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Effect of Adding Different Levels of Glycine Amino Acid on Performance, Growth, Genes Expression and Immune Status of Broiler Chickens

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Abstract

A total number of 360, day old male broiler chicks were divided into four groups (90 birds each), then each group was subdivided into three replicated (30 bird each), birds were allocated as follow; G1) served as control which fed on the basal diet only (corn-soya based diet) without any dietary treatments, G2) was fed on basal diet fortified with 0.25% glycine from premix, G3) was fed on basal diet fortified with 0.17 % glycine and G 4) was fed on basal diet fortified with 0.08 % glycine from premix. During the observation period (35 days), mortalities and growth performance parameters were weekly recorded as well as, serum samples were collected for detection of antibody titer against (NDV) vaccine. At the end of the trial, the carcass weights, breast muscle & thigh muscle yield, internal organs weights as well as, immune organs weights (spleen, bursa, and thymus) were recorded. Also, liver samples were collected and subjected for RNA extraction to detect IL6, IL1B as inflammatory response indicators, Myogenin, Insulin-like Growth Factor 1 (IGF1) as muscular structure indicators and glutathione peroxidase (GSH-PX) as an antioxidant capacity indicator by using (real-time PCR). The result revealed that, at day 35, the BW, BWG, FI were significantly elevated in G2. G2, G3 and G4 showed better gene expression of Myogenin, IGF1and GSH-Px comparing to G1. Moreover, they appeared the lowest genes expression of IL6 and IL1B. In conclusion, addition of glycine to broiler diet, improves their productivity, increase the muscular structure, enhance antioxidant capacity, and improve immune response.

KEYWORDS
Broiler chickens, Glycine, Growth performance, Real-time PCR.

INTRODUCTION

World poultry production has actively selected for features that maximize bird output while also enhancing feed utilization and profitability (Abd El-Hack *et al*., 2022). Recently the world directed to use natural feed additives especially amino acids to improve broiler performance (Abou-Kassem *et al*., 2021; Alagawany *et al*., 2021; Arif *et al*., 2021). Lysine, isoleucine, valine, histidine, leucine, methionine, tryptophan, arginine, threonine, and phenylalanine are the ten essential amino acids that cannot be synthesized by chickens and must be supplied in their ration to maintain their growth and production demands (Siegert, 2016). Glycine is the most important nonessential amino acid in poultry diet (Dean *et al*., 2006; Ospina-Rojas *et al*., 2012) and its ability to boost broiler growth has been recognized for decades (Almquist *et al*., 1940). Waguespack *et al.* (2009) mentioned that the glycine is the fourth limiting of all proteinogenic amino acid after methionine, lysine, and threonine in a diet based on corn and soybean meal for broilers during first 14 days of bird age. Ospina-Rojas *et al*. (2014) mentioned that the valine and glycine act as equally limiting after Methionine, Lysine, and Threonine in a diet based on corn and soybean meal for broilers during first three weeks of rearing. Glycine is deemed to be essential for the modern broiler chickens, since the rate of its synthesis is insufficient to support broiler chicken's development and maximum muscular growth (Dean *et al*., 2006). The integrity of the bird immune system, without a doubt, necessitates healthy and balanced ration (Butcher and Miles, 2002). Glycine is an antioxidant, boosts the bird immune response and it has a role in glutathione formation that improves the antioxidative capacity of leucocytes as well as, glycine plays a role in glycine-gated chloride channel in leucocytes cells aiding in cytokines release and elicit immune response (Fang *et al*., 2002; Zhong *et al*., 2003). Glycine has protective effects, including anti-inflammatory, immunomodulatory, and direct cytoprotective actions. Glycine acts on inflammatory cells such as macrophages to suppress activation of transcription factors and the formation of free radicals and inflammatory cytokines (Wheeler *et al*.,1999). Therefore, the current study aimed to evaluate the addition of different concentrations of glycine in broiler chicken ration and study its effects on bird performance and immune response.

MATERIALS AND METHODS

Experimental design

Three hundred and sixty, day old male Arbor Acres broiler chicks obtained from Cairo Company for poultry, were divided into four groups (90 birds each), then each group was subdivided into three replicates (30 bird each). The groups were classified as

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follow; Group1 (G1) was served as control and was fed only on the basal diet (corn-soya based diet) without any dietary treatments, Group 2 (G2) was fed on basal diet fortified with 0.25% glycine from premix, Group 3 (G3) was fed on basal diet fortified with 0.17% glycine and Group 4 (G4) was fed on basal diet fortified with 0.08% glycine from premix. Physical composition and calculated analysis of the basal diets are shown in Table 1. The diets were formulated according to the Arber Acres Manual for recommended nutrient requirements (Aviagen, 2019). Birds were raised on deep litter system with fresh wood shavings bedding on a concrete floor and they reared with optimal conditions of temperature, humidity, ventilation and maintained on 24 h constant-light system during the observation period (5 weeks). The birds were received balanced diet (including starter, grower, and finisher) and fresh clean water ad libitum. All birds were vaccinated with Hitchner-IB through eye drop technique at 5 days of age, followed by inactivated H5N1 by subcutaneous injection at 9 days of age then, Gumboro intermediate plus at 14 days of age by eye drop method then, Lasota at 21 days of age through eye drop method.

Ethical approval

All experimental techniques and bird handling were carried out in accordance with the guidelines of the institution animal care and use, and approved by the ethical committee (IACUC) at Faculty of Veterinary Medicine, Cairo University (Vet CU 2305202246), Egypt.

Clinical signs and mortality

Clinical signs and mortalities were daily observed in all experimental groups along the observation period (5 weeks).

Growth performance responses

The growth performance of broiler chickens was evaluated in terms of body weight (BW), body weight gain (BWG), feed intake, (FI) and feed conversion ratio (FCR). Individual BWG of the birds were recorded at the beginning of the experiment and on a weekly basis thereafter. Weekly records of FI for each replicate were also maintained to calculate FCR (feed: gain) according to Ghazi *et al*. (2012).

Carcass evaluation

At the end of trial, three birds were randomly selected from each replicate (nine birds per group) for carcass and internal organ weights evaluation. The birds were fasted overnight then slaughtered by severing the jugular vein with sharp knife, defeathered then eviscerated. The live weights and carcass weights were recorded. Also weights of internal organs (liver, heart, and gizzard) and immune organs weights (spleen, bursa, and thymus) were taken and recorded and expressed as percentage of live weight according (Akinola *et al*., 2015). Breast muscle and thigh muscle yield were recorded as percentage of live weight (Faria *et al*., 2010).

Humoral immunity

Anti- Newcastle disease (ND) vaccine antibody titers were evaluated by collecting serum samples at 0 and 7- days post ND vaccination of randomly selected 10 birds/group and blood samples were ethically collected from the jugular vein on plain tubes then centrifugated at 1500 rpm to separate sera. Sera samples were evaluated by hemagglutination inhibition (HI) test according to (Swayne, 1998).

Quantification of mRNA by real-time-PCR.

Liver was taken at the end of the trial from three birds per replicate (nine from each group). All samples were kept at -80°C for Insulin-like growth factor type I (IGF-I) as indicator for growth, muscle marker gene MyoG, glutathione peroxidase (GPX) as an-

Table 1. Experimental diet

Feed ingredient %	Starter	Grower	Finisher
Yellow corn	54.39	57.22	61.21
SBM 46% (Soybean bean meal)	35	32.6	28.2
CGM 60%) corn gluten meal)	4	3	2.5
Methionine	0.14	0.12	0.12
Lysine	0.17	0.11	0.12
Soya oil	2.2	3.2	4.3
MCP (Monocalcium phosphate)	1.55	1.3	1.2
Limestone	1.8	1.7	1.6
Sodium Chloride	0.4	0.4	0.4
*Premix	0.3	0.3	0.3
Toxin binder	0.05	0.05	0.05
Total	100	100	100
Calculated / chemical analysis			
ME(Kcal/kg)	3009	3095	3197
Crude protein%	23	21.5	19.5
Crude fat%	2.65	2.7	2.77
Crude fiber%	3.02	2.94	2.8
Calcium%	1	0.93	0.87
Non-phytate phosphorus%	0.5	0.44	0.41

*Per Kg premix: 1200000 IU vit A, 350000 IU vit. D3, 4000 mg vit. E, 250 mg vit. B1, 800 mg vit. B2, 600 mg vit. B6, 3.2 mg vit. B12, 450 mg vit. K3, 4.5 g nicotinic acid, 1.5 g Ca pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8g Zn, 120 mg I, 40mg Co

Table 2. Primers sequences, target genes and cycling conditions for taqman Rt-PCR.

Target gene	Primers and probes sequences (5'-3')	Reverse transcription	Primary denaturation	Amplification (40 cycles)		Reference
				Secondary denaturation	Annealing and extension	
28S rRNA	GGCGAAGCCAGAGGAAACT GACGACCGATTTGCACGTC (FAM)-AGGACCGCTACGGACCTCCACCA -(TAMRA)	50˚C 30 min.	94˚C 5 min.	94˚C 15 sec.	60˚C 1 min.	Suzuki *et al*. (2009)
IL6	GCTCGCCGGCTTCGA GGTAGGTCTGAAAGGCGAACAG (FAM)-GGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)					
IL1B	GCTCTACATGTCGTGTGTGATGAG TGTCGATGTCCCGCATGA (FAM)-CCACACTGCAGCTGGAGGAAGCC-(TAMRA)					Samy *et al*. (2015)

Table 3. Primers sequences, target genes and cycling conditions for SYBR green Rt-PCR.

Target gene	Primers sequences (5'-3')	Reverse transcription	Primary denaturation	Amplification (40 cycles)				Dissociation curve (1 cycle)		Reference
				Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation	
ß. Actin	CCACCGCAAATGCTTCTAAAC AAGACTGCTGCTGACACCTTC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	51˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	55˚C 1 min.	94˚C 1 min.	Yuan *et al.* (2007)
Myogenin	CACCACAGCTAGAGACCCACATC CCCACCGGCTCAAACTGC				57˚C 30 sec.			57˚C 1 min.		Naji *et al.* (2014)
GSH-PX	TTGTAAACATCAGGGGCAAA ATGGGCCAAGATCTTTCTGTAA				60˚C 30 sec.			60˚C 1 min.		Akbarian *et al.* (2014)
IGF1	CAACGAGCGGTTCAGGTGT TGGAGTTGAAGGTGGTCTCG				61˚C 30 sec.			61˚C 1 min.		Song *et al.* (2013)

tioxidant biomarker and pro inflammatory cytokines gene as IL6 and IL1B. RNA was extracted from liver using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). 30 mg of sample was added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml. For homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the Qiagen tissue lyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. One volume of 70% ethanol was added to the cleared lysate, and the steps were completed according to the Purification of Total RNA from Animal Tissues protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). Oligonucleotide Primers. Primers used were supplied from Metabion (Germany) are listed in (Tables 2 and 3). Taqman rt-PCR. PCR amplifications were performed in a final volume of 25 µl containing 3 µl of RNA template, 12.5 µl of 2x QuantiTect Probe RT-PCR Master Mix, 8.125 µl PCR grade water, 0.5 µl of each primer of 20 pmol concentration and 0.125 µl of each probe (30 pmol conc.) and 0.25 µl of QuantiTect RT Mix. The reaction was performed in a Stratagene MX3005P real time PCR machine. SYBR green rt-PCR. Primers were utilized in a 25 µl reaction containing 12.5 µl of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl of RevertAid Reverse Transcriptase (200 U/µL) (Thermo Fisher), 0.5 µl of each primer of 20 pmol concentration, 8.25 µl of water, and 3 µl of RNA template. The reaction was performed in a Stratagene MX3005P real time PCR machine. Amplification curves and ct values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method stated by Yuan *et al*., 2006 using the following ratio: (2^{-DDct}). ß. actin and 28S rRNA gene mRNA as the housekeeping gen

Statistical analysis

All data collected were statistically analyzed using SPSS® version 18 software PC (2008). Means are compared by one way ANOVA ($p < 0.05$) according to (Snedecor and Cochran, 1980).

RESULTS

During the observation period, no significant abnormal clinical signs have been noticed among all the experimental groups as well as, no significant mortalities were recorded among all the experimental groups.

As seen in Table 4, At 15 days old, G2 showed significant elevation in BW (471.66±6.06), followed by G1 and G3 while G4 revealed the lowest BW (437.16±5.09). At days 28 and 35, G2

showed significant increase in BW.

G2 showed significant elevation in cumulative BWG than the rest of experimental groups.

FCR, no significant differences were observed during all the experimental duration except at day 15 old. The overall BW, BWG, FI were significantly elevated in G2, while no significant differences were observed in FCR between all the experimental groups.

At the end of the experiment, no significant differences were detected between the different experimental groups in the dressing yield, breast muscle, thigh muscle, liver, gizzard, and heart percentages while, G3 revealed the lowest spleen % but no significant difference was recorded between the rest experimental groups (Table 5). The bursa %, revealed no significant difference between G1, G2, G4 also, G3 revealed no significant difference with G4 (Table 5).

Regarding the antibody titers against ND vaccine, at the two time points only numerical differences were observed between the four groups but no significant statistical differences were observed among all groups in comparison with the control (Figure 1).

Regarding the different genes expression (Figure 2), the statistical analysis of the data revealed that all concentrations of glycine in G2, G3 and G4 showed significant dose dependent increase in the gene expression of Myogenin and IGF1 in compar-

Table 4. Effect of dietary glycine fortification on growth performance of broiler chickens.

	G1	G2	G3	G4
Body weight (g)				
Initial weight	45±1.5	45±1.0	46.6±0.66	46.6±0.88
7 days	196.7±2.6	192.9±2.74	189.94±2.2	190.44±2.07
15 days	453.38±5.98^{b}	471.66±6.06^{a}	453.83±5.06 ^{b}	437.16±5.09 ^{c}
21 days	878.11±12.16^{ab}	897.61±12.37^{a}	847.76±10^{b}	868.09±5.78^{b}
28 days	1251.27±20.25^{b}	1301.44±16.64^{a}	1220.27±15.74^{b}	1195.44±17.61^{b}
35 days	1840.50±29.58^{b}	1962.61±17.18^{a}	1817.11±18.40^{b}	1777.16±17.26^{b}
BWG (g)				
Days 1- 7	151.66±5.93	147.94±2.1	143.28±4.09	143.78±.29
Days 7- 14	256.72±16.0	278.72±3.1	263.89±8.6	246.72±7.8
Days 14- 21	424.72±8.28	425.94±19.6	392.81±10.16	413.88±6.7
Days 21- 28	333.02±4.88^{b}	403.83±22.7^{a}	373.63±22.27^{ab}	380.9±21.9^{ab}
Days 28- 35	629.37±17.7	661.17±16.13	596.83±18.60	568.27±24.24
Days 1- 35	1795.50±80.75^{b}	1917.61±11.75^{a}	1770.44±89.6 ^{b}	1753.61±18.92^{b}
FI (g)				
Days 1- 7	146.50±3.4	146.50±5.7	139.44±5.1	146.27±2.11
Days 7- 14	374.17±0.5	376.66±5.09	374.27±2.3	378.33±2.5
Days 14- 21	836.61±21.1	887.94±5.0	858.89±24	857.27±16.29
Days 21- 28	812.12±10.6	827.27±17.74	844.89±29.36	808.33±32.19
Days 28- 35	1189.27±14.13^{a}	1202.72±13.7^{a}	1104.05±16.59^{b}	1111±8.5^{b}
Days 1- 35	3505.18±13.33^{b}	3587.61±28.7^{a}	3461±19.24 ^{b}	3447.5±22.9^{b}
FCR (feed/gain)				
7 days	0.97±0.034	0.99±0.03	0.97±0.03	1.01±0.035
15 days	1.5±0.09^{ab}	1.4±0.02^{b}	1.42±0.04^{ab}	1.53±0.039^{a}
21 days	1.97±0.07	2.09±0.08	2.2±0.07	2.07±0.00.02
28 days	2.4±0.04	2.06±0.1	2.2±0. 2	2.1±0.1
35 days	1.95±0.2	1.82±0.02	1.95±0.1	1.97±0.14
Days 1- 35	1.96±0.09	1.87±0.02	1.96±0.1	1.96±0.02

Data are expressed as Mean±Standard error. G1: Control - basal diet; G2 basal diet +0.25% glycine; G3: basal diet + 0.17% glycine; G4 basal diet +0.08% glycine.
^{a,b,c} Different superscripts in the same row indicate significant difference ($p \leq 0.05$).

Table 5. Effect of glycine fortification on carcass traits of broiler chickens.

Items	G1	G2	G3	G4
Dressing yield (%)	67.19±0.87	67.087±0.808	67.02±0.82	67.74±0.77
Breast (%)	31.36±1.0	31.34±0.56	32.39±0.49	32.69±0.29
Thigh (%)	42.3±0.53	43.03±0.63	41.82±0.43	41.91±0.56
Liver (%)	3.58±0.13	3.84±0.14	3.49±0.105	3.48±0.18
Gizzard (%)	3.63±0.11	3.52±0.098	3.72±0.16	3.47±0.17
Heart (%)	0.88±0.040	0.88±0.026	0.79±0.32	0.789±0.038
Spleen (%)	0.23±0.016^{a}	0.26±0.014^{a}	0.19±0.013^{b}	0.22±.02^{a}
Bursa (%)	0.22±0.020^{b}	0.20±0.02^{b}	0.31±0.02^{a}	0.24±0.022^{ab}

Data are expressed as Mean±Standard error. G1: Control - basal diet; G2 basal diet +0.25% glycine; G3: basal diet + 0.17% glycine; G4 basal diet +0.08% glycine
^{a,b,c} Different superscripts in the same row indicate significant difference ($p \leq 0.05$).

ison with G1, while the highest level of these genes' expression was observed in G2 followed by G3 and G4 which indicated that highest doses of glycine added to the diet, the highest weight gain were obtained. The gene expression of GSH-Px was significantly higher in all groups which treated with glycine with different concentration in comparison with G1.

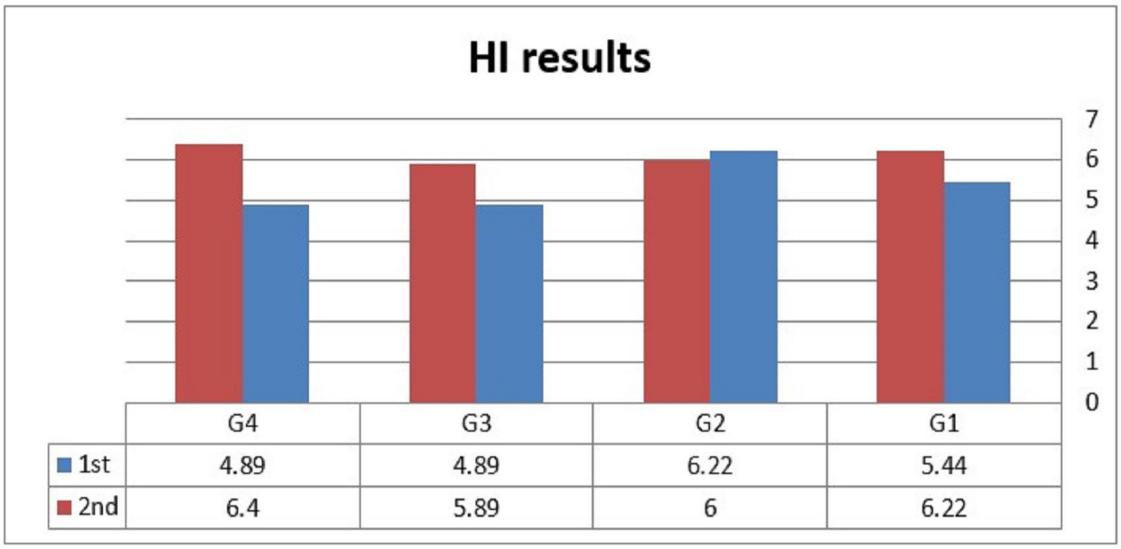
The gene expression of both IL6 and IL1B were significantly reduced in treated groups comparing to G1.

DISCUSSION

Amino acids dietary supplementation is essential for broiler performance under intensive rearing (Alagawany *et al*., 2021). Birds, especially chickens, can biosynthesis glycine but, the rate at which they do so is inadequate to satisfy the metabolic demands of the fast-growing period (Takahashi *et al*., 2008). In broiler chicks, extra glycine transformed to serine on reversible reaction pattern thus, feed requirement is expressed as the glycine + Serine level in standard avian ration (Klasing, 1998). In this study, at days 28 and 35, G2 appeared with a significant increase in BW and at day 35, the BW, BWG, FI were significantly elevated in G2, while no significant differences were observed in FCR between all the experimental groups. These results in concur with Waldroup *et al*. (2005) who found that the addition of 0.2 or 0.4% glycine to broiler diet, markedly enhance the overall productivity of the broiler chickens and they also, concluded that when glycine was added to broiler ration with 16% and 18% crude protein, they noticed significant enhancement in BW at 21 days old. On the other hand, Waldroup *et al*. (2005) noticed that the addition of 0.2 or 0.4% glycine to broiler diet with 20, 22 0r 24% crude protein, did not have any impact on broiler performance.

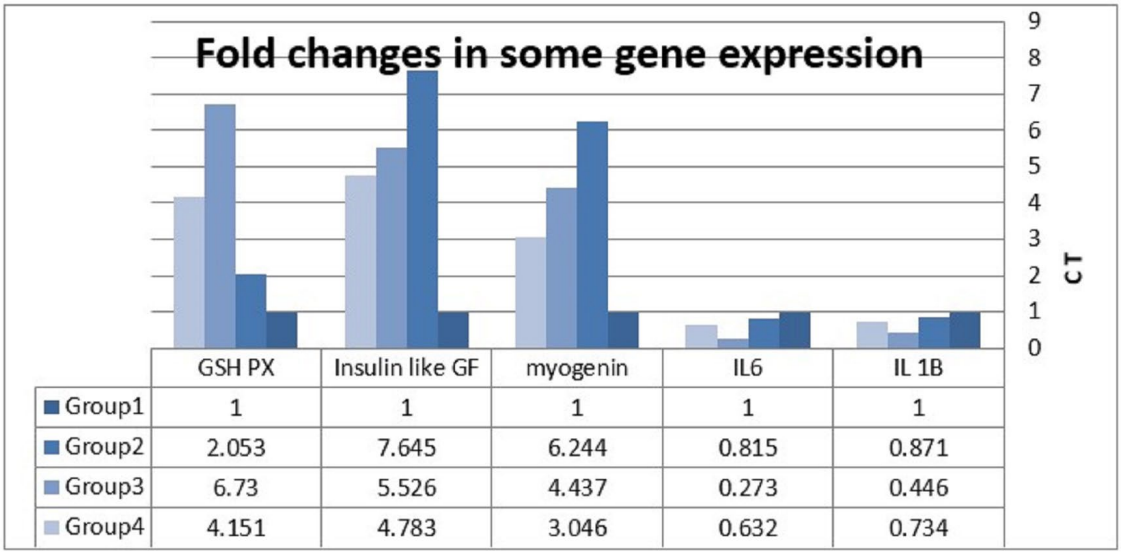
In this study, at 35 days old, no significant differences were noticed among all groups in the dressing yield, breast muscle, thigh muscle, liver, gizzard, and heart percentages while, G3 showed the smallest spleen % but no significant difference was reported among the other experimental groups. Also, the bursa %, showed no significant difference among G1, G2, G4 also, G3 revealed no significant difference with G4. Owed to the antibody titers against ND vaccine, at the two time points only numerical differences were detected among the four groups but no significant statistical differences were reported between all groups in comparison with G1 (control birds).

In this study, the antibody titers against ND vaccine, at the two time points only numerical differences were observed between the four groups but no significant statistical differences were observed among all groups in comparison with the control. These results disagreed with Takahashi *et al*. (2008) who noticed that the addition of glycine to broiler diet above levels



	G4	G3	G2	G1
1st	4.89	4.89	6.22	5.44
2nd	6.4	5.89	6	6.22

Fig. 1. Hemagglutination inhibition (HI) titer in the different experimental groups (Log 2).
G1: Control - basal diet; G2 basal diet + 0.25% glycine; G3: basal diet + 0.17% glycine; G4 basal diet + 0.08% glycine



	GSH PX	Insulin like GF	myogenin	IL6	IL 1B
Group1	1	1	1	1	1
Group2	2.053	7.645	6.244	0.815	0.871
Group3	6.73	5.526	4.437	0.273	0.446
Group4	4.151	4.783	3.046	0.632	0.734

Fig. 2. Effect of glycine fortification on changes in some gene expression.
G1: Control - basal diet; G2 basal diet +0.25% glycine; G3: basal diet + 0.17% glycine; G4 basal diet + 0.08% glycine

recommended for growth lowered the inflammatory reaction and accompanied with growth retardation reported in response to systemic reaction following lipopolysaccharide injection, also, reported that the alterations in TLR4 expression due to addition of glycine to broiler diet may explain its probable mode of action.

In this study, the statistical analysis of the results of genes expressions showed that all levels of glycine in G2, G3 and G4 revealed significant dose dependent increase in the gene expression of Myogenin and IGF1 in comparison with control birds, while the highest level of these genes' expression was observed in G2 (highest concentration) followed by G3 and G4 which indicated that highest doses of glycine added to the ration, the highest weight gain were obtained. The gene expression of GSH-Px was significantly higher in G3 followed by G4 and G2 in comparison with G1. And these results are equivalent to results of BW, BWG in this study. These results were parallel to these recorded by Elahi *et al*. (2020) who found that protein content may be lowered up to 4.5% with 1% glycine and 0.1% cysteine fortification in broiler diet, which has the potential to stop the negative impact of lowered protein levels and reach the standard productivity. Parallel results also, have been recorded by Hofmann *et al*. (2019) who found that the addition of glycine elevate average daily gain by (7.9 g/d) and gain: feed ratio by (0.07 g/g) at crude protein 132, the average daily gain by (2.4 g/d) and gain: feed ratio by (0.02 g/g) at crude protein 147 also, they concluded that the levels of blood plasma metabolites, involving amino acids and lipids, were affected by crude protein and glycine levels in broiler chickens diet.

Because the influence of glycine on broiler growth performance has been extensively studied, using chicks to investigate the possible anti-inflammatory properties of dietary glycine on anti-inflammatory characteristics are beneficial, the triggering of innate immune-related cells like macrophages is thought to cause systemic biological changes like fever, anorexia, and muscle tissue protein building, which heavily influences growth (Takahashi *et al*., 2008). These procedures are perhaps mediated by pro-inflammatory cytokines in chicks as in mammals (Humphrey and Klasing, 2004).

From our results, the gene expression of both IL6 and IL1B were significantly reduced in G3 followed by G4 and G2 in comparison with G1. These results parallel to those reported by Takahashi *et al*. (2008) who found that the addition of glycine to broiler chicken diet influences the inflammatory reaction via affecting the production of pro-inflammatory cytokines like IL-1, IL-6, IFN-gamma and TL1A. Also, these results could be explained by those inflammatory diseases may raise the glycine demand of birds during a point in life when they have an innate need for this nutrient, it's also thought that adding glycine to broiler ration could aid counteract the catabolic alterations that come with immune activation (Takahashi *et al*., 2008). In addition to, the lowered inflammatory reaction in broilers chickens reared on glycine-supplemented diet could be explained by the modulation of TNF-α and IL-1β and IL-10 secretion in mammals (You *et al*., 2006).

CONCLUSION

In this study, the highest concentration of glycine fortification (0.25%) reveals the best body performance. All glycine treated groups have proper gene expression of Myogenin and IGF1, a significant increase in the gene expression of GSH-Px, and the lowest genes expression of IL6 and IL1BFurther studies are recommended to investigate the role of glycine in the improvement of the immune response during microbial experimental infection with correlation to its impact on gut microbiota.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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