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The Ameliorative Performance of Proanthocyanidin against Florfenicol Toxicity in Broiler Chickens

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ABSTRACT

The usage of antibiotics in poultry sector is still controversial. Florfenicol (FF) is a popular antibiotic and feed additive for poultry. The immunosuppressive effects of toxic florfenicol cannot be ignored. So, a guide for its proper use is important for assurance food safety. Our study focused on the effects of florfenicol toxicity on growth performance, hepato-renal function, immune response, and florfenicol residue in chicken tissues either raw or boiled, as well as a trial to lessen the negative impact of toxicity by providing proanthocyanidin. The chickens were divided into 4 groups: the first group (G1) was control group, and the other three groups were given a toxic oral dose of florfenicol at 15 days old (40 mg/kg twice daily for five successive days). The second group (G2) serves as a positive group. The third group (G3) received proanthocyanidin as a therapy (250 mg/kg of ration) post the last dose of FF till the end of experiment, whereas fourth group (G4) received the same dose of proanthocyanidin as a prophylactic from one day old until the completion of the trial (35 days). The outcome data expresses the optimistic consequences of proanthocyanidin as a prophylactic in G4 via boosting growth characteristics, reducing ALT and AST, bedside restoring normal levels of urea at 14 days post florfenicol final dose, and levels of globulin, and total protein at 1 and 14 days post florfenicol final dose. Additionally, proanthocyanidin had suc-

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ceeded in lowering oxidative stress in G4 earlier than those of G3. Nitric oxide and HI antibody titers had fruitfully restored to normal levels at 14 days post FF last dose and at week 4 post vaccination respectively. Better ameliorative effects on lysozyme and IgG concentrations were detected in G4 than those of G3. Florfenicol and its metabolites in G4 were not detectable in muscle and were below the maximum residual limit (MRL) in kidney and liver at fifth day post florfenicol last dose. Therefore, using of proanthocyanidin will be of add value in improving antioxidant properties, liver and renal function, mending immunity in addition to lowering FF residues in tissues.

INTRODUCTION

Florfenicol (FF) is classified as a third-generation derivative of chloramphenicol antibiotic. Its chemical designation is 2,2-dichloro-N-[1-(fluoromethyl)-2-hydroxy-2-[(methylsulfonyl) phenyl] ethyl] acetamide, belonging to amphenicol antibiotic group. FF is among the most commonly utilized antibiotics in broiler farms, characterised by its high oral bioavailability and its ability to mitigate the adverse reactions associated with chloramphenicol **(Said et al. 2016)**. FF is a bacteriostatic antibiotic that inhibits protein synthesis at the ribosome by binding to the A site of the peptidyl transferase center in bacteria. It is widely used in treating pneumonia in animals because of rapidly absorbed and widely distributed in various animal tissues, including kidney and lung tissues (**Anadon et al. 2008**; **Shah et al. 2016)**. Several studies had proved the ability of FF in inducing strong immunosuppression in the body. Despite its widely used in veterinary medicine, its hematopoietic toxicity, immunotoxicity, genotoxicity and embryotoxicty properties were observed **(Al-Shahrani and Naidoo, 2015; Guan et al. 2011; Hu et al. 2016; Hu et al. 2014; Ren et al. 2017)**. Florfenicol amine (FFA) and florfenicol alcohol are main metabolites of FF in animals and are mainly excreted via the kidney. FF has a short-term hepatorenal toxicity in piglets but returns to normal with prolonged drug withdrawal. The detection of florfenicol amine determines FF metabolism in animals **(Hu et al. 2014)**.

Proanthocyanidin, a naturally arising polyphenolic antioxidant found in flowers, fruits, vegetables, nuts, and seeds, has various pharmacological and nutraceutical benefits, including reducing chronic diseases like cancer and cardiovascular ones, and antibacterial, antiviral, and antifungal activities. Its free radical scavenging capability is 20-fold higher to other antioxidants such as β-carotene, and vitamin C and E **(Shi et al. 2003; Park et al. 2011; Rajput et al. 2017)**.

FF is administered in drinking water to control disease incidence and enhance growth performance in broiler chickens. Because of the random use of antibiotics and inability to adhere to the prescribed dose and duration, severe side effects and antibiotic resistance occur. As a result, the study sought to investigate the role of proanthocyanidin as a therapeutic or prophylactic in lowering the adverse effects of florfenicol toxicity, modulating immunity, and reducing florfenicol tissue residue.

MATERIALS AND METHODS:

Ethical approval:

The protocol and conduct of the research was approved by the Animal Health Research Institute, Agriculture Research Centre, Egypt. The Approval Number was 81/24 dated June 6, 2024 according to the guidance of the Egyptian Ethics Committee in compliance with the NIH Guidelines for the Care and Use of Laboratory Animals.

Chemicals and reagents:

Florfenicol drug was purchased from Medizen pharmaceutical industries for veterinary use. Commercial grape seed powder extract contain 95% Proanthocyanidin was purchased from MD Pharma Co. All reagents were of analytical grade. Water and acetonitrile were obtained from Thermo Fisher Scientific. Kits

used for biochemical analyses were purchased from Spectrum Co. Florfenicol amine (FFA) standard (97.6%) was bought from Sigma-Aldrich Co. (St. Louis, MO).

Experimental design:

160 one-day-old broiler chicks were given unlimited access to food and water. All the birds were vaccinated using Hitchner B1live vaccine for ND, H5N2 influenza vaccine, IBD vaccine, and Lasota vaccine on the 7th, 10th, 14th, and 18th days of age, respectively. Chickens were classified into four groups (40 chicks each). First group (G1) is the control group, and the three other groups were administered a toxic double dose of florfenicol at 15 days old (40 mg/kg orally twice daily for five successive days), according to the prior study of **Said et al. (2016**). Second group (G2) acts as a positive group. Third group (G3) was treated with proanthocyanidin (250 mg/kg ration) post-FF administration (starting from 20 days old) till the end of the experiment (**Rajput et al. 2017**).

The fourth group (G4) was fed on proanthocyanidin as a prophylactic with the same previous dose from one day old until the end of the trial (35 days). Four birds from each group were humanely euthanized at different intervals from the last dose of FF administration till the end of experiment. Serum samples and Tissues (muscle, liver, and kidney) were collected to estimate some biochemical and immunological parameter in serum and florfenicol tissues residues. For Hemagglutination inhibition (HI) antibody titers, serum samples were collected weekly post NDVV vaccination.

Laboratory methods:

Biochemical parameters:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, urea, creatinine, albumin, total proteins, globulins, malondialdehyde (MDA), and superoxide dismutase (SOD) were estimated at 1 and 14 days post florfenicol last dose, according to **Lopez et al. (2013)**.

Immunological parameters:

Serum lysozyme activity and nitric oxide (NO) concentrations were estimated at 1,7 and 14 days post FF last dose according to **Schultz (1987) and Lee et al. (2011),** respectively.

Hemagglutination inhibition (HI) test against Newcastle Disease Virus Vaccine (NDVV) was carried out as described by **Spackman and Sitaras (2020)** at 1,2,3 and 4 weeks post vaccination with NDVV. Serum samples were incubated with 4Hemagglutinating units of NDV antigen for 30 minutes before adding 1% chicken RBCs. Then the plates were incubated at room temperature for half hour. Positive results were obtained when hemagglutination was inhibited.
Serum immunoglobulin G (IgG) was estimated at 1 and 14 days post FF last dose using the Chicken IgG ELISA kit Bethyl Laboratories, Inc., Cat. No. E33-104.

Boiling of tissue samples:

Tissue samples (muscles, livers, and kidneys) were taken one day following the last FF dose from G2. Samples were put in a strainer and submerged in a water bath heated to 100 ^{o}C. The liver was then cooked for 9 minutes, and the muscle and kidney portions for 24 minutes, then left to cool **(Javadi et al. 2011).**

Estimation of florfenicol residues (raw and cooked tissues) by HPLC:

Florfenicol and its metabolites are converted to florfenicol amine salts in chicken tissues through cathodic hydrolysis. The residues were estimated using HPLC assay **(EMEA 1999; Zhang-Jing et al. 2017**). The collected organ samples were minced and extracted as described by **Abd-Elhafeez et al. (2021)**. The standard FFA solutions were primed in the mobile phase at concentrations with the range of 4.88 to 9.76 ppb. FFA was separated with a retention time of 11.214 minutes as shown in figure 1 .

RT	Signal	Compound	Lvl	Amt[ppm]	Area
11.214	MWD1 A	FFA	1	4.8800e-1	37.504
			2	9.6700e-1	75.332
			3	1.952	154.800
			4	4.880	376.400
			5	9.760	753.300

Figure 1: Concentration of florfenicol amine and their corresponding area under the peak.
RT: Retention Time, Amt: Amount, MWD1: Multi Wave Detector, FFA: Florfenicol Amine

Statistical analysis:

The gathered data were statistically analyzed using IBM SPSS Statistics 20 program using a one-way ANOVA and T. TEST at p-value ≤ 0.05 (**SPSS, 2020**).

RESULTS:

Clinical signs:

Throughout the trial, the birds in the first (G1) and fourth (G4) groups behaved normally and showed no clinical symptoms. During the administration of florfenicol, the second (G2) and third (G3) groups exhibited symptoms of malnourishment, watery diarrhea, depression, and increased water intake. The third (G3) group had gradually subsided symptoms post-proanthocyanidin administration.

Growth performance:

Florfenicol had the ability to lower feed intake, body weight, and weight gain in G2 and G3 compared to G1. Administration of proanthocyanidin post-florfenicol toxicity (G3) had the ability to increase feed intake, body weight, and weight gain at the fourth and fifth weeks compared to G2. Supplementation of proanthocyanidin as prophylactic (G4) showed increasing values of feed intake, body weight, and weight gain at the third, fourth, and fifth weeks compared to G2. Comparing between G3 and G4 displayed a significant increase in feed intake, body weight, and weight gain of G4 at the third, fourth, and fifth weeks. Weekly FCR was increased in G2, G3, and G4 from the third week until the end of the experiment compared to G1. On the other side, G4 had significantly lower FCR values at the fourth and fifth weeks compared to G2 and G3, but without reaching the values of G1, as reported in Table 1.

Table 1. Effect of dietary supplementation of proanthocyanidin on nutritional parameters in florfenicol intoxicated broiler chickens

	G1	G2	G3	G4
Feed intake(g/bird/week)				
1st week	159.27±1.32^{a}	161.14±3.01^{a}	155.67±2.75^{a}	159.87±0.49^{a}
2nd week	421.45±7.26^{a}	428.32±2.87^{a}	432.67±5.24^{a}	425.02±6.59^{a}
3rd week	643.67±15.37^{a}	628.26±10.25^{b}	582.18±5.88^{c}	648.65±9.88^{a}
4th week	859.37±25.19^{a}	463.17±23.27^{d}	765.83±13.75^{c}	809.46±14.86^{b}
5th Week	1011.50±32.67^{b}	702.50±36.63^{d}	1005.67±12.74^{c}	1178.27±45.43^{a}
weekly body weight (g)				
Day 1	45.60±0.23^{a}	45.33±0.22^{a}	46.80±0.24^{a}	45.24±0.21^{a}
1st week	179.89±7.62^{ab}	180.34±6.10^{ab}	187.09±3.36^{a}	188.78±1.29^{a}
2nd week	416.71±3.98^{b}	413.31±4.73^{b}	419.26±5.45^{ab}	422.99±6.31^{a}
3rd week	941.65±15.17^{a}	815.47±6.33^{c}	807.30±7.38^{c}	921.33±11.18^{ab}
4th week	1528.61±12.82^{a}	1025.38±11.92^{d}	1259.83±10.33^{c}	1439.45±16.48^{b}
5th Week	2156.52±14.13^{a}	1276.87±13.68^{d}	1748.77±15.96b^{c}	1989.37±13.51^{b}
weight gain (g)				
1st week	134.29±6.58^{a}	137.01±5.21^{a}	140.29±5.37^{a}	144.55±4.27^{a}
2nd week	236.83±6.56^{a}	232.97±7.82^{a}	232.17±6.29^{a}	234.21±7.54^{a}
3rd week	524.94±8.80^{a}	402.16±16.18^{c}	388.04±11.55^{cd}	498.34±7.79^{b}
4th week	586.96±12.63^{a}	209.91±16.34^{d}	425.53±15.56^{c}	518.12±14.76^{b}
5th Week	627.91±18.23^{a}	251.49±19.45^{d}	488.94±14.87^{c}	549.92±13.65^{b}
FCR				
1st week	1.19 ±0.021^{a}	1.18 ±0.023^{a}	1.16 ±0.028^{ab}	1.15 ±0.022^{ab}
2nd week	1.78±0.024^{a}	1.83±0.034^{a}	1.86±0.031^{a}	1.81±0.032^{a}
3rd week	1.23±0.050^{c}	1.56±0.027^{a}	1.54±0.032^{a}	1.30±0.020^{b}
4th week	1.46±0.041^{d}	2.21±0.075^{a}	1.80±0.049^{b}	1.56±0.101^{c}
5th Week	1.61±0.099^{d}	2.79±0.148^{a}	2. 41±0.171^{b}	1.83±0.138^{c}

Mean ±SD
Different superscript letters in the same raw showed significant differences at p≤0.05

Biochemical parameters:

Malondialdehyde (MDA) and superoxide dismutase (SOD):

Administration of a double dose of FF had the ability to significantly elevate MDA and lower SOD at 1 and 14 days post-florfenicol last dose (G2, G3, and G4) compared to G1. Dietary supplementation of proanthocyanidin could reduce oxidative stress expressed as MDA and elevate antioxidant activities (SOD) at 14 days post-florfenicol last dose in G3, and 1 and 14 days post-florfenicol last dose in G4 compared to G2. Group 4 displayed a better effect than those of group 3 represented by lowering levels of MDA and increasing levels of SOD at both measuring times (table 2).

Table 2. Effect of dietary supplementation of proanthocyanidin on serum MDA and SOD in florfenicol intoxicated broiler chickens .

	G1	G2	G3	G4
MDA(nmol/l)				
1d post FF last dose(20 d old)	4.98±1.68^{c}	11.75±2.03^{a}	12.57±2.06^{a}	7.91±1.34^{b}
14d post FF last dose(34 d old)	5.8±1.16^{d}	13.93±2.15^{a}	9.38±1.68^{b}	6.56±1.07^{cd}
SOD (U/l)				
1d post FF last dose(20 d old)	43.35±3.58^{a}	27.59±1.01^{c}	28.74±1.84^{c}	32.74±2.86^{b}
14d post FF last dose (34d old)	47.64±2.68^{a}	23.86±2.86^{d}	34.21±1.92^{c}	38.89±4.01^{b}

Mean ±SD
Different superscript letters in the same raw showed significant differences at $p \leq 0.05$.

Hepatorenal function parameters:

Values of ALT, AST, urea, and creatinine were significantly elevated, while albumin, globulins, and total proteins were declined in florfenicol-intoxicated chicken groups (G2, G3, and G4) at 1 and 14 days post-florfenicol last dose compared to G1. On the other side, the G3 had a significant decrease of ALT, AST, urea, and creatinine at the opposite of albumin; globulin and total protein were increased at 14 days post-florfenicol last dose compared to G2. Supplementation of proanthocyanidin as prophylactic from 1 day (G4) showed decreasing levels of ALT, AST, urea, and creatinine, while albumin, globulins, and total proteins were increased at 1 and 14 days post-florfenicol last dose compared to G2 and G3. Proanthocyanidin as prophylactic in G4 had the ability to restore the normal values of urea, creatinine, total proteins, and globulins at 14 day post-florfenicol last dose as recorded in table 3.

Table 3. Effect of dietary supplementation of proanthocyanidin on hepatorenal function in florfenicol intoxicated broiler chickens.

	G1	G2	G3	G4
ALT (U/l)				
1d post FF last dose at 20 d old	32.02±2.53^{c}	57.76±3.61^{a}	54.92±4.23^{a}	36.45±1.64^{b}
14d post FF last dose at 34d old	30.12±2.21^{d}	59.31±4.52^{a}	42.67±1.58^{b}	34.67±3.21^{c}
AST(U/I)				
1d post FF last dose at 20 d old	57.32±1.59^{d}	92.70±2.04^{a}	85.44±8.67^{ab}	78.82±3.14^{c}
14d post FF last dose at 34d old	63.45±2.61^{d}	80.76±4.53^{a}	75.63±1.84^{ab}	72.38±1.46^{c}
Urea (mg/dl)				
1d post FF last dose at 20 d old	25.77±0.66^{d}	40.54±1.14^{b}	47.81±1.02^{a}	35.68±1.08^{c}
14d post FF last dose at 34d old	26.13±0.72^{cd}	35.49±1.38^{a}	32.67±1.14^{b}	27.57±0.84^{c}
Creatinine (mg/dl)				
1d post FF last dose at 20 d old	0.207±0.01^{d}	0.354±0.010^{a}	0.302±0.011^{b}	0.254±0.026^{c}
14d post FF last dose at 34d old	0.221±0.011^{d}	0.392±0.012^{a}	0.277±0.011^{b}	0.239±0.012^{c}
Albumin (g/dl)				
1d post FF last dose at 20 d old	1.82±0.15^{a}	1.14±0.07^{cd}	1.23±0.03^{c}	1.73±0.12^{b}
14d post last dose at 34d old	2.10±0.67^{a}	0.95±0.08^{d}	1.59±0.07^{bc}	1.72±0.21^{b}
Globulins (g/dl)				
1d post FF last dose at 20 d old	2.39±0.19^{ab}	1.73±0.02^{cd}	1.83±0.05^{c}	2.91±0.35^{a}
14d post FF last dose at 34d old	2.58±0.14^{a}	1.59±0.06^{c}	2.13±0.04^{b}	2.26±0.23^{ab}
Total proteins (g/dl)				
1d post FF last dose at 20 d old	4.23±0.42^{a}	2.87±0.09^{c}	3.06±0.11^{b}	4.14±0.39^{a}
14d post FF last dose at 34d old	4.68±0.12^{a}	2.54±0.03^{c}	3.72±0.03^{b}	3.96±0.43^{ab}

Mean ±SD
Different superscript letters in the same raw showed significant difference at $p \leq 0.05$.

Immunological investigations:

Lysozyme assay:

Data of lysozyme concentrations (figure 2) showed a significant increase in lysozyme in FF -intoxicated group (G 2) in comparing with G 1 at 1, 7, and 14 days post FF last dose. Those of G 3 showed a numerical, non-significant decrease compared with values of G 2 at the same periods. G 4 expressed a significant decrease in lysozyme concentration in comparing with G2 and G3 at all measuring times, but it failed to restore lysozyme to its normal values in comparing with G1.

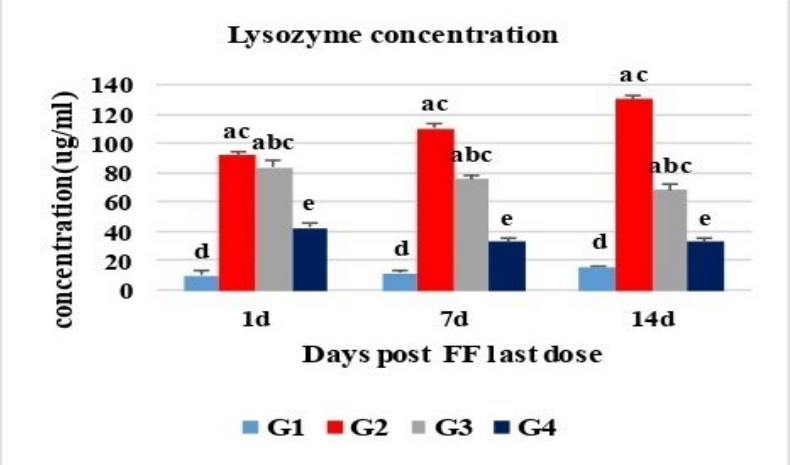

Figure (2): Effect of dietary supplementation of proanthocyanidin on lysozyme concentration in florfenicol intoxicated broiler chickens.
Data expressed as mean ± SE at $p \leq 0.05$.
Small different letters indicate significance between different groups at the same period.

Nitric oxide:

Concerning the results of nitric oxide, figure (3) data exposed a significant increase in nitric oxide concentration in G2 compared with G1 at all intervals. G 3 and G 4 convey significantly lower values of nitric oxide in comparing with G2 at 1, 7, and 14 days post FF last dose. The ameliorative effect of proanthocyanidin in G3 and G4 has succeeded in returning nitric oxide levels (5.35 and 5.81umol/ml) to be similar to those of G1 at 14 days post FF last dose.

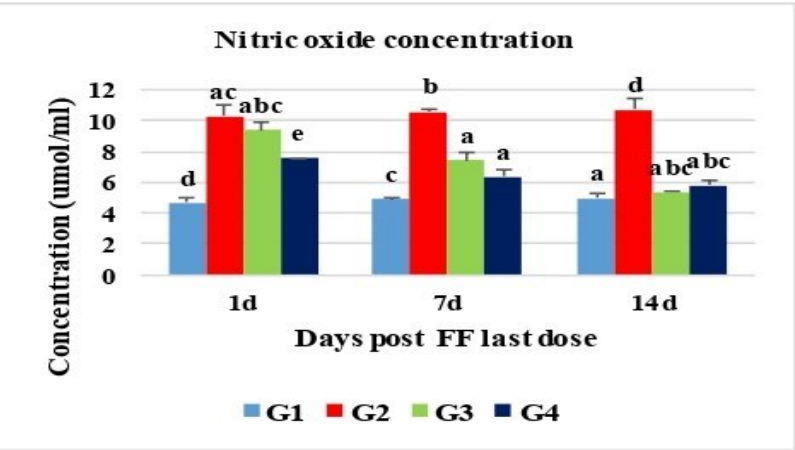

Figure. (3): Effect of dietary supplementation of proanthocyanidin on nitric oxide concentration in Florfenicol intoxicated broiler chickens.
Data expressed as mean ± SE at $p \leq 0.05$.
Small different letters indicate significance between different groups at the same period

Heamagglutination inhibition (HI) assay:
Regarding to figure (4), results of HI presented a significant decrease in HI antibody titer in G2 at W3 and W4 in comparing with G 1. Although G 3 and G 4 showed a higher significant HI antibody titer at W3 in comparison with G 2, it was still lower than G 1. The modulating effect of proanthocyanidin was proclaimed in G 3 and G4 at W4, where it returned the HI antibody titer to its normal values as in G 1.

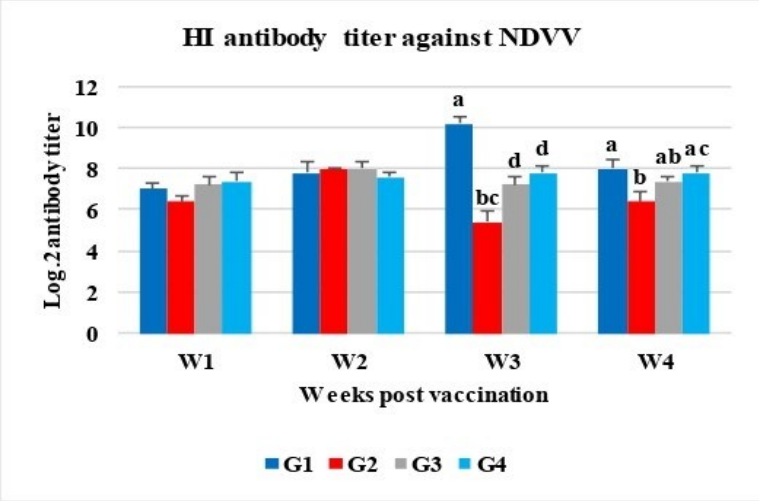

Fig. 4. Effect of dietary supplementation of proanthocyanidin on HI antibody titer against NDVV in florfenicol intoxicated broiler chickens.
Data expressed as mean ± SE at $p \leq 0.05$.
Small different letters indicate significance between different groups at the same period.

Immunoglobulin G (IgG) test:

Figure (5) exposed a significant decrease in IgG concentration in G 2 at 1 and 14 days post FF last dose in comparing with G 1. G 4 displayed significant high levels of IgG at 1 and 14 days post FF last dose compared with that of G 2. Using of proanthocyanidin as prophylactic (G 4) gave rise to better effect on IgG than using as treatment in G 3 at both time intervals.

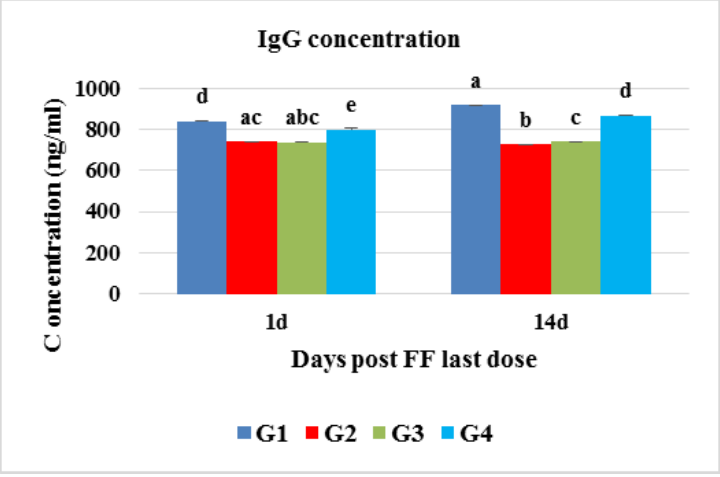

Fig 5. Effect of dietary supplementation of proanthocyanidin on IgG concentration in florfenicol intoxicated broiler chickens.
Data expressed as mean ± SE at $p \leq 0.05$.
Small different letters indicate significance between different groups at the same period.

Tissue residues of FF:

There is a significant decrease in residues across all chicken tissues after receiving the proanthocyanidin in Table 4 and Figures 6,7&8. The highest drug residue was found in the liver, although the lowest concentration was in the muscles. However FF remained detectable in the liver and kidneys for up to 5 days post-FF last dose in G2 and G3. All tissues were below the MRL standards and remained safe for human consumption on the 7 day post-FF last dose in G 2, and G 3. However, in G 4, the residue levels in all tested tissues were below the MRL on the 5th day post-FF last dose.

The effect of boiling on livers, kidneys, and muscles FF residue in group 2 at 1day post FF last dose were illustrated in Table 5. After boiling the tissues, it was found that the levels of FF residues decreased significantly, with a reduction percentage ranging from 86.49±2.31 to 95.33±9.14 %.

Table 4. Concentrations of florfenicol residues (µg/kg) in treated chickens at different time intervals post-last oral dose.

Tissues	Group	1st day	3rd day	5th day	7th day	9th day	14th day	MRL
	G2	310.70± 3.53^{a}	121.32±2.91^{a}	90.34±0.62^{a}	Nd	Nd	Nd	
Muscle	G3	304.01±6.12^{a}	122.74±2.32^{a}	87.32±3.11^{a}	Nd	Nd	Nd	100 µg/kg
	G4	192.72± 8.11^{b}	72.04± 3.03^{b}	Nd	Nd	Nd	Nd	
	G2	3074.03 ± 64.22^{a}	1419.31 ± 20.03^{a}	780.03 ± 30.52^{a}	159.71 ± 10.63^{a}	51.73 ± 2.92^{a}	Nd	
Kidneys	G3	2952.72±128.51^{a}	1325.72±117.72^{a}	749.32±31.53^{a}	151.32±16.33^{a}	54.31±3.051^{a}	Nd	750 µg/kg
	G4	2065.71±68.34^{b}	948.73±13.31^{b}	511.31±29.02^{b}	82.33±2.34^{b}	Nd	Nd	
	G2	15598.33±213.33^{a}	9254.32±124.21^{a}	4000.02±100.52^{a}	1026.32±73.32^{a}	389.32±20.52^{a}	Nd	
Liver	G3	15786.71±215.5^{2a}	9556.71±501.97^{a}	3859.05±123.73^{a}	993.71±11.51^{a}	399.35±19.01^{a}	Nd	2500 µg/kg
	G4	9942.03±147.44^{b}	5071.34±120.24^{b}	1023.71±74.81^{b}	119.02±15.61^{b}	Nd	Nd	

Data expressed as mean ± SD Nd: non detected MRLs: The maximum residue limits

Different superscript letters in the same column mean significant difference using ANOVA at p-value ≤ 0.05

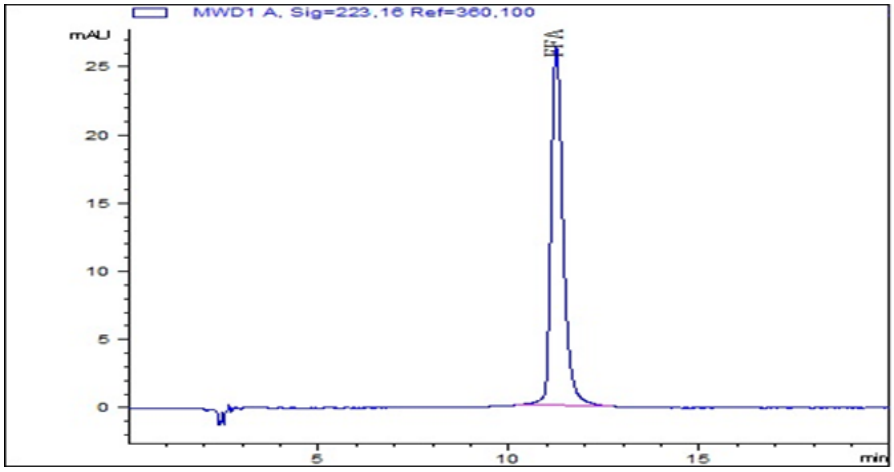

Figure 6. Chromatogram of a liver sample with FFA residue at 9341 ppb

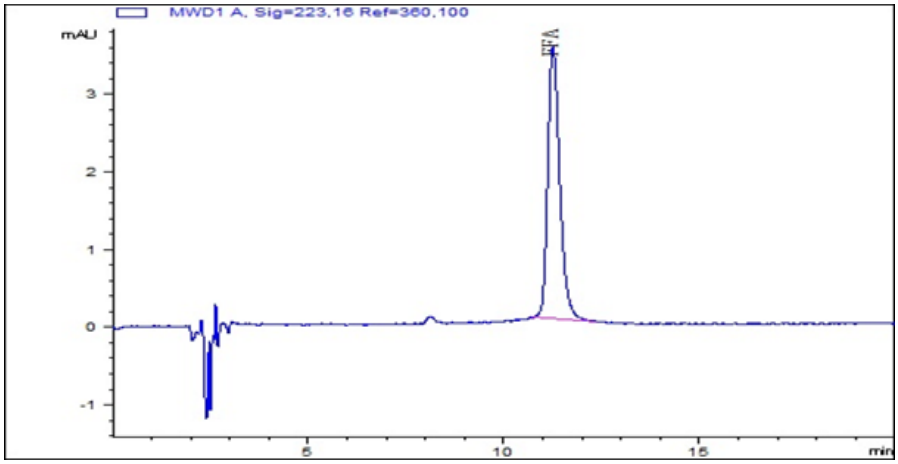

Figure 7. Chromatogram of a kidney sample with FFA residue at 950 ppb.

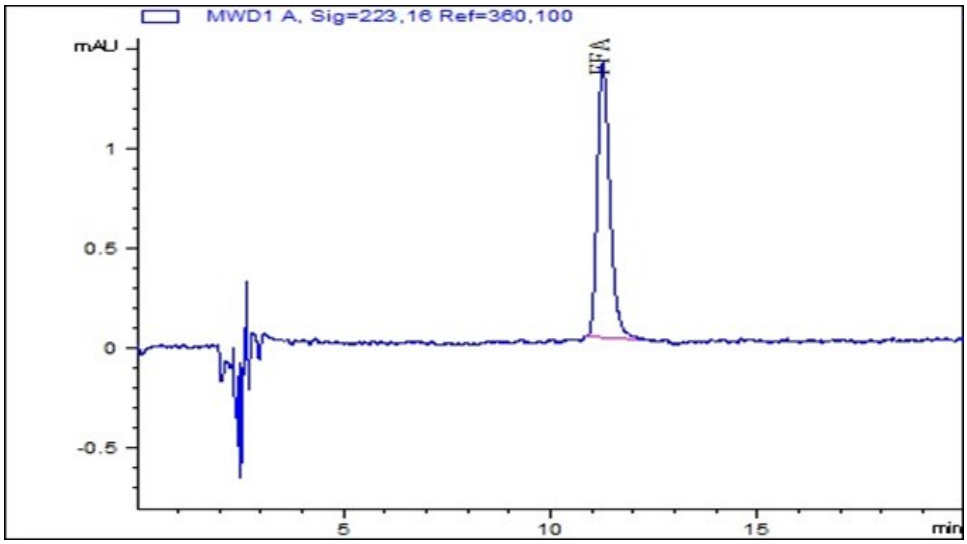

Figure 8. Chromatogram of a muscle sample with FFA residue at 195 ppb.

Table 5. Effect of boiling on FF and its metabolites residues (µg/kg)

	Muscle	Kidneys	Liver
Raw	310± 3.53	3074± 64.21	15598± 213.33
Boiled	42± 2.21*	300± 19.24*	727± 102.23*
Reduction %	86.49±2.31	90.24±3.23	95.33±9.14

Data expressed as mean ±SD .
* Mean significant difference using T. test at $p\text{-}value \leq 0.05$

DISCUSSION:

Antibiotics treat and inhibit bacterial infections in chickens and lately administered as feed additives to promote feeding efficiency and growth. However, higher doses of florfenicol may impair growth performance. Florfenicol may interfere with mitochondrial protein synthesis since prokaryotic and mitochondrial ribosomal subunits in eukaryotes are similar. It has the ability to reversibly bind to the large subunit of bacterial mitoribosomes and ribosomes, and inhibit peptidyl transferase in both prokaryotic organisms and mitochondria, resulting in antibacterial effects, mitochondrial dysfunction, and inhibition of mitochondrial protein synthesis **(Tsuyama et al. 2017)**. The florfenicol impact on nitrogen metabolism is related to its systemic anti-anabolic action that lowers amino acid assimilation into protein and hence boosts blood urea levels **(Shah et al. 2016; Fan-liang et al. 2023)**.

Stomach acid breaks down proanthocyanidin, which is made of C4-C8 units, into smaller molecules that absorbed in the small intestine and can prolong antioxidant action in the blood. Proanthocyanidin can keep the intestinal mucosa from oxidative stress and selectively inhibit the formation of pathogenic microorganisms. Dietary supplementation of proanthocyanidin improves broiler chicken growth performance by enhancing intestinal microbiota and gut architecture **(Viveros et al. 2011; Yang et al. 2014)**. The imbalance between oxidation and antioxidation, which results in liberation of reactive oxygen species, may be the cause of the increasing serum MDA and decreasing antioxidant activity of SOD in G2 and G3 (Table 2), subsequently leading to kidney and liver failure **(Abd Elghany et al. 2012; Jin et al. 2020; Wang et al. 2021)**. Proanthocyanidin reduces oxidative stress, improves antioxidant activity, and lowers lipid peroxidation in broilers. Adding of proanthocyanidin has a time-dependent cumulative influence and can reinstate the oxidant-antioxidant equilibrium, which accounts for the higher results in the G4 **(Bagchi et al. 2014; Ola et al. 2014)**. High dosages of florfenicol cause hepatic hypertrophy and damage hepatocyte cells. This is frequently accompanied by elevated transaminase enzyme levels in the blood. Hepatic hypertrophy boosts enzyme production and modifies their half-life in the blood, resulting in elevated serum transaminases **(Shah et al. 2016)**.

Higher serum urea and creatinine levels in G2 and G3 at 1d and 14d post FF last dose and in G 4 at 1d post FF last dose, were indicating impaired renal function, as shown in Table 3. These effects could be mediated by changes in the renal blood flow, renal tubular reabsorption threshold, and glomerular infiltration rate because of their inability to excrete FF and its metabolite, FFA **(Abdelhalim et al. 2020)**.

High dosages of florfenicol may reduce protein levels by increasing protein breakdown and interfering with urea excretion in the kidneys, resulting in urea retention in the blood **(Salomon et al. 2003)**. Florfenicol may stimulate creatine synthesis in muscles, boosting creatinine levels in the circulation while inhib-

iting creatinine excretion. Florfenicol produces degenerative changes in renal tubular cells, resulting in tubular obstruction, changes in renal microcirculation, and, finally, maintaining blood creatinine levels **(Liu et al. 2009; Shah et al. 2016; Wang et al. 2021**).

The high lysozyme concentrations in FF intoxicated group (G 2) could be attributed to the increasing percentage of monocytes and heterophils and in the ratio of heterophil/ lymphocyte (H/L) **(Abo-Sriea et al. 2024)**.

The ameliorative effect of proanthocyanidin in decreasing lysozyme either in G 3 or in G 4 reflects its anti-inflammatory and immune regulatory functions **(Ma and Zhang, 2017)**. Moreover, proanthocyanidin has proved its capability in competing inflammation via binding with excess inflammatory enzymes **(Rauf et al. 2019)**.

The overproduction of NO in G2 results from the activation of NO synthase (NOS) enzyme **(Bardhan et al. 2023)**. The oxidative damage that results from FF leads to stimulation of the toll-like receptor (TLR) signaling pathway, triggering the immune cells, and subsequently secretion of inflammatory factors **(Yun et al. 2023)**. The down-regulatory effect of proanthocyanidin on NO expression in G3 and G4 is compatible with **Zhang et al. (2022)**. Proanthocyanidin has scavenging activity against reactive nitrogen species **(Tulini et al. 2017)**. Additionally, it can inhibit overexpression of the inducible nitric oxide synthase (iNOS) enzyme by modulating nuclear factor kappa B, NF-kB **(Jiang et al. 2017)**. The FF has inhibitory effect on the bursa of Fabricius development that is the central motive in suppressing specific antibodies against NDVV **(Fan-liang et al. 2023)**. The mending effect of proanthocyanidin in G 3 and G4 reflects its ability to improve specific immune responses (**Araya-Sibaja et al. 2022**). The talent influence of proanthocyanidin could restore the induced apoptotic cells in bursa of Fabricius induced by aflatoxin B1 **(Rajput et al. 2019)**. The immunosuppressive effect of FF on IgG production is compatible with its attitude toward the production of antibodies against NDVV. The capability of proanthocyanidin in correcting IgG concentration is agree with **Rajput et al. (2017)** who stated an elevation in IgG, IgM, and IgA production in poultry exposed to aflatoxin B1. The highest concentration of FF residues in liver reflects its central role in drug metabolism and is agree with **Khalil et al. (2012)**. Such elevation had suggested the significant metabolic activity, converting florfenicol into its metabolites, which are then filtered and excreted by the kidneys **(Hu et al. 2014**; **Mallik et al. 2023)**. The muscular tissue had lower blood flow and metabolic activity, so it exhibited the lowest residual concentration. This accumulation pattern is crucial for understanding how the drug maintains therapeutic levels and is eventually metabolized and excreted.

The amount of FF residue in all examined tissues was below the MRL seven days post FF administration. Although the withdrawal time for the main form of florfenicol was approximately five days, the true withdrawal time for FFA would be longer (**EMEA 1999)**.

The residual levels of FF and FFA in chicken decreased gradually with longer cooking times. Within 25 minutes, the depletion rate of chloramphenicol in chickens ranged from 39.31 to 50.19%. This is agreed with the findings of **Shakila et al. (2006) and Filazi et al. (2015),** who found a substantial drop in chloramphenicol residues in shrimps, as well as FF and FFA residues in eggs, with increasing cooking time. HPLC study revealed that boiling greatly decreased oxytetracycline, gentamicin, and tilmicosin residues, with comparable reduction percentages seen in Turkey **(Heshmati et al. 2013**; **Wu et al. 2022**). Additionally, **Filazi et al. (2015)** found a significant reduction in FF and FFA residue levels in eggs after cooking (frying and boiling). While FF and FFA residues are sensitive to heat, the reduction was not enough to eliminate all residues.

CONCLUSION:

From the above results, Proanthocyanidin can reduce the toxic effects of florfenicol through its antioxidant actions, improving hepatorenal function, restoring immunity,

and decreasing the withdrawal times of FF and its metabolites from tissues. Proper boiling following antibiotic treatment is critical to reduce FF residue in chicken products. It is worthy to use proanthocyanidin as a prophylactic in broilers farms to overwhelm the hostile consequences of florfenicol.

REFERENCES

Abd EL-ghany MA, Ramadan AM, Ghozy SF. 2012. Nutraceutical effects of curcuma, ginger, celery, yeast and honey on side effects of gentamicin induced nephrotoxicity in rats. W A S J. 16(5): 646-655.

Abd-Elhafeez MS, Sabra SMM, Elshater NS. 2021.Impact of Acidifier on Florfenicol Pharmacokinetics and their Tissue Residues in Escherichia coli O78-Infected Chickens. Trop. J. Nat. Prod. Res.5(4):635-642.

Abdelhalim MAK, Qaid HA, Al-Mohy YH, Ghannam MM. 2020. The protective roles of vitamin E and alpha-lipoic acid against nephrotoxicity, lipid peroxidation, and inflammatory damage induced by gold nanoparticles. Int. J. Nanomed. (15) :729-734.

Abo-Sriea TM, Elshaimaa I, Sobhi BM, Neven H H, Elleithy EMM, Omar SA, Soliman AM, Fahmy KN, Ramadan A. 2024. Impact of dietary-nucleotides and Saccharomyces cerevisiae-derivatives on growth-performance, antioxidant-capacity, immune -response,small intestine histomorphometry, caecal Clostridia, and litter-hygiene of broiler-chickens treated with florfenicol. Int J of Vet Science and Med. 12(1): 11–24.

Al-Shahrani S, Naidoo V. 2015. Florfenicol induces early embryonic death in eggs collected from treated hens. BMC Veterinary Research.11, 213.

Anadon A, Martínez MA, Martinez M, Rios A, Caballero V, Ares I, Martínez-Larrañaga M R. 2008. Plasma and tissue depletion of florfenicol and florfenicol-amine in chickens. J of Agri and food Chem. 56(22): 11049-11056.

Araya-Sibaja AM, Wilhelm-Romero K, Vargas -Huertas F, Quirós-Fallas MI, Alvarado-Corella D, Mora-Román JJ, Vega- Baudrit JR, Sánchez-Kopper A, Navarro-HoyosM. 2022.Hybrid Nanoparticles of Proanthocyanidins from Uncaria tomentosa Leaves: QTOF-ESI MS Characterization, Antioxidant Activity and Immune Cellular Response. Plants (11): 1737.

Bagchi D, Swaroop A, Preussc HG, Bagchi M. 2014. Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview .Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis. (768):69-73.

Bardhan A, Abraham TJ, Singha J, Rajisha R, Krishna EKN, Panda SK, Patil PK. 2023. Impacts of Oral Florfenicol Medication and Residues on the Kidney and Liver of Nile Tilapia Oreochromis niloticus (L.). Vet. Sci. 10(1): 36.

EMEA. 1999. European Medicines Agency. Committee for veterinary medicinal products. MRL, Florfenicol (extension to chicken) summary report (3) (Europa.eu) .

Fan-liang M, Kui-hao L, Ye-sheng S, Pei-xun L, Tai-long W, Yi-ran Z, Si-dang L, Mengda L, Gang W. 2023. Florfenicol can inhibit chick growth and lead to immunosuppression. J of Integrative Agri. https:// doi.org/10.1016/j.jia.2023.11.040.

Filazi A, Sireli UT, Dikmen BY, Aydin FG, Kucukosmanoglu AG. 2015. The effect of cooking and storage on florfenicol and florfenicol amine residues in eggs. Italian Jof Food Sci. 27(3): 351-356.

Guan S, Song Y, Guo WX, Chu X, Zhang XZ, Wang D C, Lu J , Deng XM. 2011. Immunosuppressive activity of tilmicosin on the immune responses in mice. Immunopharmacol Immunotoxicol. (33):323-328.

Heshmati A, Kamkar A, Salaramoli J, Hassan J , Jahed G. 2013. The effect of two methods cooking of boiling and microwave on tylosin residue in chicken meat. Iranian J. Nut. Sci. & Food Technol. (8): 231-240.

Hu DF, Han ZQ, Li CL, LvL, Cheng ZL, Liu SD. 2016. Florfenicol induces more severe hemotoxicity and immunotoxicity than equal doses of chloramphenicol and thiamphenicol in Kunming mice. Immunopharmacol Immunotoxicol. (38): 472-485.

Hu D, Zhang T, Zhang Z, Wang G, Wang F, Qu Y, Niu Y, Liu S. 2014. Toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of florfenicol. Vet Immunol. Immunopathol. (162) : 122-131.

Javadi A, Mirzaie H, Khatibi SA. 2011. Effect of roasting, boiling and microwaving cooking methods on Enrofloxacin residues in edible tissues of broiler. African journal of pharmacy and pharmacology. 5(2): 214-218.

Jiang X, Liu J, Lin Q, Mao K, Tian F, Jing C, Wang C, Ding L, Pang C. 2017. Proanthocyanidin prevents lipopolysaccharide-induced depressive-like behavior in mice via neuroinflammatory pathway. Brain Res. Bull. (135): 40–46.

Jin F, Chen X, Yan H, Xu Z, Yang B, Luo P, He Q. 2020. Bisdemethoxycurcumin attenuates cisplatin-induced renal injury through anti-apoptosis, anti-oxidant and anti-inflammatory. Eur. J. Pharmacol. (874) : 173026.

Khalil S, Hamed E, Hassanin O. 2012. Residue withdrawal of florfenicol from the serum and edible tissues of broiler chickens. J Am. Sci. 8(12): 514-524.

Lee KW, Lillehoj HS, Jang SI, Li GX, Bautista DA, Phillips K, Ritter D, Lillehoj EP, Siragusa GR. 2011. Effects of Coccidiosis Control Programs on Antibody Levelsagainst Selected Pathogens and Serum Nitric Oxide Levels in Broiler Chickens. Journal of Applied Poultry Research (20): 143–152.

Liu KY, Li XX, Teng H, Li SB, Guo JY, Li DM, Hu YP. 2009. Toxicity of florfenicol to immune organs and its effect on physical and chemical characteristics of muscle in mice. J. Anhui Sci. Technol. Univ. (23):6–10.

Lopez J, Carl A, Burtis Edward R, Ashwood David E, Bruns (eds). 2013. Tietz Textbook of Clinical Chemistry and Molecular Diagnosis, 5th Edition. Ind. J .of Clin. Biochem.(28):104-105.

Ma ZF, Zhang H. 2017. Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants. (6) 71.

Mallik SK, Shahi N, Pathak R, Kala K, Patil PK, Singh B, Pandey PK. 2023. Pharmacokinetics and biosafety evaluation of a veterinary drug florfenicol in rainbow trout, Oncorhynchus mykiss (Walbaum 1792) as a model cultivable fish species in temperate water. Frontiers in Pharmacology, 14, 1033170.

Ola H, Fatma A, Ashraf A. 2014. Effects of florfenicol on the immune responses and the interferon-inducible genes in broiler chickens under the impact of E. coli infection. Vet. Res. Commun. (38):51 –58.

Park IJ, Cha SY, Kang M, So YS, Go HG, Mun SP, Ryu KS , Jang HK. 2011. Effect of proanthocyanidin-rich extract from Pinus radiata bark on immune response of specific-pathogen-free White Leghorn chickens. Poultry Science. 90(5):977-982.

Rajput SA, Sun L, Zhang N, Khalil MM, Gao X, Ling Z, Zhu L, Khan FA, Jiacai Zhang Qi D. 2017. Ameliorative Effects of Grape Seed Proanthocyanidin Extract on Growth Performance, Immune Function, Antioxidant Capacity, Biochemical Constituents, Liver Histopathology and Aflatoxin Residues in Broilers Exposed to Aflatoxin B1.Toxins. 9(371):1-16.

Rajput SA, Zhang C, Feng Y, Wei XT, Khalil MM, Rajput IR, Baloch DM, Shaukat A, Rajput N, Qamar H, Hassan M, Qi D. 2019. Proanthocyanidins Alleviates AflatoxinB1-Induced Oxidative Stress and Apoptosis through Mitochondrial Pathway in the Bursa of Fabricius of Broilers. Toxins.(11): 157.

Rauf A, Imran M, Abu-Izneid T, Iahtisham-Ul -Haq, Patel S, Pan X, Saima Naz, Silva AS, Saeed F, Suleria HAR. 2019. Proanthocyanidins: A comprehensive review. Biomedicine and Pharmacotherapy. 116 (108999):1-6.

Ren XY, Wang ZQ, Gao BQ, Liu P, Li J. 2017. Effects of florfenicol on the antioxidant status, detoxification system and bio-

molecule damage in the swimming crab (Portunus trituberculatus). Ecotoxicology and Environmental Safety.(143): 6-11.

Said A, El-Nabtity S, Selim A, Fadel M. 2016.

Effect of Florfenicol on Hematology, Cardiac Enzymes and its Residues in Broiler Chickens by HPLC. Zagazig Vet J. 44(1):56-66.

Salomon L, Levu S, Deray G, Launay-Vacher V, Brücker G, Ravaud P. 2003. Assessing residents prescribing behavior in renal impairment. Int. J. Qual. Health Care. (15):235–240.

Schultz A. 1987. Methods in Clinical Chemistry, Pesce, A. and L.A. Kaplan (Eds.). CV Mosby, St. Louis, MO., USA., ISBN-13: 978-0801638299: 742-746.

Shah JM, Qureshi TA, Shah T, Shah QA, Arain MA, Bhutto ZA, Saeed M, Siyal, FA. 2016. Impact of therapeutic and high doses of florfenicol on kidney and liver functional indicators in goat. Veterinary World, 9(10): 1135-1140.

Shakila RJ, Vyla SAP, Kumar RS, Jeyasekaran G, Jasmine GI. 2006. Stability of chloramphenicol residues in shrimp subjected to heat processing treatments. Food Microbiol. (23):47–51.

Shi J, Yu J, Pohorly PE, Kakuda Y. 2003. Polyphenolics in grape seed: biochemistry and functionality. J of Med Food. 6 (4): 291-299.

Spackman E, Sitaras I. 2020. Hemagglutination inhibition assay. Animal Influenza Virus: Methods and Protocols:11-28.

SPSS Statistics Version. 2020. Statistical package for social sciences. IBM®SPSS Statistics Data Editor 25.0 version 26.0 LicenseAuthorization Wizard. Chicago. USA.

Tsuyama T, Tsubouchi A, Usui T, Imamura H, Uemura T. 2017. Mitochondrial dysfunction induces dendritic loss via eIF2alpha phosphorylation. J. Cell Biol .216(3):815-834.

Tulini FL, Souza VB, Thomazini M, Silva MP, Massarioli AP, Alencar SM, PalloneEMJA, Genovese MI, Favaro-Trindade CS. 2017. Evaluation of the release profile, stability and antioxidant activity of a proanthocyanidin-rich cinnamon (Cinnamomumzeylanicum) extract co-encapsulated with α-tocopherol by spray chilling. Food Res. Int. (95): 117–124.

Viveros A, Chamorro S, Pizarro M, Arija I, Centeno C, 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.

Wang X, Han C, Cui Y, Li S, Jin G, Shi W, Bao Y. 2021. Florfenicol causes excessive lipid peroxidation and apoptosis induced renal injury in broilers. Ecotoxicol and environmental safety (207): 111282.

Wu M, Cheng X, Wu X, Qian H, Wang W. 2022. Effect of cooking methods on amphenicols and metabolites residues in livestock and poultry meat spiked tissues. Foods, 11(21): 3497.

Yang JY, Wang J, Wu S, Yue HY, Qi GH, Zhang HJ. 2014. Combination effects between grape procyanidins and wheat-based diet on the growth performance and immunity in broilers. Chinese Jof Ani Nutri. (26): 2270-2280.

Yun X, Zhou J, Wang J, Qi Li, Wang Y, Zhang W, Fan Z . 2023. Biological toxicity effects of florfenicol on antioxidant, immunity and intestinal flora of zebrafish (Danio rerio). Ecotoxicology and Environmental Safety. 265 (115520):1-16.

Zhang L, Chen J, Liang R, Liu C, Chen M, Chen J. 2022. Synergistic Anti-Inflammatory Effects of Lipophilic Grape Seed Proanthocyanidin and Camellia Oil Combination in LPS-Stimulated RAW264.7 Cells. Antioxidants. 11(2):289.

Zhang-jing ZJ, Gao yushi GY, Zhou qian ZQ, Wan yu WY, Tang mengjun TM, Tang xiujun TX, Chen dawei CD. 2017. Determination of chlorampheincol, thiamphenicol and florfenicol residues in chicken and egg by high performance liquid chromatographyelectrospray ionization-tandem mass spectrometry. Journal of Food Safety and Quality.8(6): 2211-2219 ref. 20.