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Role of Early Feeding by Technically Modified Diets on Physiological Traits and Gene Related to It in Newly Hatched Quails

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The aim was to investigate the effect of glycerin and whole egg powder supplements in the diet on physiological traits and gene related to it in newly hatched quails. The current study included one experimental period. 150 chicks were used in the experiment randomly distributed into 5 treatments: T1: Fasting(24h), T2: Control, T3: Glycerin (2.5%), T4: Whole egg powder (2.5%), and T5: Glycerin (2.5%) with whole egg powder (2.5%). The results of this study showed the highest significant difference ($P \leq 0.05$) was found in the treatment of birds treated with a 2.5% glycerin supplement to the diets in the concentration of total protein, albumin, and globulin, Amylase showed the highest significant difference in the expression of the Mucin2 gene. Finally, this study concluded that the use of glycerin as a nutritional supplement effectively contributes to improving growth performance of Japanese quail used as an alternative source of poultry meat.

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Keywords: Physiological; glycerin; egg powder; gene; quail.

1. INTRODUCTION

In addition, several studies have shown that early feeding after hatching accelerates the growth of the digestive tract (Sarfraz *et al.,* 2019). Early feeding increases the relative weight of the crop. As demonstrated by (Cengiz *et al*., 2012). Compared to post-hatching feeding, the pancreas also increases in relative weight at 6 days of age and the intestine at 10 days of age. Villus width increases slightly after hatching, but cell length more than doubles in the duodenum and reaches about 50% in the jejunum and ileum within 6 days (Alsereah et al., 2022). Villi per intestinal villus increase significantly after hatching in the duodenum and to a lesser extent slowly in the jejunum and ileum (Cruz *et al.,* 2019). However, when feeding is delayed, intestinal villi grow less in all regions of the intestine because intestinal epithelial cells proliferate when the hatch and are rapidly replaced within 48 hours (Roto et al., 2016). These proliferating cells settle primarily in the intestinal lumen (Uni et al., 2005). Compared to chicks that received feed, the proportion of cells proliferating in these lumen decreases when feed is delayed (Alimardani et al., 2023). According to their structure and functional role in the areas of their synthesis within the digestive organs , the various digestive enzymes are ready at the hatch (Gaber *et al.,* 2023). According to the study, newly hatched chicks have a reserve of pancreatic enzymes. However, this reserve is insufficient to fully digest food components in the intestine while maintaining a constant concentration of these enzymes in the intestinal tract, so the concentration of these enzymes decreases once the chicks hatch, For example the activity of disaccharide enzyme is low throughout embryonic development due to the low carbohydrate reserve; however, it increases by about 2-4 times during the first 2 days of life and becomes more stable as the bird ages. Recent studies have shown that adding glycerol-containing nutritional supplements to bird diets significantly enhances MUC2 gene expression. This is attributed to glycerol's anti-inflammatory and growth-promoting properties, as it improves the intestinal environment by increasing the thickness of the mucosal layer and reducing oxidative stress (Livak & Schmittgen, 2001). In a study conducted on quails by (Liu et al. 2022), it was shown that glycerol supplements increased MUC2 gene expression by 30% when added at concentrations ranging from 2-4% of the diet weight. This effect is believed to result from stimulation of TLR4 receptors in intestinal epithelial cells, leading to increased production of anti-inflammatory cytokines such as IL-10, which support the construction of the mucosal barrier (Smirnov et al., 2006). A study by Zhao et al. (2019) also indicated that glycerol supplementation reduces the prevalence of pathogens such as Salmonella and E. coli in the gastrointestinal tract of birds, indirectly promoting intestinal health by improving the internal environmental conditions. The research objectives were to identify effective methods for producing a technically enhanced feed mixture by improving the healthiness of the existing feed formulations.

2. MATERIALS METHODS

2.1 Management of Experiment

The chicks were placed in wooden cages consisting of two floors for each cage, each floor had 3 boxes with dimensions of 75×70×45 cm in length, width and height respectively (Nasiri Foomani et al., 2014). In the second experiment (1-42 day) the chicks where randomly distributed into 5 groups with three replicates for each group (10 birds per replicate). The chicks were fed on the growing diet during the age of (1-21) days which contains 2223% protein and 2950 kcal/kg metabolizable energy, while the production diet which contains 21.22% crude protein and 3050 kcal/kg metabolizable energy from the age of (22-42) days. also, temperature and humidity controllers, temperature of about (33°C) during the first week of the experiment, then the temperature was gradually reduced at a rate of (2°C) to reach (23°C) in the sixth week. Ventilation was adopted using suction fans installed on top of the walls of the experiment hall, and a continuous lighting system was used for 24 hours (Salman, 2023).

2.2 Preparation of Glycerin, Whole Egg Powder, Corn Starch and Carragenan for Use

Glycerin, whole egg powder, carrageenan, and corn starch were procured from Baghdad, as detailed in the subsequent classifications and compositions:

2.2.1 Glycerin

Liquid glycerin (Glycerin USP-Grade) with a purity of 99.5% was supplied by BOJAGRO S.A.

Company. Located in Germany, It serves as energy source and addition to enhance texture and moisture in the ingredients.

2.2.2 Whole egg powder

Dried whole egg powder, with a protein content of 48% and fat content of 42%, was procured from Ovostar Union company , based in the Ukraine.

2.2.3 Carrageenan

The refined carrageenan utilized in the formulation was acquired from Carrageenan Co, a company headquartered in the Philippines, exhibiting a purity of 90%. Carrageenan is derived from seaweed and utilized as a gelling agent.

2.2.4 Corn starch

Modified Corn Starch of 98% purity from CornTech, manufacturer in the united states, and is utilized as a binder and thickening agent.

2.3 Method of Preparation

Glycerin, whole egg powder, carrageenan, and corn starch were combined in precise proportions based on the calculated dosages and included into the bird diet.

2.4 Study Design

150 chicks were used in the experiment randomly distributed into 5 treatments (30 chicks each) with three replicates of 10 chicks each, the subjects were classified as follows: T1: Fasting group: where chicks coming directly from the hatchery were fasted for 24 hours, T2: Control group: birds were fed standard rations, T3: Glycerin group: birds were fed standard rations fortified with (2.5%) glycerin, T4: Whole egg powder group: birds were fed standard rations with (2.5%) egg powder, T5 Glycerin group with whole egg powder: birds were fed standard rations with a mixture of (2.5%) glycerin and (2.5%) egg powder.

2.5 Sample Collection

2.5.1 Blood samples

was collected from birds during slaughter at the age of six weeks, 3 birds from each group at a rate of 2 ml of blood and placed in Gel tubes to estimate some biochemical properties of the serum. The plastic tubes, free of anticoagulant and containing 2 ml of blood were placed in the centrifuge. The centrifuge was operated at a speed of 3000 rpm for 15 minutes to separate the blood serum and store it at a temperature of -20°C until the end of the analyses (Rhehab, 2024).

2.6 Measurements and traits studied

2.6.1 Biochemical parameters

Biochemical of parameters analysis was done in veterinary medicine college by spectrophotometers and special kits for each parameter as following

Total Protein Concentration: The total protein concentration in blood serum was quantified utilizing a kit from the Spain react S.A.U /Spain company, adhering to the manufacturer's instructions. The absorbance of the samples and standard solution was assessed using a spectrophotometer at a wavelength of 550 nanometers, applying the subsequent equation:

The total protein (g/100 ml) = the sample absorbance / the absorbance of the standard solution × 6 (Henry *et al.,* 1974).

List 1. Compositions and chemical analyses of the diet utilized in the experiments

Components	Growth diet (%)*(1-21)*day	Productive diet (%) (*22* to end the experiment)
yellow corn	48	46
wheat	11.5	23
protein concentrated (50% protein)	5.5	8.5
soybean meal,(48% protein)	32	18
limestone	1.5	2
vegetable oil	1.5	-
mixture of vitamins and minerals	-	2.5
Total	100	100

Components	Growth diet (%)(1-21)day	Productive diet (%) (22 to end the experiment)
Total metabolic energy (kcal/kg)	2950	3050
protein content(%)	22.23	21.22
calcium percentage (%)	0.85	0.93
Methionine percentage (%)	0.45	0.58
Phosphorus percentage (%)	0.58	0.8

NRC (1994)

Albumin Concentration: The total albumin concentration in blood serum was quantified utilizing a kit from the Spain react S.A.U /Spain Company Support Technical, following the methodology outlined by the supplying company. The absorbance of samples and standard solutions was measured using a spectrophotometerat a wavelength of 630 nm. Subsequently, the absorbance of each sample was documented, and the following equation was employed:

Total Albumin (g/100 ml) = Sample absorbance / Absorbance of standard solution x 5 (Henry *et al.,* 1974).

Globulin Concentration: Determine globulin concentration by subtracting albumin from total protein using the following equation:

Globulin concentration (g/100 ml) = total protein concentration - Concentration of albumin. (Henry *et al.,* 1974).

Glucose Concentration: Blood glucose levels were assessed using a kit provided by Spain react S.A.U /Spain company. Analyses were conducted according to the companies procedures, using a spectrophotometer at a wavelength of 550 nm. The absorbance of each sample was measured, followed by the application of the equation:

Blood glucose (mg/100ml) = Absorption sample / Standard absorbance solution × 100 (Sayah, 2022)

Cholesterol Concentration: The absorbance of the samples was determined using a spectrophotometer at a wavelength of 500 nm, following the instruction provided by the Spain react S.A.U /Spain company.

Cholesterol concentration (mg/100 ml) = Sample absorbance / Absorbance of standard solution x 200. (Henry *et al.,* 1974).

Triglycerides Concentration: The absorbance of the samples was determined using a spectrophotometer at a wavelength of 540 nm, following the instructions provided by Spinreact S.A.U /Spain company.

Triglyceride concentration (mg/100 ml) = Sample absorbance / Absorbance of standard solution × 200 (Henry *et al.,* 1974).

2.7 Digestive Enzymes

2.7.1 Lipase enzyme activity

Lipase activity was assessed via a BIOLABO kit. The reaction entailed the transformation of 1,2-diglyceride into glycerol and free fatty acids, accompanied by quinoneimine production, which was quantified at 550 nm (Rhehab,2024).

2.7.2 Amylase enzyme activity

Amylase activity was assessed via the in BIOREACT device a BIOLABO kit. The, correlated with α-amylase activity, was assessed at 405 nm (Rhehab,2024).

2.7.3 Protease enzyme activity

Protease activity was assessed via a BIOLABO kit. Proteases engage with protein substrates, leading to elevated levels of the resultant peptides Enzyme activity was assessed at a wavelength of 405 nm (Rhehab, 2024).

2.8 Gene Expression

2.8.1 Tissue samples collection

After slaughtering the birds at the age of (6) weeks, (15) pancreatic tissue samples were collected, with (3) samples for each treatment and (1) sample for the replicate. These samples were kept in special sealed containers and deep frozen at a temperature of (-80) C in the Biotechnology Laboratories of the Department of Life Sciences, College of Science, University of

Basra until the sample crushing process was carried out.

2.9 Sample Crushing and Preservation Process Using Trizol

The sample crushing process was carried out in Al-Ghari Research Laboratory in Najaf Governorate after preparing a sterile place for work and according to the following steps: A sample of pancreatic and intestinal tissue was taken after thawing it from freezing and with equal weight for all samples and placed in a special ceramic mortar. An amount of liquid nitrogen was added to crush the sample and completely convert it into a frozen powder. The powder was transferred to Eppendorf with the addition of (900) microliters of Ttizol solution. The sample was covered with dark tape to avoid damage to the sample. All steps were repeated on all samples, all samples were numbered and stored in a freezer at (-18) degrees until the RNA extraction process was completed.

2.10 Dissolving Primers

According to the manufacturer's instructions, the primers were dissolved by adding sterile deionized water as stated on the package and mixing them well. 100 pmol of each primer was prepared as stated on the label of the primer tube, thus forming the Stock Solution which was stored at -20°C.

2.11 Dilution of Primers

To dilute the primer, 10 pM of the 100 pM primer was taken in a new tube and 90 µl of sterile deionized water ddH2O was added to it and stored at -20°C. 1 µl of the last dilution was taken and added with the components of the RT-PCR tube.

2.12 The RNA Extraction and Quantitative Real-Time PCR

The Musin2 were extracted after grinding the tissue by utilizing the TransZol microRNA extraction Mini-Kit (TransGen Biotech, China), adhering strictly to the procedural guidelines outlined by the manufacturer as stated below:1- The 500 µl of triazole was Added to 500 µl of Tissue then mix by vortex.2- The 100 µl of chloroform was added to mixture. Shaked the tube vigorously by hand for 30 second and incubated at room temperature for 3 minutes.3- The sample was centrifuged at 10,000 rcf for 15 minutes at 2-8℃. The mixture separated into a lower pink organic phase, an interphase, and a colourless upper aqueous phase which containing the RNA. The volume of the aqueous upper phase is around 60% volume of triazole reagent. 4- The colourless upper phase that contain RNA transferred to a fresh RNase-free tube. 5- About 250 µl of isopropanol was added (to precipitate RNA) and mixed thoroughly by inverting tube. Incubate at room temperature for 10 minutes. 6- Centrifuge the sample at 10,000 rpm for 10 minutes at 2-8°C. Discard the supernatant. Colloidal precipitate can be seen at the wall and the bottom of the tube.7- Add 500 µl of 75% ethanol (prepared with RNase-free water), vertexing vigorously.8- Centrifuge the sample at 7,500 rpm for 5 minutes at 2-8°C.9- Discard the supernatant. Air-dry the RNA pellet 55-60°C (about 5 minutes).

10- The RNA pellet is dissolved in 50 µl of injection water (DDW).11. Incubate at 55-60°C for 10 minutes then the purified RNA was used with kit content and primers in qRT-PCR. The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was carried out to assess the expression level of Musin2 . The genotyping occurred within a 20µl reaction mixture comprising of 4µl of RNA template, 10µl of SYBR-Green 2X Master mix (Promiga, USA), and 2µl (10pmol/µl) of each specific forward and reverse primers respectively. Amplification was conducted using an MJ Mini Thermocycler (Applied Biosystems, UK). The process was subjected to a holding stage for 15 minutes at 37℃. Cycling parameters included an incubation period of 10 minutes at 95°C, 45 cycles at 95°C for 15 seconds, 58°C for 1 minute, and 72°C for 30 seconds. This was followed by the melting curve stage. The U6 gene (housekeeping gene) served as the endogenous control to standardize the expression value of. Musin2 The primers employed are as follows:

Musin2	F	CCACAAGTCGTCCAGTACCTACA	108
	R	AGGTTTCATAGTCACCACCATCTTC	

2.13 Statistical Analysis

The study data were analyzed employing a completely randomized design (CRD) utilizing (SPSS, 2019). To assess the significance of the differences among the studied means, Duncan's Multiple Range Test (P≤ 0.05) was applied, as per (Duncan, 1955), alongside a mathematical model for data analysis.

3. RESULTS

3.1 Effect of Glycerin, Whole Egg Powder and Gel Materials (Carragennan and Cornstarch) to the Calculating Some Biochemical Parameters for Birds (1-6) Weeks

Table 1 demonstrated significant differences (P≤0.05) in the majority of assessments. The glycerol group (T3) had markedly elevated levels of total protein (5.70), albumin (2.60), and globulin (3.1), whereas the lowest significant values were noted for triglycerides (111.70) and cholesterol (160.33). No notable disparities were detected in glucose levels among the groups.

3.2 Effect of Glycerin, Whole Egg Powder and Gel Materials (Carragennan and Cornstarch) to the Calculating Some Digestive Enzymes for Birds (1-6) WEEKS

Table 2 demonstrated substantial variations (P≤0.05) in amylase activity. The glycerol group (T3) exhibited the highest significant amylase activity (3.10), although no significant differences (N.S.) were noted in lipase and protease activity across the experimental groups.

Table 1. Calculating some biochemical parameters for birds (1-6) weeks (Mean ± Standard error)

Traits	T1	T2	T3	T4	T5	Sig. level
TP	4.17 ± 0.17^{d}	4.20 ± 0.19^{d}	5.70 ± 0.30^{a}	5.01 ± 0.25^{b}	4.50 ± 0.20^{c}	*
Alb.	1.59± 0.12^{c}	1.78 ± 0.19^{c}	2.60 ± 0.10^{a}	2.16 ± 0.22^{b}	2.19 ± 0.18^{b}	*
Glob.	2.58 ± 0.05^{c}	2.42 ± 0.0^{c}	3.1 ± 0. 1^{a}	2.85 ± 0.03^{b}	2.31 ± 0.02^{d}	*
Gluc.	1.75 ± 0.77	1.76 ± 0.44	1.75 ± 0.65	1.76 ± 0.34	1.76 ± 0.66	N.S
Trigly.	130.20±5.12^{a}	122.44±2.88 ^{ab}	111.70±4.20 ^{d}	116.50±9.21 ^{b}	115.54±9.30 ^{b}	*
Chol.	184.84 ± 3.77^{a}	179.39 ± 3.66^{b}	160.33 ± 4.75^{a}	169.44 ± 4.77^{c}	170.40 ± 4.70^{c}	*

*The letters on the numbers symbolize a significant difference between the groups in (p≤0.05). N.S referred to no significant difference . * referred to significant difference, T1: Fasting 24 h, T2: control, T3: 2.5% glycerin,T4: 2.5% whole egg powder, : 2.5% glycerin + 2.5% whole egg powder, TP: Total Protein, Alb: Albumin, Glob: Globulin, Gluc: Glucose, Trygly:Tryglyceride, Chol: Cholestrol*

Table 2. Calculating some digestive enzymes for birds (1-6) weeks (Mean ± Standard error)

Parameters	T1	T2	T3	T4	T5	Sig. level
lipase	8.44 ± 0.73	8.41 ± 0.75	8.48 ± 0.77	8.42 ± 0.76	8.42 ± 0.71	N.S
amylase	1.66 ± 0.10^{d}	1.64 ± 0.11^{d}	3.10± 0.10^{a}	2.88 ± 0.04^{b}	2.20 ± 0.06^{b}	*
protease	202.30 ± 0.02	202.51 ± 0.04	204.08 ± 0.02	204.34 ± 0.04	201.35 ± 0.05	N.S

*The letters on the numbers symbolize a significant difference between the groups in (p≤0.05). N.S referred to no significant difference . * referred to significant difference, T1: Fasting 24 h, T2: control, T3: 2.5% glycerin,T4: 2.5% whole egg powder, : 2.5% glycerin + 2.5% whole egg powder*

Table 3. Effect of glycerin, whole egg powder and gel materials (carragennan and cornstarch) the gene expression to MUC2 (Mean ± Standard error)

Parameters	Mean ± SD					P-value
	T1	T2	T3	T4	T5	
Mucin 2	0.81^{b} ± 0.28	0.14^{c} ± 0.02	2.06^{a} ± 0.45	1.21^{b} ± 0.07	0.91^{b} ± 0.43	0.002

*The letters on the numbers symbolize a significant difference between the groups in (p≤0.05). N.S referred to no significant difference . * referred to significant difference, T1: Fasting 24 h, T2: control, T3: 2.5% glycerin,T4: 2.5% whole egg powder, : 2.5% glycerin + 2.5% whole egg powder*

3.3 Effect of Glycerin, Whole Egg Powder and Gel Materials (Carragennan and Cornstarch) to the Gene Expression to MUC2

The gene expression of Mucin 2 exhibited significant variations (P≤0.05) as indicated in Table 3. The glycerol group exhibited the highest gene expression of Mucin 2 (2.06), whereas the fasting group demonstrated the lowest gene expression (0.14). The control group, the glycerol and egg powder mixture, and the whole egg powder occupied intermediate places, suggesting that glycerol exerts a favourable and significant influence on the enhancement of Mucin 2 gene expression.

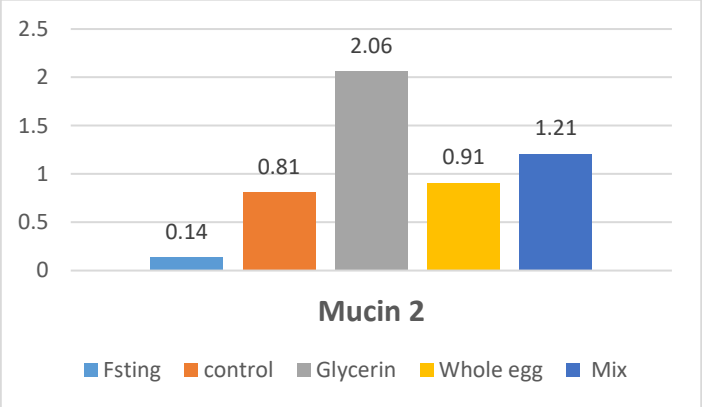

Fig. 1. Effect of glycerin, whole egg powder and gel materials (carragennan and cornstarch) The gene expression to MUC2

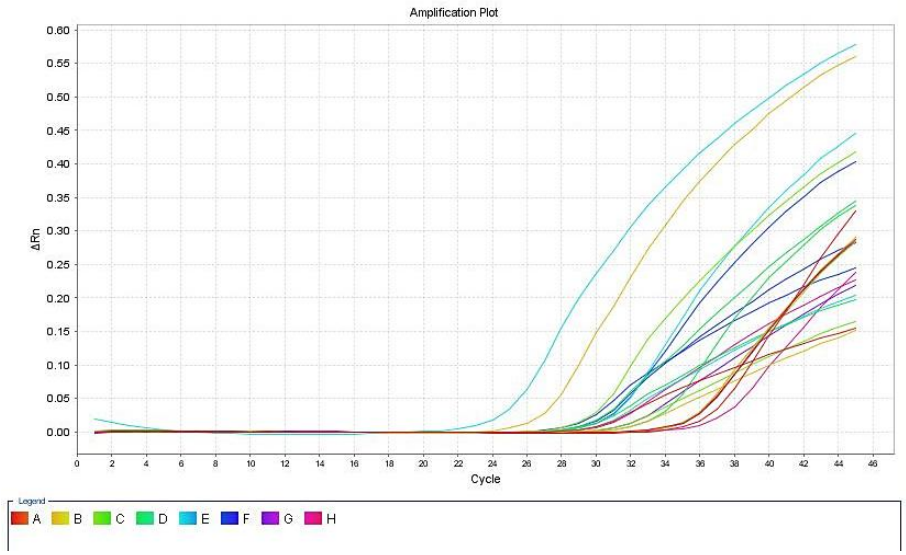

Fig. 2. Cycle troubleshoot curve of the Mucin2 gene expression that showing the number of cycles

Table 4. Pearson correlation between MUC2 with biochemical and glycerin in birds

Correlations		Total protein	Albumin	Globulin	Glucose	Triglyceride	Cholesterol
Glycerin	Pearson Correlation	0.92	0.92	-0.84	0.92	-0.92	-0.92
	N	Very strong direct relationship	Very strong direct relationship	strong inverse relationship	Very strong direct relationship	Very strong inverse relationship	Very strong inverse relationship

3.4 The Binding Relationship between MUC2 with Biochemical and Glycerol

Table 4 presents an assessment of the link between MUC2 protein levels and other significant biochemical parameters, alongside glycerol, utilising the Pearson correlation coefficient. The findings demonstrated robust correlations, which can be articulated as follows:

A robust positive association was identified between MUC2 protein and total protein levels, with a Pearson correlation coefficient of +0.92. Albumin had a robust positive connection with MUC2, evidenced by a Pearson correlation coefficient of +0.92. A robust inverse link was identified between MUC2 and globulin levels, evidenced by a Pearson correlation coefficient of -0.84. MUC2 had a robust positive association with glucose levels, indicated by a Pearson correlation coefficient of +0.92. A robust inverse link between triglycerides and MUC2 was identified, evidenced by a Pearson correlation coefficient of -0.92. A robust inverse association was identified between MUC2 and cholesterol levels, evidenced by a Pearson correlation coefficient of -0.92.

The results delineate the direct relationships identified between MUC2 and each biochemical marker, as well as glycerol, based on statistical analysis.

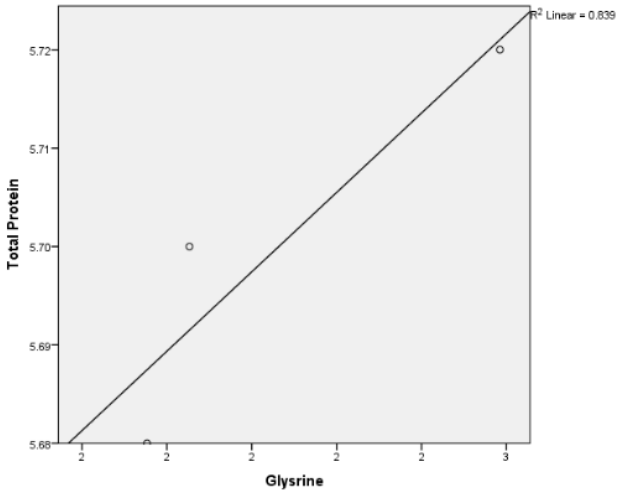

Fig. 3. Binding relationship between Mucin2, glycerin and total protein

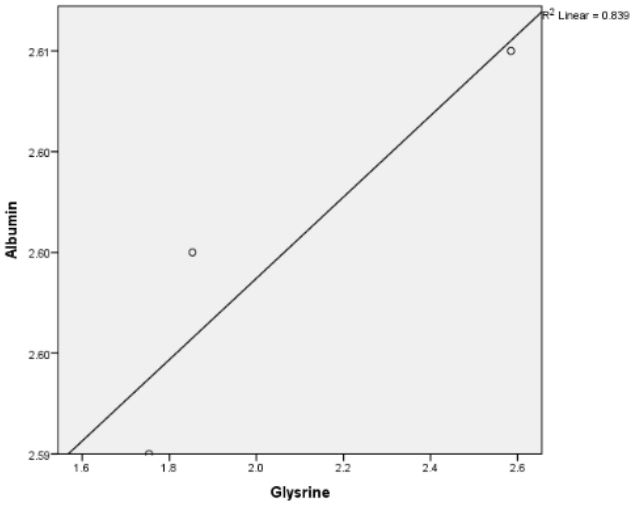

Fig. 4. Binding relationship betweenMucin2, glycerin and albumin

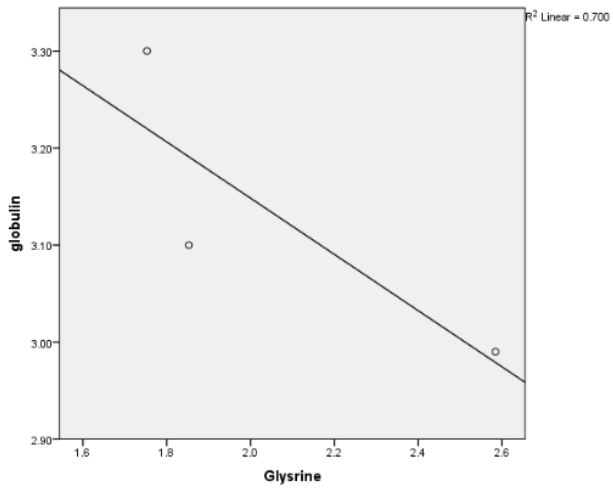

Fig. 5. Binding relationship between Mucin2, glycerin and globulin

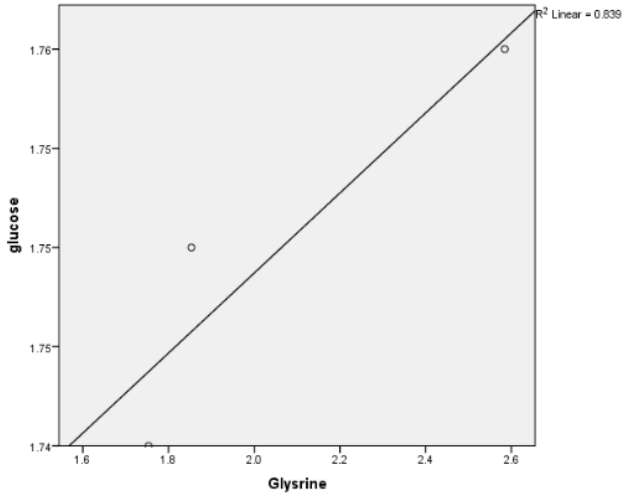

Fig. 6. Binding relationship between Mucin2, glycerin and glucose

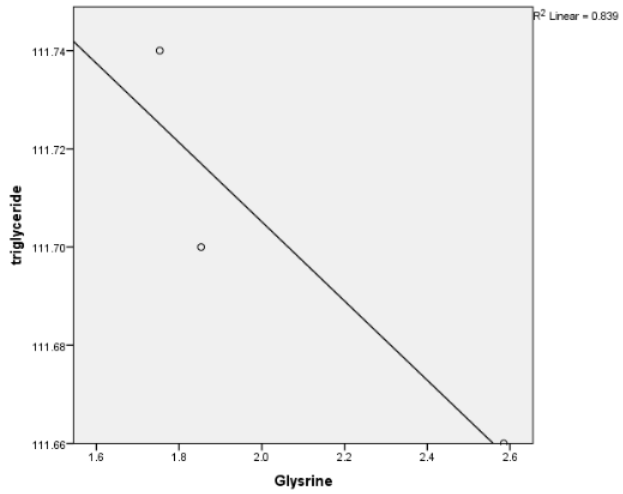

Fig. 7. Binding relationship between Mucin2, glycerin and tryglyceride

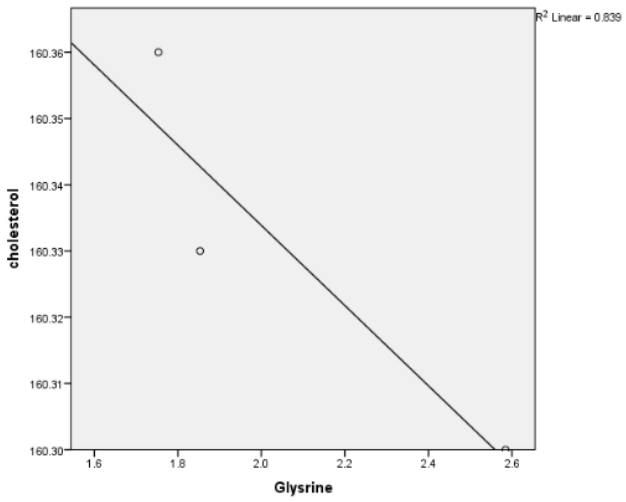

Fig. 8. Binding relationship between Mucin2, glycerin and colesterol

Table 5. Pearson correlation between MUC2 with digestive enzymes and glycerin

	Mean	Std. Deviation	N
Glysrine	2.06369774	.453966048	3
lipase	8.4800	.03000	3
amylase	3.1000	.02000	3
protease	204.0800	.05000	3

Table 6. Correlation indicating the relationship between glycerin and digestive enzymes

Correlations				
		lipase	Amylase	Protease
Glycerin	Pearson Correlation	-0.92	-0.92	-0.92
	N	Very strong inverse relationship	Very strong inverse relationship	Very strong inverse relationship

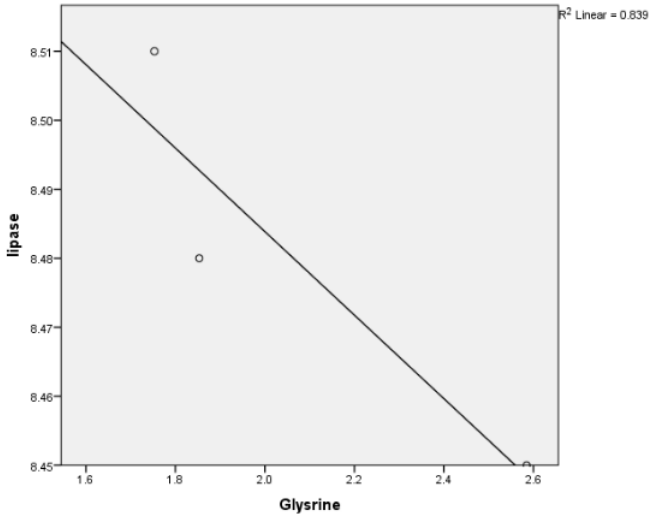

Fig. 9. Binding relationship between Mucin2, glycerin and lipase enzyme

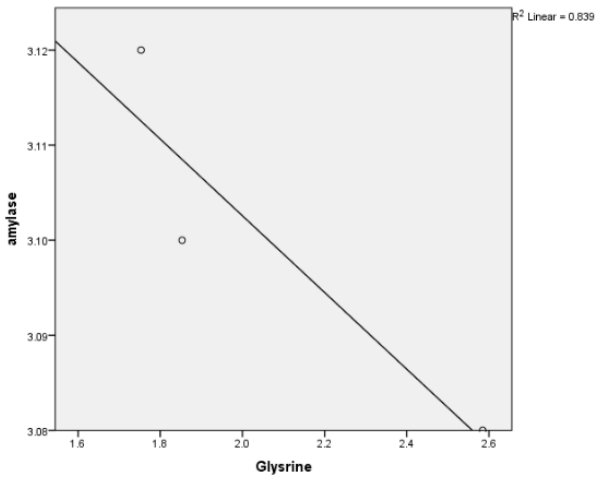

Fig. 10. Binding relationship between Mucin2, glycerin and amylase enzyme

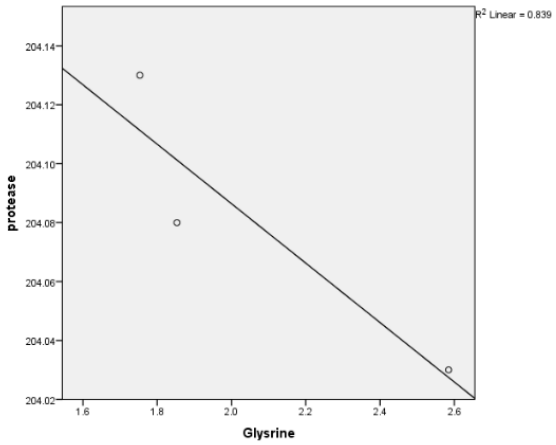

Fig. 11. Binding relationship between Mucin2, glycerin and protease enzyme

3.5 The Binding Relationship between MUC2 with Digestive Enzymes and Glycerol in Birds

3.5.1 Table 5 pearson correlation between MUC2 with digestive enzymes and glycerin

The relationship between glycerol and a set of digestive enzymes (lipase, amylase, and protease) was assessed by Pearson's correlation analysis. The findings shown in Table 5 demonstrated robust inverse associations between glycerol and each of the digestive enzymes examined. Glycerol had a Pearson's correlation coefficient of -0.92 with lipase, signifying a robust inverse association. Glycerol had a robust inverse association with amylase, evidenced by a Pearson's correlation coefficient of -0.92. An very robust inverse correlation was discovered between glycerol and protease, with a correlation coefficient of -0.92. These robust correlations indicate constant inverse relationships between glycerol levels and the levels of lipase, amylase, and protease.

4. DISCUSSION

In Table 1, the findings indicated a significant difference ($P < 0.05$) in the glycerin group in concentrations of total protein, albumin, and globulin compared with the control group. These components significantly enhance the biochemical performance of birds, demonstrating their beneficial effects on growth rate and avian health. Research indicates that glycerol serves as a significant energy source and aids in alleviating oxidative stress, hence improving the overall efficacy of the immune system in avian species (Nasser et al., 2021). Recent research

indicates that glycerol enhances metabolism by facilitating cellular energy synthesis, hence augmenting birds' capacity to adapt to varying environmental situations (Pedroso et al., 2019). No significant difference was observed in the glucose, while the variation in triglyceride levels and cholesterol levels was observed, demonstrating the stability in these critical parameters. This stability indicates that the diet including glycerin helps to maintain balanced blood energy and cholesterol levels, which enhance the health performance and nutrition of birds. These results demonstrate the nutritional biology perspective on the critical role of glycerin in promoting the biochemical parameters of bird species. These results support the formulation of new ways to enhance food effectiveness in the poultry sector. The research indicates that the inclusion of glycerin in feed can reduce production costs by enhancing the efficiency of feed conversion to meat (Patel & Stokes, 2022). The data in Table 2 showed the glycerin group is highest in the amylase activity, while the control group showed low significant differences. while lipase and protease enzymes didn't show significant differences in all groups. The results indicate that glycerin enhances activity of the digestive enzyme in birds, likely leading to enhanced digestion efficiency and feed intake. The integration of 2.5% glycerin results in a marked enhancement of the amylase activity. These results are consistent with other research suggesting that the surrounding environment rich in glycerin or dietary additives like glycerol can increase enzyme activity in the intestine. The favorable impact of glycerin is attributed to its humectant characteristics and ability to promote the proliferation of advantageous bacteria in the gastrointestinal tract. Some research shows the importance of the glycerin in enhancing tolerance and digestion in bird species (Medina et al., 2021). The results in Table 3 show that the glycerin significantly enhances Mucin2 gene expression, thus promoting intestinal mucus formation. Mucin 2 is the important protein that plays a significant role in the generation of the mucus and serves as the primary defense mechanism of the intestinal mucosa. Research indicates that enhancing Mucin2 synthesis strengthens protective bowel barriers against the infections. Glycerin, because of its moisture and nourishing properties, enhances intestinal health by augmenting the intestine's capacity to generate mucus. Research indicate that glycerin enhance efficacy of the digestiveenzymes, such lipase and amylase, result in improved digestion efficiency andnutrient absorption (Li et al., 2020). Enhancing digestive efficiency is essential for bird growth, as it promotes food absorption necessary for weight gain and muscle growth. A combination of glycerin and whole egg powder enhances gene expression of the Mucin2 gene, hence improving intestinal function. The studies show that mucus produced by Mucin2 is critical for preserving intestinal health and improving elasticity against environmental irritants and infections, thus supporting birds immune function (Kim et al., 2023). Mucus formation acts as a crucial defense against infections, and an increase in mucin 2 results in beneficial effects on immunological function.

Reports indicate that increased Mucin2 synthesis enhances the balance of intestinalnal microbiota, hence contributing to general intestinal health (Mao et al., 2021). Digestive enzymes are essential for breaking down food and transforming it into useable ingredients in the body. Studies indicate that integrating glycerin and chicken eggs into the diet enhances enzyme secretion, thus improving feed efficiency and bird growth (Li et al., 2020). The integration of these processes fosters optimal bird growth, as nutrient absorption efficacy relies on the proficiency of digestive enzymes facilitated by the glycerin (Zhao et al., 2021). Table 4 depicts the relationship between Mucin2 and other biochemical indicators in bird species. The Pearson correlation coefficient was used to quantify these correlations. Results revealed highly substantial relationships between Mucin2 and many indicators. A correlation coefficient of 0.92 between Mucin2 and total protein shows a strong positive correlation. These results indicate that elevated Mucin2 levels signify support for essential protein-dependent biological activities. Total protein acts as a biomarker for the nutritional condition of birds, providing a practical basis for enhanced enzyme synthesis and tissue growth (Pérez-Johnston et al., 2022). This indicates that Mucin2 may enhance the nutritional efficacy and health of birds. The table showed a strong association with albumin (correlation coefficient 0.92), indicating Mucin2's capacity to enhance levels of this essential protein. Albumin is essential for fluid balance control and enzyme activation, and it serves as an indicator of nutritional quality in bird species. These results support the hypothesis that Mucin2 may promote the general health of bird species. The value of the globulin, -0.84, indicated a strong positive correlation. Globulin is a crucial ingredient of immunological proteins, contributing to a bird's defense against infections. This finding

indicates that Mucin2 may boost the increase in immune response against harmful substances (Martinez et al., 2020). The results indicated a strong inverse correlation between Mucin2 and triglyceride and cholesterol, with values of -0.92 for both. This relationship indicates elevated Mucin2 levels associated with decreased cholesterol and triglyceride levels, highlighting Mucin2's potential to enhance cardiovascular health and mitigate risk associated with metabolic disease in bird species (Wang et al., 2022). The correlation value for glucose with Mucin2 was 0.92, a significant positive association. This discovery indicates that heightened Mucin2 levels may correlate with increased glucose levels, which are essential for energy regulation and metabolic functions in the birds. Results show that Mucin2 significantly affects biochemical indicators in bird species, enhancing health benefits including elevated protein and albumin levels, while relieving the risks related to cholesterol and fat. Table 5 shows strong inverse correlations between glycerin concentrations and the amounts of digestive enzymes, including lipase, amylase, and protease. The correlations were determined using percent correlation coefficients and revealed a substantial inverse correlation with lipase (r = -0.92), amylase (r = -0.92), and protease (r = -0.94). These numbers indicate complex interactions between glycerin and digestive enzymes, suggesting that the body's response to glycerin concentrations may considerably influence digestive efficiency. Glycerin, as a component of biological processes, is vital for the health and growth of bird species. Its inclusion in the diet augments the efficacy of the digestive enzymes and facilitates enhanced deterioration and absorption of nutrients. Glycerin enhances digestive efficacy by facilitating enzyme activity in the breakdown of food molecules, thus increasing the availability of vital nutrients for growth, including proteins and lipids.

The Mucin2 gene, responsible for encoding intestinal mucin, is associated with increased glycoprotein production, which is essential for intestinal protection and digestion enhancement. The inclusionof glycerin as a nutrient increases mucin2 secretion and modifies the intestinal mucosa formation, thereby increasing nutrient absorption and promotingdigestive health. Digestive enzymes are essential for food absorption, as they cooperate with glycerol to improve the efficiency of the digestive system. As glycerinconcentrations decrease, the activity of these enzymes may amount to guaranteeing the essential energy supply (Rambold et al., 2015). The specific negativeconnection between glycerin and enzymes indicates that elevated glycerin concentrations may enhance digestion and reduce the requirement for substantialenzyme output, signifying a strong digestive system. Research indicates that incorporating glycerin-rich components intomeals can promote general growth and development (Varela et al., 2020). These results underscore the significance of correlating nutrients likeglycerin, the Mucin2 gene, and digestive enzymes to enhance the birds development and production efficiency. Understanding the interaction of these elements helps toimprove dietary strategies to more effectively promote birds health and production efficiency (Wang et al., 2019).

5. CONCLUSION

We concluded that, the provide study results show that Mucin2 gene expression which creates an ideal environment for the growth of beneficial bacteria and promoter, overall digestive health, thus facilitate healthy growth in birds. The inclusion of glycerin as a food was observed to increase mucin secretion, and change the formation of the intestinal mucosa, hence boosting nutritional absorption and promoting health of the digestive. Negative relationship between glycerin and digestive enzymes was determined. elevated glycerin concentrations improve digestion, and reduce the necessity for substantial enzyme production, effective digestive system.

The study found that the research findings unequivocally indicate that:

1. Mucin2 gene expression correlates with enhanced digestive health
2. The research established that Mucin2 gene expression is crucial for fostering an ideal environment for the proliferation of beneficial gut bacteria. This beneficial bacterial proliferation enhances the overall digestive health of avians. Consequently, this enhancement of the intestinal environment promotes overall healthy avian growth.
3. The Mucin2 gene governs the synthesis of mucin proteins, which are essential constituents of the intestinal mucus layer, The mucus layer serves as a protective barrier against pathogens and fosters an

environment for the proliferation of beneficial bacteria that aid in food digestion and the synthesis of important vitamins. Augmenting the expression of this gene fortifies this barrier and enhances the microbial equilibrium in the gastrointestinal tract.

4. The incorporation of glycerol and its impact on mucin secretion and nutritional absorption: The addition of glycerol to avian meal resulted in enhanced mucin secretion. It also induced alterations in the intestinal mucosa.

These modifications, consequently, enhanced nutrient absorption and contributed to the improvement of overall digestive health. Glycerol, an organic substance, seems to enhance the secretion of mucin by intestinal cells, hence augmenting the thickness and integrity of the mucosal barrier. This enhances protection and may also affect the architecture of the intestinal villi, which are crucial for nutritional absorption. Enhancing mucosal architecture and augmenting mucin secretion may yield a more efficient absorption surface, thereby improving the capacity to assimilate vitamins, minerals, and other nutrients from food.

Inverse correlation between glycerol and digestive enzymes:

An inverse correlation has been established between glycerol and digestive enzymes.

Elevated glycerol concentrations enhance digestion.

Diminishes the necessity for extensive enzyme production.

Facilitates an efficient digestive system.

Expansion: The term "negative relationship" indicates that an increase in glycerol corresponds to a decrease in the requirement for digestive enzymes. This indicates that glycerol may function as a digestive aid, potentially by enhancing the efficacy of existing enzymes or by creating a more conducive digestive environment that promotes food breakdown, so alleviating the need on the digestive system to generate substantial quantities of enzymes. This may have favorable economic and health outcomes by decreasing reliance on digestive enzyme supplements and potentially enhancing food digestion efficiency. This result elucidates the impact of glycerol on avian digestive health through various processes, such as fortifying the intestinal barrier, enhancing nutrient absorption, and facilitating more efficient digestion.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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