12127) on a LightCycler instrument (Roche Diagnostics Corporation, USA).

**Table 2.** The primer sequences of the target and housekeeping genes

Genes	Accession number	Orientation	Primer sequences (5' -3')	Product length (bp)
SLC7A9	XM_046899466.1	Forward	GCGGTCTGGATATTTTATGGT/ TCAGGTGCGGTGATAAATTGGT	161
		Reverse		
SLC6A19	XM_419056.8	Forward	TTGGAACCCTAAATACGAGGA/ ACGATCCCTTCTCTTCTGAC	163
		Reverse		
Glut2	NM_207178.2	Forward	TGATCGTGGCACTGATGGTT/ CCACCAGGAAGACGGAGATA	171
		Reverse		
SLC7A6	XM_015292305.4	Forward	CTCATAGCTCACTGGCTGAA / AGAACTCCTTTGGGTGAGAC	193
		Reverse		
SLC7A5	NM_001030579.3	Forward	GCTTCACAAGACCAATCCAC/ AAAGGGTCACCAAGAGGACA	146
		Reverse		
SI	XM_015291762.4	Forward	CAAGAACCTGCTAATACAACC/ TCAATGCGAACCCATCATC	119
		Reverse		
β-actin	NM_205518.2	Forward	AGACATCAGGGTGTGATGGTT/ CAGTTGGTGACAATACCGTGT	122
		Reverse		
GAPDH	NM_204305.2	Forward	CTATCTCCAGGAGCGTGAC/ GGTTGACACCCATCACAAAC	186
		Reverse		

### 2.5 Statistical analysis

The data were analyzed as a factorial arrangement in a completely randomized design (CRD) using SAS software (SAS, 2004). Mean comparisons among the 12 treatments (two strains × six diets) were performed using Tukey’s test. Due to the initial body weight differences between Ross and Arian chicks, initial weight was included as a covariate in the statistical models. The statistical model was as follows:

$Y_{ijk}$  is individual observations;

$\mu$  is the experimental mean;

$T_i$  is the diet effect (6 diets);

$G_j$  is the genetic strain effect (2 strains);

(TG)  $ij$  is the interaction effect between diet and genetic strain (6×2);

$b$  is the regression coefficient of the dependent variable based on initial body weight;

$BW_{ijk}$  is the observed initial body weight of individual birds.

$B\bar{W}$  is the average initial body weight across all birds.  
 $e_{ijk}$  is the experimental error.

## 3 Results

### 3.1 Performance measurements

Statistical analysis of the main factors (genetic strain and diet) in the present experiment for the three important performance traits, plus total intestinal length, is presented in Table 3. For all of these traits, the genetic strain factor was significant, which resulted in the Ross strain having higher performance than the Arian strain. Moreover, the interaction between the two factors was not significant ( $p>0.05$ ). In other words, the genetic strain and diet did not have a significant effect on these traits. Therefore, weekly monitoring was conducted to study the effects of strain and diet on the three important performance traits, including daily weight gain (DWG), daily feed intake (DFI), and FCR (Table 4).

**Table 3.** Mean squares of live weight (LW), total feed intake (TFI), average feed conversion ratio (AFCR), intestinal length (IL) of broiler chickens under experimental treatments (two strains × six diets) at 42 days of age

Sources of Variation	Degrees of Freedom	Mean Square			
		LW	TFI	AFCR	IL
BW- 0 day	1	12298.2 ns	263.4 ns	0.013 ns	268.1 ns
Strain	1	391658.6**	376766.1*	0.047 *	2622.5 *
Diet	5	136813.9**	124392.9 ns	0.041*	869.0 ns
Strain ×Diet	5	52883.5 ns	548959.46 ns	0.009 ns	436.7 ns

ns, \* and \*\* are non-significant and significant at the 0.05 and 0.01 probability levels, respectively

**Table 4.** Daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) of broiler chicks under different treatments for the first three weeks

No <sup>1</sup>	Treatments	First week			Second week			Third week		
		DWG(g/d)	DFI(g/d)	FCR(g/g)	DWG(g/d)	DFI(g/d)	FCR(g/g)	DWG(g/d)	DFI(g/d)	FCR(g/g)
1	Arian/Control	14.89	18.36	1.25 <sup>ab</sup>	18.48 <sup>cd</sup>	25.42 <sup>b</sup>	1.38 <sup>ab</sup>	38.56 <sup>d</sup>	71.92	1.62 <sup>ab</sup>
2	Arian/Antibiotic	14.50	17.73	1.23 <sup>ab</sup>	23.92 <sup>abcd</sup>	32.24 <sup>ab</sup>	1.35 <sup>ab</sup>	49.52 <sup>abcd</sup>	80.31	1.61 <sup>ab</sup>
3	Arian/Probiotic	19.04	19.56	<b>1.02<sup>b</sup></b>	17.82 <sup>d</sup>	24.10 <sup>b</sup>	1.34 <sup>ab</sup>	38.63 <sup>cd</sup>	67.42	1.75 <sup>a</sup>
4	Arian/Prebiotic	17.39	20.66	1.20 <sup>ab</sup>	18.28 <sup>cd</sup>	25.04 <sup>b</sup>	1.40 <sup>ab</sup>	39.21 <sup>cd</sup>	62.49	1.59 <sup>ab</sup>
5	Arian/Organic acid	15.88	20.90	1.35 <sup>ab</sup>	21.95 <sup>abcd</sup>	25.66 <sup>b</sup>	<b>1.17<sup>b</sup></b>	52.13 <sup>ab</sup>	77.94	1.50 <sup>ab</sup>
6	Arian/Phytobiotic	24.14	19.47	1.41 <sup>a</sup>	19.78 <sup>bcd</sup>	25.26 <sup>b</sup>	1.28 <sup>ab</sup>	44.37 <sup>bcd</sup>	65.22	1.70 <sup>a</sup>
7	Ross/Control	18.06	21.28	1.20 <sup>ab</sup>	21.11 <sup>abcd</sup>	33.12 <sup>ab</sup>	1.58 <sup>a</sup>	51.65 <sup>ab</sup>	72.00	1.39 <sup>abc</sup>
8	Ross / Antibiotic	20.85	22.47	1.07 <sup>ab</sup>	25.75 <sup>ab</sup>	33.07 <sup>ab</sup>	1.31 <sup>ab</sup>	55.52 <sup>ab</sup>	70.69	1.30 <sup>bc</sup>
9	Ross / Probiotic	18.23	21.82	1.23 <sup>ab</sup>	20.61 <sup>abcd</sup>	32.09 <sup>ab</sup>	1.56 <sup>a</sup>	55.49 <sup>ab</sup>	71.18	1.28 <sup>bc</sup>
10	Ross / Prebiotic	17.43	19.06	1.10 <sup>ab</sup>	21.81 <sup>abcd</sup>	32.72 <sup>ab</sup>	1.49 <sup>a</sup>	53.31 <sup>ab</sup>	76.94	1.44 <sup>ab</sup>
11	Ross / Organic acid	17.20	20.98	1.25 <sup>ab</sup>	25.33 <sup>abc</sup>	<b>36.26<sup>a</sup></b>	1.41 <sup>ab</sup>	50.97 <sup>abc</sup>	67.14	1.31 <sup>bc</sup>
12	Ross / Phytobiotic	15.09	18.07	1.21 <sup>ab</sup>	<b>27.28<sup>a</sup></b>	<b>37.28<sup>a</sup></b>	1.36 <sup>ab</sup>	<b>58.26<sup>a</sup></b>	62.96	<b>1.08<sup>c</sup></b>
	SEM	1.42	1.15	0.07	1.47	1.95	0.06	2.54	4.35	0.07
	P-value	0.076	0.095	0.040	0.0002	0.030	0.004	0.0001	0.087	0.0001

<sup>1</sup> Nos. 1 to 6: Six dietary treatments in the Arian strain (1) wheat and meat powder without additives, (2) wheat and meat powder with antibiotics, (3) wheat and meat powder with probiotics, (4) wheat and meat powder with prebiotics, (5) wheat and meat powder with organic acids, and (6) wheat and meat powder with phytobiotics. Nos. 7 to 12: The same six dietary treatments in the Ross strain <sup>a-d</sup>. Within columns, mean values with common superscript (s) are not different ( $P>0.05$ ).

**Continued Table 4.** Daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) of broiler chicks under different treatments for the last three weeks

No	Treatments	Fourth week			Fifth week			Sixth week		
		DWG(g/d)	DFI(g/d)	FCR(g/g)	DWG(g/d)	DFI(g/d)	FCR(g/g)	DWG(g/d)	DFI(g/d)	FCR(g/g)
1	Arian/Control	55.29 <sup>e</sup>	93.09 <sup>e</sup>	1.70 <sup>ab</sup>	73.51 <sup>d</sup>	155.75 <sup>abc</sup>	2.14	86.82 <sup>bc</sup>	177.42	2.11
2	Arian/Antibiotic	73.54 <sup>abc</sup>	113.14 <sup>abcde</sup>	<b>1.53<sup>b</sup></b>	81.68 <sup>bcd</sup>	149.64 <sup>bc</sup>	1.83	91.87 <sup>bc</sup>	171.66	2.31
3	Arian/Probiotic	57.48 <sup>de</sup>	101.92 <sup>cde</sup>	<b>1.58<sup>b</sup></b>	69.65 <sup>bcd</sup>	145.87 <sup>c</sup>	1.74	74.69 <sup>b</sup>	214.45	2.33
4	Arian/Prebiotic	59.50 <sup>cde</sup>	107.15 <sup>bcde</sup>	1.80 <sup>ab</sup>	78.83 <sup>cd</sup>	155.30 <sup>abc</sup>	1.96	81.02 <sup>bc</sup>	180.98	2.25
5	Arian/Organic acid	62.90 <sup>bcde</sup>	96.43 <sup>de</sup>	<b>1.54<sup>b</sup></b>	88.57 <sup>abcd</sup>	172.74 <sup>abc</sup>	1.96	87.30 <sup>bc</sup>	193.81	2.22
6	Arian/Phytobiotic	64.66 <sup>bcde</sup>	95.57 <sup>de</sup>	1.66 <sup>ab</sup>	83.67 <sup>cd</sup>	139.03 <sup>c</sup>	2.00	81.96 <sup>bc</sup>	195.90	2.42
7	Ross/Control	70.38 <sup>abcd</sup>	<b>135.76<sup>a</sup></b>	1.93 <sup>a</sup>	92.90 <sup>abc</sup>	173.05 <sup>abc</sup>	1.86	92.78 <sup>bc</sup>	207.79	2.25
8	Ross / Antibiotic	<b>82.30<sup>a</sup></b>	125.43 <sup>abc</sup>	<b>1.53<sup>b</sup></b>	100.65 <sup>ab</sup>	178.37 <sup>abc</sup>	1.77	<b>119.15<sup>a</sup></b>	207.27	1.74
9	Ross / Probiotic	70.90 <sup>abcd</sup>	118.76 <sup>abcd</sup>	1.68 <sup>ab</sup>	97.59 <sup>abc</sup>	171.81 <sup>abc</sup>	1.76	92.78 <sup>bc</sup>	218.76	2.38
10	Ross / Prebiotic	70.71 <sup>abcd</sup>	128.39 <sup>ab</sup>	1.82 <sup>ab</sup>	97.47 <sup>abc</sup>	191.39 <sup>ab</sup>	1.97	98.98 <sup>abc</sup>	184.64	1.86
11	Ross / Organic acid	70.70 <sup>abcd</sup>	126.67 <sup>abc</sup>	1.79 <sup>ab</sup>	93.01 <sup>abc</sup>	176.23 <sup>abc</sup>	1.90	100.20 <sup>ab</sup>	205.61	1.98
12	Ross / Phytobiotic	76.25 <sup>ab</sup>	125.41 <sup>abc</sup>	1.65 <sup>ab</sup>	<b>103.20<sup>a</sup></b>	<b>198.02<sup>a</sup></b>	1.93	104.65 <sup>ab</sup>	228.16	2.28
	SEM	3.02	5.12	0.06	3.95	9.03	0.09	5.02	13.08	0.16
	P-value	0.0006	0.006	0.003	0.0001	0.0005	0.294	0.002	0.001	0.086

No significant differences were observed for DWG in the first week, and DFI in the first, third, and sixth weeks; and also for FCR in the last two weeks ( $p>0.05$ ). To put it differently, the genetic strain and diet did not have a significant impact on these traits during these weeks.

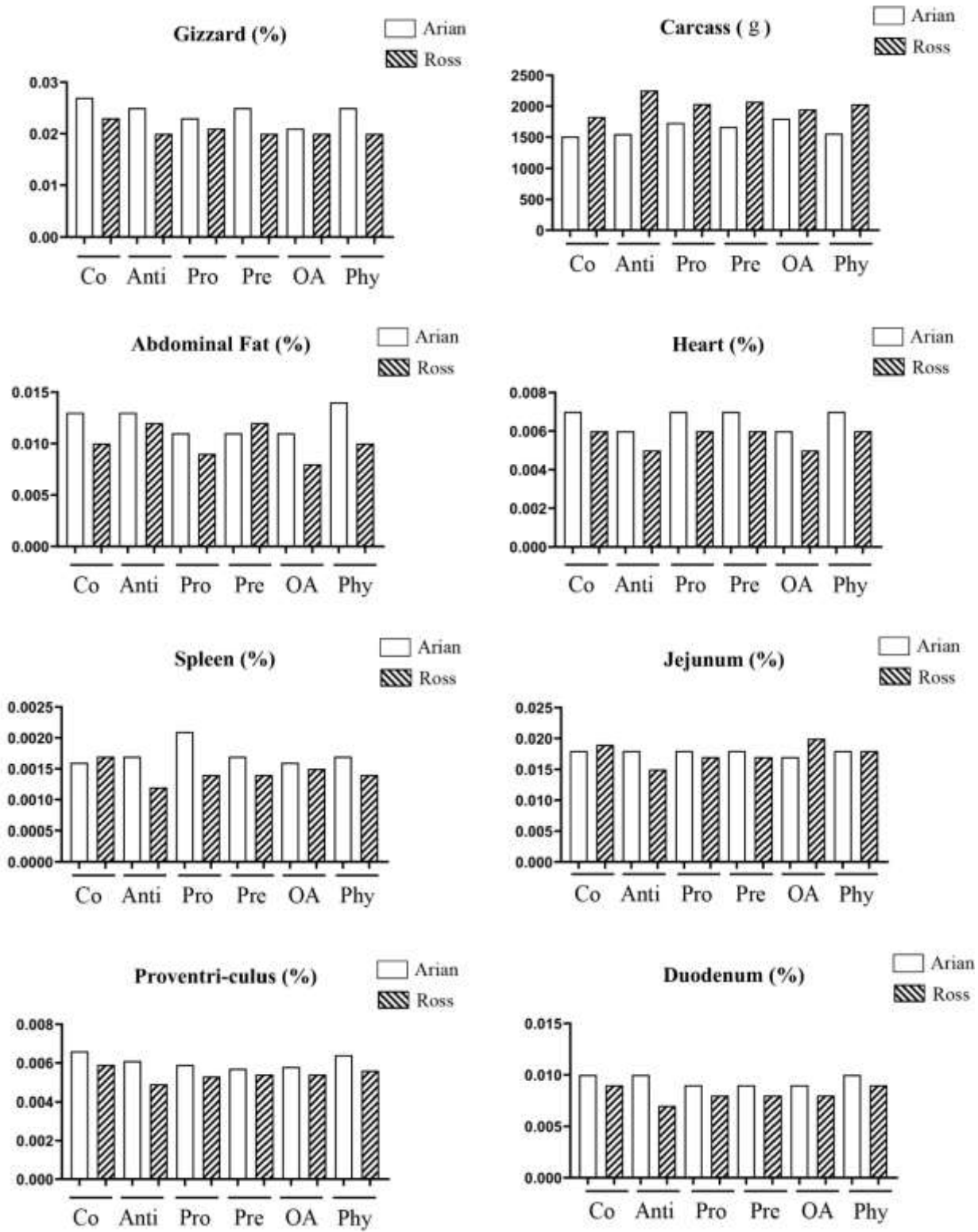
The lowest performance was achieved by the basal diet (without additives) among all treatments. Regarding the basal diet, the Ross strain had a higher DWG than the Arian strain. Furthermore, treatments 8 and 12 (Ross strain) displayed the highest DGW, while treatments 1 and 3 (Arian strain) displayed the lowest DGW ( $p<0.05$ ). Significant differences in DFI were observed only in the second, fourth, and fifth weeks. During these weeks, the Ross strain exhibited higher DFI than the Arian ( $p<0.05$ ). The impact of feed supplementation on FI was not consistently evident during these weeks; however, phytobiotic notably improved feed intake performance in the Ross strain.

Over 42 days, the Ross strain consumed 12.5% more feed than the Arian strain (4543.5 g vs. 3977.3 g), yet demonstrated a superior FCR throughout the period. The overall FCR in the Ross was 1.66, while for the Arian strain it was 1.76. No clear superiority was observed for the

treatments in other weeks. The best FCR was recorded for treatment 3 in the first week, treatment 5 in the second week, treatment 12 in the third week, and treatments 2, 3, 5, and 8 in the fourth week ( $p<0.05$ ). However, treatment 8 (supplemented with antibiotics) demonstrated consistently favorable FI, DWG, and FCR across all weeks.

### 3.2 Carcass characteristics

Differences in carcass weight and the relative weight of internal organs at 42 days of age are presented in [Figure 1](#). The Ross strain was found to have a superior carcass weight. The average carcass weight for the Arian and Ross strains was 1637 g and 2030 g, respectively. The antibiotics followed by the prebiotic supplement treatments had the most pronounced effect on carcass weight in the Ross strain; however, this positive impact was not observed in the Arian strain. In contrast, the diet containing organic acids had a more substantial effect on total carcass weight in the Arian strain. The lowest carcass weight (1511.6 g) was recorded in Arian broilers that were fed only a basal diet.



**Figure 1.** Carcass weight (g) and the relative weight of internal organs (%) under different treatments:(Con) wheat and meat powder without additives, (Anti) wheat and meat powder with antibiotics, (Pro) wheat and meat powder with probiotics, (Pre) wheat and meat powder with prebiotics, (OA) wheat and meat powder with organic acids, and (Phy) wheat and meat powder with phytobiotics

Genetic strain and diet had an impact on the relative weight (%) of the gizzard, spleen, heart, proventriculus,

abdominal fat, duodenum, and jejunum ( $p < 0.05$ ); however, the weight percentage of pancreas, liver, crop, bursa

Fabricius, thymus, and ileum did not have any significant differences ( $p>0.05$ ). The Arian strain had a higher relative weight for the gizzard, proventriculus, spleen, liver, heart, duodenum, and abdominal fat ( $p<0.05$ ). The highest relative weight of the duodenum was observed in treatments 2 and 6 (Arian strain), and the lowest was for treatment 8 related to the Ross strain. The genetic strain effect influenced the total intestinal length. The length of the whole intestine in the Ross strain (226.1 mm) was significantly ( $p<0.05$ ) longer than that of the Arian strain (202.9 mm).

The effects of experimental treatments on the morphometric measures of the intestine were statistically

analyzed. While the genetic strains and the interaction between strain and diet for all morphometric measures were not significant ( $p>0.05$ ), dietary treatments had a significant effect on some of these indices (Table 5). Among the morphometric measures, VH, CD, and GC were affected by the dietary supplements. While VH and GC increased with the probiotic supplement, CD increased with the organic acid ( $p<0.05$ ). VH and CD are marked in Figure 2 by the difference in VH and CD for probiotic and organic acid treatments, respectively.

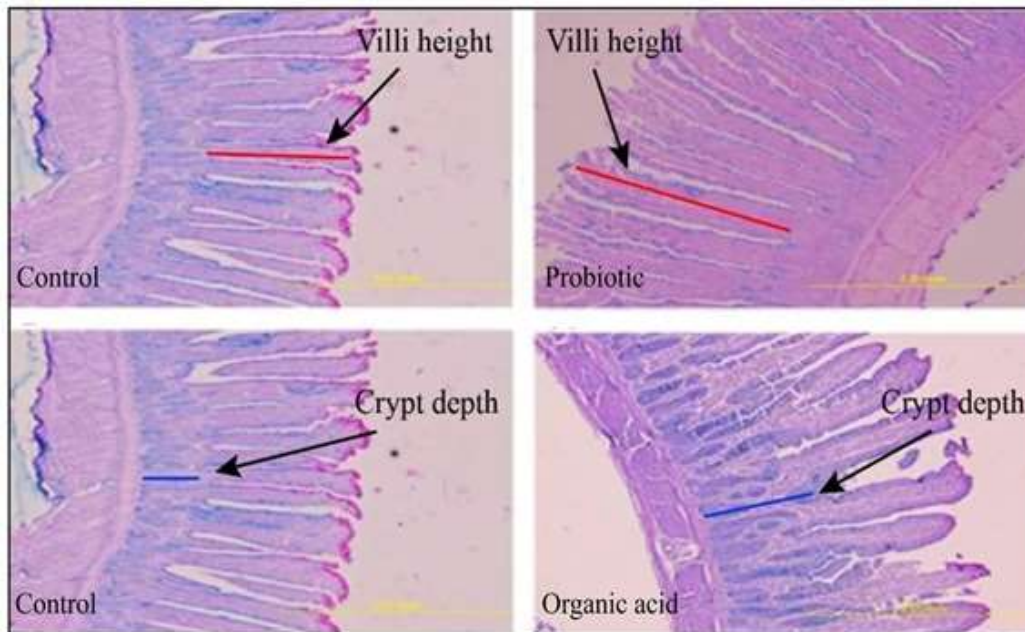
**Table 5.** Differences in six morphometric measures of the broiler intestine under feeding treatments<sup>1</sup>

Feed Treatments	Morphometric Measures <sup>2</sup>					
	VH	VW	CD	GC	MT	LP
Control	3.071 <sup>abc</sup>	2.27	2.47 <sup>ab</sup>	0.65 <sup>ab</sup>	2.46	1.71
Antibiotic	3.041 <sup>c</sup>	2.20	2.41 <sup>b</sup>	0.77 <sup>ab</sup>	2.44	1.69
Probiotic	3.148 <sup>a</sup>	2.24	2.48 <sup>ab</sup>	0.80 <sup>a</sup>	2.48	1.72
Prebiotic	3.055 <sup>bc</sup>	2.19	2.41 <sup>b</sup>	0.53 <sup>b</sup>	2.46	1.69
Organic Acid	3.070 <sup>abc</sup>	2.26	2.53 <sup>a</sup>	0.66 <sup>ab</sup>	2.50	1.73
Phytobiotic	3.136 <sup>ab</sup>	2.22	2.50 <sup>ab</sup>	0.67 <sup>ab</sup>	2.49	1.70
SEM	0.21	0.15	0.22	0.37	0.23	0.23
P-value	0.025	0.338	0.031	0.04	0.70	0.56

<sup>1</sup> The log transformation was applied to all data.

<sup>2</sup> Villus Height (VH), Villus Width (VW), Crypt Depth (CD), Goblet Cells (GC), Muscle Thickness (MT), and Lamina Propria (LP).

<sup>a-c</sup>: Within columns, mean values with common superscript (s) are not different ( $p>0.05$ ).



**Figure 2.** Differences in the two intestinal morphometry measures (villi height and crypt depth) under probiotic and organic acid treatments.

Clinical examination of intestinal tissue revealed no significant differences between the Ross and Arian strains for gastrointestinal disorders, including dysbacteriosis (14 items for scoring) and necrosis (12 items for scoring). This result was the same for both analyses, including absolute numbers and a percentage of total scores. These results were further corroborated by histomorphological examinations of the intestine, in which both strains showed no obvious differences.

The ileum bacterial population data were statistically analyzed. There were no significant differences between feed treatments or interaction between genetic strains and feed treatments ( $p>0.05$ ). However, the intestinal microbial populations were affected by two broiler strains. Three microbial groups (*Escherichia coli*, *Lactobacillus*, and *Clostridium perfringens*) had a higher microbial population in the Ross strain.

### 3.3 Gene expression

The expression of the *GLUT2*, *SLC6A19*, *SLC7A9*, *SLC7A5*, *SI*, and genes is presented in

**Table 6.** The results showed that both *SLC6A19* and *SLC7A9* genes were highly expressed only in the Ross strain under phytobiotic and antibiotic treatments. The expression of the *SLC7A6* gene was higher under phytobiotic and organic acid treatments in both broiler strains. Except for phytobiotic and organic acid treatments, the other feed additives did not affect the expression of all six genes in the Arian strain. The *SLC7A5* gene expression was identical to *SLC7A19*, with the superiority of phytobiotic treatment in the Ross strain. *SI* gene expression was higher in the Ross strain than in the Arian strain, except for the prebiotic treatment. The Organic acid supplement greatly affected the *GLUT2* gene expression.

**Table 6.** The expression of studied genes under different genetic strains and dietary supplements

Strains/ Feed supplements	SI	GLUT2	SLC7A5	SLC7A6	SLC7A9	SLC6A19
Arian/ Antibiotic	0.301 <sup>b</sup>	1.165 <sup>bcd</sup>	0.428 <sup>b</sup>	3.44 <sup>b</sup>	0.162 <sup>b</sup>	0.469 <sup>b</sup>
Arian/ Probiotic	0.133 <sup>b</sup>	0.458 <sup>cd</sup>	0.139 <sup>b</sup>	4.04 <sup>b</sup>	0.155 <sup>b</sup>	0.897 <sup>b</sup>
Arian/ Prebiotic	0.570 <sup>b</sup>	0.293 <sup>d</sup>	0.158 <sup>b</sup>	0.32 <sup>b</sup>	0.168 <sup>b</sup>	0.301 <sup>b</sup>
Arian/ Organic Acid	0.234 <sup>b</sup>	11.293 <sup>a</sup>	0.311 <sup>b</sup>	27.04 <sup>ab</sup>	0.704 <sup>ab</sup>	0.454 <sup>b</sup>
Arian/ Phytobiotic	0.066 <sup>b</sup>	3.936 <sup>bcd</sup>	1.465 <sup>b</sup>	4.63 <sup>b</sup>	0.571 <sup>ab</sup>	0.267 <sup>b</sup>
Ross / Antibiotic	1.131 <sup>b</sup>	7.958 <sup>ab</sup>	0.812 <sup>b</sup>	0.93 <sup>b</sup>	1.577 <sup>a</sup>	1.422 <sup>b</sup>
Ross / Probiotic	2.522 <sup>ab</sup>	0.190 <sup>d</sup>	0.733 <sup>b</sup>	1.14 <sup>b</sup>	0.385 <sup>ab</sup>	0.518 <sup>b</sup>
Ross / Prebiotic	0.267 <sup>b</sup>	3.582 <sup>bcd</sup>	0.529 <sup>b</sup>	1.38 <sup>b</sup>	0.290 <sup>ab</sup>	0.149 <sup>b</sup>
Ross / Organic Acid	10.576 <sup>a</sup>	7.338 <sup>abc</sup>	0.476 <sup>b</sup>	23.65 <sup>ab</sup>	0.099 <sup>b</sup>	0.293 <sup>b</sup>
Ross / Phytobiotic	2.284 <sup>ab</sup>	0.420 <sup>cd</sup>	40.463 <sup>a</sup>	58.43 <sup>a</sup>	1.449 <sup>a</sup>	14.210 <sup>a</sup>
SEM	1.573	1.434	2.060	3.300	0.420	1.109
P-value	0.0036	0.0001	0.0001	0.0001	0.0014	0.0001

<sup>a-d</sup>: Within columns, mean values with common superscript (s) are not different ( $p>0.05$ ).

## 4 Discussion

The Arian broiler chicken has been genetically selected for its various characteristics over two decades in Iran. The Ross 308 strain is also used more frequently than other strains. Genetic variations in growth rate, feed intake, and feed efficiency among different broiler chicken genotypes

have been widely documented (Jawasreh et al., 2019; Menchetti et al., 2024; Sam, 2023). In this study, while the Ross 308 strain consumed 756 grams more feed than Arian, its average FCR was better than Arian's (1.66 vs. 1.76). The results of other comparative studies showed that the FI in the growth period of the Ross strain was higher than that of the Arian strain (Varmaghany et al., 2020). A study on three commercial strains (Ross, Cobb, and Arian) reported no

significant differences in FI, but the highest WG and the lowest FCR belonged to the Ross strain. This study, similar to our experiment, showed that the FCR for the entire rearing period was 1.65 in the Ross strain, whereas it was 1.76 in the Arian strain (Mehdizadeh et al., 2022). Despite this report and our study, some other reports have given different results (Samadian et al., 2023; Varmaghany et al., 2020). In a study comparing five broiler strains, including Arian and Ross 308, no significant differences in FI were observed when birds were raised for 42 days. However, when the rearing period was extended to 49 days, FI in the Ross strain was significantly higher than in the Arian strain. Despite this, WG and FCR did not differ significantly, except for the first week (Varmaghany et al., 2020). A previous study on six broiler chicken strains found that when birds were reared until 56 days of age, FI was significantly higher in the Arian strain compared to the Ross 508 strain. However, there were no significant differences in BW or BWG at 42 days of age (Rahimi et al., 2006). A study examining four commercial broiler strains (Ross, Arbor Acres, Marshall, and Cobb) found that, except for the Marshall breed, which had lower initial weight, final weight, total weight gain, and average daily gain, the other three breeds had fairly similar weights (Kareem-Ibrahim et al., 2021).

While most previous studies were conducted using corn- and soybean-based diets, the present study utilized wheat- and meat-meal-based diets. The basal diet, formulated with high concentrations of wheat and meat meal, is rich in non-starch polysaccharides (NSPs) and other components that can act as dietary stressors, potentially causing imbalances in gut microbiota and impairing nutrient utilization in broiler chickens. The probiotics, prebiotics, organic acids, and phytogetic feed additives were selected as alternatives to antibiotic growth promoters to evaluate their potential in supporting gut health under dietary stress. By comparing these feed additives against an antibiotic control, we aimed to determine whether they could mitigate the negative effects of such challenging diets, maintain gut microbial balance, and improve overall gut health and performance. A diet containing 45-55% wheat and 9-10% meat meal did not significantly induce changes in intestinal tissue integrity, including necrosis and dysbacteriosis. Moreover, the maximum FCR was also 1.71, which was not an acceptable result. Another study using wheat-based diets (wheat and fishmeal) for just the Ross strain showed that the FCR was 1.81 (Ghiamatiun et al., 2022). Therefore, both broiler strains had effective adaptation to these diets.

Carcass weight was higher in the Ross strain. This result is in agreement with Samadian et al. (Samadian et al., 2025) and contrary to other reports (Varmaghany et al., 2020). These researchers suggested that the lower weight of the legs in the Ross strain may contribute to its superior carcass weight. The intestinal length of the Ross strain was longer, which indicates a greater ability to absorb nutrients. The relative weight of the gizzard was higher in the Arian strain, a finding also reported by Samadian et al. (Samadian et al., 2025), which indicates the greater ability of feed processing in the Arian strain. In a study on the gastrointestinal tract of a dual-purpose male chicken, Lohmann Dual (LD), and a broiler line, Ross 308, it was found that LD birds had a significantly heavier gizzard and shorter intestine (Metzler-Zebeli et al., 2018).

The Arian strain had a lower villus width than the Ross strain. Another study on these broiler strains (Samadian et al., 2023) indicated that the villus thickness and villus surface area were greater in the Arian strain in both the jejunum and ileum. Still, the crypt depth was greater in the Arian strain just in the ileum. The use of probiotics, prebiotics, and fermented foods has been reported to increase the height of the villus and the ratio of villus height to crypt depth (Chae et al., 2012), which is in line with the results of this study.

The microbial populations of *Escherichia coli*, *Lactobacillus*, and *Clostridium perfringens* were significantly higher in Ross birds than in Arian. Thus, the superior DWG and DFI observed in the Ross strain in the present study may be attributed to its higher intestinal microbial population. However, this study showed that nutritional supplements had minimal impact on the microbial population of the cecum; other studies have demonstrated that such supplements can increase bacterial populations in the ileum (Aljumaah, Alkhulaifi, et al., 2020) and jejunum (Kan et al., 2021).

In the present study, the expression of certain intestinal nutrient transporter genes in response to induced intestinal microbiota imbalance was compared between two commercial broiler strains with different genetic backgrounds (Ross and Arian strains). Several studies have investigated the expression of genes related to nutrient absorption in different broiler strains with varying growth rates (Mott et al., 2008). The transducing genes and their expression in the broiler intestine may have an impact on both overall nutrient status and growth performance. For instance, reduced expression of *GLUT2* and *SI* has been linked to impaired carbohydrate transport to tissues,

resulting in weight loss in broiler chickens (Su et al., 2014). Nutrient absorption is further regulated by specific transporters across the plasma membrane of the small intestine, and alterations in these transporters may contribute to reduced BW and FCR under nutritional challenges (Zeng et al., 2011). *GLUT* gene expression did not show an increasing trend in wheat-based diets (Veluri et al., 2024). However, high-viscosity diets (e.g., wheat-based diets) can significantly influence gene expression patterns. These changes include both overexpression and lower expression in processes related to nutrient transport, intestinal defense mechanisms, apoptosis, and immune responses (Chen et al., 2015).

The results showed the influence of phytobiotic on the expression of 4 out of 6 studied genes. A study showed that phytobiotics could effectively compete with antibiotics as feed additives (Aljumaah, Suliman, et al., 2020). Also, higher mRNA expression of glucose and amino acid transporter genes was reported by adding phytobiotics to the diet (Musa et al., 2023). Moreover, phytobiotic ingredients changed the fluidity and permeability of the cell membrane, making it easier to absorb nutrients. (Amad et al., 2013).

## 5 Conclusion

In conclusion, both broiler strains showed good adaptability to a wheat-based diet, with minimal signs of intestinal damage. However, Ross 308 strain outperformed the Arian strain in growth performance traits, carcass weight, intestinal length, microbial population, and nutrient transporter gene expression under the dietary challenge. Among feed additives, phytobiotics showed the most promising effects by enhancing the expression of key amino acid transporter genes, particularly in Ross birds. These findings suggest that strategic use of functional feed additives, especially phytobiotics, can support gut health and nutrient absorption under challenging dietary conditions. Moreover, the results highlight the genetic differences in physiological response between strains, providing practical insight for strain-specific diet formulation and management strategies in broiler production.

## Acknowledgements

The authors wish to express their appreciation to everyone who assisted in this study.

## Conflict of Interest

We declare that no conflict of interest.

## Author Contributions

S.H.H.M. designed and directed the project; M.M.A. planned the experiments and feed formulation; S.O.Z. and S.H.H.M. carried out the experiments; A.G.L. evaluated intestinal dysfunction and veterinary supervision; N.G.H. analyzed the data.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethical Considerations

The authors confirm that this study was conducted following the animal welfare and ethical guidelines established by the Animal Science Department's Ethical Committee at the University of Guilan. The research proposal's code of ethics, which was approved by the Ethics Committee of the University of Guilan, is ETHICS-2402-1085.

## Funding

This work was supported by The Vice-Presidency for Science, Technology, and Knowledge-Based Economy Affairs under grant No. 00/14403.

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