THE EFFECTS OF GLUTAMINE AND GLUTAMIC ACID VS MICROORGANISM PRODUCING GLUTAMIC ACID SUPPLEMENTATION ON PERFORMANCE AND INTESTINAL MORPHOLOGY OF BROILER CHICKENS

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SUMMARY

This study evaluated the effects of dietary supplementation with glutamine (Gln), glutamic acid (Glu), and glutamic acid-producing bacteria on broiler chickens, focusing on growth performance, intestinal morphology, carcass characteristics, some blood measurements and economic efficiency. A total of 324 unsexed Ross 308 broiler chicks (average initial weight: 41.11 g) were assigned on day 5 to 42 floor pens (12 birds per pen), with three replicate pens per treatment. Birds were managed under a three-phase feeding program: starter (days 5–10), grower (days 11–24), and finisher (days 25–42), with diets formulated to meet broiler nutritional requirements. The treatments were as follows G1 (Control): Chicks were fed the basal diet, G2 : G1 plus 0.05% Glu, G3 : G1 plus 0.1% Glu, G4 : G1 plus 0.05% Gln, G5 : G1 plus Gln 0.1%, G6 : G1 plus 1cm^3\ Kg diet biological sources (2-*Bacillus subtilis*), G7 : G1 plus 1cm^3\ Kg diet biological sources (2-*Bacillus* zero), G8 : G1 plus 1cm^3\ Kg diet biological sources (*Enterococcus faecium*) and G9 : G1 plus 1cm^3\ Kg diet biological sources (*Enterococcus* zero). Results indicated that 0.05% Gln (G4) supplementation numerically enhanced body weight gain (BWG) and feed conversion ratio (FCR), while also improving intestinal health, as evidenced by increased villus height (VH) and a higher VH-to-crypt depth (VH/CD) ratio. Carcass characteristics were not significantly affected in all groups. Also, results indicated that the liver functions (ALT, AST), serum antioxidant (SOD, GPX) and the European production efficiency factors (EPEF) improved by 0.05% Gln supplementation.

Keywords: *Glutamine, glutamic acid, probiotic, broilers, performance, intestinal health, blood measurements*

INTRODUCTION

The global demand for poultry meat has driven significant expansion in broiler production in recent years. To meet this growing market demand, optimization of production systems through advanced genetic selection (Ensminger *et al.*, 2004), enhanced nutritional strategies and improved herd management practices are essential (He *et al.*, 2021). However, despite these advances, broilers remain particularly vulnerable to pathogenic challenges that can compromise growth performance and production efficiency. Today, the modern poultry industry faces numerous challenges in maintaining optimal intestinal health, a critical factor influencing overall flock performance. The gastrointestinal tract serves as the primary interface for nutrient absorption while simultaneously functioning as a major immunological barrier. Its structural and functional integrity directly determines feed efficiency, growth performance, and disease resistance in broilers (Kogut and Arsenault, 2016). Environmental stressors represent another critical challenge in modern poultry production. Fluctuations in temperature, humidity, and air quality can disrupt gut microbiota composition and intestinal barrier function, leading to systemic inflammation and reduced nutrient absorption (Dai *et al.*, 2009).

Amino acids serve as fundamental components of poultry nutrition, functioning not only as the building blocks of proteins necessary for growth and development, but also as key regulators of metabolic pathways (Wu, 2009). Specific functional amino acids - including glutamine, arginine, leucine, proline, cysteine, and tryptophan - have been shown to modulate critical physiological processes such as immune function regulation, protein synthesis and accretion, intestinal mucosa development and nutrient utilization efficiency. These pleiotropic effects make targeted amino acid supplementation a powerful tool for optimizing broiler health and productivity, particularly in supporting gut function and stress resilience (Li *et al.*, 2007). Glutamine, a conditionally essential amino acid with well-documented benefits

in human medicine (Newsholme *et al.*, 2003), has shown significant potential in broiler nutrition. Numerous studies demonstrate its positive effects on growth performance, immune function, and gut health (Bartell and Batal, 2007; Murakami *et al.*, 2007; Sakamoto *et al.*, 2006 and Yi *et al.*, 2005). Glutamine supplementation plays a critical role in avian health as a conditionally essential amino acid during inflammatory states. It functions as a gut-protective agent by maintaining intestinal barrier integrity and reducing inflammation, thereby potentially preventing enteric diseases (Newsholme *et al.*, 2003 and Reeds *et al.*, 2000). Research demonstrates that glutamine enhances intestinal morphology through increased villus height and improved mucosal maintenance. Glutamine and glutamate are interconvertible in various organs, including the intestine, liver, and kidney, and both play a role in the development of the gastrointestinal tract in broiler chickens (Wu, 2009; Tapiero *et al.*, 2002 and Newsholm *et al.*, 2003). Glutamine (Gln) and glutamic acid (Glu) serve as crucial energy substrates for rapidly proliferating cells, including those in the immune system and intestinal mucosa (Windmueller and Spaeth, 1974; Newsholme *et al.*, 1985, 2003). Beyond their role as protein and peptide constituents, these amino acids contribute to metabolic pathways such as the synthesis of nucleotides and other amino acids (Wu, 1998; Newsholme, 2001). Supplementing broiler diets with glutamine (Gln) has been shown to enhance immune function and intestinal development. Bartel and Batal (2007) observed higher antibody levels in broilers receiving Gln supplementation compared to non-supplemented groups. Furthermore, studies by Sakamoto *et al.* (2006) and Murakami *et al.* (2007) demonstrated that a 1% dietary Gln inclusion improved intestinal mucosa development in one-week-old broilers, enhancing nutrient digestion and absorption, which may subsequently boost overall performance. Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, offer a promising alternative for enhancing gut health. Strains such as *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Bifidobacterium lactis* not only support intestinal integrity but may also modulate glutamine metabolism, potentially boosting endogenous glutamine availability while promoting overall digestive and immune function. Given these multifunctional roles, this study aims to evaluate the efficacy of probiotic supplementation as a potential source of glutamic acid in poultry nutrition, comparing its effects against conventional crystalline glutamate and glutamine supplementation in broiler diets.

MATERIALS AND METHODS

Experimental design, chicks, and diets:

The experiment was carried out at the Experimental Farm of the Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, from late October 2022 to December 2022. The objective of this study was to evaluate the influence of dietary glutamine (Gln) and glutamic acid (Glu) supplementation versus probiotic producing glutamic acid strain on productive performance and gut health. A total of 324-day-old unsexed Ross-308 broiler chicks were used (the mean initial body weight of the chicks was 41.11 g). On the fifth day, the chicks were weighed individually and randomly assigned to 27-unit groups. Each treatment has three replicate floor pens, with 12 birds per pen (1.25 m^2), and serving as the experimental unit. The treatments were distributed across three-phase feeding program: a broiler starter diet phase (5 to 10 days of age), a broiler grower diet phase (11 to 24 days of age), and a broiler finisher diet phase (25 to 42 days of age). The birds were fed a crumbled diet (corn- and soybean meal-based) from 1 to 4 days' post-hatch. From day 5 until the end of the experiment, a mash diet was provided. A corn and soybean meal basal diet without additive supplementation was formulated for each production phase according to Ross 308 (Aviagen, 2019 guide) during the experimental period no anticoccidial agents or antibiotics were added. Two probiotic strains, *Bacillus subtilis* and *Enterococcus faecium* (identified as the main probiotic bacteria), were isolated, identified, and tested for their ability to produce glutamic acid at the Regional Centre for Food and Feed (RCFF), Agricultural Research Centre. Extracellular glutamic acid (ECG) and intracellular glutamic acid (ICG) production from the untreated selected strains were quantified using the LC-MS/MS technique and the research was published in 2025 by (Hend et all., 2025). These two strains were used in this study as sources of probiotics producing glutamic acid at a level of 1×10^8cfu\ Kg diet.

The treatments were as follows G1 (Control): Chicks were fed the basal diet, G2 : G1 plus 0.05% Glu, G3 : G1 plus 0.1% Glu, G4 : G1 plus 0.05% Gln, G5 : G1 plus Gln 0.1%, G6 : G1 plus $1cm^3$\ Kg diet biological sources (2-*Bacillus subtilis*), G7 : G1 plus $1cm^3$\ Kg diet biological sources (2-*Bacillus* zero), G8 : G1 plus $1cm^3$\ Kg diet biological sources (*Enterococcus faecium*) and G9 : G1 plus $1cm^3$\ Kg diet biological sources (*Enterococcus* zero). The effects of dietary treatment on broiler performance were determined. Feed intake, body weight gain and feed conversion ratio were assessed in the periods from 5 to 10 days, 11 to 24 days, 25 to 42 and 5 to 42 days of age.

Table (1): Composition and calculated analysis of experimental basal diet of broiler chicks.

Ingredient	Starter diet (5-10 days)	Grower diet (11-24 days)	Finisher diet (25-42 days)
Yellow corn 7.5%	52.525	54.500	59.800
Soybean Meal 44%	33.900	33.300	26.850
corn gluten meal 60%	5.350	3.650	4.250
HCL-Lysine %	0.340	0.255	0.270
DL-Methionine %	0.415	0.350	0.335
L-threonine %	0.175	0.130	0.120
L-Arginine %	0.146	0.070	0.085
L-Valine %	0.085	0.040	0.040
L-isoleucine%	0.050	0.020	0.040
Vegetable oil %	2.900	4.300	5.000
Sodium Chloride (NaCl) %	0.250	0.250	0.250
sodium bicarbonate	0.165	0.165	0.180
Limestone	1.000	1.000	0.930
Di Calcium Phosphate	2.400	1.670	1.550
Premix *	0.300	0.300	0.300
Total	**100.00**	**100.00**	**100.00**
Calculated **			
Protein %	23	21.5	19.5
Metabolizable energy (Kcal/Kg)	3000	3100	3200

**Each 3 Kg of vitamins mixture contains: Vitamina,13000000IU; Vit. D3,6000000IU; Vit. 80000mg; Vit.K_3 ,4000 mg; Vit.B_1,5000 Vit.B_2 9000; Vit.B_6,5000 mg; Vit.B_{12} 35 Mg; Pantothenic Acid. 20000mg, Niacin.70000 mg Folicacid.2000 mg; Biotin.250 mg, Choline Chloride.1000 g, Manganese. 120 g, Zinke110 g, Iron. 40 g, Cooper. 16 g, Iodine. 1.25 g Selenium. 0.3 g. Cobalt. 0.15g. **Calculation based on (CLFF 2001).*

All chicks had free access to feed and water (*ad libitum*) throughout the experimental period. Fresh and dried wood shavings were used as litter at a depth of approximately 10 cm in all pens over a concrete floor. Lighting was provided 24 hours daily using incandescent bulbs, and the temperature schedule was followed according to the breeder's recommendations (Ross 308, 2019 edition). The birds in all experimental treatments were subjected to vaccine against disease.

Growth performance evaluation:

The chicks were weighed at the start of the experiment (fifth day old) intervals to nearest gram (±1.1gm) in each group. Under commercial conditions, it may take 36 to 48 hours for newly hatched broiler chickens to be transported and provided access to feed and water in production facilities. The live body weight of chicks was individually recorded and measured in three phases: from 5 to 10 days, 11 to 24 days, 25 to 42 days, and 1 to 42 days of age. Body weight gain, feed intake, feed conversion ratio (g feed /g gain) were recorded.

Sample collection and sacrifice:

At 42 days of age, four birds from each replicate pen were selected for slaughter and sample collection, based on their fasted live weight. The birds were fasted for approximately 2 hours prior to slaughter. Each bird was individually weighed, slaughtered to ensure complete bleeding, and then processed by plucking the feathers. The weight was recorded. The slaughtered birds were used to determine carcass weight and carcass yield (excluding feet, heads, and necks). The weight of edible organs (liver) and dressing percentage were calculated relative to live body weight, following the methodology of (Silva *et al.* 2023a). Additionally, live body weight (LBW), carcass, and liver were measured by gram.

Blood parameters:

Blood samples were collected from six birds per treatment group at 42 days of age. Blood was obtained from the jugular vein of each bird and collected in heparinized tubes. The samples were then centrifuged at 3000 rpm for 15 minutes to separate the plasma. Plasma was subsequently collected and stored at -20°C until analysis. Biochemical

analysis of plasma was performed to quantitatively determine blood parameters using spectrophotometric methods. The parameters assessed included: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by using a commercial kit produced by Spectrum Diagnostics (S.A.E), Cairo, Egypt based on kinetic method as described by (Breuer, 1996). The glutathione peroxidase (GPX), and superoxide dismutase (SOD) using a commercial kit produced by Bio- Diagnostic Company for Diagnostic and Research Reagents (29 Tahreer St., Dokki, Giza, Egypt)as a colorimetric method described by Paglia and Valentine 1967 and Nishikimi *et al.* 1972, respectively. All blood chemical analyses were conducted using spectrophotometric techniques.

Histomorphometry intestinal:

Fixed jejunum samples were processed, and 4-µm-thick tissue sections were prepared from paraffin-embedded tissue blocks. These sections were stained with hematoxylin and eosin following the protocol outlined by Bancroft and Gamble (28). The stained tissues were examined using a light microscope (Leica DM300 equipped with a Leica FLEXACAM C1), and representative fields were photographed for morphometric analysis using Leica LAS X dedicated software. Villus height (VH), villus width (VW), crypt depth (CD), and muscular layer thickness were measured. These parameters were determined as the mean of 10 randomly selected regions per sample. Additionally, villus surface area was calculated by modeling the villus as a cylindrical structure (29) using the following equation: [(2π) × (villus width/2) × (villus height)].

European production efficiency factor (EPEF):

The European Production Efficiency Factor (EPEF) was calculated using the following equation: EPEF = [mean body weight in kg × livability (100 - mortality)) ÷ (experimental period in days × feed conversion rate)] × 100 (Kryeziu *et al.*, 2018).

Cumulative mortality rates were calculated by subtracting the total number of live birds at the end of the experiment from the total number of birds in the same experimental group. These values were then expressed as a percentage of the total initial number of chicks.

Statistical analysis:

Data were subjected to analysis of variance (ANOVA) using a one-way experimental design in SPSS (Statistical Package for the Social Sciences), also known as IBM SPSS Statistics, version 2023. The analysis was performed according to the following model:

$$Yij = \mu + Tri + eij$$

Where:
Y_{ij} = observation of the ith chick within the jth treatment,
µ = overall mean,
Tr_i = effect of the ith treatment (i = 1–9),
e_{ij} = residual error.

Duncan's New Multiple Range Test was used to assess differences between means at a significance level of $P \leq 0.05$ (Duncan, 1955). Analysis of covariance (ANCOVA) was employed to analyze weight gain, using feed intake and live body weight as covariates (Snedecor, 1955). Also, liver weight was corrected to BW as a covariate, (Brown *et al.*, 1985). The mathematical model is represented by the following equation:

$$Yij=\mu+\alpha i+\beta(Xij-\bar{X})+\epsilon ij$$

Where:
• Y_{ij} = dependent variable,
• X_{ij} = independent variable or covariate (e.g., Sex, FI, BW),
• X = mean of the covariate,
• B = regression coefficient,
• E_{ij} = random error,
• µ = overall mean.

RESULTS

Effects of (Glu) and (Gln) on productive performance:

The effects of dietary supplementation of different levels of glutamine, glutamic acid and probiotic produced glutamic acid on BWG of broilers at different periods of age statistically corrected for sex are shown in Table 3. The body weight gain was not affected significantly among the experimental groups in all different periods except the 24-d period where significant differences were found between G1 and G3. Total body weight gain was increased numerically by G4 group compared to the other groups.

Table (2): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on live body weight gain corrected for sex (M±SE).

Treatments	Items			
	BWG 10 day	BWG 24 day	BWG 42 day	Total BWG (5-42 day)
G1	166±5.68	732.3±17.18	1623.4±60.57	2509.3±61.85
G2	179.2±6.39	709.2±17.11	1673.8±65.29	2566.07±66.66
G3	167.4±5.88	628±15.69	1796.9±61.62	2593.19±62.92
G4	174.8±6.18	687.6±16.54	1749.1±63.23	2611.34±64.56
G5	170±5.88	691.7±15.69	1638±59.95	2503.16±61.21
G6	168±5.68	669.4±15.17	1600.7±60.57	2445.01±61.85
G7	168.9±5.70	687.2±15.23	1582.1±58.06	2440.61±59.28
G8	161.4±5.88	648.3±15.69	1677.2±61.62	2487.52±62.92
G9	156±5.68	658.9±15.46	1582.9±58.02	2392.71±59.24
Sig.	N.S	*	N.S	N.S

G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5:0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7:1cm^3\ Kg diet 2-Bacillus Zero, G8: Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean. BWG: body weight gain. N.S: Not significant.

Dependent Variable: BWG 24 day				
(I) Treat	**(J) Treat**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**
1	3	104.321*	23.796	0.022

Table (3): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on live body weight gain corrected for sex and feed intake (M±SE).

Treatments	Items			
	BWG 10 day	BWG 24 day	BWG 42 day	Total BWG (5-42 day)
G1	159.569	726.166	1584.882	2456.614
G2	173.054	716.008	1637.414	2468.361
G3	172.642	630.683	1720.464	2438.602
G4	171.305	691.229	1761.847	2609.519
G5	170.486	690.677	1664.555	2558.463
G6	171.022	670.058	1602.751	2463.160
G7	164.939	684.983	1604.130	2493.024
G8	169.650	646.340	1714.229	2567.212
G9	159.666	656.855	1634.395	2494.047
Sig.	N.S	*	N.S	N.S

G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean. BWG: body weight gain. N.S: Not significant.

The effects of dietary supplementation of different levels of glutamic acid and glutamine on BWG of broilers at different periods of age with statistically corrected for sex and feed intake are shown in Table 4. Observed that there are differences in arrange between groups for total body weight gain trait then recorded higher numerically value by G4 group compared to the other groups.

Groups from G1 to G9 received the basal diet supplemented with (G1 0.0 control), (G2, 0.05 Glutamic acid), (G3, 0.1 Glutamic acid), (G4, 0.05 Glutamine), (G5, 0.1 Glutamine), (G6, 1cm^3\ Kg diet 2-Bacillus sucrose), (G7, 1cm^3\ Kg diet 2-Bacillus Zero), (G8, 1cm^3\ Kg diet Entero sucrose), (G9, 1cm^3\ Kg diet Entero zero), /kg. SE: Standard error of mean; M: mean. BWG: body weight gain. N.S: Not significant.

Dependent Variable: BWG 24 day				
(I) Treat	**(J) Treat**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**
1	3	104.321*	23.796	0.022

The effects of dietary supplementation of different levels of glutamic acid and glutamine on FI of broilers at different periods of age statistically corrected for sex are shown in Table 5. The FI was not affected significantly among the experimental groups in (10 d and 24 d) periods. There is a significant increase in FI for G3 compared to G8 and G9 in the 42-d period. But there is not a significant difference between G3 and other groups. It was also noted that total FI was significantly increase for G3 compared to G5, G7, G8, and G9 which recorded the significantly lowest value. While there are no significant differences between G3 and other groups.

Table (4): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on feed intake corrected for sex (M±SE).

Treatments	**Items**			
	FI 10 day	**FI 24 day**	**FI 42 day**	**Total FI (5-42)**
G1	252.15±4.70	1090.06±33.48	3440.85±76.84	4816.02±77.45
G2	252.10±5.29	1253.69±33.33	3432.64±82.83	4911.63±83.49
G3	234.27±4.87	1201.56±30.57	3586.14±78.17	5032.53±78.79
G4	248.05±5.11	1213.31±32.22	3244.56±80.21	4707.80±80.85
G5	242.37±4.87	1155.22±30.57	3191.59±76.05	4586.38±76.65
G6	237.78±4.70	1176.05±29.56	3285.52±76.84	4665.36±77.45
G7	248.83±4.72	1140.33±29.68	3208.78±73.65	4592.53±74.24
G8	230.14±4.87	1142.56±30.57	3151.48±78.17	4534.53±78.79
G9	237.08±4.70	1141.86±30.12	3096.07±73.60	4488.52±74.18
Sig.	N.S	N.S	*	*

G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean. FI: feed intake. N.S: Not significant.
1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet

Dependent Variable: FI 42 day				
(I) Treat	**(J) Treat**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**
3	8	434.667*	103.146	0.031
3	9	490.071*	109.53	0.019
Dependent Variable: Total FI (5-42 day)				
(I) Treat	**(J) Treat**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**
3	5	446.158*	104.983	0.029
3	7	440.009*	105.606	0.034
3	8	498.000*	103.961	0.01
3	9	544.019*	110.396	0.008

The effects of dietary supplementation of different levels of glutamic acid and glutamine on FCR of broilers at different periods of age statistically corrected for sex are shown in Table 6. The FCR was not affected significantly between all groups in (10d, 42d and total 5-42d) periods. But in 24d the FCR was significantly increased for G3

compared to G1 group which recorded the lowest value. The G4 was lower numerically value of FCR compared to other groups during (42d and total 5-42d) periods.

Table (5): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on feed conversion ratio corrected for sex (M±SE).

Treatments	Items			
	FCR (10 day)	FCR (24 day)	FCR (42 day)	Total FCR (5-42 day)
G1	1.51±0.045	1.49±0.066	2.13±0.071	1.92±0.038
G2	1.40±0.051	1.77±0.066	2.04±0.077	1.91±0.041
G3	1.40±0.047	1.90±0.060	1.99±0.072	1.94±0.038
G4	1.41±0.049	1.76±0.064	1.86±0.074	1.80±0.039
G5	1.42±0.047	1.68±0.060	1.95±0.071	1.83±0.037
G6	1.41±0.045	1.75±0.058	2.05±0.071	1.90±0.038
G7	1.48±0.045	1.65±0.059	2.03±0.068	1.88±0.036
G8	1.43±0.047	1.75±0.060	1.87±0.072	1.82±0.038
G9	1.53±0.045	1.73±0.059	1.95±0.068	1.87±0.036
Sig.	N.S	*	N.S	N.S

G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean. FCR: feed conversion ratio. N.S: Not significant.
1cm^3\ Kg diet1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet

Dependent variable : FCR 24 day				
(I) Treat	**(J) Treat**	**Mean Difference (I-J)**	**Std. Error**	**Sig.[b]**
1	3	-.417*	0.091	0.016

Table (6): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on live body weight, carcass and liver (M±SE).

Treatments	Items			
	LBW	Carcass (g)	Carcass (%)	Liver (g)
G1	2749±63.34	2045±41.73	74.47±**0**.657	3.01±.079
G2	2784±49.43	2125±47.04	76.41±1.453	2.77±.176
G3	2705±45.48	2014±30.56	74.48±**0**.454	2.57±.105
G4	2939±62.93	2207.±52.87	75.08±**0**.621	2.68±.097
G5	2846±116.3	2079.4±55.38	73.71±1.898	2.88±.110
G6	2741±61.47	2098.6±45.76	77.02±2.572	2.75±.081
G7	2701±69.74	2069.80± 61	76.95±2.465	2.75±.228
G8	2652±54.43	2012.1±33.612034.9±39.66	76.30±2.242	2.69±.110
G9	2642±51.179	0.085	77.37±2.244	2.96±.106
P-value	0.052		0.819	0.308

G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean.
1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet mean; LBW: live body weight.

Carcass characteristics:

Data presented in Table (7) showed that all live body weight, carcass and liver weight were not significantly affected in all groups compared to the control group. The highest numerical value for LBW and carcass (g) recorded by G4 and the lowest numerical value for LBW recorded by G9 and for carcass (g) by G8. In the same table the G9 recorded

the highest numerical value for carcass (%) while and G5 recorded the lowest value. On the other hand, the liver weight recorded the height value by G1, and the lowest value recorded for G3.

Histomorphometric parameters:

Data in table (8) showed that there was no significant effect on intestinal length, crypt depth and villus: crypt ratio. Then, the intestinal length recorded the higher numerical value by G2 while G9 recorded the lower numerical value. Concerning the crypt depth, the G6 recorded the highest numerical value, in contrast to G7 the villus: crypt ratio G3 was the highest numerical value compared to G5 which recorded the lower numerical value. The villus height showed significantly increased for G3, G4, G6, G8, G1, G2, and G5 respectively compared to G7 which significantly decreased. The villus width showed highly significantly for G2 followed by decreasing order G6, G7, and G5 while the G9 and G1 were recorded the lower significant values.

Table (7): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on Hematological Parameters (M±SE).

Treatments	Items				
	Intestinal length (IL)	Villus Height (µm)	Villus Width (µm)	Crypt Depth (µm)	Villus: Crypt Ratio
G1	213±4.51	2067^{ab}±112.4	210^{c}±17.8	221±12.9	9.5±0.46
G2	237.5±4.83	2042^{ab}±132.0	317^{a}±20.1	222±9.2	9.2±0.44
G3	210.7±19.90	2332^{a}±94.5	244^{bc}±13.2	202±24.5	13.2±1.86
G4	212.6±19.41	2190^{a}±58.5	232^{bc}±7.0	228±28.2	10.7±1.24
G5	202.5±18.57	2037^{ab}±144.2	267^{abc}±7.3	242±23.1	8.8±0.85
G6	235.5±6.21	2155^{a}±117.1	289^{ab}±38.4	245±19.6	9.4±1.09
G7	218.8±4.54	1679^{c}±42.6	272^{abc}±26.0	180±8.2	9.4±0.39
G8	204.1±3.68	2079^{ab}±70.4	225^{bc}±15.3	226±29.6	10.6±1.43
G9	201.6±3.16	1814^{bc}±55.9	209^{c}±15.7	196±17.0	9.8±0.89
P-value	0.262	0.001	0.002	0.386	0.161

a, b, and c mean the same row having different superscripts is significantly different (P < 0.05) and (P <0.01). G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean. 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet; IL: intestinal length. VH: Villus height. VW: Villus width. CP: crypt depth. VCR: villus crypt ratio.

Table (8): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on liver function and serum antioxidant parameters (M±SE).

	Items			
Treatments	AST (U/L)	ALT (U/L)	SOD (U/ml)	GPX (mU/ml)
G1	35.20^{a}±1.45	19.16^{b}±0.86	254.3^{c}±11.16	32.01^{ab}±9.38
G2	33.96^{a}±1.65	18.11^{b}±0.67	276.8^{b}±2.38	21.99^{b}±3.55
G3	31.17^{ab}±3.43	18.00^{b}±0.81	292^{b}±16.68	47.53^{a}±10.35
G4	30.49^{ab}±2.84	19.70^{b}±0.97	279.8^{b}±6.48	15.13^{b}±3.86
G5	26.15^{bc}±1.38	24.96^{a}±1.81	352.8^{a}±1.87	19.84^{b}±3.64
G6	23.18^{c}±0.74	15.91^{b}±1.67	361.7^{a}±1.38	14.66^{b}±1.16
G7	22.35^{c}±1.43	10.91^{c}±0.96	362.9^{a}±1.87	15.00^{b}±0.13
G8	24.11^{c}±1.90	16.24^{b}±1.45	361.3^{a}±1.24	15.89^{b}±0.97
G9	23.21^{c}±1.82	16.58^{b}±1.02	360.9^{a}±2.29	14.56^{b}±0.83
P-value	<0.01	<0.01	<0.01	0.003

a, b, and c mean the same row having different superscripts is significantly different (P < 0.05) and (P <0.01). G1 : control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean.1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet AST: Aspartate aminotransferase. ALT: alanine aminotransferase. SOD: Superoxide dismutase. GPX: Glutathione peroxidase.

Blood biochemical parameters:

Analysis of variance of blood serum parameters as affected with various doses glutamic acid and glutamine fed table 9 showed that the AST values for the G1, G2, G3 and G4 groups recorded the highest significant values compared to the other groups (G5, G6, G7, G8, and G9) which recorded the lowest significant values. However, the differences between (G3, G4 and G5) and between (G5, G6, G7, G8, and G9) were insignificant. Concerning the value of ALT the G5 recorded the highest significant value compared to the other groups. While the lowest significant value recorded by G7. However, the differences among the other groups were insignificant. In the same table SOD values increased significantly for G5, G6, G7, G8, and G9 followed significant decreasing order by G3, G4, G2 and G1, respectively. The GPX value significantly increased for G3, which recorded the highest value compared to the other groups. While the differences among other groups were insignificant.

Table (9): Effect of supplementation different levels of glutamine, glutamic acid and probiotic produced glutamic acid on European production efficiency factors.

Treatments	Items				
	BWG Kg	Livability	FCR	Age	EPEF*
G1	2.51	91.7	1.92	42	285
G2	2.57	94.4	1.91	42	302
G3	2.59	97.2	1.94	42	309
G4	2.61	100	1.8	42	345
G5	2.50	97.2	1.83	42	317
G6	2.45	91.7	1.9	42	281
G7	2.44	97.2	1.88	42	301
G8	2.49	100	1.82	42	325
G9	2.39	94.4	1.87	42	288

*G1: control, G2: 0.05% Glutamic acid, G3: 0.1% Glutamic acid, G4: 0.05% Glutamine, G5: 0.1% Glutamine, G6: 1cm^3\ Kg diet 2-Bacillus sucrose, G7: 1cm^3\ Kg diet 2-Bacillus Zero, G8: 1cm^3\ Kg diet Entero sucrose, G9: 1cm^3\ Kg diet Entero zero. SE: Standard error of mean; M: mean.1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet 1cm^3\ Kg diet *EPEF: European production efficiency factor.*

Economics Efficiency

Results of the table (11) illustrated that G4 Achieved the best economic value according to EPEF compared to other treatments.

DISCUSSION

Data of body weight gain at 10, 42 days and during the hole experimental period corrected for sex and FI are given in tables (2, 3, 4 and 5). Statistical analysis indicated insignificant differences between treatments during the hole experimental periods tested (P>0.05). However, there are clear numerical differences between treatments, which are economic meaning. Compared to the control group we observe that a 0.05% glutamine supplementation yields the best results (+102 gm), followed by 0.1% glutamic acid (+84 gm) and then 0.05% glutamic acid (+45gm). The superior performance observed with 0.05% glutamine supplementation may be attributed to its direct role in supporting intestinal integrity, reducing oxidative stress and immune function, leading to better nutrient absorption and overall health (Zhang, *et al.*, 2022, Bai, *et al.*, 2023 and Cruzat, *et al.*, 2018). While glutamic acid also contributes positively, its effects might be more pronounced at higher inclusion levels, such as 1%, due to its role in various metabolic pathways.

The diminished performance at 0.05% glutamic acid could be due to insufficient availability to elicit significant physiological benefits. While both glutamine (Gln) and glutamate (Glu) are important amino acids in poultry nutrition, the specific physiological roles of Gln particularly in supporting intestinal health, enhancing stress responses, and boosting antioxidant activity and the found that during the first-pass through the small intestine into the portal circulation, dietary Glu is extensively oxidized to CO_2, but dietary Gln undergoes limited catabolism in birds (He, *et*

al., 2021) may contribute to the superior performance observed in broilers supplemented with Gln compared to those receiving Glu. This result aligns with findings from several studies Jazideh, *et al.*, (2014) found that no significant differences were observed between the treatments for feed intake and feed conversion ratio during the starter period, grower period, or entire experiment (P> 0.05). During the grower period, only 0.05% glutamine-fed birds had higher body weight gain than others (P < 0.05). For the entire period, body weight gain of 0.05% glutamine-fed birds was greater than that of 0.00% and 0.25% glutamine-fed ones (P< 0.05). Also, Wu *et al.* (2023) investigated the effects of glutamine on broilers challenged with *Salmonella pullorum*. The study found that dietary glutamine supplementation improved growth performance and enhanced systemic innate immune responses in the challenged broilers. Specifically, broilers receiving 0.05% glutamine showed improved average daily gain and feed conversion ratios compared to the control group.

On the other hand, data given in table (2, 3, 4 and 5) indicate that, while glutamine is beneficial for broilers, higher doses (like 1%) may not necessarily provide better results and can sometimes cause adverse effects like excessive nitrogen load or metabolic imbalances. This mean that any increase in glutamine level beyond a threshold where it becomes harmful. The first possible reason for this negative effect on BWG is the role of Gln in ammoniagenesis, which increases the energy requirement for excretion of uric acids. The Gln acts as a precursor for ammoniagenesis in the gut and kidneys (Tapiero *et al.*, 2002). In birds, ammonia is excreted in feces in the form of uric acid and is involved in uric acid synthesis (Soltan, 2009). The second reason is possibly the production of high ammonium ions in 1% Gln-fed birds. The Gln is converted to α-ketoglutarate and thus generates ammonium ions (NH4). Although excretion of ammonium ions helps buffer metabolic acidosis in normal temperatures (Chasiotis et al 1983), it negatively affects the blood acid base balance during HS-induced alkalosis and hence does not improve BWG. These results agreement with Bartell and Batal (2007), Soltan (2009), Maslami *et al.*, (2019), Alsogair *et al.*, (2024) and Nassiri Moghaddam and Alizadeh-Ghamsari (2013) reported that, Overall, poor performance was related to a diet containing levels higher than 1% of glutamine and these results support the results of the present studies.

In the same table also revealed that broilers consuming probiotics that produce glutamic acid may grow less compared to those consuming a control diet or a diet with crystal glutamic acid. This results underscore the need for careful assessment of probiotics genetic content before their inclusion in animal feed. In this connection, Cui *et al.*, (2020) performed a systematic evaluation of the safety of commercial Bacillus probiotics intended for usage in humans, animals, plants, aquaculture and environment in China. Nearly half of the 65 isolated Bacillus spp. strains from these commercial probiotic products were capable of producing hazardous toxins. Infections with representative isolates could cause sepsis, intestinal inflammation and liver injury in different mouse models. Additionally, these isolates harbor multiple antimicrobial resistance genes coupled with mobile genetic elements. Collectively, the capability of producing various toxins and harboring mobile antimicrobial resistance genes in Bacillus probiotics indicates a potential risk for one health. The results also showed that in tables (4 and 5) the highest feed intake was recorded for the group fed 1% glutamic acid, which may be due to the fact that glutamic acid is more palatable than other additives in the remaining treatments. It has been proven that salt the sodium salt of glutamic acid, has two effects in foods: one is to induce a unique taste called umami, which is one of the five basic tastes, and the other is to make food palatable (Korytko., 2024, Yamamoto and Inui-Yamamoto, 2023). The best FCR was recorded for G4 which contains 0.05 glutamine this is a result of improvement in BWG and FI for birds.

In general results of investigation showed that 0.5 and 0.1 % glutamic acid and glutamine have resulted in improved carcass traits. These results may be due to glutamine help to metabolise fats, glutamine also plays a role in the synthesis of arginine in the body, which increases the size of the liver and pancreas (Adjei *et al.*, 1994). Therefore, according to the above positive effects of glutamine may be happiness improvement of carcass characteristics. These results are consistent with Mendanha *et al.* (2014) and Gholipour, *et al.* (2019) which found that using 0.5 or 1% glutamine in broilers diets improve carcass characteristics. In general results for histomorphometric traits observed that Gln supplementation improvement the histomorphometric of intestinal due to glutamic acid and glutamine may be play a substrate or energy source for maturation of fast proliferating cells such as enterocytes and hence its supplementation in the first days of age may have activated cell mitosis and caused higher VH (Kelly., et all 1991 and Nkukwana *et al.*, 2015). The protective effect of Gln on alleviating intestinal lesions may also be associated with enhanced development of the intestinal mucosa. L-glutamine is responsible for retaining the mucosal structure (Khan *et al.*, 1999) and for reconstruction after damage (Rhoads *et al.*, 1997). Souba *et al.* (1990) suggested that glutamine is an important AA for maintenance of gut metabolism, structure, and function especially during critical illness when the gut mucosal barrier compromised based on human research. These results supported by many previous studies by (Silva *et al.*, 2023b, Hassan *et al.*, 2022 and Abdulkarimi *et al.*, 2019) As well as Gholipour *et al.* (2019) and Zulkifli *et al.* (2016) are disagreement with our results. Blood results can be attributed to the fact that results of serum

antioxidant parameters may be due to the fact that glutathione is a tripeptide composed of the amino acids glutamate, cysteine, and glycine. Therefore, glutamine and glutamic acid may play a role in glutathione synthesis (Koga *et al.*, 2011). Furthermore, glutamine could increase SOD, CAT, and GPX levels in cells by modulating the nuclear factor erythroid 2-related 2/Kelch-like ECH-associated protein 1 (NRF2-Keap1) pathway (Hu *et al.*, 2016). NRF2-Keap1 is a transcription factor pathway that activates the gene expression of the antioxidant response element gene cluster, including the antioxidant enzymes. The results of the economic study showed that the highest economic value was achieved by the treatment with a 0.5 % glutamine level. This was due to the increased body weight gain and feed conversion in this treatment. It can be concluded that adding a level of (0.05%) of the amino acid glutamine to broiler chicken feed improved growth performance and achieved the best economic production without negatively affecting body functions under Egyptian conditions.

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تأثير إضافة الجلوتامين وحمض الجلوتاميك والكائنات الدقيقة المنتجة لحمض الجلوتاميك على أداء ومورفولوجيا الأمعاء في دجاج التسمين

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أجريت هذه الدراسة في محطةِ بحوث الدواجنَ، كليّة الزراعة، جامعة الأزهر، القاهرة، جمهورية مصر العربية. الهدف من هذة الدراسة معرفة تأثير إضافة مستويات مختلفة من الجلوتامين وحمض الجلوتاميك والبكتيريا المنتجة لحمض الجلوتاميك فى علائق دجاج التسمين، مع التركيز على أداء النمو والقياسات المورفومترية للأمعاء وخصائص الذبيحة وبعض قياسات الدم والكفاءة الاقتصادية لدجاج التسمين (روس 308) من عمر (5-42) يوم. تم تقسيم إجمالي 324 كتكوت تسمين غير مجنس إلى 9 مجموعات تجريبية كل مجموعة في ثلاث مكررات تحتوي كل منها على 12 طائرًا. وكانت المعاملات كالاتى: مجموعة التحكم وغذّت الكتاكيت على النظام الغذائي الأساسي وباقى المعاملات (0.05%، 1.0% حمض الجلوتاميك، 0.05%، 1.0% الجلوتامين، ١٪ (Bacillus subtilis-٢)، ١٪ (Bacillus zero-٢)، ١٪ (Enterococcus sucrose)، ١٪ (Enterococcus zero) من مصادر بيولوجية). حيث سجلت المجموعة التى تغذت على 0.05% من الجلوتامين على أعلى زيادة فى الوزن وأفضل معدل تحويل غذائى تليها المجموعات الأخرى والتي كانت الفروق بينهم غير معنوية. أظهر ت النتائج أن قيم الألانين أمينوترانسفيراز وأسباراتاتي أمينوترانسفيراز ضمن المستوى الطبيعي وانزيمات الاكسدة وتحسنت مع جميع المعاملات مقارنة بالكنترول. لوحظ عدم وجود فرق معنوي بين المجموعات من حيث وزن الذبيحة فارغة والكبد. أظهرت الكتاكيت المغذاة على 0.05% من الجلوتامين تحسنا في طول وعمق يين خملات الامعاء بالمقارنة بباقى المعاملات. كما أن معامل كفاءة الإنتاج الأوروبي (EPEF) قد زاد مع اضافة 0.05% من الجلوتامين.

يمكن الاستنتاج أن إضافة مستوى (0.05%) من الحمض الامينى الجلوتامين فى علائق دجاج التسمين حسن أداء النمو وحقق أفضل إنتاج إقتصادي دون التأثير السلبي على وظائف الجسم.

الكلمات الاسترشادية: *الجلوتامين، حمض الجلوتاميك، البروبيوتيك، دجاج التسمين، قياسات الدم*