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Efficacy of Early Feeding with Probiotic-Fermented Feed in Promoting Growth Performance, Immunity, Antioxidant Activity, Gene Expression, and Gut Integrity in Ostrich Chicks (*Struthio camelus*)

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Simple Summary

Ostrich embryos face many challenges during their last few days pre-hatching and the first few days post-hatching, including oxidative stress and increased mortality rates, which may be related to microbial imbalances, digestive disorders, and weakened immune functions. Fermented feeds have many positive effects on the health and performance of chickens, for example, breaking down anti-nutritional factors, strengthening the immune system, and promoting intestinal health. The introduction of beneficial probiotics and reduction in pathogens makes nutrients more available and creates a healthy intestinal environment, thereby improving the growth rate and feeding efficiency. Therefore, early feeding with probiotic-fermented feed may contribute to promoting the growth of gut bacteria and the development of the immune and digestive systems, leading to improved health and performance of newly hatched ostrich chicks. The primary objective of this study was to evaluate the effects of early feeding of probiotic-fermented feed on the health and performance of ostrich chicks. Early feeding with probiotic-fermented feed may represent a promising strategy for supporting the health and performance of newly hatched ostrich chicks.

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Abstract

The purpose of this study was to evaluate the effect of early feeding with probiotic-fermented feed on growth performance, intestinal microbiota structure, immune responses, and gene expression. Two hundred and forty-one-day-old African ostrich chicks were randomly divided into three groups (eight replicates/group). The control group was fed a basal diet (CON), whereas the PELF3 and PELF6 groups were fed the probiotic-fermented feed for the first 3 or 6 days post-hatching, respectively, after which, all chicks were fed the basal diet for 56 days. The results showed that adding PELF3 or PELF6 significantly enhanced body weight gain and the feed conversion ratio. Chicks fed PELF had higher superoxide dismutase (SOD, $p < 0.05$), immunoglobulin A (IgA), and IL-10 levels and lower IL-6 and malondialdehyde (MDA, $p < 0.05$) levels than those fed CON. Plasma cholesterol, low-density lipoprotein (LDL), creatinine, uric acid, and alanine aminotransferase (ALT) levels decreased; however, high-density lipoprotein (HDL, $p < 0.05$) levels increased in the PELF groups. The addition of PELF reduced the pathogenic counts in the intestines of chicks ($p < 0.05$). Moreover, increased expression of IGF-1 and MUC-2 genes was observed in the PELF3 and PELF6 groups, whereas the expression of SLC15A1 increased in the PELF6 group. In conclusion, growth performance, immunity, gene expression, oxidative stability, and gut microbiota can all be significantly enhanced by early feeding with PELF. This study demonstrated an effective technique for applying early feeding of PELF in ostrich chicks.

Keywords: early feeding; ostrich performance; fermentation; immunity; gene expression; intestinal integrity

1. Introduction

The conditions during the first few hours before or after hatching are among the most important factors affecting the life of chickens and their health and performance [1]. The long duration of hatching, sorting, and receiving at the farm, which can reach up to 36–50 h, in addition to delayed feeding, has detrimental effects on the health of newly hatched chicks [2,3]. These events make chicks more susceptible to deteriorating health and performance and increased mortality rates [4]. This may be because during this stage, the immune and digestive systems are not fully developed, making them more sensitive to environmental changes both inside and outside the egg [5,6] and thus exposing them to numerous pathogens and depressing growth [7,8]. Therefore, early feeding before and after hatching plays a pivotal role in promoting the growth and development of the digestive system and muscles, and supporting the digestion and absorption of nutrients, skeletal muscle growth, and chick performance [7]. Furthermore, early feeding has been demonstrated to promote immune system maturation, satellite cell proliferation, and lymphoid organ development [8,9], thereby improving chick health. Despite these well-documented benefits in poultry, early post-hatching nutritional strategies in ostrich production systems remain largely unexplored.

Ostrich farms (*Struthio camelus*) have recently spread in North Africa since the climate is suitable for raising ostriches and this large desert bird can withstand different environmental conditions [10]. Additionally, the increasing demand for ostrich products, including meat, feathers, and skin, as well as the advantages of ostrich meat (low cholesterol and fat contents), ref. [11] have expanded the ostrich market and led to increased ostrich breeding. Environmental factors, including suitable rearing conditions, stress, and nutrition, are crucial [12], especially because ostrich chicks are highly susceptible to stress and disease. Therefore, the appropriate egg storage, incubation, feeding, and hygiene

conditions are critical for chicken quality and survival [13]. However, breeders suffer high mortality rates during the early post-hatching stages, which may be attributed to digestive and immune system disorders, causing huge economic losses [14]. A previous study described the digestive system of ostrich chicks that died during the first three months compared to healthy ostrich chicks and reported that the cause of death was due to microbial imbalance, which is an indicator of enteritis [15–17]. However, this finding contrasts sharply with the high mortality rate of chicks observed in the early post-hatching stages, highlighting a critical gap that requires targeted nutritional intervention during this sensitive stage of growth. Furthermore, promoting intestinal integrity through early nutritional interventions is of critical importance as it supports the growth of the digestive and immune systems [8,17,18] in ostriches. This leads to enhanced nutrient absorption, the development of a strong immune system, microbiota formation, and protection against pathogens, thereby reducing mortality rates and improving growth performance in young ostriches [1,2,9]. Although some studies have explored improving the performance of ostrich chicks through in ovo injection [18,19], research on early post-hatch feeding strategies, particularly those using fermented feeds to enhance growth and reduce mortality in modern ostrich chicks, remains very limited. Previous interventions have not consistently improved growth performance or survival, highlighting the need for targeted nutritional approaches during this critical period.

Microbial fermentation of feed is an advanced technology for feed processing and for supporting animal health. Several reports indicate that feed fermentation improves nutrient bioavailability and nutritional value, and reduces antinutrients in chickens [20,21]. Fermented feed or probiotic supplementation improves immune responses and digestive health [22,23]. Additionally, fermented feed enhances the gut microbiota and lowers pH, which improves nutrient digestion and absorption [19,24,25]. Fermented feed technology has been adopted because of its various benefits for poultry nutrition.

Therefore, feeding fermented feeds to ostrich chicks immediately after early hatching may be an effective strategy for providing easily digestible nutrients that support the development and growth of digestive and immune systems. The present study aimed to investigate the influence of early feeding with probiotic-fermented feeds on the immune responses, antioxidant status, gene expression, lipid profile, gut microbiota, and growth performance of ostrich chicks.

2. Materials and Methods

2.1. Experimental Design, Management, and Diets

Two hundred and forty healthy ostrich chicks were obtained immediately after hatching (one day old, average initial body weight: 912 ± 18.6 g) from a commercial farm in Menoufia, Egypt. They were randomly divided into three experimental groups with eight replicates each (10 chicks per replicate): CON, chicks fed the control diet; PELF3, chicks fed the fermented diet during the first 3 d post-hatching (0–3 d); and PELF6, chicks fed the fermented diet during the first 6 d post-hatching (0–6 d). Each group had eight replicates, each containing ten chicks, and the experiment lasted for 56 days. Table 1 shows the composition of the commercial feed purchased from the farm where the chicks were obtained. The proximate composition was determined according to the AOAC INTERNATIONAL Official Methods of Analysis: dry matter (≠934.01≠), crude protein (≠990.03≠), ether extract (≠920.39≠), crude fiber (≠978.10≠), and ash (≠942.05≠) [26]. All ostrich chicks were fed experimental diets and provided with water ad libitum. The chicks were placed in a sealed room with a concrete floor (to prevent leg injuries) that was divided into 2 × 2 m ground-level pens, with one pen for each replicate. Before division, the room was thoroughly cleaned and disinfected. The chicks exhibiting congenital defects or diseases

were excluded. The temperature was maintained at 32–33 °C for the first two days, then gradually reduced by 2–3 °C every week, with adequate ventilation provided without direct drafts to avoid exposing the chicks to cold shock. The room windows were open during the day to let in the sun and the floors were cleaned. A 24 h lighting program was implemented during the first week, followed by a gradual reduction to 16 h of light and 8 h of darkness with a high light intensity (30–50 lux) throughout the experiment. The average relative humidity was 53%, and the relative temperature ranged from 31.6 °C to 34.4 °C throughout the experimental period. The chicks' health management included monitoring for common ostrich diseases, recording daily mortality, and immediately removing dead birds. The vaccination program followed at the farm where the ostriches were purchased (as per the veterinarian's instructions) was as follows: (1) Newcastle disease: the first dose was given at one week of age via eye drops, and subsequent booster doses were given; (2) Infectious Bursal disease: the first dose was administered at 13 days of age, followed by a booster dose at 23 days of age; and (3) Fowl Pox: it was given at 30 days of age using the wing-pricking method.

Table 1. The basal diet's composition and chemical analysis of ostrich chicks.

Ingredient	Starter (1 to 56 Days)
Yellow corn	49.4
Soybean meal (44%)	26.3
Alfalfa	4.00
Sunflower meal	3.00
Corn Gluten meal (62%)	3.00
Wheat bran	5.00
Soybean Oil	3.50
Dicalcium phosphate	2.40
Calcium carbonate	1.63
Vit. & min. premix *	1.00
HCL lysine	0.12
DL-methionine	0.30
NaCl	0.25
Sodium bicarbonate	0.10
Chemical composition	
ME (Kcal/kg)	2813
Crude protein	19.94
NDF	13.40
ADF	6.311
Calcium	1.205
Available phosphorus	0.602
Lysine	1.117
Methionine	0.572

* Each 1 kg of vitamin–mineral premix contains Vit. D3, 4500 I.U; Vit. A, 13,000 I.U.; Vit. E, 21 IU; Vit. K3, 3.7 mg; Vit. B2, 8 mg; Vit. B12, 17 mg; D3, 4500 I.U; Vit. B1, 4 mg; Vit. B6, 5 mg; D-biotin, 200 mg; niacin, 60 mg; folic acid, 2.1 mg; calcium, 16.18 mg; $CuSO_4H_2O$, 31.18 mg; iron, 80.0 mg; manganese, 100.0 mg; $ZnSO_4H_2O$, 200 mg; selenium, 250.0 mg; cobalt, 500.0 mg; and iodine, 2.0 mg. NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber.

2.2. Preparation of Fermented Feed

Lactobacillus acidophilus (*L. acidophilus*, ATCC 8014) and *Bacillus subtilis* (*B. subtilis*, ATCC 19659) were obtained from the Microbiology Department, Faculty of Agriculture, Ain Shams University, Egypt. These experimental probiotic strains were selected based on their safety, stability, and documented functional efficacy in avian nutrition. Although data on ostriches are limited, these strains are widely used in chicken feeding studies [18,21,23] and are considered biologically viable in ostriches because of the similarity in gastroin-

testinal characteristics between avian species. *B. subtilis* was prepared and activated by inoculating it on Lu–ria–Bertani (LB) agar (LB contains 10.0 g/L tryptone, 5.0 g/L yeast extract, and 10.0 g/L NaCl) and incubating it at 37 °C for 24 h. *L. acidophilus* was prepared and activated by inoculating it on de Man, Rogosa, and Sharpe (MRS) agar (MRS contains tryptic digest of casein, yeast extract, beef extract, glucose, manganese sulfate, di-potassium hydrogen orthophosphate, magnesium sulfate, sorbitan monooleate, sodium acetate, and distilled water) and incubating it at 37 °C for 72 h. Both strains were revived and subcultured for no more than two successive passages before fermentation to ensure high viability and genetic stability. After ribbon culture on specialized agar plates, a single colony of each strain was selected to initiate fermentation. Approximately 5 kg of previously prepared feed was weighed, and both *L. acidophilus* and *B. subtilis* cultures were added at a concentration of approximately 1 × 109 CFU/kg feed [27]. Viable cell counts were determined using the standard serial dilution (10^{-1}–10^{-7}) and plate count methods on selective agar media. Colony-forming units (CFU) were counted on plates containing 30–300 colonies. To achieve a moisture content of 60% in the prepared feed microbial culture mixture, sterile water was added to facilitate microbial activity. The moistened mixture was then transferred to tightly sealed sterile plastic bags (breathing bag with a one-way breathing valve) and incubated at 32 °C for 5 days to complete fermentation following the protocol of Zhu et al. [28]. After fermentation, the feed was air-dried at room temperature and then stored at 4 °C until use. The fermentation process and its quality were verified by monitoring the pH over time (PB-20, Sartorius, Gottingen, Germany), determining the final number of colony-forming units (*L. acidophilus* and *B. subtilis*) on selective media, and performing microbiological analyses to identify spoilage or contaminating microorganisms (e.g., *Salmonella* and *E. coli*) [29]. pH was measured after fermentation; it ranged from 4.5–5.0.

2.3. Growth Performance

After randomly assigning chicks to the experimental groups, the initial live body weight (IBW, g) of each chick was recorded. The feed conversion ratio (FCR, g:g), average daily feed intake (ADFI, g/day), and average daily weight gain (ADG, g/day) were calculated at 28 and 56 days of age following the protocol of Elbaz et al. [22]. Feed intake of each replicate was recorded (each replicate contained 10 chicks). To minimize feed waste, feed was provided in appropriately sized feeders and filled continuously three to four times daily at low rates to prevent spillage while maintaining an ad libitum feed supply throughout the trial period. Feeders were cleaned and refilled every morning. The feed provided and any leftovers were accurately weighed to calculate the weekly feed consumption. Mortality was recorded daily for each experimental group throughout the study. The exact mortality rate for each group was calculated and expressed as the percentage of chicks that died relative to the initial number of chicks per replicate.

2.4. Plasma Biochemistry

Blood samples were drawn from eight chicks per group (one chicks/replicate) to obtain pre-slaughter plasma from the jugular vein (at 56 days). The blood samples were placed in anticoagulant tubes, centrifuged at 3400× *g* for 9 min, and sent directly to the laboratory for analysis. Liver enzymes (including alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), lipid profile (including high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, and triglycerides), kidney function (uric acid and creatinine), and glucose levels were assessed using commercial kits and calorimeters following the protocols of Elbaz et al. [19] and Pu et al. [30]. Using commercially available ELISA kits (MyBioSource, San Diego, CA, USA, and Life Diagnostics Inc., West Chester, PA, USA), the levels of interleukin-6 (IL-6), interleukin-10 (IL-10), immunoglobulin G

(IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) were analyzed according to the manufacturer' protocols. Additionally, the activity of oxidative enzymes, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA), was assessed using the protocol of Elbaz et al. [18]. The concentrations of stress markers, including triiodo-thyronine (T3) and corticosterone (COR), in the plasma, were also assessed using the protocol of Abdel-Moneim et al. [31].

2.5. Microbial Community

Intestinal digests from 24 ostrich chicks (eight chicks per group) and 5 g of ceca from each chick were collected and placed into sterile bags. Upon arrival at the Microbiology Laboratory at Ain Shams University, Egypt, the necessary dilutions of the samples were prepared for bacteriological testing. A portion of the bacteria was transferred into Petri dishes containing the required agar for each microorganism: total coliform (EMB agar), Enterococcus (Slanetz and Bartley Agar), *Escherichia coli* (*E. coli*; MacConkey agar), *Clostridium perfringens* (*C. perfringens*; TSC agar), *Salmonella* (XLD agar), and *Lactobacillus* (MRS agar). The plates were incubated at 37 °C for 48 h for *Lactobacillus* (anaerobic), 37 °C for 24 h for *C. perfringens* (anaerobic), 37 °C for 48 h for *E. coli* (aerobic), 37 °C for 48 h for Enterococcus (aerobic), and 37 °C for 24 h for *Salmonella* (aerobic), according to the protocols of Ghaseminejad et al. [32] and Czerwiński et al. [33].

2.6. Gene Expression

Twenty-four ostrich chicks (8 chicks per group) were slaughtered, and the liver and small intestine were collected immediately. Total RNA was isolated using TRIzol reagent (Tiangen Bio-tech Co., Ltd., Beijing, China) following the manufacturer's protocol to ensure RNA integrity. Using the PrimeScript RT Reagent Kit, the extracted RNA was reverse-transcribed into complementary DNA (cDNA) according to the man-ufacturer's protocol (Takara Biotechnology Co., Ltd., Beijing, China). Then, using the SYBR Premix Ex Taq kit, quantitative real-time polymerase chain reaction (qRT-PCR) was performed according to the instructions of Takara Biotechnology Co., Ltd. (Beijing, China).

Specific primers targeting the selected genes were designed using Primer3 software and verified for specificity against the *Struthio camelus* genome using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/, accessed on 15 February 2025). The primers used were as follows: IGF-1 forward (5′-GCCATCTGCAGGATACTTTGC-3′) and reverse (5′-CTGGGAGAATGCCCATTGGT-3′) (Accession No. AB035804.1); SLC15A1 forward (5′-GACCAGCAGGGGTCAAGATG-3′) and reverse (5′-CTGAATCTGAATAGCTCCAA-3′) (Accession No. XM_068928558.1); and MUC-2 forward (5′-CCACAGTGCTCTTCAGTCGT-3′) and reverse (5′-TGGCAGCATAGAC-CTGCAAA-3′) (Accession No. XM_068944759.1). The ß. actin gene primer sequences were 5′-CATCACAAGGGTGTGGGTGT-3′ and 5′-GCG-CAAGTACTCTGTCTGGA-3′ (Accession No. KJ729106.1).

Primer specificity was confirmed by melt curve analysis using RT-qPCR, which showed a single peak for each primer pair, indicating the amplification of a single product. The amplification efficiency ranged from 95% to 105%, and the correlation coefficients (R^2) were >0.99. The relative gene expression levels of MUC-2, SLC15A1, and IGF-1 were quantified using the $2^{-\Delta\Delta Ct}$ method and normalized to β-actin expression as an endogenous control based on the protocol by Livak and Schmittgen [34].

2.7. Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) and SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL, USA). To examine the significance of group differences, Tukey's multiple comparison test was used, and differences were considered statistically significant if $p < 0.05$. Pearson's correlation was used to evalu-

ate the synergistic interaction between IGF-1, SLC15A1, and MUC-2. Consideration was given to the *p*-value and correlation coefficient (r). The results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Growth Performance

Early feeding with probiotic-fermented feed boosted growth performance, as shown in Table 2. Compared with the control group, ostrich chicks fed PELF3 and PELF6 showed significantly increased BWG at 28 and 56 days ($p < 0.05$). Additionally, enhanced FCR was observed in PELF3- and PELF6-fed ostrich chicks compared to the control group ($p < 0.05$); however, feed intake was not affected ($p > 0.05$). There was also a reduced mortality rate (Table 2) in the PELF3- and PELF6-fed groups compared to the control group ($p < 0.05$), as shown in Figure 1.

Table 2. Impact of early feeding with probiotic-fermented feed on growth performance in ostrich chicks at 28 and 56 days.

	Parameter	CON	PELF3	PELF6	*p*-Value
1–28 days	ADG, g	109.2 ± 1.43 [b]	117.8 ± 1.33 [a]	118.6 ± 1.72 [a]	0.001
	ADFI, g	186.3 ± 2.54	185.6 ± 3.48	185.2 ± 2.25	0.197
	FCR, g/g	1.705 ± 0.04 [a]	1.576 ± 0.05 [b]	1.565 ± 0.03 [b]	0.001
28–56 days	ADG, g	210.5 ± 4.91 [b]	223.1 ± 3.06 [a]	224.7 ± 3.12 [a]	0.001
	ADFI, g	533.2 ± 7.32	525.6 ± 7.88	521.5 ± 7.41	0.104
	FCR, g/g	2.539 ± 0.06 [a]	2.356 ± 0.02 [b]	2.321 ± 0.07 [b]	0.001
1–56 days	ADG, g	159.6 ± 22.7 [b]	170.4 ± 24.5 [a]	171.5 ± 21.9 [a]	0.001
	ADFI, g	359.7 ± 34.1	355.6 ± 31.2	353.4 ± 35.2	0.317
	FCR, g/g	2.253 ± 0.07 [a]	2.089 ± 0.05 [b]	2.064 ± 0.09 [b]	0.001
	MOR, %	15.00	8.750	7.500	-

[a,b] Means with different lowercase letters in the same row are significantly different ($p < 0.05$). ADG, average daily weight gain; FCR, feed conversion ratio; ADFI, average daily feed intake; MOR, mortality rate; CON, chicks fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days.

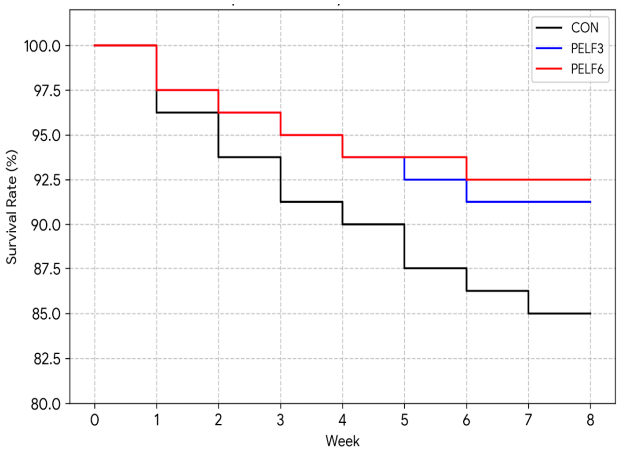


Figure 1. Impact of early feeding with probiotic-fermented feed on the mortality rate (%) in ostrich chickens at 1–56 days (1–8 weeks). CON, chicks were fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days.

3.2. Kidney and Liver Functions

The effect of early feeding with probiotic-fermented feed on kidney and liver function at eight weeks is shown in Table 3. Ostrich chicks fed PELF3 and PELF6 had significantly

lower uric acid, creatinine, and ALT levels than chicks in the control group ($p < 0.05$). However, plasma AST levels were not affected in chicks in all treatment groups ($p > 0.05$).

Table 3. Impact of early feeding with probiotic-fermented feed on kidney and liver functions, and plasma lipid profile of ostrich chicks at 56 days.

	Parameter	CON	PELF3	PELF6	*p*-Value
Kidney and liver functions	Creatinine, mg/dL	4.77 ± 1.21 [a]	3.05 ± 1.06 [b]	3.13 ± 1.54 [b]	0.001
	Uric Acid, mg/dL	3.26 ± 0.57 [a]	2.71 ± 0.62 [b]	2.64 ± 0.71 [b]	0.001
	AST, U/L	84.1 ± 6.33	83.7 ± 5.01	83.3 ± 7.54	0.107
	ALT, U/L	65.4 ± 3.51 [a]	63.9 ± 2.88 [b]	63.1 ± 3.26 [b]	0.013
Lipid profile	Glucose, mg/dL	82.27 ± 2.5	82.59 ± 1.9	82.63 ± 2.3	0.255
	TRI, mg/dL	276 ± 8.6	271 ± 9.5	268 ± 6.9	0.083
	CHO, mg/dL	196.1 ± 5.1 [a]	187.2 ± 6.4 [ab]	179.1 ± 5.7 [b]	0.015
	LDL, mg/dL	165 ± 3.4 [a]	149 ± 2.8 [b]	143 ± 3.3 [b]	0.001
	HDL, mg/dL	82.1 ± 1.9 [b]	96.9 ± 2.1 [a]	96.1 ± 2.4 [a]	0.001

[a,b] Means with different lowercase letters in same row are significantly different ($p < 0.05$). AST, aspartate aminotransferase; ALT, alanine aminotransferase; TRI, triglyceride; CHO, cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. CON, chicks fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days.

3.3. Lipid Profile

Table 3 shows the effect of early feeding with probiotic-fermented feed on the lipid profile of the growing ostrich chicks. Early feeding with PELF3 and PELF6 resulted in significant changes in lipid profiles, increased HDL levels, and decreased LDL levels ($p < 0.05$), whereas a tendency towards decreased CHO levels was observed in the PELF6 group compared to the PELF3 and control groups. However, the glucose and TRI levels were unaffected by the experimental treatments ($p > 0.05$).

3.4. Immunity

IgG and IgM levels were unchanged between the experimental groups (Table 4); however, IgA levels were significantly higher in PELF6-fed ostrich chicks than in those fed PELF3 or the control diet ($p < 0.05$). Additionally, IL-10 levels were significantly increased and IL-6 levels were significantly decreased in the PELF3 and PELF6 groups compared to the control group ($p < 0.05$), while IL-10 levels were highest in the PELF6 group ($p < 0.05$).

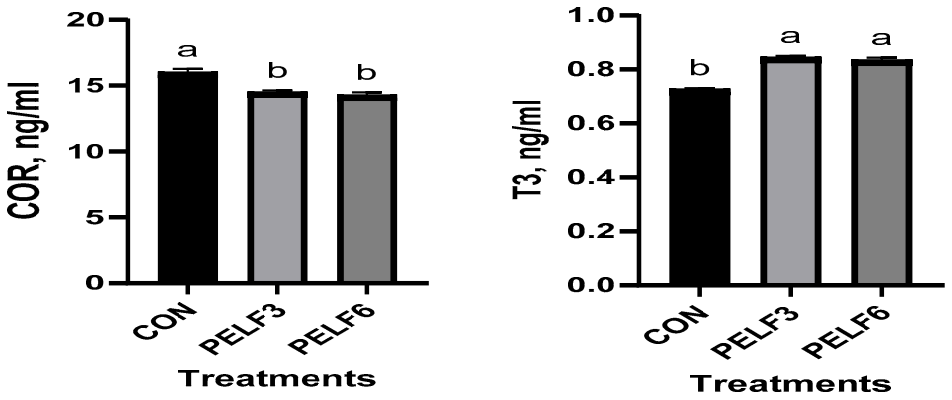
Table 4. Impact of early feeding with probiotic-fermented feed on plasma antioxidative ability and immunity of ostrich chicks at 56 days.

	Parameter	CON	PELF3	PELF6	*p*-Value
Antioxidative ability	SOD, U/mL	165.8 ± 3.4 [b]	194.2 ± 3.7 [a]	198.8 ± 4.1 [a]	0.002
	GPx, U/mL	41.2 ± 1.32	42.6 ± 1.41	41.9 ± 1.06	0.109
	MDA, nmol/mL	1.741 ± 0.05 [a]	0.943 ± 0.07 [b]	0.924 ± 0.04 [b]	0.001
Immunoglobulin	IgG, mg/dL	512 ± 7.52	529 ± 6.26	521 ± 6.80	0.204
	IgM, mg/dL	151 ± 1.88	156 ± 1.35	155 ± 1.73	0.162
	IgA, mg/dL	232 ± 3.06 [c]	244 ± 2.61 [b]	265 ± 3.14 [a]	0.011
Immune cytokines	IL-10, pg/dL	39.6 ± 0.51 [c]	44.8 ± 0.34 [b]	53.2 ± 0.47 [a]	<0.001
	IL-6, pg/dL	87.4 ± 1.35 [a]	80.3 ± 0.97 [b]	81.5 ± 1.09 [b]	0.030

[a–c] Means with different lowercase letters in the same row are significantly different ($p < 0.05$). IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G; IL-10, interleukin 10; IL-6, interleukin 6; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; CON, chicks fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days.

3.5. Stress and Oxidant Markers

Early feeding with probiotic-fermented feed showed mixed effects on antioxidative ability (Table 4). SOD levels increased and MDA decreased ($p < 0.05$) in PELF3- and PELF6-fed ostrich chicks compared to the control group, whereas GPx levels were unaffected. Blood T3 levels increased and COR levels decreased ($p < 0.05$) in PELF3- and PELF6-fed ostrich chicks compared to those in the control group (Figure 2A,B).



(**A**) (**B**)

Figure 2. Impact of early feeding with probiotic-fermented feed on corticosterone COR (**A**) and triiodothyronine T3 (**B**) in ostrich chickens at 56 days. CON, chicks fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days. The data is represented as the mean ± SE. Different lowercase letters (a, b) indicate statistically significant differences among groups ($p < 0.05$).

3.6. Intestinal Microbiota

Early feeding with probiotic-fermented feed significantly modified the gut microbiome (Table 5). *Lactobacillus* counts increased, while *C. perfringens*, *Salmonella*, coliforms, and *E. coli* decreased ($p < 0.05$) in PELF3- and PELF6-fed ostrich chicks compared to the control group. The highest *Lactobacillus* counts were observed in the PELF6 group ($p < 0.05$), whereas lower *E. coli* counts were observed in the PELF6 group. However, the Enterococcus population was not affected by feeding on PELF3 and PELF6 compared to the control ($p > 0.05$).

Table 5. Impact of early feeding with probiotic-fermented feed on cecal microbiota (log10 CFU/g) in ostrich chicks at 56 days.

Bacterial Strain	CON	PELF3	PELF6	*p*-Value
Lactobacillus	5.34 ± 0.17 ^{c}	6.51 ± 0.14 ^{b}	7.82 ± 0.19 ^{a}	<0.001
Enterococcus	6.68 ± 0.32	6.34 ± 0.27	6.41 ± 0.35	0.343
C. perfringens	3.74 ± 0.10 ^{a}	1.93 ± 0.08 ^{b}	1.86 ± 0.04 ^{b}	0.002
E. coli	2.81 ± 0.21 ^{a}	1.74 ± 0.23 ^{b}	1.43 ± 0.18 ^{c}	0.001
Salmonella	1.79 ± 0.13 ^{a}	1.15 ± 0.16 ^{b}	1.02 ± 0.14 ^{b}	0.030
Total coliform	4.62 ± 0.17 ^{a}	3.09 ± 0.25 ^{b}	2.95 ± 0.20 ^{b}	0.001

$^{a–c}$ Means with different lowercase letters in the same row are significantly different ($p < 0.05$). *C. perfringens*, *Clostridium perfringens*; *E. coli*, *Escherichia coli*; CON, chicks fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days.

3.7. Gene Expression

Figure 3 illustrate the effect of early feeding with probiotic-fermented feed on the expression of the IGF-1, SLC15A1, and MUC-2 genes. The expression of IGF-1 and MUC-2 genes was higher in PELF3- and PELF6-fed ostrich chicks ($p < 0.05$) than in the control

group. SLC15A1 expression was higher in PELF6-fed ostrich chicks than in those fed the control and PELF3 diets ($p < 0.05$). The mRNA levels of SLC15A1 were positively correlated with IGF-1 and MUC-2 mRNA levels (r = 0.760, $p = 0.001$, and r = 0.586, $p = 0.02$, respectively). The mRNA levels of IGF-1 were positively correlated with the mRNA levels of MUC-2 (r = 0.823, $p = 0.001$).

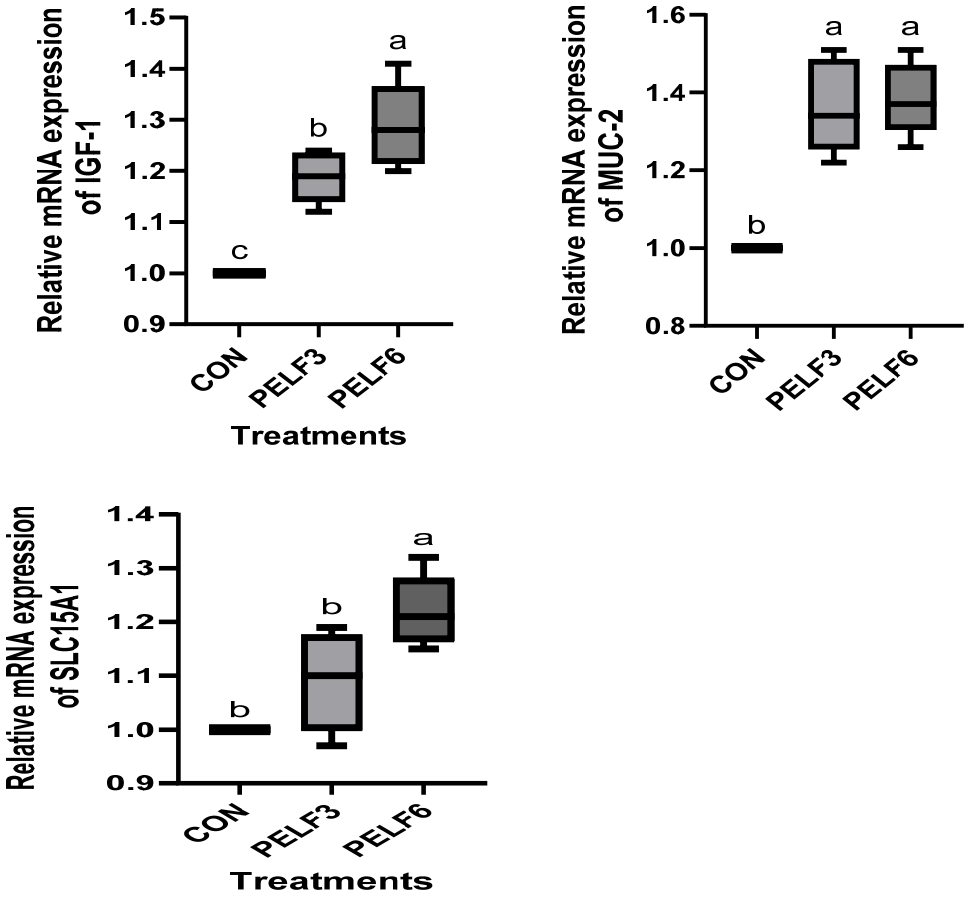


Figure 3. Impact of early feeding with probiotic-fermented feed on the mRNA expression of IGF-1, MUC2, and SLC15A1 genes in ostrich chickens at 56 days. CON, chicks were fed the control diet; PELF3, chicks fed probiotic-fermented feed for 3 days; PELF6, chicks fed probiotic-fermented feed for 6 days. The data is represented as the mean ± SE. Different lowercase letters (a–c) indicate statistically significant differences among groups ($p < 0.05$).

4. Discussion

Ostrich chicks are characterized by high growth rates and limited digestive capacity at an early age, resulting in high nutritional requirements, particularly for proteins and energy. In addition, their body weight increases eleven-fold during the first three months, and the relative weight of the small intestine peaks at 41 days of age [11]. Additionally, shortened villi have been observed in the duodenum of ostriches, which may indicate a decrease in the overall activity of membrane-associated enzymes, resulting in low digestive efficiency at an early age [11]. Interestingly, ostrich chicks are born without intestinal bacteria; however, some microbes begin to appear after 10 days, indicating that they have a weak fermentation capacity at very early ages [11,13], making them more susceptible to pathogens. Fermented feed can enhance nutrient availability, improve digestibility, and increase the population of beneficial gut microbiota [17,21,23], which supports more efficient nutrient utilization and enhances overall health. This is consistent with the results of the current study, where modern feeding strategies, including early feeding, supported the health, performance, and growth of newly hatched chicks by improving oxidative

stability, immunity, and intestinal integrity, thus enhancing nutrient utilization [2,4,18,20]. These positive effects are particularly pronounced in ostrich chicks due to their elevated metabolic demands, which likely explains why their growth performance is more sensitive to fermented feed compared with other poultry species with slower early growth rates and lower nutritional requirements.

The results of the present study indicate improved performance in ostrich chicks receiving probiotic-fermented feed, as evidenced by higher live body weights, and lower feed conversion ratios and mortality rates. Several previous studies have reported that feeding fermented feeds enhances the growth performance of broiler chickens, which is consistent with our findings [17,22]. This may be due to the beneficial effects of the fermentation process, including reducing anti-nutritional factors, thus enhancing nutrient bioavailability and nutritional value during the early life stages [35]. Additionally, it significantly increases the protein content of the feed, which enhances nitrogen utilization, digestive enzyme activity [36], small peptide formation, and amino acid synthesis, and increases villus height [37], further contributing to enhanced nutrient digestion and absorption. Furthermore, the fermentation of feeds produces short-chain fatty acids (SCFAs; especially acetate, propionate, and butyrate), which support the supply of energy to intestinal cells, enhance the integrity of the intestinal barrier, inhibit the growth of pathogenic bacteria, regulate inflammatory responses, and enhance immune defense. SCFAs also support the proliferation and differentiation of intestinal cells, which improves the growth of intestinal villi and the ability to absorb nutrients [35,38], thereby enhancing intestinal integrity and contributing to the growth of ostrich chicks. The improvement in the performance of ostrich chicks in our study could be attributed to the addition of *L. acidophilus* and *B. subtilis* bacteria, which are able to promote the growth of lactic acid bacteria and produce a variety of vital enzymes [39,40]. Therefore, the use of probiotic-fermented feed in early feeding may be effective in promoting growth performance in ostrich chicks, providing a new method for use in research on the importance of probiotic-fermented feed during the early life of chickens.

Blood uric acid and creatinine levels indicate kidney function [41]. Our study showed a significant decrease in uric acid and creatinine levels in the probiotic-fermented feed-treated groups compared to the control group. Consistent with the current study, some studies have reported that probiotic intake significantly reduces uric levels [42]. Creatinine is primarily filtered and excreted by the kidneys as a by-product of muscle metabolism. An increased creatinine level is indicative of kidney dysfunction. High blood uric acid levels are also indicators of kidney dysfunction [41]. In addition, AST and ALT activity levels reflect liver cell injury, with elevated levels indicating liver cell damage [42]. Our results indicate improved liver function and performance in growing ostriches treated with PELF, as evidenced by decreased ALT levels, despite unchanged AST levels. In contrast, Mohamed et al. [42] and Elbaz et al. [36] did not detect differences in AST and ALT activity levels in chickens fed with probiotics. This variation in the effects of probiotics on AST and ALT levels across reports may be due to the type of microbes, concentration, or method of supplementation. The improved liver and kidney function resulting from fermented feeds may be due to multiple biological mechanisms, including the promotion of the growth of beneficial microbes and the production of their metabolites, which have been shown to effectively bind to toxins [43,44], reducing the transfer of endotoxins into the bloodstream, and thus improving liver and kidney function. Additionally, the hepatoprotective effect of probiotics could be attributed to their anti-inflammatory and antioxidant properties, which alleviate oxidative stress in the liver and kidneys, enhance cell integrity, and promote liver function [45]. Moreover, it enhances nutrient availability by breaking down complex carbohydrates and anti-nutritional factors [46], and reduces metabolic stress in liver and

kidney tissues. Collectively, these effects promote kidney and liver function and health in ostrich chicks receiving probiotic-fermented feeds.

Our results indicated that early feeding with probiotic-fermented feed significantly decreased cholesterol and LDL-cholesterol levels, while increasing HDL cholesterol levels, compared with the control group. This is in agreement with the results reported by Zhang et al. [47], who found that adding fermented feed to chicken diets significantly decreased cholesterol. Studies have shown that probiotic and fermented feed affect chicken lipid profiles by altering gene expression, enzymes, or signaling pathways related to fat synthesis, distribution, breakdown, and transport in various body tissues such as liver, muscles, and abdominal fat tissues [48]. Furthermore, several previous reports have indicated that feeding chickens a diet containing probiotics had a direct effect (through gut microbes) on intestinal bile salt recycling, as well as an inhibitory effect on the activity of the hepatic enzyme 3-hydroxy-3-methylglutaryl coenzyme reductase by slowing down the synthesis of this steroid from acetyl-CoA. This reduces cholesterol and LDL cholesterol in the blood, which is excreted in the feces [42,49], and can affect cholesterol excretion and absorption. Additionally, the beneficial effect of fermented feeds on lipid profiles may be due to modification of gut microbes, which play a crucial role in nutrient digestion and metabolism [22,47].

Chicks are exposed to many stressors while they are in the hatching chamber before they are given feed and water, which can impair their physiological development [50]. Changes in blood T3 and COR levels are among the stress markers that have been assessed [51]. Elevated COR and decreased T3 levels were observed in the current study, which may be due to the exposure of the chicks to stress during hatching. Interestingly, our results showed increased T3 levels and decreased COR levels in the probiotic-fermented feed-fed chicks. This low COR may be due to the fermentation process enhancing feed digestion and nutrient absorption, thereby meeting the body's needs and reducing the secretion of stress hormones, such as COR, to compensate for nutrient deficiencies, enhancing the growth and overall health of the chicks. Furthermore, the enhanced production and function of thyroid hormones may result from improved nutrient availability and gut health during fermentation, which may help enhance chick performance [51,52]. In particular, thyroid hormones are crucial to numerous physiological functions, most notably the regulation of metabolism. Consistent with our findings, several reports have shown that adding probiotics to feed enhances thyroid function by increasing the production of the thyroid hormone T3 [53], which promotes growth by enhancing the physiological performance of stressed chicks. This explains how adding fermented feed reduces the impact of stress during the early life stages of ostrich chicks.

The current study examined immunological markers, as many reports have indicated that the immune system is not fully functional during the post-hatching period [4]. Immunoglobulins are antibodies that protect poultry from pathogens; therefore, immunoglobulin levels in the blood are key indicators of the humoral immune status of chickens [54]. In addition, providing multiple nutrients is crucial for promoting the development of immune organs, as most immune tissue development occurs during the late brooding and early post-hatching periods in chicks [1]; in our study, enhanced immune function was achieved through giving probiotic-fermented feed. Early feeding of PELF improved the immune response of ostrich chicks, increased IgA and IL-10 levels, and decreased IL-6 levels. Consistent with our findings, other studies have found that early feeding or supplementation with fermented feed enhances the immune response by promoting antibody production [55], the production of anti-inflammatory cytokines and immunoglobulins, and increased organ weight [56]. Early feeding or supplementation with fermented feed may contribute to immune stimulation by supplying essential minerals and substrates during the initial days

after hatching, thereby promoting gut-associated lymphocyte proliferation [4,38], which is essential for strengthening the immune system in chicks. Previous studies have shown that during fermentation, some compounds such as small peptides are produced, which in turn leads to increased concentrations of immunoglobulins [28,57]. These immunoglobulins encourage quicker yolk sac absorption immediately after hatching, which is necessary for the absorption of nutrients and maternal immunoglobulins [58], supporting the immune system of chicks and enhancing their disease resistance. Cytokines (T-cell types I and II) are also believed to play a role in cellular immune responses, such as inducing IL-6 and IL-10 production, stimulating B-cell growth and differentiation [39], and activating humoral immune responses. Our results showed an alteration in the concentration of T-cell type II cytokines, which could be attributed to the increased abundance of *Lactobacillus bacteria* in the fermented feed groups [59]. This suggests that the addition of fermented feed improves immune function in ostrich chicks.

Newly hatched chicks are more susceptible to oxidative stress, particularly from hatching until they receive feed and water [60]. This stress can lead to cell and tissue damage, increased susceptibility to disease, impaired immunity, and poor growth [21]. Adding probiotics and fermented feed to post-hatching chicks was found to be an effective way to support their antioxidant defenses and enhance chick vitality and health [18,53]. This finding is consistent with our study, which showed that early feeding with PELF decreased the MDA content and increased SOD activity. Thus, experimental supplements or fermented feed can stimulate antioxidant enzymes [57,61], enhancing the animals' ability to maintain an antioxidant–peroxide balance. Additionally, antioxidant compounds released during fermentation support antioxidant resistance [57,62], thereby enhancing immune function and growth performance. As MDA is an end product of lipid oxidation, it can be used to assess the extent of lipid oxidation [39]. As previously mentioned, feed fermentation produces several biologically active byproducts, most notably SCFAs (acetate, propionate, and butyrate) [35,38,63], which are involved in regulating oxidative and antioxidant balance in chickens. Butyrate, in particular, enhances oxidative stability by reducing the production of reactive oxygen species (ROS) [63,64] in intestinal and liver cells, thus supporting their integrity and function. Furthermore, butyrate enhances mitochondrial efficiency by reducing electron leakage from the respiratory chain, which is a major source of ROS generation [65], thus improving mitochondrial function. These results indicate that feeding ostrich chicks probiotic-fermented feed has beneficial effects such as protecting tissues from lipid peroxidation and oxidative instability.

Maintaining a healthy gut is extremely important because of its pivotal role in physiological processes, including immune responses, metabolism, and the digestion and absorption of nutrients [66]. Enhancing the gut microbiota is important for maintaining gut health, given its role in metabolic processes and its ability to secrete a range of digestive enzymes that facilitate the breakdown of organic matter in feed [38,67]. It also influences the normal functional and structural development of the mucosal immune response [68]. The composition of the intestinal microbiome in chickens is influenced by a wide range of factors, including genotype, age, diet, and environmental conditions, with diet being particularly important. In the current study, probiotic-fermented feed modified the gut microbiota by increasing *Lactobacillus* and reducing *C. perfringens*, *Salmonella*, total coliform, and *E. coli*. Similarly, numerous reports have demonstrated that probiotic supplements or feed fermentation modify the composition of the gut microbiota [69–72], strengthening the immune system and alleviating intestinal disorders [38,47] by allowing beneficial microbes to colonize the gut and produce SCFAs [37]. SCFAs play numerous roles in supporting gut health [67,72,73], for example, by maintaining metabolic balance, secreting hormones, regulating intestinal motility, and enhancing the activity of the amino acid metabolism

pathway [65,74,75]. Additionally, beneficial probiotic microbes compete with pathogenic bacteria for resources and space in the chicken intestine, thereby reducing the likelihood of disease [69,76,77]. Furthermore, probiotic supplementation has been shown to rebuild the intestinal structure by increasing villus height, strengthening the mucous barrier, and stimulating the proliferation of crypt cells, thus increasing the surface area for absorption and helping to maintain gut integrity and enhance nutrient absorption [69,78,79]. Probiotic supplementation can also support the physiological functions necessary for intestinal homeostasis in chickens and thus gut performance. *Lactobacillus bacteria* can produce lactic acid, which plays a crucial role in inhibiting the colonization of pathogenic microorganisms, such as by reducing *Campylobacter* colonization in the intestines of chickens [80]. The beneficial effects of fermented feed are likely due to *B. subtilis* and *Lactobacillus* bacteria, which have been reported to inhibit harmful microbes and increase beneficial microbes [38]. Fermented feed can serve as a strategy for modifying the intestinal microbiota in ostrich chicks by modulating mucosal balance, immune responses, and feeding efficiency.

One of the benefits of gut microbes is their ability to modify the expression of numerous genes through the production of SCFAs, which directly or indirectly affect adipose tissue in the intestine or liver [69,81]. In the current study, PELF-fed ostrich chicks showed modified gene expression such as increased expression of the IGF-1, SLC15A1, and MUC-2 genes. Consistent with these results, Salehizadeh et al. [81] found an increase in the expression of IGF-1 in chickens fed probiotics, which enhanced growth and skeletal development. Several reports have indicated a close correlation between increased IGF-1 expression and body weight gain in chickens [82]. Additionally, a previous study found increased expression of MUC-2 in chickens fed a diet containing *Bacillus subtilis* or *Lactobacillus plantarum* [31]. In addition, increased MUC-2 gene expression resulting from feeding on probiotic-fermented feed has positive effects due to its function as a physical barrier; it also enhances the formation of IgA-mediated immune defenses by binding to a variety of bacterial species and triggering IgA production [83]. This selectively contributes to the growth of normal intestinal bacteria and prevents the invasion of gut bacteria into intestinal epithelial cells, indicating that increased MUC-2 gene expression supports intestinal integrity [80]. Consistent with our results, Salehizadeh et al. [81] reported increased IGF-1 expression in chickens receiving probiotics. Numerous reports have demonstrated a close link between growth hormone (GH) and IGF-1 levels. Increased IGF-1 expression leads to increased GH production [31,84], which promotes normal tissue and bone growth, and thus has a significant impact on chick growth and development [85]. The increased expression of the IGF-1 gene may be because the gut microbiota is capable of producing SCFAs, which affect adipose tissue and the liver through multiple mechanisms to increase blood IGF-1 levels [82] and thus enhance the development and growth of the host skeleton. Additionally, probiotic-fermented feed supplementation significantly upregulated SLC15A1, which encodes a transporter responsible for the absorption of the di- and tripeptides that result from protein digestion in the small intestine. Consistent with our study, Fernandez-Alarcon et al. [86] found that adding *Bacillus* spp. to chicken feed increased SLC15A1 expression. These results suggest that probiotic fermentation can enhance the trans-cellular transport of amino-peptide molecules [87], which can stimulate the absorption of free amino acids and increase protein digestibility. Furthermore, our results showed positive correlations between the expression of SLC15A1, IGF-1, and MUC-2 in probiotic-fermented feed-fed chicks, which could improve epithelial cell growth, mucosal barrier function, and intestinal nutrient transport. In poultry, including ostriches, probiotic supplementation has been shown to modify the intestinal microbial environment and increase the production of microbial metabolites (such as short-chain fatty acids) [88]. These changes in the intestinal lumen support goblet cell function, epithelial metabolic status, and mucin synthesis [89–92];

mucin shields the intestine from pathogen invasion, leading to increased MUC-2 and IGF-1 gene expression [93]. IGF-1 gene expression is closely associated with intestinal growth, epithelial renewal, and nutrient utilization [94]. Additionally, improved intestinal epithelial function and proliferation reflect higher nutrient and digestive efficiency due to improved regulation of mucin production, which protects the epithelial surface against pathogens [95], leading to increased expression of SLC15A1, which stimulates the expression of growth factor-stimulating factor (IGF-1). Therefore, the observed correlations between the gene expression changes suggest that probiotic-fermented feeds promote goblet cell maturation and mucosal immunity, protect the intestinal epithelial surface from pathogens and mechanical damage, stimulate intestinal growth, increase the production of the intestinal mucus layer and its integrity, and enhance nutrient availability. The results of this study show that a diet supplemented with probiotic-fermented feed during the early feeding of ostrich chicks stimulates the expression of IGF-1, SLC15A1, and MUC-2, which supports the growth of ostrich chicks by modifying the gut microbiota. This in turn activates various signaling pathways and secreted chemical factors and regulates mucin production [96,97], thereby supporting intestinal integrity, improving nutrient absorption, and strengthening the immune system, and improving the growth performance and well-being of ostrich chicks.

5. Conclusions

Early feeding with probiotic-fermented feed improved growth performance in ostrich chicks by enhancing the gut microbiota, oxidative stability, and immunity, as well as upregulating IGF-1 gene expression. Furthermore, early feeding with probiotic-fermented feed enhanced gut health by modifying the gut microbiota and upregulating MUC-2 and SLC15A1 gene expression. Therefore, probiotic-fermented feeds can be used as a nutritional strategy for enhancing the performance and health of growing ostriches.

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Institutional Review Board Statement: All animal handling procedures complied with the guidelines of the Institutional Animal Care and Use Research Ethics Committee at the Faculty of Agriculture, Ain Shams University and Desert Research Center, Cairo, Egypt, which approved this study under protocol #5-2025-47, approved on 5 February 2025.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Abbreviations

The following abbreviations are used in this manuscript:

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ADG	average daily weight gain
IgA	immunoglobulin A
COR	corticosterone
T3	triiodothyronine
SOD	superoxide dismutase
MUC-2	mucin 2
IGF-1	Insulin-Like Growth Factor 1
SLC15A1	Solute Carrier Family 15 Member 1
C. perfringens	*Clostridium perfringens*
GPx	glutathione peroxidase
MDA	malondialdehyde
IgM	immunoglobulin M
IgG	immunoglobulin G
IL-10	interleukin 10
IL-6	interleukin 6
FCR	feed conversion ratio
TRI	triglyceride
CHO	cholesterol
HDL	high-density lipoprotein cholesterol
LDL	low-density lipoprotein cholesterol
ADFI	average daily feed intake
MOR	mortality
CON	chicks fed the control diet
PELF3	chicks fed probiotic-fermented feed for the first 3 days post-hatching
PELF6	chicks fed probiotic-fermented feed for the first 6 days post-hatching

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