52

Egypt. J. Vet. Sci. **Vol. 56,** No. 3, pp. 543-557 (2025)

Egyptian Journal of Veterinary Sciences

Efficacy of *Astragalus Polysaccharide* versus Enrofloxacin on *Campylobacter Jejuni* Colonization in Broiler Chickens

Maha S. Abd Elhafeez[1], Fatma H. Amro[2], Heba E. Farhan[3], Pierre E. Mehanny[4], Farida H. Mohamed[5], Gomaa Y. Elhaddad[6], Eman Elkhawaga[7], Amany Abdelnaby[8] and Fady Sayed Youssef[9*]

[1]*Pharmacology and Pyrogen Unit, Biochemistry, Toxicology, Feed Deficiency Department, Animal Health Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt.*
[2]*Food Hygiene Dep., Animal Health Research Institute, Agriculture Research Center, Giza 12618, Egypt.*
[3]*Bacteriology Dept., Animal Health Research Institute, Dokki, Giza 12618, Egypt.*
[4]*Biochemistry, Toxicology, Feed Deficiency Department, Animal Health Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt.*
[5]*Immunology Dep., Animal Health Research Institute, Agricultural Research Center, Giza 12619, Egypt.*
[6]*Biotechnology Dep., Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute Damanhur Branch, 22511 , Agriculture Research Center, Egypt.*
[7]*Food Hygiene Dept., Animal Health Research Institute, Mansoura, Agricultural Research Center, Egypt.*
[8]*Toxicology Dep., Animal Health Research Institute, Banha Branch, 13512, Agricultural Research Center, Egypt.*
[9]*Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.*

Abstract

Campylobacteriosis is the world's most common zoonotic disease, affecting broiler chickens. A study was conducted to assess the effectiveness of *Astragalus polysaccharides* (APS) versus the antibiotic enrofloxacin (ENR) in controlling *Campylobacter jejuni*. A total of 160 Hubbard broiler chicks (1 day old) were randomly divided into eight groups: healthy chicks (H), healthy chicks given ENR (HA), APS (HP), both (HAP), experimentally infected chicks (I), infected chicks given ENR (IA), APS (IP), and both (IAP). On days 18 and 35, the *Campylobacter* load was assessed in the cecal contents. The study also evaluated serum immune parameters at various intervals post-infection. Three chickens were slaughtered after the last oral dose of ENR to determine ENR and its metabolite ciprofloxacin (CIP) residues by HPLC in different tissues. The experiment involved estimating the antioxidants of breast muscles, as well as their physico-chemical characteristics, along with measuring serum liver enzymes. The treated groups showed a significant decrease in bacterial count, with no growth observed in the IA and IAP groups after 35 days. ENR and APS significantly reduced serum interleukin-6 (IL-6) and nitric oxide (NO) levels in infected groups while increasing lysozyme levels in all experimental groups. APS lowered MDA levels and increased both SOD and GSH levels in breast muscle. Serum ALT and AST activities were higher in I- and IA- groups compared to healthy and APS-administered groups. APS did not affect marker residue concentrations in the HAP group tissues, but increased concentrations in the IAP group tissues without an increase in drug withdrawal time (3 days). These findings suggest that APS is necessary to improve meat quality and immunity, reduce oxidative damage induced by enrofloxacin and infection, and demonstrate anti-*Campylobacter* action in vivo.

Keywords: Enrofloxacin, *Campylobacter*, *Astragalus polysaccharides* (APS), withdrawal time, oxidative damage.

Introduction

Campylobacteriosis is the most prevalent zoonotic disease affecting humans. *Campylobacter spp.* is a member of the saprophytic microflora that exists in the digestive tracts of a variety of wild and domestic animal species, particularly poultry [1]. Species that can infect humans include *C. jejuni, C. lari, C. coli,* and *C. upsaliensis*, with *C. jejuni* being the predominant zoonotic pathogen implicated in foodborne bacterial gastroenteritis [2]

The highest concentrations of this Gram-negative bacterium are found in poultry, particularly broilers, which are considered a natural reservoir for *C. jejuni*. Most broilers carry the bacterium in their digestive tract without showing any clinical symptoms, posting a significant risk during slaughtering and meat processing due to intestinal content contamination leading to cross-infection of broiler carcasses [3].

These thermotolerant *Campylobacter* species can be transmitted from the broilers to humans through direct contact or by consuming or handling contaminated food products or water. Therefore, broilers and broiler products are believed to be the primary food sources of human illness [4].

The situation raises the possibility of implementing mandatory control measures, such as antibiotics, which were frequently used in chicken feed or water to reduce or eliminate *C. jejuni* from poultry flocks, thus decreasing the occurrence of human infections [5].

Enrofloxacin (ENR) is one of the most widely prescribed fluoroquinolone antibiotics for *Campylobacter* infections [6]. Because of its potent anti-pathogen properties and appropriate pharmacokinetic profile, this second-generation fluoroquinolone synthetic antimicrobial is frequently used in chickens.

As the demand for antibiotic-free poultry products grows, it is critical to find novel methods of combating these foodborne pathogens in broilers, such as using a variety of safe alternatives to lower or eliminate the infection load in poultry [7].

Green feed additives, such as plant polysaccharides, appear to be the most intriguing of these alternatives because of their safety properties, lack of resistance, and minimal toxic and negative effects. It is rational to add these to the feedstuff to enhance production efficiency, intestinal micro ecology, antioxidant activity, and the quality of poultry flocks and products [8].

Astragalus polysaccharides (APS) are active components and the crucial constituent of a water-soluble heteropolysaccharide obtained from the Chinese medicinal plant *Astragalus membranaceus* stems or dried roots. Meanwhile, *Astragalus membranaceus* is an annual herb that belongs to the *Leguminosae* family. Interestingly, APS exhibits extensive biological properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, anti-stress, anticancer effects along with immunomodulatory capabilities [9].

Additionally, APS is frequently employed as an immunological adjuvant, being recognized as a type of macromolecule that significantly affects the immune system [10]. Supplementation of APS in chicken feed additives has been proven to significantly improve average daily gain, feed conversion ratio, growth performance, physiological function, immunity, and antioxidant activity. Despite the active components and outstanding biological attributes of *Astragalus polysaccharides*, not much research has been done on its effectiveness in controlling *Campylobacter* infections [11].

Considering the information presented above and the importance of food safety and human health, a study was established and executed to investigate the potential effectiveness of Enrofloxacin and the herbal feed additive *Astragalus polysaccharides* against *Campylobacter jejuni* in broiler chickens.

Material and Methods

Ethical statement

The current protocol was permitted by the Institutional Animal Care and Use Committee in the Veterinary Medicine Faculty, Agriculture Research Center (IACUC Protocol number: ARC/AHRI/4/24).

Drugs

Enrofloxacin (Avitryl®): Oral solution (10%), obtained from Pharma-Swede Co., Egypt.

APS product (Mega Immune®): Poultry Immune Booster *Astragalus Polysaccharide* purchased from Shijiazhuang ZDHF Stock-raising Co., LTD (Shijiazhuang City, Hebei, China).

Experimental design

The trial was conducted in Animal Biosafety Level 2, a facility authorized to perform animal testing (no.C-22-745-1). It was carried out following the general principles and detailed recommendations outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching [12]. Prior to the start of the trial, the room, food system, and drinking systems were thoroughly cleaned and disinfected.

One hundred and sixty Hubbard broiler chicks (one day old) were obtained from Al-Kahira Poultry Company, located on the 10th of Ramadan City, Egypt. They were randomly divided into eight groups, with 20 broilers in each group. The study design is shown in the Table (1).

Isolation, identification, and confirmation of C. jejuni

To isolate *Campylobacter spp.* as described by [14], 1 mL of cloacal content from diseased broilers collected from different farms in the Giza government, was suspended in nine milliliters of Preston enrichment broth, which includes supplements for *Campylobacter* growth (CM067, SR048, SR117, and SR232; Oxoid Ltd., Cambridge, UK). For 48 hours, the suspensions were incubated at 42°C in microaerobic conditions (5% O_2, 10% CO_2, and 85% N2). A hundred µL was distributed on chromogenic selective culture medium plates (CHROMagar™ *Campylobacter* base) (NORTHEAST LABORATORY SERVICES Maine ME 04901, USA) and incubated for 48 hours at 42°C under microaerobic circumstances. Before being used, the developed *Campylobacter* cultures were then frozen at -80°C in MH broth enhanced with 30% glycerol (v/v). The boiling procedure was utilized to extract the DNA of *C. jejuni* [15] for conventional PCR analysis.

Chick colonization assay:

On day 11, all the birds were given I mL of 1×10^8 CFU of *C. jejuni* / mL in a suspension of phosphate buffer saline (PBS) by oral gavage for challenge [16].

The basal diet was supplemented with 0.3 g/kg feed of APS [8] from the first day of age. Enrofloxacin was orally administered at 10 mg/kg body weight for 5 consecutive days [17] via stomach tube after infection symptoms, such as diarrhea, appeared and were confirmed with cloacal swabs for the isolation of *C. jejuni*.

Sample collection:

For the residue study, three chickens were slaughtered on the 1st, 3rd, 5th, 7th, and 9th day following the last oral dosage of the ENR antibiotic. Sections of liver, kidneys, and muscular tissue were taken from the slaughtered birds for residue quantification of enrofloxacin and its metabolite ciprofloxacin by HPLC analysis. The samples were homogenized separately using a blender, and 1 gram of each sample was accurately weighed, and transferred into a polypropylene centrifuge tube (50 mL), then saved at -20ºC for further chromatographic analysis.

After taking blood samples from the wing vein, they were transferred instantly to centrifuge tubes. Following 20 minutes of centrifugation at 4000 Xg at 4°C, the serum was collected and stored at -20°C for immune parameter analysis on the 3rd, 7th, 14th, and 21st dpi and hepatic enzymes (ALT and AST) on day 35.

On day 35, the breast muscles were harvested after the broilers were euthanized. They were then homogenized in 0.75% cold saline buffer and centrifuged for ten minutes at 2500 Xg/ 4°C. The

supernatant was frozen (-18°C) till the investigation of antioxidant function.

For bacterial count, three chickens from infected groups (I-, IA-, IP-, and IAP- groups) at 24 hr. after the last oral dose of enrofloxacin (day 18) and on day 35 birds were euthanized by cervical dislocation after anesthesia. Each chicken was dissected, and the internal organs were removed. Cecal contents were collected and transferred to the laboratory immediately to count *C. jejuni*.

C. jejuni count in caeci contents:

The counting was done according to [18]. One gram of cecal contents was collected and added to a 9mL sterilized physiological saline solution (0.9%) then 10-fold serially diluted. The dilution was spread on CHROMagar™ *Campylobacter* base medium and incubated for 48 hours at 42°C in a microaerophilic setting. The plates were examined to look for typical colonies of *C. jejuni*, and their number was totaled and expressed as log 10 colony forming units (cfu) per gram.

Evaluation of the immune parameters:

Serum interleukin-6 (IL-6) was measured using a commercial ELISA kit, catalog number: ECH0046 (Fine Test, Wuhan Fine Biotech Co., Ltd., Wuhan, China). The procedure followed the manufacturer's instructions.

Serum nitric oxide (NO) was assayed according to [19] using Griess reagent after serum deproteinization with zinc sulfate and sodium hydroxide, and nitrate reduction to nitrite with copper-plated cadmium. Following that, an ELISA plate reader was used to measure the optical density at 545 nm. By utilizing a standard curve and various sodium nitrite concentrations, the NO concentration was calculated.

Serum lysozyme was measured using the agarose gel plate lyses assay as described in [**20**]. Briefly, *M. lysodeikticus* bacteria (50 mg/100 ml agarose) was dissolved in 1% agarose in PBS, 67 mmol/L at pH 6.3, to create lysoplates. Each well was supplemented with 25 µL of serum samples. Eighteen hours later, the inhibition zone diameters were measured. By preparing a logarithmic curve with various concentrations of a standard lysozyme solution, the level of the lysozyme was determined.

Liver enzymes determination:

Alanine transaminase (ALT) and aspartate aminotransferase (AST) were estimated quantitatively according to [21] and [22].

Physicochemical Characterization of carcass breast muscle:

Using a pH meter (Jenway 3,505, Staffordshire, United Kingdom), the pH of breast meat was

determined. Two buffers with pH values of 4 and 7 were used to calibrate the pH probe, and the process was repeated between samples. The chemical constituents of broiler breast meat including protein [23], fat [24], and ash [25] contents were estimated. The Association of Analytical Chemists' guidelines were followed in the analysis of the contents of specimens of breast muscle.

Breast Muscle Antioxidant Function

Using commercial kits from Nanjing Jincheng Bioengineering Institute (Nanjing, China), the levels of glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) were measured in breast muscles.

Residues of enrofloxacin and its metabolite, ciprofloxacin, in various tissues:

The tissue level of ENR and its metabolite, ciprofloxacin (CIP), was determined according to [26]. This was done using solid phase extraction followed by detection with the Agilent HPLC Series 1200, which included an autosampler, quaternary pump, MWD detector, and Chemstation software for the Agilent system (Hewlett-Packard, Les Ulis, France). The method was verified following the ICH Q2(R1) guidelines [27].

The mobile phase consisted of acetonitrile and 0.05M sodium phosphate monobasic in ratio of 35:65 (v/v), with a pH of 2.5 using 3.5mM sodium lauryl sulfate. The flow rate was maintained at 0.8 milliliters per minute. Chromatography was carried out at 20°C using a UV wavelength of 278 nm. An Agilent-C18 stationary phase (4.6 × 250 mm, 5μm i.d.) was used for separation. Reference standards of enrofloxacin and its metabolite ciprofloxacin from Sigma Aldrich Co. were initially dissolved in 0.1 N caustic soda to prepare a stock standard solution, then diluted in the mobile phase to prepare the intermediate level (10 ppm) and the working levels ranging from 10-1000 ppb) for calibration plots. To ensure the quality control of the chromatographic method, blank tissues from the (H group) were fortified with various levels (1/2 MRL, MRL, 2 MRL) in triplicate to calculate recovery and precision. The limit of detection and quantification (LOD& LOQ) were calculated based on the standard deviation of the intercept (S) and the slope (a) $[LOD = 3.3 * \frac{S}{a} \ \& \ LOQ = 10 * \frac{S}{a}]$. Before analysis, all spiked matrices were left at room temperature for 30 minutes.

To extract from tissues, mix 1 gram of each sample with 5 milliliters of 0.3 percent metaphosphoric acid in cyanomethane (1:10, v/v), then homogenize for three minutes. Sonicate the mixture at 200 mA, then centrifuge for 10 minutes at 20000 rpm. Collect the upper layer for purification via SPE Cartridge after preconditioning with 1 mL of carbinol and 1 mL of HPLC purified water, then

elution using 1 mL of mobile phase. Filter the eluent using 0.45 μm acrodiscs (Micropore). Introduce 50 microliters of the filtered eluent into the HPLC apparatus.

Statistical Analysis

SPSS software (version 20.0, SPSS Inc.) was used to analyze the data (Chicago, Illinois). For repeated group comparisons, a one-way analysis of variance with an LSD post hoc test was employed. The standard deviation (SD), the standard error (SE) plus the mean were used to express the values. *P-values* were regarded as statistically significant if they were less than 0.05 [28].

Results

Symptoms & Gross lesions of infected chickens:

The symptoms appeared 48 hours after the experimental infection. Diarrhea, pasty vent plumage, and depression were the three clinical signs observed in the affected birds. Gross lesions showed focal hepatic necrosis in some cases, as well as distension from the jejunal region to the two caeci with an accumulation of watery fluid.

Isolation, identification, of C. jejuni:

Figure 1 shows the isolated strains of *C. jejuni* from the collected samples on CHROMagar™ *Campylobacter* base selective medium. It was observed that *C. jejuni* appeared at 816 bp (Figure 2) using conventional PCR.

C. jejuni count in caeci contents:

Table (2) shows a significant decrease in the count was achieved in the IAP-group received followed by the IA-group and then the IP-group. It was found that there was no *C. jejuni* found (no growth) on 35 days in both IA and IAP groups (Fig. 3).

The serum lysozyme level:

As depicted in Fig. 4, the I group had significantly higher lysozyme levels early post-infection at 3 dpi compared to the H and HA groups. However, these levels decreased significantly in comparison to the other experimental groups at 14 and 21 dpi. In the IP and IAP groups, lysozyme levels were significantly upregulated compared to the I group at 14 and 21 dpi, approaching normal values. The upregulation in the infected groups was higher in the IAP group than in the IA and IP groups. Furthermore, in the healthy groups, the HP and HAP groups displayed the highest values of lysozyme among the other healthy groups. They exhibited a significant increase compared to the I group at 14 dpi and the H, HA, and I groups at 21 dpi.

The serum IL-6 and NO levels

The results of the IL-6 and NO assays, shown in Figures 5 and 6, revealed that the I-group induced significantly higher IL-6 and NO values compared to

all other groups throughout the experiment. However, all treated infected groups exhibited significantly lower levels of IL-6 and NO compared to the I group. In the IAP group, IL-6 reached normal values at the 7th dpi, and NO reached normal values in the IA-, IP-, and IAP- groups, at the 14th and 21st dpi. The downregulation was more pronounced in the IAP- group than in the IA- and IP- groups. Additionally, in the healthy groups, APS in the HP- and HAP- groups induced slightly increased IL-6 and NO levels compared to the other healthy groups throughout the experiment.

Breast muscle antioxidants:

The inclusion of APS in feed significantly reduced MDA levels in all chicken groups ($p<0.05$), while also significantly increasing SOD and GSH levels in chicken breast muscle at $p<0.05$. The addition of ENR did not have a significant impact on breast muscle antioxidants in both healthy and experimentally infected groups, with the exception of the MDA levels which significantly decreased in the IA group ($p<0.05$), as displayed in Table 3.

Liver enzyme levels

The average serum ALT and AST activities of various groups are tabulated in Table 4, showing significantly higher levels in infected groups and groups treated with ENR antibiotic ($p<0.05$) in compared to healthy and APS- administered groups.

Physicochemical characterization

Regarding the physicochemical analyses of carcass breast muscle, the average pH ranged from 5.7 to 6.1, with no significant differences among different groups. The chemical composition of the breast muscle indicated a significant decrease in protein levels and a significant increase in fat and ash percentages in experimentally infected broilers compared to healthy ones. The addition of APS in the feed ration significantly increased protein% in all supplemented groups and increased fat and ash percentages only in infected groups as shown in Table (5).

Intra-lab verification results of HPLC assay

Enrofloxacin and ciprofloxacin standards ranging from 10-1000 ppb were diluted in the mobile phase, showing a correlation coefficient of 0.99998 for ENR and 0.99991 for CIP as depicted in Figure 7. The retention times (RT) for ENR and CIP were 4.193 and 5.688, respectively (Figure 8). The recovery rates of ENR and CIP in different tissues ranged from 98-101.5% and 93-95.2%, respectively. LOD for ENR in muscle, liver, and kidneys are 1.2, 1.8, and 2 ppb, and for CIP are 1.6, 1.1, and 0.8 ppb. LOQ for ENR in muscle, liver, and kidneys are 3.8, 5.6, and 6.1 ppb and for CIP are 4.8, 3.3, and 2.5 ppb.

Concentration of enrofloxacin and its metabolite ciprofloxacin

Table 6 shows a significant decline in the marker residues of ENR (sum of ENR &CIP) in the IA group compared to the HA group. APS supplementation did not affect the concentration of the marker residue in analyzed tissues of the HAP group, However, in the IAP group, the concentrations increased significantly without affecting the withdrawal time of the drug from tissues to be safe for human consumption (< MRL set by [29]; 100, 200, and 300 ppb for muscle, liver, and kidneys).

Discussion

Multidrug-resistant bacteria can be reduced by APS [30], potentially lowering the risk of foodborne pathogen infections in the gastrointestinal tract. In this study, we assessed APS's ability to inhibit the colonization of the digestive tract by C. jejuni in commercial broilers. APS could stop colonization and lower the load of *C. jejuni* in broilers ceci when administered alone and in combination with the enrofloxacin antibiotic.

The isolated strains of *C. jejuni* from diseased broilers collected from different farms were isolated on CHROMagar™ *Campylobacter* base selective medium as mentioned by [14] and confirmed using conventional PCR after extraction of DNA by boiling procedure at 816 bp as described by [15].

Eleven-day-old chicks were inoculated with Campylobacter, a closer match to the actual conditions seen during chicken rearing [31]. At 48 hours post-infection, diarrhea, pasty vent plumage, and depression were the three clinical indications of the afflicted birds [32]. *C. jejuni* colonization was assessed in broiler chickens at 24 hours after the last oral dose of enrofloxacin and on day 35 of age. There was a significant decline in all treated groups, as APS has an antibacterial effect [33].

Because APS increases serum lysozyme levels, which are strong antimicrobial agents that combat bacterial, fungal, and viral infections. Lysozyme is a crucial element of the innate immune system. Along with making other medications work better, it also prevents infections, strengthens immunity, and acts as a natural antibiotic [34].

The innate immune system responds to *C. jejuni* infection by activating a series of intracellular signaling pathways that lead to the creation of pro-inflammatory mediators such as IL-6 and NO [35, 36]. In this study, *C. jejuni* infection (I group) resulted in significant increases in serum IL-6 and NO levels compared to the other groups. The elevated levels of IL-6 were consistent with previous research [37]. IL-6, a crucial pleiotropic cytokine, shows a crucial role in promoting the inflammatory and immune responses during bacterial infections

[38]. The increased levels of NO in the I group were also in line with previous findings [36], which showed that *C. jejuni* stimulated nitrite and iNOS production in chickens. Nitric oxide, a powerful antimicrobial molecule, can also induce apoptosis in host cells at higher concentrations [36]. The elevated IL-6 and NO levels can be attributed to the *C. jejuni* effect on stimulating the IL-6 and NO production from M1 macrophages and intestinal epithelial cells [38, 39, 40]. Lysozymes are essential antibacterial elements of the non-specific innate immune response that break down the peptidoglycan layer, a major constituent of bacterial cell walls [41]. The *C. jejuni* control group (I-group) showed significantly higher lysozyme levels at the 3rd dpi, but by the 14th and 21st dpi, the levels were significantly reduced compared to most other groups. The initial increase in lysozyme levels could be owing to the rapid innate immune response, however, the subsequent decline could be due to the apoptotic effects of prolonged infection on immune cells through *C.jejuni's* cytolethal distending toxin [42].

The supplementation of APS, either alone (IP) or combined with ENR (IAP), in infected groups can modulate the detrimental effect induced by *C. jejuni* and downregulate excessive IL-6 and NO levels. This is supported by previous studies that showed APS downregulated IL-6 in necrotic enteritis in chickens [43], shifted macrophages from the M1 to M2 phenotype [44], and reduced the inflammatory NF-kB pathway [45]. Furthermore, APS in IP- and IAP-groups can counteract the immune-suppressive effect of *C. jejuni* infection and significantly increase lysozyme levels at 14th and 21st dpi compared to the I-group. In healthy groups (HP and HAP), APS enhanced innate immune parameters (IL-6, NO, and lysozyme) compared to H- and HA- groups. These findings are consistent with previous research showing that APS increased IL-6 [46], NO [47], and lysozyme [48] levels by stimulating the TLR4-NF-kB pathway in immune cells [49]. The current results demonstrate that APS not only improves the chicken's innate immune response but also reduces excessive inflammatory mediators caused by *C. jejuni* infection. This is in line with [50] showing that APS has diverse immune regulatory effects under different physiological conditions due to its complex structure and various pharmacological effects [51]. Additionally, treatment with antibiotics in parallel with APS (IAP), showed the best results among the challenged groups as enrofloxacin was able to eradicate the bacteria and enhance the immune modulating effects of APS in alleviating the detrimental effects of *C. jejuni* infection [52].

APS has long been thought of as an antioxidant that prevents oxidative damage [53]. The concentration of MDA has been employed to measure lipid peroxidation, which represents the oxidative stress level. Numerous investigations have shown that dietary APS decreased MDA concentration, increased total antioxidant capacity, and improved the activities of antioxidant enzymes in farmed animals [54]. All APS groups demonstrated a significant decline in muscle MDA content and an improvement in total antioxidant capacity, which is consistent with our study. These findings suggested that APS enhanced the broilers' antioxidant capacity, as demonstrated by the results in zebrafish [33]. In broilers, [55] demonstrated that supplementing the feed with 500-1000 mg/kg APS might enhance the performance of growth and serum levels of GSH–Px, SOD, IgM, IgG, and IgA while reducing MDA. APS significantly increased total antioxidant capacity (T-AOC) activity and decreased MDA levels significantly in the serum and breast muscle of chickens [8]. The level of MDA was significantly increased in experimentally infected broilers; however, those administered enrofloxacin antibiotic (IA- and IAP- groups) showed marked improvement compared to the infected group (I group).

The current investigation also showed that in comparison to the H group, the serum MDA levels increased in the enrofloxacin-treated group. Free radicals produced by enrofloxacin bind covalently to microsomal lipids and proteins initiating the peroxidation of membrane polyunsaturated fatty acids [56]. The elevated MDA level suggested increased lipid peroxidation due to tissue damage and an ineffective antioxidant defense system.

Aspartate aminotransferase (ALT) and alanine transaminase (AST) are enzymes used to identify liver damage and liver failure. Increased ALT and AST levels indicate liver disease, Oxidative stress, caused by imbalanced reactive nitrogen species and reactive oxygen species, damages cells and molecules [57].

In the current investigation, the I group was inoculated with *C. jejuni* caused a significant rise in liver enzymes (ALT and AST) due to degenerative alterations and necrotic processes caused by *campylobacter* toxins. The findings published by [58], who reported that *C. jejuni* elevated uric acid, creatinine, and liver enzymes in broilers, corroborated these findings. These effects were accompanied by gross lesions showing focal hepatic necrosis.

In the HA- and IA- groups, which were administered enrofloxacin antibiotic, there was a significant increase in liver enzyme levels. Enrofloxacin works by blocking the catalytic activities of DNA gyrase and topoisomerase IV, affecting nucleic acid processes, and resulting in liver damage. Stable fluoroquinolone protein and cation complexes, long-term oxidative stress, and enduring changes in gene regulation are the causes of this [59].

The results of this research demonstrate that APS is effective in enhancing antioxidant status and preventing oxidative stress caused by C. *jejuni* and enrofloxacin by reducing lipid peroxidation, raising TAC, and lowering ROS generation. Hepatic enzymes were controlled in all APS-treated groups. Additionally, the data demonstrated that APS might protect the liver from intracellular oxidative damage by restoring normal liver function enzyme activity, which is in agreement with [60]. By stabilizing plasma membranes and preserving hepatocyte structural integrity, APS may be able to restore the hepatoprotective effect and heal liver tissue damage that has occurred after infection and enrofloxacin treatment demonstrating the regenerative property of APS.

No significant variations were seen in the pH of the pectoral muscle across any of the experimental groups. Similarly, prebiotics delivered In Ovo did not affect the pH of the flesh of Kuroiler chicks infected with Eimeria species [61]. APS improves meat quality as it increases the crude protein in healthy and C. *jejuni* infected broilers and decreases fat and ash percentages in infected groups (IP and IPA) [62]. According to [62], broiler meat quality can be improved by adding 0.5 g/kg of Chinese yam polysaccharide (CYP) to the diet. This can be achieved through increases in shear force, antioxidant capacity, carcass quality, and meat color. [63] found that APS improves the meat quality of *Furong crucian* carp after supplementation with 0.05%, 0.10%, and 0.15% APS. The low-fat content may be due to reduced lipase activity and fat absorption in the small intestine of broilers.

Enrofloxacin and its metabolite, ciprofloxacin, were found in high concentrations in liver, kidney, and muscle tissues, with the highest concentrations observed in the kidney and liver. Throughout the study, the liver consistently had higher enrofloxacin content compared to the muscles, in line with previous findings [64]. This difference is attributed to enrofloxacin's lipophilicity and limited protein binding capacity [65].

Enrofloxacin undergoes hepatic metabolism, primary producing ciprofloxacin as well as minor metabolites, like oxo-ciprofloxacin, and enrofloxacin amide [66]. Contrary to previous research [64], our study found a high degree of enrofloxacin transformation to ciprofloxacin. Tissue samples from enrofloxacin-treated chickens were analyzed to track this metabolism. In both the HA- and HAP- groups, the withdrawal period for ENR antibiotic to be safe for human consumption is 5 days. Although the addition of APS did not impact the drug's withdrawal period, in infected chickens, the APS increased the concentration of the residue in the analyzed tissues, as APS can enhance drug absorption and protect it from degradation in the gastrointestinal system. Polysaccharides particles possess the ability to adhere to surfaces of mucosal epithelium, aiding in drug trafficking through the mucosal epithelium and increasing their bioavailability [67].

Conclusion

According to recent studies, *Astragalus polysaccharides* (APS) have shown anti-*Campylobacter* activity in vivo, which may help prevent colonization of *Campylobacter jejuni* in broiler chickens. APS is a helpful antioxidant that can reverse oxidative stress and tissue damage caused by enrofloxacin antibiotic and infection, whether administered alone or combined with enrofloxacin. Additionally, APS is necessary to improve the immunity of the bird and the meat quality of the carcass. Based on these encouraging results, it is recommended to administer these herbs in feed in suitable ways.

TABLE 1. Identification of experimental groups

Group		Infection	APS	AB
Healthy	H	-	-	-
Healthy+ Antibiotic	HA	-	-	+
Healthy+ Polysaccharides	HP	-	+	-
Healthy+ Antibiotic+ Polysaccharides	HAP	-	+	+
Infected	I	+	-	-
Infected+ Antibiotic	IA	+	-	+
Infected+ Polysaccharides	IP	+	+	-
Infected+ Antibiotic+ Polysaccharides	IAP	+	+	+

TABLE 2. *C. jejuni* count (log 10 CFU/gm) in cecal contents of experimentally infected broilers after supplementation with ENR and APS.

Group	On day 18	On day 35
I	8.4 ± 0.4^a	8.64 ± 0.6^a
IA	4.2 ± 0.4^b	No growth
IP	7.3 ± 0.3^c	5.5 ± 0.5^b
IAP	2.7 ± 0.4^d	No growth

The data that carry different alphabetical letters within the same column is considered significant at $p<0.05$.
Data expressed as mean $\pm$ SD & n= 6.

TABLE 3. Effect of APS versus ENR on breast muscle antioxidants on 35th day in experimentally infected chickens with *C. jejuni*.

Group	MDA (nmol/mg)	SOD (U/mg)	GSH (µmol/mg)
H	0.25 ± 0.01^{abf}	33.1 ± 0.56^{ab}	3.50 ± 0.11^{ab}
HA	0.25 ± 0.01^{ab}	31.9 ± 1.42^{ab}	3.55 ± 0.11^{abe}
HP	0.22 ± 0.02^c	$36.5 \pm 0.5c$	3.74 ± 0.12^{be}
HAP	0.20 ± 0.01^c	$36.7 \pm 0.5c$	3.76 ± 0.15^{be}
I	0.30 ± 0.02^d	25.9 ± 0.9^d	2.24 ± 0.08^c
IA	0.32 ± 0.01^e	26.7 ± 1.0^d	2.35 ± 0.14^c
IP	0.27 ± 0.01^{abf}	33.7 ± 0.42^a	3.22 ± 0.18^d
IAP	0.27 ± 0.01^{af}	33.7 ± 0.47^a	3.29 ± 0.18^d

The data that carry different alphabetical letters within the same column is considered significant at $p<0.05$.
Data expressed as mean $\pm$ SD & n= 6.

TABLE 4. Effect of APS versus ENR on hepatic enzyme activities in experimentally infected chickens with *C. jejuni*.

Group	ALT (U/L)	AST (U/L)
H	3.75 ± 0.05^a	110 ± 3^a
HA	5.23 ± 0.06^b	142.3 ± 10.7^b
HP	3.39 ± 0.16^c	102.7 ± 4^a
HAP	3.42 ± 0.24^c	104.7 ± 5^a
I	7 ± 0.17^d	248.3 ± 20.8^c
IA	10.03 ± 0.21^e	289.7 ± 17.1^d
IP	3.87 ± 0.25^a	102 ± 8^a
IAP	3.94 ± 0.07^a	100.3 ± 8.7^a

The data that carry different alphabetical letters within the same column is considered significant at $p<0.05$.
Data expressed as mean $\pm$ SD & n= 6.

TABLE 5. Effect of APS versus ENR on physicochemical characterization of broiler breast muscle in experimentally infected chickens with *C. jejuni*.

Group	pH	Protein%	Fat%	Ash%
H	5.87 ± 0.16	20.4 ± 0.09^a	1.67 ± 0.03^a	0.77 ± 0.01^a
HA	5.9 ± 0.01	20.6 ± 0.48^a	1.67 ± 0.02^a	0.78 ± 0.03^a
HP	5.96 ± 0.09	24.7 ± 0.26^b	1.68 ± 0.02^a	0.77 ± 0.03^a
HAP	5.93 ± 0.12	24.8 ± 0.21^b	1.69 ± 0.02^a	0.78 ± 0.09^a
I	6.02 ± 0.11	18.2 ± 0.3^c	1.94 ± 0.06^b	1.24 ± 0.15^b
IA	6.03 ± 0.05	18.6 ± 0.45^c	1.96 ± 0.15^b	1.27 ± 0.2^b
IP	5.99 ± 0.09	21.2 ± 0.25^d	1.69 ± 0.2^a	0.86 ± 0.05^a
IAP	5.98 ± 0.03	21.1 ± 0.1^d	1.41 ± 0.07^c	0.91 ± 0.06^a

The data that carry different alphabetical letters within the same column is considered significant at $p<0.05$.
Data expressed as mean $\pm$ SD & n= 6.

TABLE 6. Concentrations of ENR and its metabolite CIP in experimentally infected broilers with *C. jejuni* (µg/kg).

Day post last dose			1st	3rd	5th	7th	9th
Muscle	HA	ENR	867±13[a]	76.7±3.1[a]	39.1±0.8		
		CIP	74.3±3.5[a]	17.3±1.2	nd		
		Total	941±11.9*[a]	94±3.5[a]	39.1±0.8		
	HAP	ENR	868±46[a]	80±2[a]	38±2.6		
		CIP	76.3±5.1[a]	20±2	nd		
		Total	945±47*[a]	100±4*[b]	38±2.6	nd	
	IA	ENR	569±41[b]	38±1.7[b]	nd		
		CIP	58±2.6[b]	nd	nd		
		Total	627±42*[b]	38±1.7[c]	nd		
	IAP	ENR	691±11[c]	59±2[c]	nd		
		CIP	64±2.6[b]	nd	nd		
		Total	755±9*[c]	59±2[d]	nd		
Liver	HA	ENR	1920.3±71[a]	127.3±7[a]	67±4.2[a]	25.2±1.7	
		CIP	1231.3±12[a]	103±3.1[a]	40±2[a]	nd	
		Total	3151.7±59*[a]	230.3±6*[a]	108±6[a]	25.2±1.7	
	HAP	ENR	1955±56[a]	127±7[a]	66±4[a]	29±2	
		CIP	1208±7[a]	101±2[a]	39±1[a]	nd	
		Total	3163±56*[a]	228±6*[a]	106±3[a]	29±2	nd
	IA	ENR	1056±61[b]	81±2.5[b]	23±2[b]	nd	
		CIP	710±15[b]	52±3[b]	nd	nd	
		Total	1766±49*[b]	133±3[b]	23±2[b]	nd	
	IAP	ENR	1527±71[c]	104±9[c]	84±3[c]	30±2	
		CIP	829±45[c]	71±2[c]	34±1[b]	nd	
		Total	2356±110*[c]	175±10[c]	118±3[c]	30±2	
Kidney	HA	ENR	1277±54[a]	101±4[a]	70±2[a]	11±0.8	
		CIP	824±14[a]	56±4[a]	15±0.7[a]	nd	
		Total	2051±59*[a]	157±7[a]	84±2[a]	11±0.8	
	HAP	ENR	1258±65[a]	119±6[b]	71±4[a]	13±1	
		CIP	977±39[b]	60±2.5[a]	15±0.5[a]	nd	
		Total	2235±92*[b]	179±6[b]	86±3[a]	13±1	
	IA	ENR	786±17[b]	71±6[c]	nd	nd	
		CIP	408±12[c]	23±1[b]	nd	nd	
		Total	1194±28*[c]	94±6[c]	nd	nd	
	IAP	ENR	973±55[c]	86±5[d]	27±1[b]	nd	
		CIP	726±28[d]	48±0.6[c]	nd	nd	
		Total	1699±36*[d]	134±5[d]	27±1[b]	nd	

*The concentration ≥ MRL set by [29].

The data that carry different alphabetical letters within the same column is considered significant at $p<0.05$.

Data expressed as mean ± SD & n= 3.
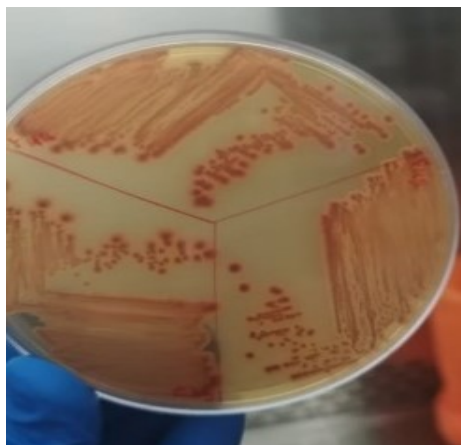
Fig. 1. *C. jejuni* on CHROMagar™ *Campylobacter* base selective medium

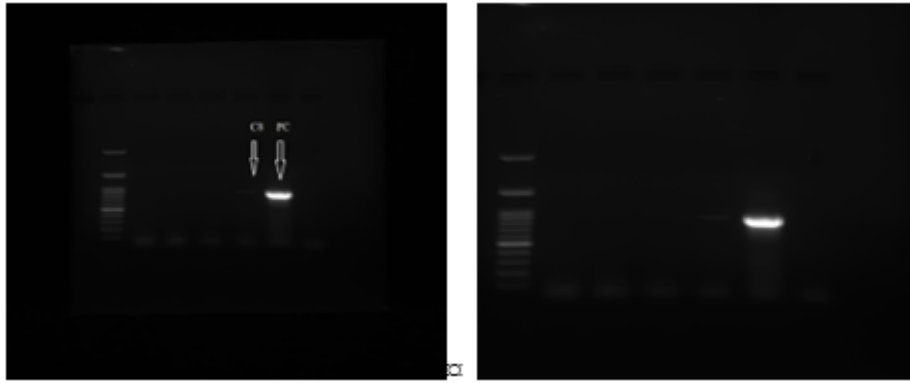**Fig. 2. Gel image for PCR detection of *C. jejuni* at 816 bp**
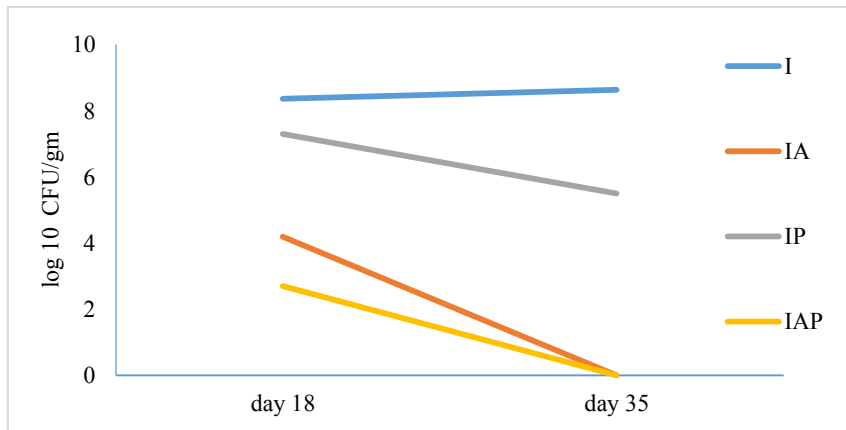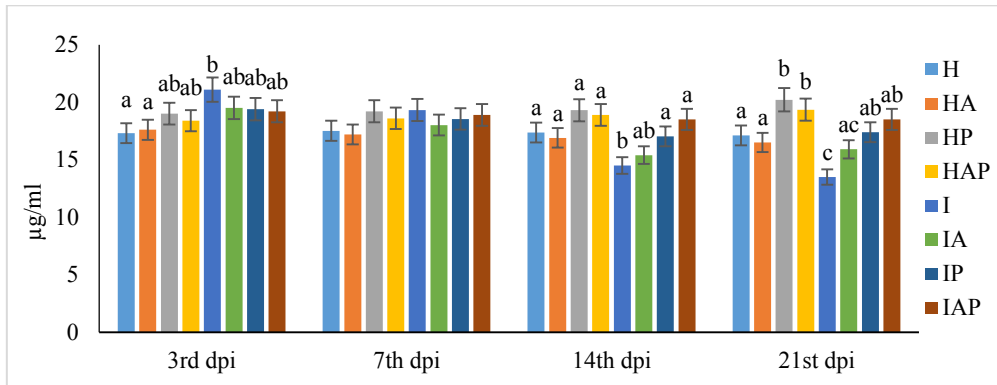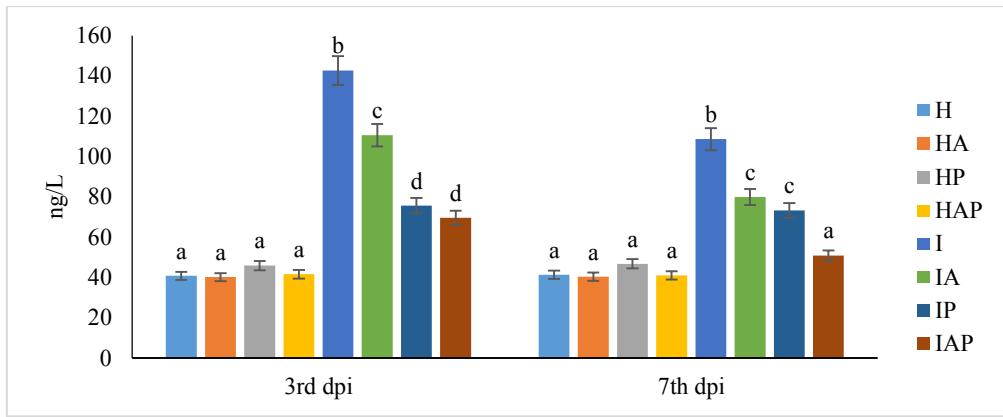

Fig. 3. Chart showing *C. jejuni* count in cecal contents after supplementation with ENR and APS in experimentally infected chickens with *C. jejuni*.
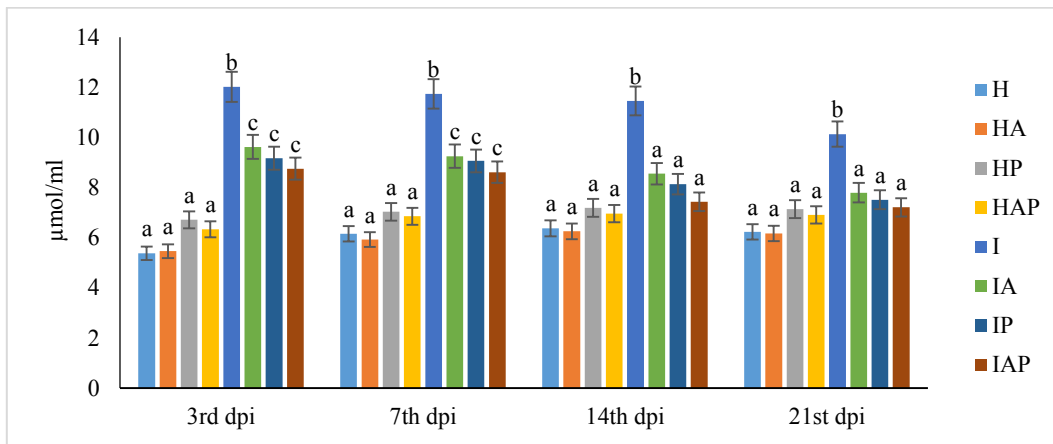

The columns that carry different alphabetical letters within the same time point are considered significant at *p*<0.05.

Fig. 4. Chart showing the serum lysozyme level (mean ± SE) after supplementation with ENR and APS in experimentally infected chickens with *C. jejuni*, n= 6.

The columns that carry different alphabetical letters within the same time point are considered significant at *p*<0.05.

Fig. 5. Chart showing the serum IL-6 levels (mean ± SE) after supplementation with ENR and APS in experimentally infected chickens with *C. jejuni,* n= 6.
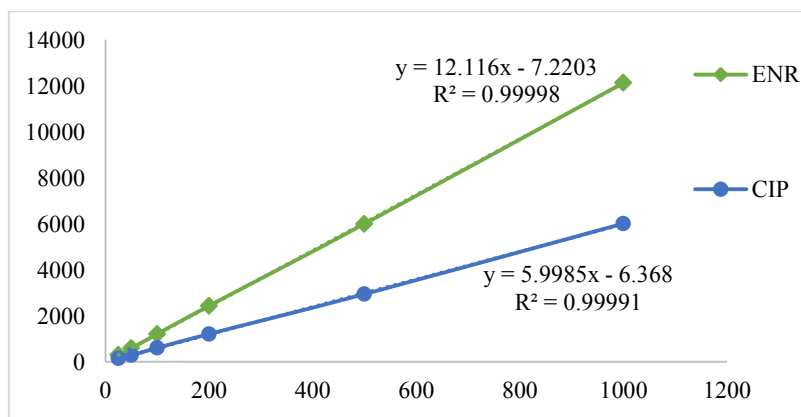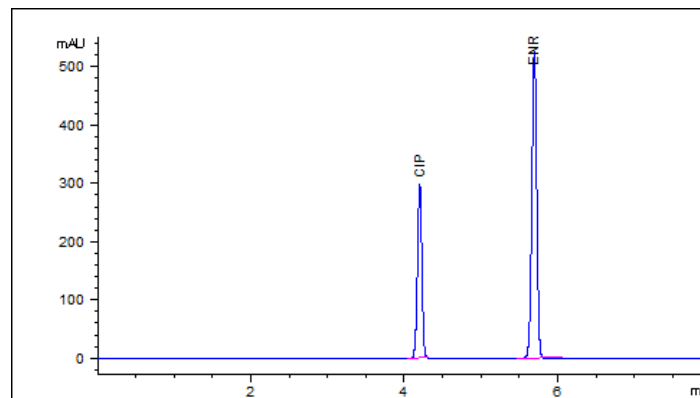

The columns that carry different alphabetical letters within the same time point are considered significant at *p*<0.05.

Fig. 6. Chart showing the serum NO level (mean ± SE) after supplementation with ENR and APS in experimentally infected chickens with *C. jejuni,* n= 6.

Fig.7. Standard curves of ENR and CIP, concentration versus area under peak.

Fig. 8. Chromatogram showing RT of 200 ppb for each ENR and CIP at 4.913& 5.

References

1. Smialek, M., Burchardt, S. and Koncicki, A. The influence of probiotic supplementation in broiler chickens on population and carcass contamination with *Campylobacter spp.*-Field study. *Research in Veterinary Science*, **118**, 312-316 (2018).

2. Hankel, J., Jung, K., Kuder, H., Keller, B., Keller, C., Galvez, E. and Visscher, C. Cecal microbiota of experimental *Campylobacter jejuni*-infected chickens at different ages. *Frontiers in Microbiology*, **10**, 2303 (2019).

3. Mileng, K., Ramatla, T.A., Ndou, R.V., Thekisoe, O.M. and Syakalima, M. Isolation and antibiotic sensitivity of *Campylobacter species* from fecal samples of broiler chickens in Northwest Province, South Africa. *Veterinary World*, **14**(11), 2929 (2021).

4. Zbrun, M.V., Rossler, E., Olivero, C.R., Soto, L.P., Zimmermann, J.A., Frizzo, L.S. and Signorini, M.L. Possible reservoirs of thermotolerant *Campylobacter* at the farm between rearing periods and after the use of enrofloxacin as a therapeutic treatment. *International Journal of Food Microbiology*, **340**, 109046 (2021).

5. Khan, M., Anjum, A.A., Nawaz, M., Awan, A.R. and Ali, M.A. Effect of Newly Characterized Probiotic *Lactobacilli* on Weight Gain, Immunomodulation and Gut Microbiota of *Campylobacter jejuni* Challenged Broiler Chicken. *Pakistan Veterinary Journal*, **39**(4) (2019).

6. Goulart, D.B., Beyi, A.F., Wu, Z., Adiguzel, M.C., Wilson, S., Xu, C. and Sahin, O. Influence of single dose enrofloxacin injection on development of fluoroquinolone resistance in *Campylobacter jejuni* in calves. *Antibiotics*, **11**(10), 1407 (2022).

7. Taha-Abdelaziz, K., Singh, M., Sharif, S., Sharma, S., Kulkarni, R.R., Alizadeh, M. and Helmy, Y.A. Intervention Strategies to Control *Campylobacter* at Different Stages of the Food Chain. *Microorganisms*, **11**(1), 113 (2023).

8. Qiao, Y., Guo, Y., Zhang, W., Guo, W., Oleksandr, K., Bozhko, N. and Liu, C. Effects of compound polysaccharides derived from *Astragalus* and *Glycyrrhiza* on growth performance, meat quality and antioxidant function of broilers based on serum metabolomics and cecal microbiota. *Antioxidants*, **11**(10), 1872 (2022a).

9. Qiao, Y., Liu, C., Guo, Y., Zhang, W., Guo, W., Oleksandr, K. and Wang, Z. Polysaccharides derived from *Astragalus membranaceus* and *Glycyrrhiza uralensis* improve growth performance of broilers by enhancing intestinal health and modulating gut microbiota. *Poultry Science*, **101**(7), 101905 (2022b).

10. Naji, A.I. and Huda, S.J. *Astragalus Polysaccharides* Enhance the Immune Response to *Newcastle* Disease Virus Vaccination in Chickens. *Annals of RSCB*, **25**(1), 586-596 (2021).

11. Wei, F., Abulahaiti, D., Tian, C.C., Chen, Y., Jiang, S.S., Lu, J.X. and Zhang, G.H. Effects of dietary *Astragalus mongholicus*, *Astragalus polysaccharides* and *Lactobacillus* on growth performance, immunity, and antioxidant status in Qingjiaoma finishing broilers. *Czech Journal of Animal Science*, **67**(7) (2022).

12. FASS; Guide for Care and Use of Agricultural Animals in Research and Teaching. 3rd Edition, Federation of Animal Science Societies, Champaign, IL (2010).

13. ISO 10272-1:2006. Microbiology of Food and Animal Feeding Stuffs–Horizontal Method for Detection and Enumeration of *Campylobacter* spp.–Part 1: Detection Method, International Organization for Standardization, Geneva, Switzerland (2006).

14. Hailu, W., Helmy, Y. A., Carney-Knisely, G., Kauffman, M., Fraga, D. and Rajashekara, G. Prevalence and antimicrobial resistance profiles of foodborne pathogens isolated from dairy cattle and poultry manure amended farms in northeastern Ohio, the United States. *Antibiotics*, **10**(12), 1450 (2021).

15. Kashoma, I.P., Kassem, I.I., John, J., Kessy, B.M., Gebreyes, W., Kazwala, R.R. and Rajashekara, G. Prevalence and antimicrobial resistance of *Campylobacter* isolated from dressed beef carcasses and raw milk in Tanzania. *Microbial Drug Resistance*, **22**(1), 40-52 (2016).

16. Stas, T., Jordan, F.T.W. and Woldehiwet, Z. Experimental infection of chickens with *Campylobacter jejuni*: strains differ in their capacity to colonize the intestine. *Avian Pathology*, **28**(1), 61-64 (1999).

17. Sureshkumar, V. and Sarathchandra, G. Withdrawal Period of Enrofloxacin and its Primary Metabolite Ciprofloxacin Residues in Broiler Chicken after Pulse Water Medication. *J. Anim. Res.*, **11**(05): 775-782 (2021).

18. Chaveerach, P., Lipman, L.J.A. and Van Knapen, F. Antagonistic activities of several bacteria on in vitro growth of 10 strains of *Campylobacter jejuni/coli*. *International Journal of Food Microbiology*, **90**(1), 43-50 (2004).

19. Yang, X., Sun, Q., Raza, Asim, A., Jiang, X., Zhong, B., Shahzad, M., Zhang, F. and Han, Y., Lu, S. Nitric oxide in both bronchoalveolar lavage fluid and serum is associated with pathogenesis and severity of antigen induced pulmonary inflammation in rats. *J Asthma*, **47**(2),135–44 (2010).

20. Peeters, T.L. and Vantrappen, G. Factors influencing lysozyme determination by lysoplate method. *Cli. Chim. Acta*, **74**, 217-255 (1977).

21. Young, D.S. Effects of drugs on clinical laboratorytests. *Ann. Clin. Biochem.* **34**(6), 579-581 (1997).

22. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**(1), 56–63 (1957).

23. AOAC. Protein content in meat. 992.15. In Official methods of analysis (17th ed.). Gaithersburgh, Maryland: Association of Official Analytical Chemists (2000).

24. AOAC. Fat content. 960.39. In Official methods of analysis (17th ed.). Gaithersburgh, Maryland: Association of Official Analytical Chemists (2000).

25. AOAC. Ashes content. 920.153. In Official methods of analysis (17th ed.). Gaithersburgh, Maryland: Association of Official Analytical Chemists (2000).

26. Verma, M.K., Ahmad, A.H., Pant, D., Rawat, P., Sharma, S. and Arya, N. Screening of enrofloxacin and ciprofloxacin residues in chicken meat by high-performance liquid chromatography. *Journal of Pharmaceutical Research International*, **32**(21), 64-69 (2020).

27. ICH Harmonised Tripartite Guideline, I. H. T. Validation of analytical procedures: text and methodology. *Q2 (R1)*, **1**(20), 05 (2005).

28. Kim, Y. Analysis of variance (ANOVA) comparing means of more than two groups, *Restorative Dentistry Endodontics*, **39**(1), 74-77 (2014).

29. EMEA (The European Agency for the Evaluation of Medicinal Products). Enrofloxacin, extension to all food producing species, Summary report (5), EMEA/MRL/820/02-FINAL (2002).

30. Ma, Y.H., Liu, C.Y., Qu, D., Chen, Y., Huang, M.M., and Li, Y.P. Antibacterial evaluation of sliver nanoparticles synthesized by polysaccharides from *Astragalus membranaceus* roots. *Biomed. Pharmacother.* **89**, 351- 357 (2017).

31. Newell, D.G. and Fearnley, C. Sources of *Campylobacter* colonization in broiler chickens. *Appl. Environ.Microb.*, **69**,4343- 4351 (2003).

32. Liz, J., Rhiannon, L., Martyd, K. and Kathr, Y. Prevalence of *Campylobacter coli* and *Campylobacter jejuni* in Retail chicken, Beef, Lamb, and Pork Products in Three Australian States. *J. Food Prot.,* **82** (12) 26- 34 (2020).

33. Li, Y., Ran, C., Wei, K., Xie, Y., Xie, M., Zhou, W., and Zhou, Z. The effect of *Astragalus polysaccharide* on growth, gut and liver health, and anti-viral immunity of zebrafish. *Aquaculture*, **540**, 736677 (2021).

34. Ragland, S.A. and Criss, A.K. Insight serial killing to immune modulation: Recent insights into the functions of lysozyme. *PLOS Pathogens*, **13**(9), e1006512 (2017).

35. Friis, L.M., Keelan, M. and Taylor, D.E. *Campylobacter jejuni* Drives MyD88-Independent Interleukin-6 Secretion via Toll-Like Receptor 2. *Infect. Immun.*, **77** (4), 1553–1560 (2009).

36. Mortada, M., Cosby, D.E., Akerele, G., Ramadan, N., Oxford, J., Shanmugasundaram, R. and Selvaraj, R.K. Characterizing the immune response of chickens to *Campylobacter jejuni* (Strain A74C). *PLOS One*, **16**(3), e0247080 (2021).

37. Li, C.Q., Trudel, L.J. and Wogan, G.N. Nitric oxide-induced genotoxicity, mitochondrial damage, and apoptosis in human lymphoblastoid cells expressing wild-type and mutant p53. *Proceedings of the National Academy of Sciences*, **99**(16), 10364-10369 (2002).

38. Iovine, N.M., Pursnani, S., Voldman, A., Wasserman, G., Blaser, M.J. and Weinrauch, Y. Reactive nitrogen species contribute to innate host defense against *Campylobacter jejuni*. *Infect. Immun.*; **76**, 986-993 (2008).

39. Korneev, K.V., Kondakova, A.N., Sviriaeva, E.N., Mitkin, N.A., Palmigiano, A., Kruglov, A.A., Telegin, G.B., Drutskaya, M.S., Sturiale, L., Garozzo, D Nedospasov, S.A., Knirel, Y.A. and Kuprash, D.V. Hypoacylated LPS from foodborne pathogen *Campylobacter jejuni* induces moderate TLR4-mediated inflammatory response in murine macrophages. *Front. Cell Infect. Microbiol.*, **8**, 58 (2018).

40. Elmi, A., Watson, E., Sandu, P., Gundogdu, O., Mills, D.C., Inglis, N.F., Manson, E., Imrie, L., Bajaj-Elliott, M., Wren, B.W., Smith, D.G., Dorrell, N. *Campylobacter jejuni* outer membrane vesicles play an important role in bacterial interactions with human intestinal epithelial cells. *Infect. Immun*., **80**, 4089-4098 (2012).

41. Moore, M.N., Allen, J.I. and McVeigh, A. Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. *Marine Environmental Research*, **61**(3), 278-304 (2006).

42. Hickey, T.E., Majam, G. and Guerry, P. Intracellular survival of *Campylobacter jejuni* in human monocytic cells and induction of apoptotic death by cytholethal distending toxin. *Infection and Immunity*, **73**(8), 5194-5197 (2005).

43. Song, B., Li, P., Yan, S., Liu, Y., Gao, M., Lv, H., Lv, Z. and Guo, Y. Effects of Dietary *Astragalus Polysaccharide* Supplementation on the Th17/Treg Balance and the Gut Microbiota of Broiler Chickens Challenged with Necrotic Enteritis. *Front. Immunol*., **13**:781934 (2022).

44. Hussell, T. and Bell, T.J. Alveolar macrophages: plasticity in a tissue specific context. *Nat. Rev. Immunol*., **14**, 81–93 (2014).

45. Dong, N., Li, X., Xue, C., Zhang, L., Wang, C., Xu, X. and Shan, A. *Astragalus polysaccharide* alleviates LPS-induced inflammation via the NF-Kappab/MAPK signaling pathway. *J. Cell Physiol*., **235**(7-8),5525.40 (2020).

46. Deng, L.L., Zeng, P.Y. and Yue, L.L. Effect of the *Astragalus polysaccharide* on the expression of MICA Gene and protein and the cytotoxicity of NK cells in Leukemia cells. *Chin. J. Hematol*., **33**,765-767 (2012).

47. Feng, S., Ding, H., Liu, L., Peng, C., Huang, Y., Zhong, F., Li, W., Meng, T., Li, J., Wang, X., Li, Y. and Jinjie, W. *Astragalus polysaccharide* enhances the immune function of RAW264.7 macrophages via the NF-κB p65/MAPK signaling pathway. *Experimental and Therapeutic Medicine*, **21**, 20 (2021).

48. Yuan, C., Pan, X., Gong, Y., Xia, A., Wu, G., Tang, J. and Han, X. Effects of *Astragalus polysaccharides* (APS) on the expression of immune response genes in head kidney, gill, and spleen of the common carp, *Cyprinus Carpio* L. *International Immunopharmacology*, **8**(1), 51-58 (2008).

49. Wei, W., Xiao, H.T., Bao, W.R., Ma, D.L., Leung, C.H. and Han, X.Q. TLR-4 may mediate signaling pathways of *Astragalus polysaccharide* RAP-induced cytokine expression of RAW264.7 cells. *J Ethnopharmacol*. **179**, 243-252 (2016).

50. Zheng Y., Ren W., Zhang L., Zhang Y., Liu D. and Liu Y. A Review of the Pharmacological Action of *Astragalus Polysaccharide*. *Front. Pharmacol*., **11**, 349 (2020).

51. Gao, Y.F. and Gao, N. Research progress on physiological effects of *Astragalus membranaceus*. *New Agric*. 01, 20-21 (2017).

52. Wu, Y., Gong, X., Shen, J. and Zhu, K. Post antibiotic leukocyte enhancement-mediated reduction of intracellular bacteria by macrophages. *Journal of Advanced Research*, S2090-1232 (2023).

53. Chen, R. Z., Tan, L., Jin, C.G., Lu, J., Tian, L., Chang, Q.Q. and Wang, K. Extraction, isolation, characterization, and antioxidant activity of polysaccharides from *Astragalus membranaceus*. *Industrial Crops and Products*, **77**, 434-443 (2015).

54. Shi, X., Wu, C., Ma, H., Liu, J., Fu, C., Zhou, R. and Zhang, R. Exploring the effects of *astragalus polysaccharide* food supplementation on growth performance, immunity, antioxidant capacity, and intestinal microflora in Chinese mitten crabs. *Aquaculture Reports*, **34**, 101908 (2024).

55. Wu, S. Effect of dietary *Astragalus membranous polysaccharide* on the growth performance and immunity of juvenile broilers. *Poultry Science*, **97**(10), 3489-3493 (2018).

56. Carreras, I., Castellari, M., Regueiro, J.G. Guerrero, L., Esteve-Garcia, E. and Sarraga, C. Influence of enrofloxacin administration and α-tocopherol acetate supplemented diets on oxidative stability of broiler tissues. *Poultry Science*, **83**(5), 796-802 (2004).

57. Pomacu, M.M., Traşcă, M.D., Pădureanu, V., Bugă, A.M., Andrei, A.M., Stănciulescu, E.C. and Pisoschi, C.G. Interrelation of inflammation and oxidative stress in liver cirrhosis. *Experimental and Therapeutic Medicine*, **21**(6), 1-9 (2021).

58. Abou Zeid, M.A., El-Gammal, G.A., Salim, A.A., Ali, G. and Fadl, S.E. Trails to decrease *Campylobacter jejuni* infection in diseased broilers. *Alexandria Journal for Veterinary Sciences*, **73**(2), 20-34 (2022).

59. Grabowski, Ł., Gaffke, L., Pierzynowska, K., Cyske, Z., Choszcz, M., Węgrzyn, G. and Węgrzyn, A. Enrofloxacin—the ruthless killer of eukaryotic cells or the last hope in the fight against bacterial infections? *International Journal of Molecular Sciences*, **23**(7), 3648 (2022).

60. Farag, M.R., Elhady, W.M., Ahmed, S.Y., Taha, H.S. and Alagawany, M. *Astragalus polysaccharides* alleviate tilmicosin-induced toxicity in rats by inhibiting oxidative damage and modulating the expressions of HSP70, NF-kB and Nrf2/HO-1 pathway. *Research in Veterinary Science*, **124**, 137-148 (2019).

61. Angwech, H., Tavaniello, S., Ongwech, A., Kaaya, A.N. and Maiorano, G. Efficacy of In Ovo delivered prebiotics on growth performance, meat quality and gut health of kuroiler chickens in the face of a natural coccidiosis challenge. *Animals*, **9**(11), 876 (2019).

62. Chang, Y., Zhang, J., Jin, Y., Deng, J., Shi, M. and Miao, Z. Effects of dietary supplementation of Chinese yam polysaccharide on carcass composition, meat quality, and antioxidant capacity in broilers. *Animals*, **13**(3), 503 (2023).

63. Li, Y., Dong, X., Zhang, Y., Xiao, T., Zhao, Y. and Wang, H. *Astragalus polysaccharide* improves the growth, meat quality, antioxidant capacity and bacterial resistance of *Furong crucian* carp (*Furong* carp♀× red *crucian* carp♂). *International Journal of Biological Macromolecules*, **244**, 124999 (2023).

64. Sureshkumar, V. and Sarathchandra, G. A HPTLC-fluorescent densitometry assay for simultaneous detection of enrofloxacin and ciprofloxacin in broiler chicken tissues. *Food Analytical Methods*, **11**, 1076-1085 (2018).

65. Papich, M.G., and Riviere, J.E. Fluoroquinolone antimicrobial drugs. *Veterinary Pharmacology and Therapeutics*, **9**, 984-1004 (2001).

66. Prescott, J.F., Baggot, J.D. and Walker, R.D. Fluoroquinolones. In: Antimicrobial Therapy in Veterinary Medicine. Prescott J.F, J.D. Baggot and R.D. Walker, 3rd Edn., Iowa State University Press, Iowa, pp. 315-339 (2000).

67. Visan, A.I. and Cristescu, R. Polysaccharide-based coatings as drug delivery systems. *Pharmaceutics*, **15**(9), 2227 (2023).

فعالية استراجلس بولي سكاريد مقابل الإنروفلوكساسين في استعمار كامبيلوباكتر جوجينى في دجاج التسمين.

مها صبرى عبد الحفيظ [1]، فاطمة أمره [2]، هبة فرحان [3]، بيير مهني [4]، فريدة محمد [5]، جمعة الحداد [6]، إيمان الخواجة [7]، أماني عبد النبي [8] وفادي سيد يوسف [9]*

[1] وحدة الأدوية والبيروجين، قسم الكيمياء والسموم والنقص الغذائى، معهد بحوث صحة الحيوان، مركز البحوث الزراعية، الدقي، الجيزة 12618، مصر

[2] قسم صحة الغذاء، معهد بحوث صحة الحيوان، مركز البحوث الزراعية، الجيزة 12618، مصر.

[3] قسم البكتريولوجى، معهد بحوث صحة الحيوان، الدقي، الجيزة 12618، مصر

[4] قسم الكيمياء والسموم والنقص الغذائى ، معهد بحوث صحة الحيوان، مركز البحوث الزراعية، الدقي، الجيزة 12618، مصر.

[5] قسم المناعة، معهد بحوث صحة الحيوان، مركز البحوث الزراعية، الجيزة 12619، مصر

[6] قسم التكنولوجيا الحيوية، المعمل المرجعي لمراقبة الجودة البيطرية على إنتاج الدواجن، معهد بحوث صحة الحيوان فرع دمنهور، 22511، مركز البحوث الزراعية، مصر

[7] قسم صحة الغذاء، معهد بحوث صحة الحيوان، المنصورة، مركز البحوث الزراعية، مصر

[8] قسم السموم، معهد بحوث صحة الحيوان، فرع بنها، 13512، مركز البحوث الزراعية، مصر

[9] قسم الأدوية، كلية الطب البيطري، جامعة القاهرة، 12211 الجيزة، مصر.

الملخص

داء الكامبيلوباكتريوسيس هو أكثر الأمراض حيوانية المنشأ شيوعًا في العالم، ويؤثر على دجاج التسمين. قامت دراسة بتقييم فعالية استراجلس بولي سكاريد (APS ;Astragalus) مقابل المضاد الحيوي الإنروفلوكساسين في السيطرة على كامبيلوباكتر جوجينى *Campylobacter jejuni*. تم تقسيم إجمالي 160 كتكوت تسمين (عمر يوم واحد) عشوائيًا إلى ثماني مجموعات: كتاكيت صحية (H)، كتاكيت صحية أعطيت الإنروفلوكساسين (HA)، و بولي سكاريد (HP)، كلاهما (HAP)، كتاكيت مصابة تجريبيًا (I)، الكتاكيت المصابة أعطيت الإنروفلوكساسين (IA)، و بولي سكاريد (IP)، وكلاهما (IAP). في عمر 18 و35، تم تقييم حمولة العطيفة في محتويات الأعور. قامت الدراسة أيضًا بتقييم المعلمات المناعية في الدم على فترات مختلفة بعد الإصابة. تم ذبح ثلاث دجاجات بعد آخر جرعة فموية من الإنروفلوكساسين لتحديد متبقيات الإنروفلوكساسين و سيبروفلوكساسين (CIP) بواسطة HPLC في أنسجة مختلفة. تضمنت التجربة تقدير مضادات الأكسدة الموجودة في عضلات الصدر وخصائصها الفيزيائية والكيميائية، إلى جانب قياس إنزيمات الكبد في الدم. أظهرت المجموعات المعالجة انخفاضًا كبيرًا في عدد البكتيريا، مع عدم ملاحظة أي نمو في مجموعات IA وIAP بعد 35 يومًا. أدى الإنروفلوكساسين و الأستراجلس بولي سكاريد إلى خفض مستويات إنترلوكين -6 (IL-6) وأكسيد النيتريك (NO) في الدم بشكل ملحوظ في المجموعات المصابة مع زيادة مستويات الليزوزيم في جميع المجموعات التجريبية. خفضت APS مستويات MDA وزادت مستويات SOD و GSH في عضلات الصدر. كانت أنشطة ALT وAST في الدم أعلى في المجموعتين I وIA مقارنة بالمجموعات الصحية والمجموعات التي تديرها APS. لم يؤثر APS على تركيزات بقايا العلامة في أنسجة مجموعة HAP، ولكنه زاد التركيزات في أنسجة مجموعة IAP دون زيادة في وقت سحب الدواء (3 أيام). تشير هذه النتائج إلى أن APS ضروري لتحسين جودة اللحوم، وتقليل الضرر التأكسدي الناجم عن الإنروفلوكساسين والعدوى، وإظهار العمل المضاد للعطيفة في الجسم الحي.

الكلمات الدالة: إنروفلوكساسين، كامبيلوباكتر ، استراجلس بولي سكاريد (APS)، وقت السحب، الضرر التأكسدي.