10.21608/avmj.2025.332957.1455 Assiut University web-site: www.aun.edu.eg

ASSESSMENT OF SOME ESSENTIAL AMINO ACID FUNCTION AS IMMUNOMODULATOR AGAINST SALMONELLA INFECTION IN BROILERS

KHEDER Z.A.[1], RANIA GHANEM[1], GEHAN K. SALEH[1], MARWA SHALABY[2], MOHAMED ABDELRAZEK[2] AND HALA M. ISMAIL[3]

[1] Biochemistry, Nutritional Deficiency Unit and Toxicology, Animal Health Research Institute (AHRI), Mansoura Provincial Lab (AHRI-Mansoura), Agriculture Research Center (ARC), P.O. Box 264 Dokki, 12618- Giza, Egypt.
[2] Department of Bacteriology, Animal Health Research Institute (AHRI) (Mansoura Branch), Agriculture Research Center (ARC), P.O. Box 264 Dokki, 12618 – Giza, Egypt.
[3] Department of Pathology, Animal Health Research Institute (AHRI), Mansoura Provincial Lab (AHRI-Mansoura), Agriculture Research Center (ARC), P.O. Box 264 Dokki, 12618 – Giza, Egypt.

Received: 18 November 2024; **Accepted:** 30 December 2024

ABSTRACT

Salmonella is the foremost cause of food-borne zoonotic disease worldwide. This study aimed to investigate the effect of amino acids (AA) on the immune status of poultry against salmonella infection. A total of 180 one-day-old chicks (Ross 308) were reared, swapped for detection of salmonella and divided into 5 groups randomly. G1 noninfected, non-treated, G2 infected with *S.entertieds* at the 7 th day old, G3 infected with *S.entertieds* at the 7 th day old and supplemented with AA 1 cm/ liter for 3 successive days per week, G4 non-infected and supplemented with AA 1 cm/liter for 3 successive days per week and G5 infected with *S.entertieds.* The body weight gain increased significantly in G4. The IgG level was very low in G4, especially on the 35th day, while it was high in G5. IgM level was markedly low in G4, especially on the 28th day and very high in G2 on the 28th day. Glutathione peroxidase enzyme level increased significantly in G4 at age 35. A total number of 58 (12.89%) *Salmonella* isolates were recovered from 450 samples. The *sopB, bcfC,* and *stn* genes were detected in all isolates, while the *spvC* gene was absent in all isolates. The (*qnrA)* gene was detected only in one examined isolate, while (*qnrB*) was detected in two isolates. Histologically the liver and intestine were improved in the AA-supplemented groups even with infection (G3), with normal hepatic parenchyma and hepatocytes and abundant goblet cells and hyperplasia of the intestinal mucosa. Besides, the immunoreactivity (CD3 and CD79) significantly increased, indicating more lymphocytes compared to other groups. In conclusion, supplementation of amino acids improved the performance and immunity of poultry and reduction of colonization of *S. Enteritidis* in the rearing birds.

Keywords: Essential, Amino acids, immunomodulator, *Salmonella*, broiler, immunohistochemistry

INTRODUCTION

Low crude protein (CP) diets with correct amino acid (AA) supplementation are an effective way to reduce nitrogen excretion and ammonia emissions. In the poultry industry, this is the modern way (Aletor *et al.*, 2000; Roberts *et al.*, 2007). When we decrease CP, Gly+Ser levels decrease drastically. Therefore, the decrease in performance of broilers occurred when dietary CP is decreased by more than 3%. Glycine supplementation

Corresponding author: KHEDER Z.A.
E-mail address: zaghlolkheder0@gmail.com
Address: Biochemistry, Nutritional Deficiency Unit and Toxicology, Animal Health Research Institute, Mansoura Provincial Lab (AHRI-Mansoura), Agriculture Research Center (ARC), P.O. Box 264 Dokki, 12618 – Giza, Egypt.

may be due to the marginal levels of dietary Gly+Ser inducing the complete recovery of bird performance during the starter phase, when these birds were fed a low-CP diet (Ospina-Rojas *et al.*, 2012). During the starter phase, glycine is an AA for broilers and also a major component of bile salts, constitutes approximately 90% of the total bile AA (Souffrant, 2001).

Poultry byproducts are also low-cost and high-quality protein feedstuffs for livestock species, fish, and companion animals (Liu *et al.*, 2020), adequately glycine or proline in chickens is not synthesized (the most abundant AAs in the body but present in plant-source feedstuffs at low content) relative to their nutritional and physiological needs. Therefore, these two AAs must be sufficient in poultry diets (Wenliang *et al.*, 2021). *Salmonella enteritidis* are the most common food borne pathogens, resulting in cases of gastroenteritis and economic losses (Chen *et al.*, 2013). *Salmonella* enteritidis *and S.* typhimurium were transmitted vertically through trans-ovarian infection and horizontally between farms (El-Sharkawy *et al.*, 2017).

Abd El-Ghany *et al.*, 2012, recorded that both *Salmonella* enteritidis *and S.* typhimurium are the main serotypes recorded in Egyptian poultry farms. During epidemics, serotyping was used as a basic biomarker for *Salmonella* infections to assign the source of contamination (Bell *et al.*, 2016). The emergence of antimicrobial-resistant bacteria in laying hen farms resulted from the extensive use of antibiotics as growth promoters and therapeutic agents. Ammar *et al.* (2016) approved that *Salmonella* spp. in Egypt had antimicrobial resistance to cefaclor, doxycycline, penicillin, and streptomycin (Elbediwi *et al.*, 2021) and found that *Salmonella* isolates own the resistance to beta-lactams and quinolones. The pathogenesis of Salmonella inside the host is affected by virulence genes that are present in the chromosome and plasmids (Choudhury *et al.*, 2016). The presence of virulence genes that encode various proteins responsible for adhesion, recognition, invasion, internalization, iron acquisition, neutralization, proliferation, and survival, which are located on plasmids or in *Salmonella* pathogenicity islands, is the main route for *Salmonella* pathogenicity (Shah *et al.*, 2011). *Sop* gene (chromosomal gene) is a *Salmonella* outer protein. It is responsible for the invasion of host epithelial cells. While the enterotoxin production is encoded by the *stn* gene, which is the main causative agent for diarrhea (Huehn *et al.*, 2010). The survival and replication of *Salmonella* inside the host based on (*spvs*) (Rabie *et al.*, 2012). *bcfC* (fimbrial gene) is located on a fimbrial structure and has a role in cell invasion (Huehn *et al.*, 2010). The AAs play a significant role in maintaining hepatic and GIT integrity and increasing immune cells that may have growth-limiting activity against *Salmonella* (Mohamed *et al*., 2019). The objective of the present study was to evaluate AAs requirements in low-CP diets with different levels of digestibly**.** And its effect on immune states of poultry against the infection of some bacteria, especially *Salmonella* infection, and compare this to treatment with antibiotics in broiler chickens.

MATERIALS AND METHODS

Samples culture

The samples were collected to detect *Salmonella* enteritidis prevalence as follows: 50 cloacal swabs from healthy chickens, 200 samples (50 from each organ), including the crop, liver, cecum, and spleen, 50 freshly dead birds, and 200 samples (crop, liver, cecum, and spleen) from each (50 sacrified birds) suffered from ruffled feathers, lameness, retarded growth, and accumulation of whitish diarrhea around the vent for bacteriological analysis The samples (25 g) were placed aseptically into sterile Difco-buffered peptone water

(225 ml) tubes (Oxoid, UK) and pre-enriched according to Arthur *et al.* (2004) and confirmed biochemically according to Quinn and Keough (2002). Serological typing for *Salmonella* isolates by slide agglutination with monovalent and polyvalent antisera (Microgen, UK), according to the Kauffmann White Le Minor Scheme (Grimont and Weill, 2007), was done at the Animal Health Research Institute, Dokki, Giza.

Antimicrobial sensitivity test

Ten antibiotic discs, including chloramphenicol (30 µg/disk), flumequine (30 µg/disk), forfloxacin (10 µg/disk), enrofloxacin (5 µg/disk), levofloxacin (5µg/disk), nalidixic acid (30 µg/disk), neomycin (30 µg/disk), spectinomycin (10 µg/disk), ampicillin (10 µg/disk), ciprofloxacin (5 µg/disk), and amoxicillin (10 µg/disk) Oxoid, UK**.** (Clinical and Laboratory Standards Institute Guideline) was used for the test protocol and interpretation of the results.

Uniplex polymerase chain reaction (PCR*)*

DNA extraction was done according to the instructions of the QIAamp DNA mini kit, Germany.

The amplification of the PCR reaction was performed using a thermal cycler (Master cycler, Eppendorf, Germany). Oligo-nucleotide primers for virulence genes and plasmid-mediated quinolone resistance genes were described in Table (1). Cycling conditions were done according to Murugkar *et al. (*2003) and Huehn *et al.* (2010).

Experimental work

In the present experiment, 180 broiler chicks (Ros 308) (one day old) were used. Randomly five birds were sacrificed for the bacteriological examination. Broiler chicks were assigned into five groups (n = 35 birds/group) (G1, G2, G3, G4, and G5) randomly. G1 noninfected, non-treated, for five successive days at the age from the 7^{th} to the 14^{th} day old. Birds in G2, G3, and G5 were challenged orally by 1 ml of saline containing 10^5 cfu/ml. *S. enteritidis*, which was previously isolated from diseased chicks in this experiment, and was positive for *qnrB* and *qnrS* resistance genes, at the age of 7 days old (Abd El-Dayem *et al*., 2015). G2 infected without treatment, G3 infected and supplemented with amino acids mixture (ATCO PHARMA-Egypt), 1 cm/liter for three successive days per week, G4 non-infected and supplemented with amino acids, 1 cm/liter for three successive days per week. G5 infected and medicated with spectinomycin (Amoun Company, Egypt) 20 mg/kg body weight (Abu-basha *et al.,* 2007).

The weight of all birds in each group and the consumption of rations were recorded to calculate the food conversion ratio. Dead birds were necropsied immediately, and macroscopical lesions were recorded. Swabs from the cecum and crop were collected from five sacrificed birds from each group on the 2^{nd} and 4^{th} weeks for re-isolation of *S. enteritidis,* according to Arthur *et al.* (2004). Then confirmed biochemically according to Quinn and Keough (2002).

The use of broiler chicks (Ros 308) was reviewed and approved following the ethical rules of the Agriculture Research Center Institutional Animal Care and Use Committee (ARC-IACUC).

Blood samples for biochemical analysis:

Blood samples were obtained from the wing vein from five birds/group at 7, 14, 21, 28, and 35 days and divided into 2 parts: blood with heparin and the other without anticoagulant, then centrifuged at 3500 rpm for 15 min to obtain serum and plasma and stored at -20^{o}C. Commercial diagnosis kits used to determine serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The level of glutathione peroxidase enzyme (GSH-PX) was measured using commercial diagnostic kits (BIO diagnostic CO. Egypt,

Giza). IgG and IgM in plasma were measured using ELISA plates (Ameko, Shanghai), according to the protocol provided by the manufacturer. Amino acid levels (alanine, histidine, and glycine) were examined in plasma samples by using high-performance liquid chromatography (HPLC).

Histopathological examination:

Specimens from the liver and intestinal tissues were fixed in 10% buffered formalin at least for 24 hours. After fixation, samples were dehydrated and paraffin wax-infiltrated, blocks were cut into 5 um sections following common histological routine and stained by hematoxylin and eosin. In addition, Alcian blue was used for staining of goblet cells. Histological examination by a light microscope and photographed by a digital camera (C-5060, Olympus, Japan) (Bancroft and Gamble, 2008).

Immunohistochemistry (IHC)

Detection and scoring of T-lymphocytes (CD3) in the thymus and B-lymphocytes (CD79) in the spleen were performed according to Mohamed *et al.(* 2019). At the end of the experiment, the spleen and thymus paraffin sections were deparaffinized, dehydrated, and washed with distilled water. Antigen retrieval in a water bath with buffer citrate was applied for 20 min. The activity of endogenous peroxidase was removed by 4% hydrogen peroxide. Then the sections were incubated with primary antibody at room temperature for 30 min, CD3 and CD79 (Novous Biological Company). In all experimental groups, the sections were stained by secondary antibody, according to the instructions of manufacturer. The stained sections were washed for 15 minutes three times by PBS and then incubated at room temperature for 15 min in poly HRP conjugate. To visualize the antigen-antibody reaction, the sections were incubated with DAB for 10 minutes, and counterstained with hematoxylin. The mounting and scoring of positive immune cells in all groups by detection of B and T lymphocyte frequency per focus using the point and count method, in which a digital microscope faces a digital camera at 100 magnification (Weibel, 1969).

RESULTS

Salmonellosis symptoms were ruffled feathers, lameness, retarded growth, and accumulation of whitish diarrhea around the vent. The postmortem findings were septicemia, splenomegaly, and necrotic foci with enlargement of the liver, pericarditis, intestinal inflammation, congested cecum, and kidneys. Based on the morphological and biochemical features, *Salmonella* enteritidis recovered in 12.89% (58 of the 450 investigated samples) of the samples. Nine strains (18%) were isolated from 50 healthy birds (cloaca), 28 strains (56%) were identified from diseased birds, and 21 strains (42%) from freshly dead. The recovery rates of *Salmonella* were sixteen strains (16%) from the liver, 13 (13%) from the spleen, 12 (12%) from the cecum, and 8 (8%) strains from the crop.

The antimicrobial susceptibility pattern of 58 *Salmonella* strains showed resistance to flumequine and nalidixic (each 100%), amoxicillin (89.6%), ampicillin (58%), enrofloxacin (48.2%), neomycin (44.8%), and spectinomycin (39.6%). While low resistance rates against ciprofloxacin, levofloxacin, chloramphenicol, and norfloxacin (3.4%, 10.3%, 17.2%, and 18.9%), respectively.

The *sop*B, *bcf*C, *stn*, and *spvC* virulence genes were detected by uniplex PCR analysis in 8 isolates which were highly resistant to ciprofloxacin and levofloxacin. *Sop*B, *bcf*C, and *stn* genes were detected in all isolates, while the *spvC* gene has not been detected in any sample. Also, it revealed the presence of resistance genes *qnrS* in (2/8) isolates and *qnrB* in (1/8) examined isolates as shown in Fig (1).

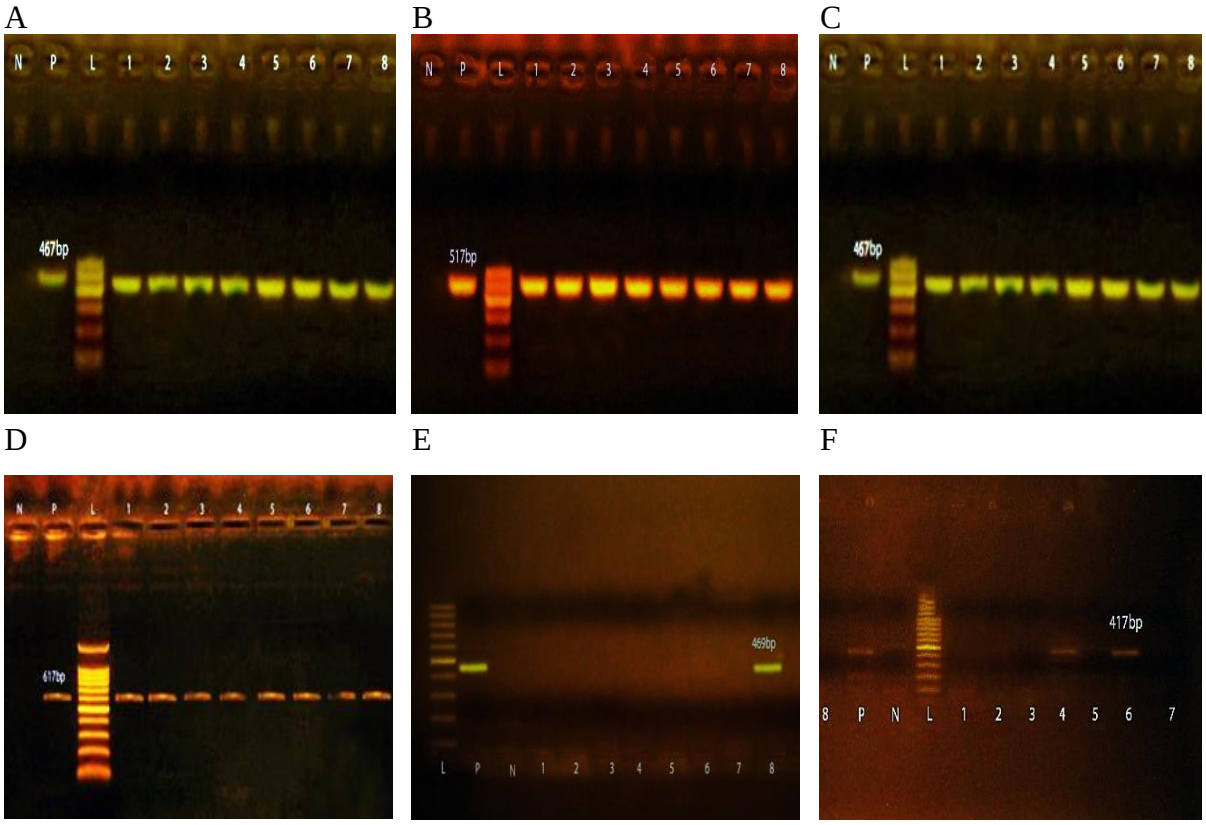

Figure (1): PCR amplified products of *spvC* (A), *sopB* (B), and *bcfC* (C) and *stn* (D) virulence genes, *qnrB* I and *qnrS* (F) antibiotic resistance genes by agarose gel electrophoresis. Lane L: DNA molecular marker (100 bp), lanes 1- 8: *S. enteritidis* isolated from chicken organs

Pathological changes*:*

Liver: Gross lesions showed swelling, congestion, and dark bronze discoloration patches (Fig. 2A). Histologically, the control group showed normal tissue parenchyma and hepatocyte cords arranged in radiation surrounding the central vein (Fig. 2B). In the *Salmonella*-infected group, there was blood vessel congestion, haemorrhages, diffuse hepatic vacuolar degeneration, and coagulative necrosis, which is surrounded by aggregation of heterophils and leucocytes (Fig. 2C). In the *Salmonella*-infected and amino acid-supplemented group, multifocal vacuolar degeneration and vascular congestion were detected (Fig. 2D). While the non-infected supplemented group with amino acids displayed normal hepatocytes and normal hepatic parenchyma (Fig. 2E). In the infected and treated with antibiotics group, the liver displayed severe vascular congestion, heterophilic infiltration, and multiple degenerative and necrotic areas (Fig. 2F).

Intestine: Grossly, distended and enlarged due to catarrhal enteritis in *Salmonella*-infected groups, which is characterized by thick mucus and slimy exudate in the intestinal lumen (Fig. 3A). In the control group, the intestinal villi appeared normally with an intact epithelial lining layer (Fig. 3B). The *Salmonella*-infected group showed severe destruction of villi tips, aggregation of lymphocytes in the intestinal lumen, and severe hemorrhage in the lamina propria (Fig. 3C). *Salmonella*-infected and amino acid-supplemented groups showed mild desquamation of lining epithelium and of intestinal glands hyperplasia (Fig. 3D). The non-infected supplemented group with amino acid displayed broad intestinal villi with hyperplasia of intestinal crypts and goblet and intact lining epithelium (Fig. 3E). The infected and treated with antibiotics group showed an increase in the thickness of lamina propria due to heterophilic and lymphocytic infiltration. Degenerated and necrotic abundant heterophils. Mild desquamation of lining epithelium with intestinal glands hyperplasia (Fig. 3F).

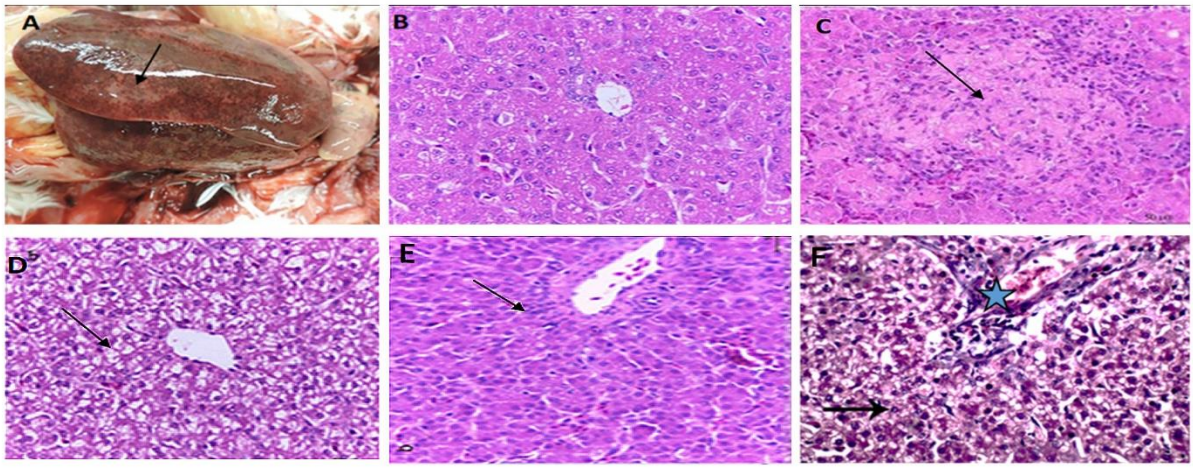

Figure (2): Liver, (A): *Salmonella*-infected group showing enlarged liver and mottled with multiple miliary necrosis, with a diameter of 1-2 cm (arrow). Histopathological examination of the liver stained with H&E, **(B):** negative control group showing normal histological structure, **(C):** *Salmonella*-infected group showing the area of necrosis, mononuclear cell proliferation, and focal hemorrhage (arrow), **(D):** *Salmonella*-infected and amino acid-supplemented group showing mild hepatocytic vacuolar degeneration (arrow), **(E):** Amino acid-supplemented group shows normal hepatocytes and normal hepatic parenchyma (arrow), **(F):** the *Salmonella*-infected and treated with antibiotics group showed severe hepatocytic vacuolar degeneration, vascular congestion, and necrosis of hepatocytes (arrow), and infiltration of heterophils (star). B, C, D, E (X200) and F (X400).

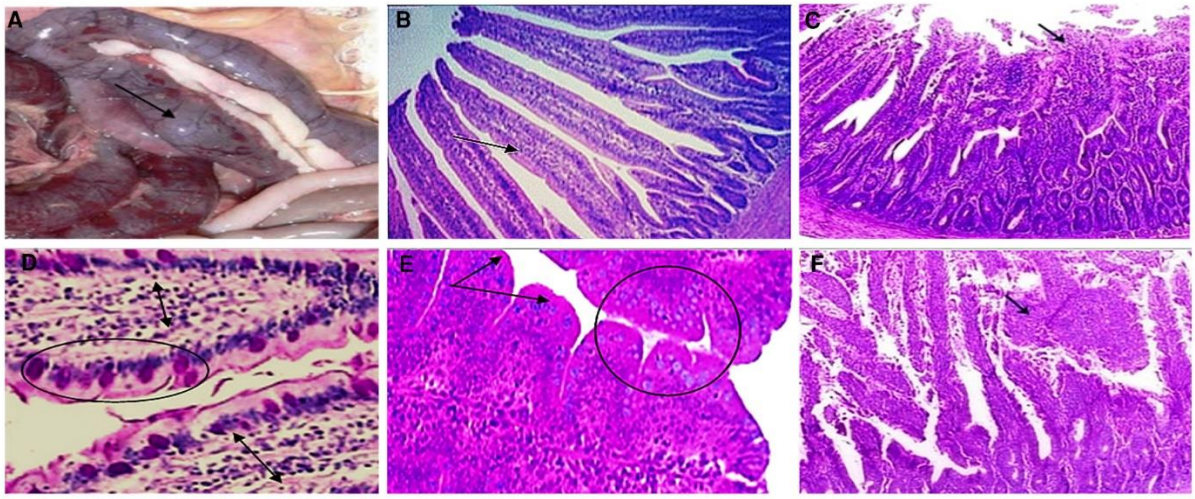

Figure (3): Intestine, **(A)**: Salmonella- infected group is characterized by catarrhal enteritis (arrow). Histopathological examination of the intestine stained with H&E with Alcian blue, **(B)**: negative Control group showing normal villi and enterocytes (arrow), **(C)**: Salmonella infected group showing interstitial hemorrhage, desquamation and sloughing of villi lining epithelium (arrow), **(D)**: Salmonella-infected and amino acids-supplemented group showing intact epithelium, increase goblet cells (circle) and inflammatory cell infiltration in lamina propria (arrow), **(E)**: non-infected amino acid supplemented group showing broad and intact intestinal villi with hyperplastic intestinal crypts (arrow) and increase of goblet cells (circle), **(F)**: infected- treated with antibiotic showing the destruction of the upper portion of villi, besides hyperemia and edema in the submucosa (arrow). H,I,and L (X200), J and K (X400).

Immunohistochemistry of CD3 and CD79 in spleen and thymus

Detection of positive immune cell reaction in cytoplasmic CD3 (T-lymphocytes) in the thymus of the experimental groups showed a significant decrease in Group 1 compared to Group 2. Comparing Group 3 and Group 5, the CD3 displayed a significant increase in Group 3. Also, in Group 4, the immunoreactivity slightly increased in the thymus, when compared to Group 1. The reactive cells, CD79 (B-lymphocytes), in the spleen had a significant increase in Groups 2 and 3. In comparison, CD79 increased in Group 4 more than in Group 1 (Fig. 4) and (Table 7).

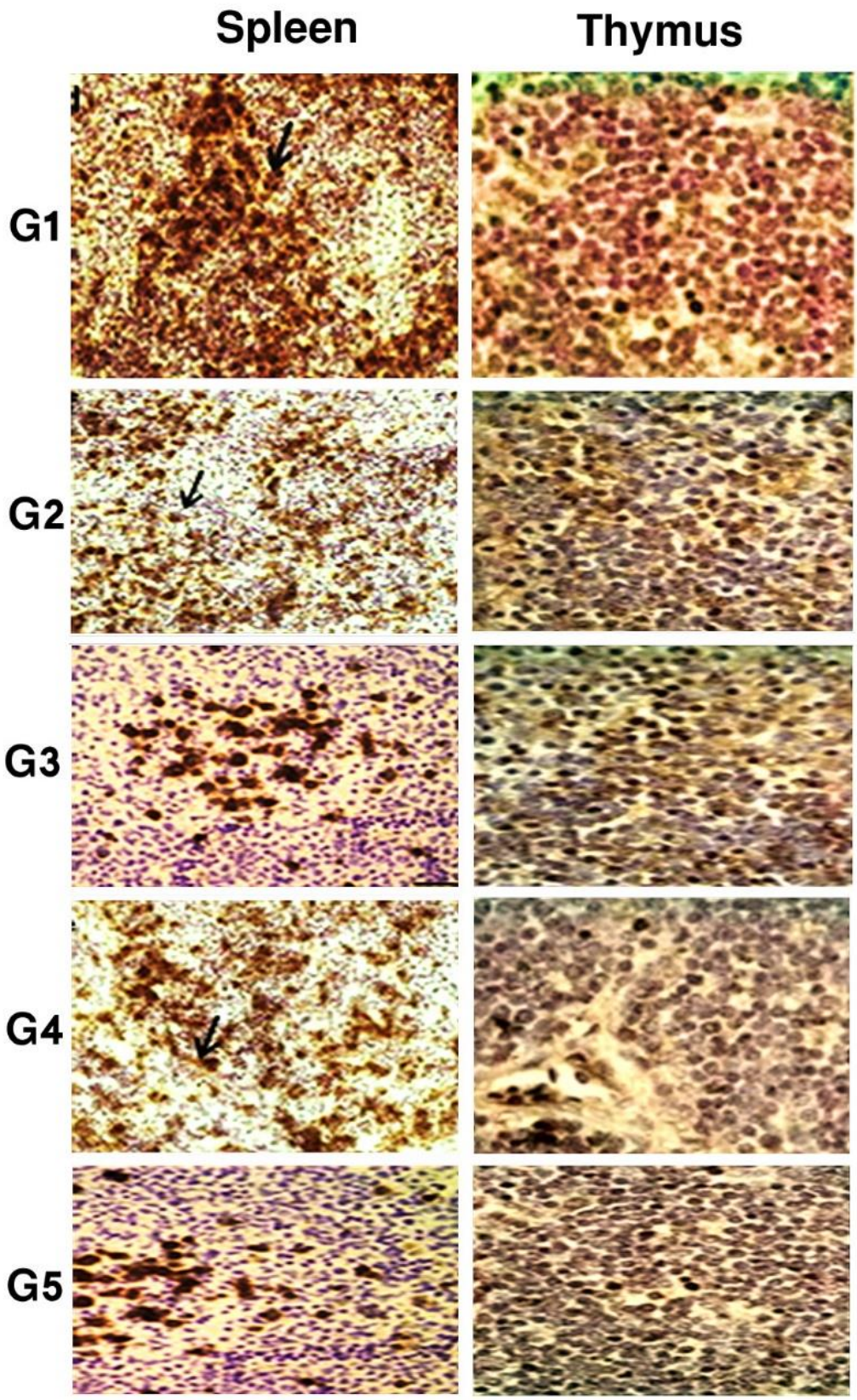

Figure (4): Immuno-stained sections in different groups showing CD 79 immune-positive cells in lymphoid follicles of the white pulp of the spleen and CD 3 immune-positive cells in the thymus.

Table 1: Oligonucleotide Primer for virulence genes and Plasmid-mediated quinolone resistance genes:

The gene	Primer sequences (5'→3')		product size (bp)	Reference
Stn	F	TTGTGTCGCTATCACTGGCAACC	617bp	**(Murugkar *et al.*, 2003)**
	R	ATTCGTAACCCGCTCTCGTCC		
*bcf*C	F	ACCAGAGACATTGCCTTCC	467bp	**(Huehn *et al.*, 2010)**
	R	TTCTGCTCGCCGCTATTCG		
sopB	F	TCAGAAGTCGTCTAACCACTC	517 bp	
	R	TACCGTCCTCATGCACACTC		
spvC	F	ACCAGAGACATTGCCTTCC	467 bp	
	R	TTCTGATCGCCGCTATTCG		
Plasmid-mediated quinolone resistance genes				
qnrS	F	ACGACATTCGTCAACTGCAA	417Bp	**(Robicsek *et al.*, 2006)**
	R	TAAATTGGCACCCTGTAGGC		
qnrB	F	GATCGTGAAAGCCAGAAAGG	469 Bp	
	R	ACGATGCCTGGTAGTTGTCC		

Table 2: Distribution of *Salmonella* serovars in the samples ($n = 58$):

Serotypes	Antigenic formula	Sample number	%
S. Enteritidis	O:1,9,12; H1:g,m; H2	19	32.75
S. Typhimurium	O:1,4,5,12; H1:I;H2:1,2	13	22.4
S.Kentucky	O:8,20; H1:I; H2:Z6	6	10.34
S.Molade	O:8,20;H1:Z10;H2:Z6	10	17.24
S. Bargny	O:8,20;H1:I;H2:1,5	3	5.17
S. Inganda	O:6,7;H1:Z10;H2:1,5	5	8.62
S. Infantis	O:6,7;H1:r;H2:1,5	2	3.44

Table 3: *Salmonella enteritidis* re-isolation from the crop and cecum of broilers infected and supplemented by amino acids.

Organs / Groups	Crop		Cecum	
	Day 14	Day 28	Day 14	Day 28
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
G1	0^{d}	0^{c}	0^{d}	0^{d}
G2	2.08×10^{4a}±15.62	1.4×10^{3a}±7.83	6.4×10^{4a} ±58.25	2.9×10^{4a} ±18.42
G3	1.2×10^{3c}±19.80	0^{c}	1.42×10^{3c} ±35.21	1.2×10^{3c}±13.55
G4	0^{d}	0^{c}	0^{d}	0^{d}
G5	3.1×10^{3b}±9. 45	1.8×10^{3b}±6.55	1.96×10^{3b}±19.32	1.7×10^{3b} ±15.34

a-c = the same letter in each column indicates a non-significant difference at $P \leq 0.05$
Different letters mean a significant difference between groups at $P < 0.05$

Table 4: The average and body weight of broiler chicken from day 1 to 35 days by (gm)

DAYS / group	(1)	(7)	(14)	(21)	(28)	(35)	BW gain	FCR
G1	42±0.5^{a}	135±5^{a}	403±22^{b}	864±57^{b}	1350±113^{b}	1805±173^{bc}	1762.5^{b}	1.8^{c}
G2	41±3^{a}	1328±8^{a}	335±30^{a}	654±56^{a}	958±305^{a}	1250±345^{a}	1209^{a}	2.6^{d}
G3	41.5±2^{a}	133±7^{a}	516±50^{b}	857±55^{b}	1150±205^{ab}	1950±250^{c}	1908.5^{C}	1.66^{b}
G4	42±2.5^{a}	134±9^{a}	658±35^{c}	932±45^{c}	1455±155^{c}	2250±250^{d}	2208^{d}	1.44^{a}
G5	41.5±1.5^{a}	131±11^{a}	645±45^{c}	815±75^{b}	105±145^{a}	1725±205^{b}	1683.5^{b}	1.88^{c}

Value of mean ± SE
a-d = the same small letter in each raw are not significantly different at P≤0.05
FCR= feed conversion ratio

Table 5: Concentration of IgG and IgM (u/ml) in plasma of broiler chickens and amino acids (Histidine, alanine, glycine) (n mol/ml) in plasma of broiler chicken

Group / Items		G1	G2	G3	G4	G5
IgG	7th	0.056±0.005^{a}	0.054±0.006^{a}	0.057±0.003^{a}	0.061±0.004^{a}	0.058±0.003^{a}
	14th	0.048±0.002^{a}	0.071+0.006^{c}	0.063±0.007^{b}	0.042±0.002^{a}	0.069±0.001^{b}
	21st	0.046±0.008^{a}	0.079±0.002^{d}	0.069±0.003^{b}	0.045±0.005^{a}	0.072±0.007^{c}
	28th	0.049±0.001^{a}	0.083±0.009^{d}	0.075±0.006^{b}	0.049±0.007^{a}	0.078±0.004^{c}
	35th	0.051±0.003^{a}	0.081±0.005^{c}	0.078±0.008^{b}	0.046±0.009^{a}	0.083±0.006^{c}
IgM	7th	0.052±0.005^{a}	0.061±0.003^{a}	0.059±0.002^{a}	0.063±0.004^{a}	0.057±0.001^{a}
	14th	0.059±0.008^{a}	0.069±0.001^{c}	0.062±0.002^{b}	0.054±0.006^{a}	0.062±0.003^{b}
	21st	0.064±0.003^{b}	0.075±0.007^{c}	0.064±0.007^{b}	0.039±0.004^{a}	0.067±0.005^{b}
	28th	0.042±0.008^{a}	0.082±0.005^{c}	0.055±0.005^{b}	0.034±0.005^{a}	0.059±0.001^{b}
	35th	0.0038±0.001^{a}	0.081±0.002^{c}	0.058±0.004^{b}	0.039±0.007^{a}	0.054±0.003^{b}
Histidine	7th	55±1^{b}	64±3^{c}	48±3^{b}	49±1^{b}	41±2^{a}
	14th	95±3^{b}	105±4^{c}	84±3^{a}	87±4^{ab}	81±3^{a}
	21st	121±4^{b}	101±5^{c}	112±3^{a}	125±4^{ab}	109±3^{a}
	28th	128±6^{ab}	112±7^{a}	129±5^{b}	152±6^{d}	138±5^{c}
alanine	7th	215±9^{a}	218±8^{a}	216±9^{a}	214±7^{a}	215±8^{a}
	14th	310±10^{a}	352±8^{b}	340±7^{b}	345±10^{b}	312±9^{ab}
	21st	460±10^{b}	480±12^{c}	430±10^{a}	418±11^{a}	455±12^{b}
	28th	530±13^{a}	525±15^{a}	548±11^{b}	583±12^{c}	575±12^{c}
Glycine	7th	280±9^{a}	282±8^{a}	281±9^{a}	278±7^{a}	285±8^{a}
	14th	320±10^{a}	370±11^{c}	345±11^{b}	350±12^{bc}	318±9^{a}
	21st	420±13^{a}	410±15^{a}	419±14^{a}	448±13^{b}	424±13^{a}
	28th	497±17^{b}	430±19^{a}	483±16^{b}	592±17^{c}	483±15^{b}

Value of mean ± SE
a-d = the same small letter in each raw are not significantly different at p≤0.05

Table 6: The concentration of GSH-Px enzyme (u/ml), AST (u/ml) and ALT (u/ml) in chicken serum groups:

Items		G1	G2	G3	G4	G5
GSH-Px	7th	325±4.9 a	331±5.3 a	327±5.1 a	329±4.2 a	334±3.8 a
	14th	339±5.3 b	311±6.2 a	342±3.9 c	345±2.7 d	341±4.7bc
	21st	345±4.8b	321±7.2 a	349±5.2bc	356±4.3 c	349±.8bc
	28th	352±6.1 b	323±6.8 a	352±6.1b	361±3.7 c	351±6.4bc
	35th	353±3.9 c	334±4.6 a	361±4.8 d	367±3.4e	348±5.6bc
AST	7th	36.8±0.8 a	38.3±0.6 a	37.2±0.9 a	36.7±0.5 a	35.5±0.7 a
	14th	49.8±0.3 b	53.4±0.6 d	49.8±0.5 b	43.9±0.4 a	50.6±0.7 c
	21st	76.4±0.9 c	83.6±0.7 d	72.3±0.2 b	64.6±0.6 a	77.9±0.3c
	28th	83.5±0.3 b	96.7±0.9 d	81.4±0.6b	72.9±0.4 a	85.6±0.5 c
	35th	88.7±0.8 b	101.9±0.9 d	89.8±0.6 b	76.4±0.7 a	93.7±0.2 c
ALT	7th	11.7±0.17 a	11.2±0.2 a	12.1±0.2 a	11.7±0.3 a	10.9±0.4 a
	14th	14.6±0.25 b	16.8±0.29d	15.8±0.17 c	13.2±0.20 a	14.5±032 a
	21st	15.3±0.12 a	18.6±0.16 c	16.3±0.28 b	14.7±0.31 a	16.8±0.27 b
	28th	19.7±0.32 b	23.6±0.28 c	19.4±0.33 b	16.2±0.21 a	19.7±0.16 b
	35th	20.6±0.35 b	27.3±0.39 d	21.2±0.21 b	16.3±0.27 a	22.9±0.19 c

Value of mean ± SE
a-d = the same small letter in each raw are not significantly different at p≤0.05

Table 7: The mean number of CD3 positive cells in the thymus and CD79 positive cells in spleen in broiler chicken in all experimental groups.

Positive immune cells	Groups				
	G1	G2	G3	G4	G5
CD79 in spleen	35.6±3.25d	56.8 ±2.41b	73.4±8.71a	46.5±2.75c	55.3±22b
CD3 in thymus	55.4 ±8.21d	88.21 ±7.3a	95.3± 2.01a	79.15± 16b	62.4±34c

Data are expressed as Mean ± S.E. values in the same row with different superscripts that differ significantly at ($p < 0.05$).

DISCUSSION

Amino acids are considered the main component needed mainly for most of the many vital processes inside the bodies of all animal kingdoms and poultry, which were the main source for protein synthesis, whether functional protein or structural protein (Awad *et al.,* 2017). Also, due to differences in genetic selection, environmental conditions, and dietary composition, modern breeds of chickens have different requirements for amino acids (AAs) than the breeds used 30 years ago (Bailey *et al.,* 2010).

So, we aimed in this study to get the latest version of rearing chickens and its need. Our results found that the average body weight gain in group G4 was high (2208 gm) with a food conversion ratio of 1.44, while in G2, which was infected with *Salmonella* and not treated either by amino acid supplementation or antibiotic treatment, it was very low (1209 gm) with a very high conversion ratio (2.6), but G5, which was treated with antibiotics, comes

after G3, which was treated by amino acid supplementation, and the FCR was (1.88, 1.66), respectively; these results were agreed with Ospina-Rojus *et al*. (2012) and He *et al.* (2021), and this was due to the fact that the chickens do not synthesize adequate amounts of both glycine and proline, the most abundant AAs in the body, but very low in the plant sources that feed to the poultry, and when we added it in adequate amounts in amino acid mixtures, it led to these results.

According to biochemical analysis results, we found that the level of histidine amino acid in the plasma of G4 was significantly different (increased) at 152 ± 6 nmol/ml, especially from the 14th day old till the end of the experiment, and low level in G2 (112 ± 7 nmol/ml) than any other group, which was (128 ± 6,129 ± 5, and 138 ± 5 nmol/ml) in G1, G3, and G5, respectively, and these results were in accordance with Oso *et al. (*2019).

These results were in accordance with the level of both glycine and alanine amino acids in the plasma of examined birds, since there was a significant increase in the level of glycine, especially at the 28th day of age in G4 (592 ± 17 nmol/ml), and its level was not very low as other amino acids in other groups, but it was low in G2 at the 28th day (430 ± 19 nmol/ml) and also the level of alanine in G4 & G5 was significantly increased (583 ± 12 & 575 ± 12 nmol/ml), respectively, than that in G2, which was (525 ± 15 nmol/ml), and this result is in agreement with Johnson *et al.* (2020), which was explained this due to elevating dietary (AAs) density supplementation leading to enhancement of feed deficiency and this based on the recent advances in nutrition and metabolism of AAS. (Wu, 2014).

According to IgG & IgM analysis, there was a significant increase in group G2, especially at the age of 35 days old. The level of IgG was 0.081 ± 0.005 u/ml in G2, while it was decreased in G4 (0.043 ± 0.009 u/ml), and these results were in accordance with the level of IgM, which was (0.081 ± 0.002 u/ml in G2 at 35 days, while in G4 at the same age it was (0.039 ± 0.007 u/ml), and this was in agreement with Jamroz *et al.* (2003) and Du *et al. (*2016), who explained this due to a dose-dependent trend with optimal response for adequate amounts of amino acids and for increased expression of brush border enzymes and improved nutrient transport systems (Viveros *et al.,* 2011). Glutathione peroxidase enzyme analysis (GSH-Px) revealed a significant increase, especially at 35 days old, in G4 (367 ± 3.4 u/ml) compared to the other groups, while it was low in G2 (334 ± 4.6 u/ml) at the same age, and this was in agreement with Zhu *et al.* (2022), as this enzyme, responsible for removing H2O2 and organic peroxides from living organisms, prevents lipid peroxides from damaging body tissues and so increases the body weight with low FCR.

According to the liver enzymes analysis, there was a significant decrease in the level of AST on the 35th day in G4 (76.4 ± 0.7 u/L), while it was increased in G2 (101.9 ± 0.9 u/L) on the 35th day. Also, the level of ALT was significantly decreased on the 35th day of age in G4 (16.3 ± 0.27 Iu/L) and was increased in G2 (27.3 ± 0.39 Iu/L) than in other groups, and this is in agreement with Sun *et al.* (2015), He *et al.* (2021), and Zhu *et al. (*2022), and this increase in the liver enzymes, especially in G2, G3, and G5, while it was decreased in G4, which may be due to lysis of red blood corpuscles and damage of intestinal villi and liver tissue as a result of the presence of *Salmonella* infection in these groups, but in G4 there was no infection, besides the presence of an adequate amount of AAs, which acts as protection for these side effects.

The recovery rate of *Salmonella spp.* based on the morphological and biochemical characteristics was 12.89%, which was closer to that obtained by Ammar *et al.* (2016), which was 17%, but lower than that

founded by El-Sharkawy *et al.* (2017) and Elkenany *et al.* (2019), who isolated *Salmonella spp.* by 28.6% and (38%), respectively. The most *Salmonella* serotypes commonly isolated from chicks were *S.* Enteritidis and *S.* Typhimurium*;* the same result was recorded by Elkenany *et al.* (2019) and Shalaby *et al.* (2022). *Salmonella* isolates (n = 58) were resistance to Enrofloxacin (48.2%), compared to the 15% that was interpreted by Donado-Godoy *et al.* (2012). Coorevits *et al.* (2015) and Amany *et al.* (2022) recorded that the sensitivity rate for spectinomycin was 44.4%, which is nearly in agreement with our result. Nhung *et al.* (2016) and Lekagul *et al.* (2019) showed resistance to ampicillin (100%), which was used against bacterial infection as the first-line drug in animal farms since the bacterial pathogenicity was affected by virulence and antimicrobial resistance genes (Huehn *et al.*, 2010). The survival of Salmonella is primarily due to the antimicrobial resistance genetic factors, which enhance their drug resistance (Yang *et al.*, 2010). Uniplex PCR analysis recognized virulence genes (*bcf*C, *sop*B, *stn, and spvC*); the *spvC* gene was not recognized in any one of the isolates in this study, similar to Khodadadipour *et al.*, *(*2016) who stated the absence of *spvC* gene from all *Salmonella* isolates. All the isolated strains were positive for the *sop*B gene, which encodes the inositol phosphate phosphatase. PCR assay was carried out for the detection of the *sop*B gene from the isolated strains. This result is in agreement with Wood *et al.* (1998), Prager *et al.* (2000), Mohammed (2014), who approved that the *sopB* gene was detected in all serovars of *Salmonella.* Concerning the bcfC gene, it presents in all strains with 100%. It is a fimbrial gene in the microbial genome and allowing bacterial protein secretion during the intestinal invasion. This result agreed with that of Alatfehy (2012). *Stn* gene was demonstrated in a percentage of 100% of the isolates. The same results were achieved by Murugkar *et al*. (2003) and Shalaby *et al. (*2012), who found it in all the isolates. In this study, the *qnrS* gene was identified in two *Salmonella* isolates, while *qnrB* was recognized in only one isolate. This result was also approved by Kehrenberg *et al. (*2006) and Ahmed *et al*. *(*2009). Amino acid supplementation induces an increase in GSH-Px, which may explain the low Salmonella count (Pilonieta *et al.,* 2012).

Amino acid addition to the broiler diet improves its normal defense barrier function and effectively alleviates the intestinal mucosal damage caused by *S.* Enteritidis infection by increasing the intraepithelial lymphocyte cells and numbers of goblet cells **(**Liu *et al*., 2020).

The histopathological examination revealed that the amino acid-supplemented groups displayed fewer inflammatory and degenerative alterations compared to the control-positive group. These results were proved by Li and Wu (2017), who reported that both glycine and proline represented 57% of total amino acids in collagen structure, which is the most important protein in the body, essential to connective tissue structure and strength, such as skin, cartilage, bones, and blood vessels. Also, the amino acid-supplemented group showed hyperplasia of the epithelium lining the intestine and increased the length of the intestinal villi and abundance of goblet cells. These results agree with the findings of Caspary (1992), who indicated that the increased height of the intestinal villi increases the intestinal surface area, which could improve the absorption of available nutrients. Also, Langhout *et al.* (1999) and Shamoto and Yamauchi (2000) suggested that increasing villus height in the intestine may indicate an enhanced function of the intestinal villi. Moreover, Brahmankar *et al.* (2011) reported an increase in mucus production, which may be due to increasing the activity of the intestinal gland. They suggested a consequent enhancement in dietary absorption, which may explain the apparently improved weight gain and

maintaining GIT integrity, which may have growth-limiting activity against Salmonella in the group supplemented with amino acids in the current study.

The immunohistochemical method is a reliable and efficient tool for accurate diagnosis and immunological detection of avian immune (Zhong *et al.*, 2017). The current results of the surface antigens in the thymus (CD3) and spleen (CD79) indicated that amino acids have a positive significant effect on activating the two immune organs, which enhance protection against *Salmonella* infection. These results agree with Field (2005) and Calder (2006), who reported that amino acids play an important role in immune responses by regulating the activation of T and B lymphocytes, macrophages, and natural killer cells. Also, the proliferation of lymphocytes and antibody production enhances immune status and cytokines thereby reducing. The detection of avian T-cell antigens was performed by using single anti T-cell antibody (CD3), while the B-cell antigens have seven antibodies for detection B-cell mainly through detection of positive reaction of corresponding cells in lymphoid tissues (Kurokawa and Yamamoto, 2023). The reduction in lesion ratings with increasing immunity in the infected supplemented group compared to the infected treated group with antibiotics increases the interest in replacing antibiotics with natural products such as amino acids.

CONCLUSION

Supplementation of amino acids improved the performance and immunity of broilers and reduced the colonization of *S.* Enteritidis in the reared birds. Also, from an economic point of view, the addition of amino acids to the ration or in the water will decrease the costs and many other logistics for rearing poultry.

REFERENCE

Abd El-dayem, G.; Khalial, M.R.; Kheder, Z.A. and Abd El-gafar, A. (2015): Evaluation of some organic acids alternative to antibiotics for control of salmonella infection in broiler chickens. *Egyptian Journal of Chemistry and Environmental Health*, 1, 1-17. DOI:10.21608/ EJCEH.2015.232157

Abd ElL-Ghany, W.A.; El-Shafii, S.S. and Hatem, M. (2012): A survey on Salmonella species isolated from chicken flocks in Egypt. *Asian journal of animal and veterinary advances*,7,489-501. DOI: 10.3923/ajava.2012.489.501

Abd El-Mohsen, A.; El-Sherry, S.; Soliman, M.A. and Amen, O. (2022): Serological and Antibacterial Characteristics of Salmonella Isolates From Chickens In Assiut. *Egypt. Benha Veterinary Medical Journal*, 41, 93-99. DOI: 10.21608/bvmj. 2021.93816.1468

Ahmed, A.M.; Younis, E.E.A.; Ishida, Y. and Shimamoto, T. (2009): Genetic basis of multidrug resistance in Salmonella enterica serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. *Acta Tropica*, 111, 144-149.https://doi.org/10.1016/ j.actatropica.2009.04.004

Alatfehy, N. (2012): Genomic comparison and characterization of Salmonella enterica serovars by the use of different molecular techniques. Ph.D. Thesis, Faculty of Veterinary Medicine: Cairo University.

Aletor, V.A.; Hamid, I.I.; Nie, E. and Pfeffer, E. (2000): Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilisation. *Journal of the Science of Food and Agriculture*, 80, 547-554. https://doi.org/10.1002/(SICI)1097-0010(200004)80:5<547

Ammar, A.M.; Mohamed, A.A.; Abd El-Hamid, M.I. and El-Azzouny, M.M. (2016): Virulence genotypes of clinical SalmonellaSerovars from broilers in Egypt. *The Journal of Infection in Developing Countries*, 10, 337-346. doi: 10.3855/jidc.7437.

Arthur, T.M.; Bosilevac, J.M.; Nou, X.; Shackelford, S.D.; Wheeler, T.L.; Kent,

M.P. et al. (2004): Escherichia coli O157 Prevalence and Enumeration of Aerobic Bacteria, Enterobacteriaceae, and Escherichia coli O157 at Various Steps in Commercial Beef Processing Plants. *Journal of Food Protection*, 67, 658-665. DOI: 10.4315/0362-028x-67.4.658

Awad, E.A.; Zulkifl, I.I.; Soleimani, A.F. and Aljuobori, A. (2017): Effects of feeding male and female broiler chickens on low-protein diets fortified with different dietary glycine levels under the hot and humid tropical climate. *Italian Journal of Animal Science*, 16, 453-461. https://doi.org/10.1080/1828051X.2017.1291288

Bailey, J.K.; Pinyon, J.L.; Anantham, S. and Hall, R.M. (2010): Commensal Escherichia coli of healthy humans: a reservoir for antibiotic-resistance determinants. *Journal of Medical Microbiology*, 59, 1331-1339. doi: 10.1099/jmm.0.022475-0.

Bancroft, J. and Gamble, M. (2008): Theory and Practice of Histopathological Techniques. 6th ed Churchill Livingstone. New York, London and Madrid.

Bell, R.L.; Jarvis, K.G.; Ottesen, A.R.; Mcfarland, M.A. and Brown, E.W. (2016): Recent and emerging innovations in Salmonella detection: a food and environmental perspective. *Microbial biotechnology*, 9, 279-292. doi: 10.1111/1751-7915.12359

Brahmankar, M.G.; Wagh, S.B.; Kale, D.B. and Joshi, M.V. (2011): Immunotoxicity of lead acetate in broiler birds. *Journal of advances in pharmacy and healthcare Research.*http://www.japhr.iaphp.in.ISSN 2231-6817

Calder, P.C. (2006): Branched-chain amino acids and immunity. *The Journal of Nutrition*, 136, 288S-293S. https://doi.org/10.1093/jn/136.1.288S

Caspary, W.F. (1992): Physiology and pathophysiology of intestinal absorption. *The American Journal of Clinical Nutrition*, 55, 299S-308S. https://doi.org/10.1093/ajcn/55.1.299s

Chen, H.M.; Wang, Y.; Su, L.H. and Chiu, C.H. (2013): Nontyphoid Salmonella Infection: microbiology, clinical features, and antimicrobial therapy. Pediatr Neonatol. 54(3): 147-152.https://doi.org/10.1016/j.pedneo.2013.01.010

Choudhury, M.; Borah, P.; Sarma, H.K.; Barkalia, L.M.; Deka, N.K. and Hussain, I., et al. (2016): Multiplex-PCR Assay for Detection of some Major Virulence Genes of Salmonella enterica Serovars from Diverse Sources. *Current Science*, 111(7): 1252-1258. https://www.jstor.org/stable/24909136

Coorevits, L.; Boelens, J. and Claeys, G. (2015): Direct susceptibility testing by disk diffusion on clinical samples: a rapid and accurate tool for antibiotic stewardship. European journal of clinical microbiology & infectious diseases : Eur J Clin Microbiol Infect Dis., 34(6): 1207-1212. DOI 10.1007/s10096-015-2349-2

Donado-Godoy, P.; Clavijo, V.; Leon, M.; Tafur, M.A.; Gonzales, S. and Hume, M. et al. (2012): Prevalence of Salmonella on Retail Broiler Chicken Meat Carcasses in Colombia. J Food Prot., 75 (6): 1134-1138. https://doi.org/10.4315/0362-028X.JFP-11-513

Du, E.; Wang, W.; Gan, L.; Li, Z.; Guo, S. and Guo, Y. (2016): Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with Clostridium perfringens. J Anim Sci Biotechnol., 7:19. DOI 10.1186/s40104-016-0079-7

El-Sharkawy, H.; Tahoun, A.; El-Gohary, A.E.A.; El-Abasy, M.; El-Khayat, F. and Gillespie, T., et al. (2017): Epidemiological, molecular characterization and antibiotic resistance of Salmonella enterica serovars isolated from chicken fams in Egypt. Gut pathog., 9, 8-8. DOI 10.1186/s13099-017-0157-1

Elbediwi, M.; Tang, Y.; Shi, D.; Ramadan, H.; Xu, Y. and Xu, S. et al. (2021): Genomic Investigation of Antimicrobial-Resistant Salmonella enterica Isolates From Dead Chick Embryos in China. Front microbiol., 12. 684400-684400. https://doi.org/10.3389/fmicb.2021.684400

Elkenany, R.; Elsayed, M.M.; Zakaria, A.I.; El-Sayed, S.A.E.S. and Rizk, M.A. (2019): Antimicrobial resistance profiles and virulence genotyping of Salmonella

enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. *BMC Veterinary Research*, 15, 124-124. https://doi.org/10.1186/s12917-019-1867-z

Field, C.J. (2005): The Immunological Components of Human Milk and Their Effect on Immune Development in Infants. *The Journal of Nutrition*; 135(1): 1-4. https://doi.org/10.1093/jn/135.1.1

Grimont, P.A. and Weill, F.X. (2007): Antigenic formulae of the Salmonella serovars. WHO collaborating centre for reference and research on Salmonella, 9th edition. 9, 1-166 .

He, W.; Li, P. and Wu, G. (2021): Amino acid nutrition and metabolism in chickens. Amino Acids in Nutrition and Health: Amino Acids in the Nutrition of Companion, Zoo and Farm Animals, 109-131.
doi: 10.1007/978-3-030-54462-1_7.

Huehn, S.; La Ragione, R.M.; Anjum, M.; Saunders, M.; Woodward, M.J. and Bunge, C., et al. (2010): Virulotyping and Antimicrobial Resistance Typing of Salmonella enterica Serovars Relevant to Human Health in Europe. *Foodborne Pathogens and Disease*, 7(5): 523-535. https://doi.org/ 10.1089/fpd.2009.0447

Jamiroz, D.; Orda, J.; Kamel, C.; Wiliczkiewicz, A.; Wertelecki ,T. and Skorupinska, J. (2003): The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. *J Anim Feed Sci*., 12(3): 583-596.
DOI:10.22358/jafs/67752/2003

Johnson, N.F.; Velasquez, N.; Restrepo, N.J.; Leahy, R.; Gabriel, N. and EL Oud, S. et al. (2020): The online competition between pro- and anti-vaccination views. *Nature*, 582(7811): 230-233.
DOI: https:// doi.org/10.1038/s41586-020-2281-1

Kehrenberg, C. (2006): Identification of the plasmid-borne quinolone resistance gene qnrS in Salmonella enterica serovar Infantis. *Journal of Antimicrobial Chemotherapy*, 58 (1): 18-22. https://doi.org/10.1093/jac/dkl213

Khodadadipour, T.; Amini, K. and Mahmoudi, R. (2016): Evaluation of virulence and enterotoxin genes in Salmonella enteritidis strains isolated from Meat and Egg samples by Multiplex-PCR. *Journal of Food Microbiology*, 3 (2): 25-33. https://sid.ir/paper/250772/en
doi:10.1292/jvms.23-0255.

Kurokawa, A., and Yamamoto, Y. (2023). Immunohistochemichal identification of T and B lymphocytes in formaline fixed paraffin embedded tissues of 35 avian species using commercial antibodies. J Vet Med Sci. 1,85(10):1121-1130. doi:10.1292/jvms.23-0255.

Langhout, D.J.; Schutte, J.B.; Van Leeuwen, P.; Wiebenga, J. and Tamminga, S. (1999): Effect of dietary high-and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *British Poultry Science*, 40 (3): 340-347. https://doi.org/10.1080/00071669987421

Lekagul, A.; Tangcharoensathien, V. and Yeung, S. (2019): Patterns of antibiotic use in global pig production: A systematic review. *Veterinary and animal science*, 7, 100058-100058.https://doi.org/10.1016/j.vas.2019.100058

Li, P. and Wu, G. (2017): Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids*, 50, 29-38. DOI: 10.1007/s00726-017-2490-6

Liu, Z.; Wu, Q.; Jiao, C.; Cheng, B.; Zhu, D. and Ma, Y. et al. (2020): Effects of Glutamine on the Mucosal Structure and Immune Cells in the Intestines of Broiler Chickens Challenged with Salmonella Enteritidis. *Brazilian Journal of Poultry Science*, 22, eRBCA-2020.https://doi.org/10.1590/1806-9061-2020-1270

Mohamed, F.; Thabet, M. and Ali, M. (2019): The use of probiotics to enhance immunity of broiler chicken against some intestinal infection pathogens. *SVU-International Journal of Veterinary Sciences*, 2(1): 1-19.
DOI: 10.21608/svu.2019.23141

Mohammed, E.E.S. (2014): Studies on recent methods for detection of Salmonella species isolated from poultry.M.V.Sc., Thesis, Benha Univ. Egypt.

Murugkar, H.; Rahman, H. and Dutta, P. (2003). Distribution of virulence genes in Salmonella serovars isolated from man &

animals. *Indian Journal of Medical Research*, 117, 66-70.

Nhung, N.T.; Cuong, N.V.; Thwaites, G. and Carrique-Mas, J. (2016): Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. *Antibiotics*, 5(4): 37. **https://doi.org/10.3390/antibiotics5040037**

Oso, A.O.; Suganthi, R.U.; Reddy, G.B.M.; Malik, P.K.; Thirumalaisamy, G. and Awachat, V.B. et al. (2019). Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. *Poultry Science*; 98 (10): 4755-4766. https://doi.org/ 10.3382/ps/pez191

Ospina-Rojas, I.C.; Murakami, A.E.; Eyng, C.; Nunes, R.V.; Duarte, C.R.A. and Vargas, M.D. (2012): Commercially available amino acid supplementation of low-protein diets for broiler chickens with different ratios of digestible glycine+serine:lysine. *Poultry Science*, 91 (12): 3148-3155. https://doi.org/10.3382/ps.2012-02470

Abu-basha, E.A.; Gehring, R. and Albwaneh, S.J. (2007): Pharmacokinetics and bioavailability of spectinomycin after i.v., i.m., s.c. and oral administration in broiler chickens Journal of Veterinary Pharmacology and Therapeutics 30(2): 139-144. https://doi.org/ 10.1111/j.1365-2885.2007.00825.

Pilonieta M.C.; Nagy, T.A.; Jorgensen, D.R. and Detweiler, C.S. (2012): A glycine betaine importer limits Salmonella stress resistance and tissue colonization by reducing trehalose production. Molecular Microbiology, 84(2): 296-309. https://doi.org/10.1111/j.1365-2958.2012.08022.x

Prager, R.; Mirold, S.; Tietze, E.; Struta, U.; Knuppel, B. and Rabsch, W., et al. (2000): Prevalence and polymorphism of genes encoding translocated effector proteins among clinical isolates of Salmonella enterica. *International Journal of Medical Microbiology*, 290 (7): 605-617. https://doi.org/10.1016/S1438-4221(00)80009-0

Quinn, G.P. and Keough, M.J. (2002): Experimental Design and Data Analysis for Biologists, Cambridge University Press.

Rabie, N.S.; Khalifa, N.O.; Radwan, M.E. and Afify, J.S. (2012): Epidemiological and molecular studies of Salmonella isolates from chicken, chicken meat and human in Toukh, Egypt. *Global Vet*., 8(2): 128-132.

Khairy, R.M.M. (2015): Anti-Microbial Resistance of Non-Typhoid Salmonella in Egypt. *Fermentol Techno.*,4(123):2. 04.DOI: 10.4172/10.2167-7972.1000123

Robicsek,A; Jacoby ;G.A. ;Hooper ;D.C. (2006). The worldwide emergence of plasmid mediated quinolone resistance. Lancet Infect. Dis. 6, 629–640.

Roberts, B.W.; Kuncel, N.R.; Shiner, R.; Caspi, A. and Goldberg, L.R. (2007): The Power of Personality: The Comparative Validity of Personality Traits, Socioeconomic Status, and Cognitive Ability for Predicting Important Life Outcomes. *Perspectives on Psychological Science*, 2(4): 313-345. https://doi.org/10.1111/j.1745-6916.2007.00047.x

Shah, D.H.; Zhou, X.; Addwebi, T.; Davis, M.A.; Orfe, L. and Call, D.R., et al. (2011). Cell invasion of poultry-associated Salmonella enterica serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. *Microbiology*, 157(5): 1428-1445. doi: 10.1099/mic.0.044461-0

Shalaby, A.; Ismail, M.M. and El-Sharkawy, H. (2022): Isolation, Identification, and Genetic Characterization of Antibiotic Resistance of Salmonella Species Isolated from Chicken Farms. *Journal of tropical medicine*, 1, 6065831. https://doi.org/10.1155/2022/6065831

Shamoto, K. and Yamauchi, K. (2000): Recovery responses of chick intestinal villus morphology to different refeeding procedures. *Poultry Science*, 79(5): 718-723. https://doi.org/10.1093/ps/79.5.718

Souffrant, W.B. (2001): Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. *Animal Feed Science and Technology*, 90 (1-2): 93-102. https://doi.org/10.1016/S0377-8401(01)00199-7

Sun, J.; Liu, D. and Shi, R. (2015): Supplemental dietary iron glycine modifies growth, immune function, and antioxidant enzyme activities in broiler chickens. *Livestock Science*, 176, 129-134. https://doi.org/ 10.1016/j.livsci.2015.03.004

Sunita, S.; Rakesh, S.; Reddy, A.; Sharma, R. and Varsha, S. (2000): Antibiotic sensitivity of Salmonella typhimurium isolates from domestic poultry. Indian Veterinary Journal,77, 998-999.

Viveros, A.; Chamorro, S.; Pizarro, M.; Arija, I.; Centeno, C. and Brenes, A. (2011): Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, 90 (3): 566-578. https://doi.org/ 10.3382/ps.2010-00889

Weibel, E.R. (1969): Stereological Principles for Morphometry in Electron Microscopic Cytology. International Review of Cytology, 26, 235-302. https://doi.org/ 10.1016/S0074-7696(08)61637-X

*Wenliang He; Li P and Wu G (*2021). Amino acid nutrition and metabolism in chickens. Amino Acids in Nutrition and Health: Amino Acids in the Nutrition of Companion, Zoo and Farm Animals; 109-131.

Wood, M.W.; Jones, M.A.; Watson, P.R., Hedges, S.; Wallis, T.S. and Galyov, E.E. (1998): Identification of a pathogenicity island required for Salmonella enteropathogenicity. *Molecular Microbiology*, 29 (3): 883-891. https://doi.org/10.1046/j.1365-2958.1998.00984.x

Wu, G. (2014): Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. J Animal Sci Biotechnol., 5, 34. http://www.jasbsci.com/content/5/1/34

Yang, B.; Qu, D.; Zhang, X.; Shen, J.; Cui, S. and Shi, Y., et al. (2010): Prevalence and characterization of Salmonella serovars in retail meats of marketplace in Shaanxi, China. *International Journal of Food Microbiology*, 141(1-2): 63-72. https://doi.org/10.1016/j.ijfoodmicro.2010.04.015

Zhong ;Z. ;Wheeler ;M.D. ;Li ;X.L. ;Froh M., S;chemmer; P. Yin ;M. Bun zendaul ;H., Bradford, B., Lemasters, J.J., 2017., Glycine: a novel anti-inflammatory, immunomodulatory, and cytoprotective agent. Curr Opin Clin Nutr Metab Care 6, 229–240.

Zhu, X.; Shang, X.; Li, N.G.; Li, H.; Feng, X. and Zhang, H. (2022): Effects of Zinc Glycinate on Growth Performance, Serum Biochemical Indexes, and Intestinal Morphology of Yellow Feather Broilers. *Biological Trace Element Research*, 200, 4089-4097. https://doi.org/10.1007/s12011-021-02990-x

تقييم دور الأحماض الأمينية الأساسية للحالة المناعية ضد الإصابة بعدوى السالمونيلا في بدارى التسمين

زغلول خضر ، رانيا غانم ، جيهان صالح ، مروة شلبى ، محمد عبد الرازق ، هاله اسماعيل

Email: zaghlolkheder0@gmail.com Assiut University web-site: www.aun.edu.eg

تعد السالمونيلا السبب الرئيسي للأمراض الحيوانية المنشأ التي تنتقل عن طريق الغذاء في جميع أنحاء العالم، لذا تسعى دراستنا إلى البحث في تأثير الأحماض الأمينية على الحالة المناعية للدواجن ضد عدوى السالمونيلا. تم تربية ١٨٠ فرخاً بعمر يوم واحد (روس ٣٠٨)، وتم أخذ مسحات للكشف عن السالمونيلا. قسمت الكتاكيت إلى ٥ مجاميع عشوائياً كما يلي: المجموعة الأولى غير المصابة وغير المعالجة، المجموعة الثانية تمت اصابتها بالبكتيريا المعوية في اليوم السابع المجموعة الثالثة G3 تمت اصابتها بالمعويات في عمر اليوم السابع وأعطيت أحماض أمينية ١سم/لتر ٣ أيام متتالية في الأسبوع. المجموعة الرابعة G4 غير مصاب ودعمت بالأحماض الأمينية ١سم/لتر لمدة ٣ أيام متتالية في الأسبوع و المجموعة الخامسة G5 مصاب بالسالمونيلا. زاد تأثير الأحماض الأمينية الأساسية على زيادة وزن الجسم بشكل ملحوظ في G4. كان مستوى IgG منخفضًا جدًا في G4، خاصة في اليوم ٣٥، بينما كان مرتفعًا في اليوم ٣٥ في G5. بالنسبة لمستوى IgM، كان منخفضًا بشكل ملحوظ في G4، خاصة في اليوم الثامن والعشرين، ومرتفعًا جدًا في G2 في اليوم الثامن والعشرين. ارتفع مستوى إنزيم الجلوتاثيون بيروكسيديز بشكل ملحوظ في G4 عند عمر ٣٥ عامًا. تم استخراج إجمالي ٥٨ (١٢,٨٩٪) من عزلات السالمونيلا من ٤٥٠ عينة. تم اكتشاف جينات sopB وbcfC وstn في جميع العزلات، بينما غاب جين spvC في جميع العزلات. كما تم اكتشاف الجين (qnrA) في عزلة واحدة فقط بينما تم اكتشاف الجين (qnrB) في عزلتين. تم تحسين التغيرات النسيجية المرضية في كل من الكبد والأمعاء في مجموعات الأحماض الأمينية المكملة حتى مع الإصابة، وهو ما تمت الإشارة إليه من خلال الأداء الجيد لحمة الكبد وخلايا الكبد، والخلايا الكأسية الوفيرة، وتضخم الغشاء المخاطي المعوي. الى جانب ذلك، زادت الفعالية المناعية (CD3A وCD79A) بشكل ملحوظ مقارنة مع المجموعات الأخرى. مكملات الأحماض الأمينية تحسن من أداء ومناعة الدواجن وتقلل من استعمار بكتيريا السالمونيلا في طيور التربية.