



Biochemical Assessment of Oxidative Status and Liver Biomarkers in Acetaminophen Associated Hepatic-Toxicity in Broiler Chicks

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ABSTRACT

The present study was designed to evaluate the antioxidant activity of the methanolic extract of coriander seeds extract in broiler chicks during induced hepato-toxicity through acetaminophen. A total of 300 broilers (Cobb) chicks were used as tested criteria. Birds were randomized and divided into six groups consisting of ten birds each in five replicates. Groups C-1, C-2, and C served as the negative, positive control, and standard groups. However, groups CME-1, CME-100, and CME-1000 were fed with methanolic extract of coriander seeds @ 1, 100, and 1000mg/kg body weight daily for two weeks. Paracetamol (Acetaminophen) was induced as a liver toxicogenic agent @ 750 mg/kg body weight bases in the experimental birds at day 21 of the rearing phase except for negative control. Performance indicators were studied (feed intake, weight gain, and feed conversion ratio). Blood biochemical analyses were performed for aspartate aminotransferase (AST), alanine transaminase (ALT), superoxide dismutase (SOD), catalase (CAT), and immunoglobulin (antibody titer against ND). It was observed that the pre-infection phase of rearing meat-type birds showed no significant effects on overall performance while the toxicogenic agent acetaminophen significantly affected the overall performance in the post-infection phase. However, the CME-III significantly improved the performance indicators of the broiler chicks when compared with the positive control, and recorded significantly the highest mortality in the +control group. Serological indicators of the broiler chicks at the post-infection phase were abruptly altered by the toxicogenic agent acetaminophen. Liver biomarkers AST and ALT were significantly normalized by methanolic extract of coriander seeds extracts at the highest level (CME-III) of supplementation. The highest level of supplementation of methanolic extract of coriander seeds significantly improved the concentration of SOD and CAT in the treatment group compared with the positive control. It is concluded that the medicinal plant Coriander seed extract significantly improved the overall performance of the broiler chicks during induced hepato-toxicity.

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MM, MS, MA, AI, AS, MIK, MS, NUAN, SAS, AM, MSU: Contributed to the study conception, design, material preparation, data collection, analysis, and drafting of the manuscript.

Key words

Broiler, Performance indicators, Liver toxicity, Acetaminophen, Liver serology

INTRODUCTION

Poultry is one of the sub-sectors of the livestock sector providing 11.5 % of the Annual GDP of Pakistan. The recent investment in poultry is about seven hundred Billion increasing up to 10 % annually (Economic Survey of Pakistan, 2021-2022). Pakistan ranked 11 in the global poultry industry in 2022. This rapidly growing industry is

contributing 19 billion eggs and 1518 thousand tons of white meat to total meat production ([Economic Survey of Pakistan, 2021-2022](#)). This major industry of Pakistan has faced several issues regarding slow growth rate, low FCR, antibiotics resistance, infectious and non-infectious diseases (feed toxic agent), and other management practices. Another issue regarding poultry production is the outbreak of infectious and non-infectious diseases. The inclusion of antibiotics (chlortetracycline, procaine penicillin, oxytetracycline, tylosin, bacitracin, neomycin sulfate, streptomycin, erythromycin, lincomycin, oleandomycin, virginamycin, and bambamycin) as a therapeutic agent, growth-promoting antibiotics, coccidiostats etc. in the formulated diet for industrial poultry may have negative effects on liver physiology ([Westphal *et al.*, 1994](#)). Antibiotic related liver injuries cover most of the clinical and pathological expressions of hepatic dysfunction, including cytotoxic hepatitis (isoniazid), intrahepatic cholestasis (macrolides, penicillin, clavulanic acid), mixed hepatitis (sulphonamides), chronic active hepatitis (nitrofurantoin), or microvesicular steatosis (tetracycline) ([Westphal *et al.*, 1994](#)). The physiological function of the liver may include the metabolism of these therapeutic and growth-promoting agents/chemicals which can increase the energy expenditure resulting in the deterioration of the productive performance parameters in broilers. Over and prolonged use of these potential feed additives in industrial poultry ration can lead to irreversible liver damage ([German, 2021](#)). Numerous reports have been published regarding the extensive uses of non-steroidal anti-inflammatory drugs (NSAIDs) and their toxicity. NSAIDs are subsequently used in human medicine. Now a day's acetaminophen is introduced in the poultry industry as therapeutic medicine (veterinary practices). Acetaminophen is one the most common therapeutic drugs used as an analgesic and antipyretic. An overdose of acetaminophen results in hepatic toxicity (cytolysis and centrilobular necrosis). Metabolism of acetaminophen occurs through the liver by glucuronidation and sulfuration pathways. At overdose, acetaminophen is subsequently metabolized into NAPQI (toxic agent). Various antioxidant enzymes can safely reduce this NAPQI to nontoxic mercaptate and cysteine compounds and the renal system removed it as a waste product. Globally people are trying to limit the use of synthetic medicine to avoid residue and reduce high cost, adverse effects, and toxicity ([Okitoi *et al.*, 2007](#)). While natural medicine has fewer adverse effects on humans and animals. In poultry farming, herbal medicine is used to prevent a lot of problems in poultry production. Herbal medicine has less toxic effects and a pathological effect on the host cell ([Wojdylo *et al.*, 2007](#); [Bajpai *et al.*, 2005](#); [Mothana and Lindequist, 2005](#)). Herbal medicine is

preferred because they have very little toxicity and inhibit pathogens in the host cell. The coriander seed is used as a flavoring agent in the food industry in the form of whole extract ([Baytop, 1999](#)). Coriander extract has no known adverse effects or toxicity and it may be used as food and is considered safe for human consumption ([Bruduck and Carabin, 2009](#)). According to recent research, linalool is been the major component of coriander essential oil ([Silva *et al.*, 2011](#)). Petroselinic acid is the most abundant fatty acid ([Masada *et al.*, 2009](#)). A typical seed is composed of gamma-terpinene camphor, limestone geraniol, and myrcene ([Matasyoh *et al.*, 2009](#)). These constituents were evaluated for different therapeutic uses (antibiotic, antifungal, antiviral and antioxidant activate) and most of the studies show positive effects ([Da-Silva *et al.*, 2013](#)). Because of the premise that non-steroidal anti-inflammatory drugs are considered to be a potential hepato-toxic agent in avian spp., the present research project was designed to evaluate the toxic effects and efficiency/potential of methanolic extract of coriander seeds against the induced hepato-toxicity through acetaminophen in broiler chicks.

MATERIALS AND METHODS

Collection, identification, and extracts preparation of coriander seeds

The plant material (Coriander seeds) was procured from the local market. The seeds were identified by the plant taxonomist Department of Weed Science, The University of Agriculture Peshawar, Pakistan. The developed protocol of [Dabur *et al.* \(2004\)](#) for methanolic extract preparation of medicinal plants was followed.

Preparation of dose rate

The preparation of the dose rate was carried out according to the developed protocol of [Jagadish and Mahmood \(2008\)](#) for hepato-protective activity. The coriander seeds in the present study was powdered and then extracted methanol extract and weighed as per the calculation of 1,100, and 1,000 mg/kg body weight.

Birds husbandry and paracetamol-induced toxicity in broilers

A total of 300 broilers (Cobb) chicks of age one day were used as tested criteria under open sided house. During the first week, the temperature was maintained at 33-34°C and was regularly reduced at the rate of 0.33°C/day (by decreasing the heating source like brooder we decrease the number of bulb to reduce the temperature). A clean and pathogenic-free environment was provided to all birds along with 23 h light (8ft candle at bird level) and fresh water and feed. The birds were vaccinated according

to the broiler vaccination program. Birds were randomized and divided into six groups consisting of ten birds each in five replicates. Group C-1 served as a negative control, group C-2 served as positive control and C-standard (treat with commercial anti-hepatic toxicity drug), while groups CME-1, CME-100, and CME-1000 were fed with methanolic extract of coriander seeds @ 1, 100 and 1000mg/kg b.w daily for two weeks. The acetaminophen (Paracetamol-GSK) as given by oral route, at the dose rate of 750mg/kg body weight to all experimental birds except the negative control group for 3 days at the age of 21 days. All the experimental chicks were closely observed for any clinical signs of illness, if any over there. Mortality was recorded and necropsy procedures were followed to know the possible cause of death.

Production performance and biochemical parameters

Overall performance parameters, feed intake, weight gain, and feed conversion ratio (FCR) were calculated according to the procedure explained by Shuaib *et al.* (2021, 2022). Three birds from each of the groups were randomly selected for serological study and blood was collected. The collected blood was centrifuged for serum separation at the rate of 4000 rpm for 15 min and was further processed for the determination of immunomodulatory parameters (antibody titer ND), liver biomarkers (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)). HA and HI tests were conducted to evaluate the antibody titer against the New Castle disease virus (NDV) in the Microbiology Lab of the College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan. Antioxidant activities

were measured through a commercial kit (LPL Activity Assay Kit, Biomedical, Inc. Sigma-Aldrich) while liver biomarkers were evaluated through spectrophotometry.

Statistical analysis

The collected data was analyzed through one-way ANOVA by using SPSS version 20.0 (SPSS, Inc., Chicago, IL). The Least Significant Difference (LSD) test was used to calculate the significance difference ($p < 0.05$) among groups. Data were expressed as mean $\pm$ SE among different groups (Steel and Torrie, 1981).

RESULTS

Table I shows the result of feed intake. During the pre-infection phase, feed intake was not affected while in the post-infection phase the feed intake was significantly decreased in positive control and CME-I groups as compared to negative control and other treatment groups. The highest ($p < 0.05$) feed intake was observed in the negative group, follow up by CME-III and standard groups, respectively. Table II indicates the result regarding the weight gain. In the pre-infection phase, weight gain was not affected while significantly marked changes were recorded for body weight gain in the post-infection phase. A significant decline was observed in weight gain at the post-infection phase in the positive control and treated group with methanolic extract @ 1mg/kg b.w. and the highest ($p < 0.05$) weight gain was observed in a negative control group followed by standard and CME-III groups, respectively. Table III explains the FCR at two different rearing phases of broiler chicks. FCR was not effected during the pre-infection phase in all the experimental groups while the post-infection phase showed a significant decreased FCR for the positive control as compared to other

Table I. Effects of methanolic extract of coriander seeds (CME) on weekly feed intake (mg) of broiler chicks at pre and post infection phase with paracetamol toxicity (Means $\pm$ SE).

Groups	Pre-infection phase			Post-infection phase		Overall
	Week-1	Week-2	Week-3	Week-4	Week-5	
NC	112.3 $\pm$ 2.84	291.6 $\pm$ 8.83	476.6 $\pm$ 9.26	784.6 $\pm$ 15.6 ^a	1008 $\pm$ 9.16 ^a	2673.3 $\pm$ 43.2 ^a
PC	114.6 $\pm$ 2.90	300 $\pm$ 5.19	478.6 $\pm$ 13.9	613.3 $\pm$ 18.4 ^c	776.3 $\pm$ 23.1 ^d	2283 $\pm$ 53.4 ^d
Standard	110.3 $\pm$ 1.85	289.6 $\pm$ 8.08	466 $\pm$ 8.08	746.6 $\pm$ 12.2 ^b	978 $\pm$ 19.2 ^b	2590.6 $\pm$ 22.2 ^b
CME-I	115.3 $\pm$ 2.40	294.3 $\pm$ 6.17	470.3 $\pm$ 7.26	616.3 $\pm$ 16.8 ^c	783.6 $\pm$ 16.8 ^d	2280 $\pm$ 38.1 ^d
CME-II	108.3 $\pm$ 1.66	299.6 $\pm$ 4.91	477.6 $\pm$ 6.17	710 $\pm$ 5.85 ^{bc}	922.6 $\pm$ 31.4 ^c	2518.3 $\pm$ 44.6 ^c
CME-III	113.6 $\pm$ 4.33	294 $\pm$ 7.50	480 $\pm$ 3.60	739.3 $\pm$ 19.6 ^b	969.6 $\pm$ 21.6 ^b	2596.6 $\pm$ 14.4 ^b
P-Value	0.5926	0.7167	0.8268	0.0001	0.0001	0.0001

Means in column having superscripts are different at $\alpha = 0.05$. NC, negative control; PC, positive control; Standard, treated with commercial anti-hepatic toxicity drug; CME-1, Methanolic extract of *Coriander* seeds @ 1 mg/kg b.w; CME-100, Methanolic extract of *Coriander* seeds @ 100 mg/kg b.w; CME-1000, Methanolic extract of *Coriander* seeds @ 1000mg/kg b.w.

Table II. Effects of methanolic extract of coriander seeds (CME) on weekly weight gain (mg) of broiler chicks at pre and post infection phase with paracetamol toxicity (Means±SE).

Groups	Pre-infection phase			Post-infection phase		Overall
	Week-1	Week-2	Week-3	Week-4	Week-5	
NC	106.6±1.76	211.6±3.84	300.3±6.38	385±4.50 ^a	462±10.2 ^a	1465.6±24.4 ^a
PC	107±2.51	212.3±2.84	300.6±2.60	238±6.42 ^c	312.6±4.63 ^f	1170.6±15.8 ^d
Standard	106.6± 0.66	213.3±3.84	300.3±25.1	351.6±13.0 ^b	450.3±5.78 ^b	1422.2±20.2 ^b
CME-I	107±1.52	212.3±2.84	304.3±4.17	263.3±5.45 ^d	332.3±10.2 ^c	1219.3±18.2 ^d
CME-II	108±1.00	210±0.57	299.6±3.17	288±1.52 ^c	377±11.9 ^d	1282.6±12.7 ^c
CME-III	106.6±0.33	213±1.52	301.6±2.84	350.3±2.02 ^b	445±7.63 ^b	1416.6±12.9 ^b
P-Value	0.9885	0.7009	0.8614	0.0001	0.0001	0.0001

Means in column having superscripts are different at $\alpha=0.05$. For group description see [Table I](#).

Table III. Effects of methanolic extract of coriander seeds on weekly feed conversion ratio (FCR) of broiler chicks at pre and post infection phase with paracetamol toxicity (Means±SE).

Groups	Pre-infection phase			Post-infection phase		Overall
	Week-1	Week-2	Week-3	Week-4	Week-5	
NC	1.05±0.01	1.37±0.02	1.59±0.01	2.03±0.01 ^c	2.18±0.02 ^c	1.82±0.01 ^b
PC	1.07±0.03	1.41±0.01	1.59±0.03	2.58±0.04 ^a	2.48±0.05 ^a	1.95±0.02 ^a
Standard	1.03±0.02	1.35±0.03	1.55±0.06	2.12±0.14 ^b	2.17±0.01 ^c	1.82±0.02 ^b
CME-I	1.08±0.01	1.38±0.02	1.54±0.01	2.33±0.02 ^{ab}	2.36±0.10 ^b	1.87±0.03 ^{ab}
CME-II	1.00±0.02	1.42±0.02	1.59±0.02	2.46±0.03 ^a	2.45±0.05 ^a	1.96±0.01 ^a
CME-III	1.06±0.04	1.38±0.03	1.59±0.02	2.11±0.05 ^b	2.18±0.08 ^c	1.83±0.02 ^b
P-Value	0.4934	0.5936	0.8152	0.0003	0.0225	0.0078

Means in column having superscripts are different at $\alpha=0.05$. For group description see [Table I](#).

Table IV. Effects of methanolic extract of coriander seeds on liver biomarkers and immunity of broiler chicks at pre and post infection phase with paracetamol toxicity (Means±SE).

Groups	Post-infection phase					
	Mortality (%)	SOD(U/ml)	CAT(U/ml)	AST (U/ml)	ALT (U/ml)	ND-Titer
NC	00.0±0.00 ^c	34.0±1.15 ^c	1.93±0.04 ^b	222±3.46 ^d	4.90±0.02 ^c	5.00±0.01
PC	1.33± 0.33 ^a	23.0±1.15 ^c	1.30±0.05 ^d	255±2.88 ^a	6.73±0.12 ^a	4.33±0.33
Standard	0.33± 0.33 ^{bc}	42.3±1.45 ^a	2.30±0.05 ^a	216.0±1.73 ^d	4.06±0.29 ^d	5.33±0.33
CME-I	1.00±0.01 ^{ab}	27.6±1.20 ^d	1.43±0.03 ^d	245.3±2.90 ^b	6.36±0.08 ^a	4.33±0.33
CME-II	0.66± 0.33 ^{abc}	31.6±0.88 ^c	1.70±0.05 ^c	234.0±3.05 ^c	5.66±0.29 ^b	4.33±0.33
CME-III	0.33± 0.33 ^{bc}	39.3±1.76 ^b	2.06±0.08 ^b	221.3±1.85 ^d	4.93±0.08 ^c	5.00±0.57
P-Value	0.0762	0.0001	0.0001	0.0001	0.0001	0.3403

Means in column having superscripts are different at $\alpha=0.05$. For group description see [Table I](#). SOD, superoxide dismutase; CAT, catalase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

experimental groups and the best FCR was calculated for the negative control, standard, and CME-III groups, respectively. [Table IV](#) shows the result regarding the biochemical parameters (metabolic activities) and mortality

percent. The highest ($p<0.05$) mortality was recorded in a positive control group, follow up by CME-I as compared to other experimental groups by the toxicogenic agent, and significantly low mortality was recorded for an un-infected

group (Negative control) follow up by standard and CME-III groups, respectively. Significantly, low concentration of SOD and CAT was recorded in positive control and CME-I groups, respectively, while the highest concentration of antioxidant enzymes was found in standard and CME-III groups. The highest ($p < 0.05$) serum concentration of AST and ALT was recorded for the positive and CME-I groups as compared to other treatment and negative control groups. Treatment with methanolic extract of coriander seeds at the highest level significantly reduced the serum AST and ALT levels as compared to the positive control group. No cellular immune-modulatory effects were recorded at the post-infection phase against the ND.

DISCUSSION

CME-III group containing toxicogenic agent (acetaminophen) and the highest concentration of methanolic extract of coriander seeds in broiler had significantly improved feed intake, weight gain, and FCR at the post-infection phase compared to the pre-infection phase than in the other treatment groups containing acetaminophen and methanolic extract of coriander seeds (group CME-I and II). A lethal dose of acetaminophen was used as a hepatic-toxic agent which caused significant mortality in the experimental groups. The findings of the present study are in line with the results of [Samantha et al. \(2021\)](#), who documented that drinking water of broiler chicks supplemented with phyto-genic extract (coriander) significantly improved body weight gain. Periodic or continuous supplementation of phyto-genic in poultry ration significantly improved the overall performance of the birds ([Abdelli et al., 2021](#)). Similarly, [Rahul et al. \(2021\)](#) reported that feed-added coriander seed powder significantly improved FCR in broiler chicks. [Chawke et al. \(2021\)](#) also described that dietary supplementation of phyto-biotics in poultry bird production significantly affects the FCR. Similarly, [Soliman and Al-Afifi \(2020\)](#) described that feed-added phyto-genic compounds significantly enhanced the overall performance biomarkers. Also feed supplementation with coriander seed powder resulted in marked improvement in the body weight of broiler chicks ([Maroof et al., 2016](#)), which supports the present investigation. The addition of phyto-genic substance (coriander) to the diet of broiler chicks stimulates the digestive enzymes and enhanced the feed intake ([Hady et al., 2016](#)). Phyto-genic (coriander) supplemented broiler ration had a positive impact on feed consumption ([Naemasa et al., 2015](#)), which supports the findings of the present study. The present investigation is in favor of the results obtained by [Esteghamat \(2014\)](#) who documented that feed-added coriander significantly brought improvement in the feed consumption in broiler

chicks. Linalool is the active metabolite of coriander which significantly stimulate digestive function, liver metabolic activities, and pancreatic enzymes resulting in enhanced feed utilization ([Nadeem et al., 2013](#)). Supplementation of the broiler diet with coriander significantly improved the feed intake as reported by [Al-Mashhadani et al. \(2011\)](#). [Rajeshwari and Andallu \(2011\)](#) reported that coriander active ingredients stimulate the digestive enzymes which further enhanced the consumed feed digestion/absorption resulting in an improvement in the feed conversion ratio. The use of an aromatic plant (coriander) in poultry production can lead to stimulating the digestive enzymes which results in an increase in appetite (Gheralin receptor) ([Hashemi and Davoodi, 2010](#)) which is in agreement with the present findings. Acetaminophen liver toxicity significantly depleted the antioxidant enzymes in the present study. Supplementation of coriander seed extract in drinking water at the highest level significantly improved the antioxidant enzyme level during induced haptic toxicity in broiler chicks. These findings are supported by the results of [Hesham \(2021\)](#) who reported that lead (Pb) significantly causes structural and functional variations in the cerebellar cortex and somatosensory cortex which were regenerated by supplementation of coriander seed extract due to its chelating and antioxidant activates by increasing the SOD and CAT levels and reduced the oxidative indicator MDA in the animal model. The findings of the present study are also in line with the results of [Iqbal et al. \(2018\)](#) who documented that oral administration of coriander extracts significantly restore liver injury/toxicity by CCl₄ in an animal model which significantly affected the metabolic activities (physiological process) of the liver. Biologically active ingredients of phyto-biotics are responsible for antioxidant activities which help in the slowdown of lipid peroxidation mechanisms in the host body and promote unfavorable conditions for oxidation and ROS production ([Abd El-Hack et al., 2020](#)). The findings of the present study are in line with the results of [Yang et al. \(2019\)](#) and [Placha et al. \(2014\)](#), who reported that supplementation of medicinal plants in the diet of broiler chicks significantly reduced the MDA level and increased the activities of antioxidant enzymes to come-up the liver injury after induced toxicity. Supplementation of phyto-biotics essential oil in the diet of meat-type birds significantly improved the host antioxidant system by increasing the antioxidant enzymes activities ([Ognik and Krauze, 2016](#)). Supplementation of phyto-biotics in the broiler chicks ration significantly inhibits the formation and secretion of oxidative damage marker MDA and decreases/weakens the peroxidation process that activated the production of free radicles that are responsible for liver injury and increased the response of the antioxidant system

(Keshvari *et al.*, 2013). The findings of the present study are in agreement with the results of a previous study by Ciftci (2011) and Al-Kassie (2009) that stress reactions (oxidation reaction) in the cell significantly decreased the level of CAT enzyme, while phytobiotics should be considered a benefit in case of liver toxicities. Phenolic compounds of phytobiotics significantly improved the CAT (antioxidant enzyme) activities resulting in the neutralization of H_2O_2 and converting the lipid hydroperoxides into non-toxic compounds that make the liver healthy (Fki *et al.*, 2005). Overdose of paracetamol (acetaminophen) disturbs the normal physiological processes of absorption and metabolism and will convert into NAPQI (active metabolite) which renders the antioxidant system of the host body and results in oxidative stress which further opens the mitochondrial membrane permeability transition pores (halt the ATPs synthesis). It initiates lipid peroxidation resulting in thiobarbituric acid reactive substances (TBARS) and the production of ROS. This destructive mechanism leads to the breakdown of the DNA, and cell membrane and ultimately results in cell death and acute inflammation (Jaeschke and Ramachandran, 2018; Jaeschke *et al.*, 2012; Jaeschke and Bajt, 2006). Disturbed metabolic activities can lead to a remarkable decrease in the overall performance parameters of the host body. The improvement in the overall performance parameters, regeneration of hepatocytes, restoration of liver injury and toxicity, and minimized mortality or death ratio in said group of the treatment might be due to coriander seed extract antioxidant (Linalool is an active compound) effects which further counters the lipid peroxidation process and restoration of TBARS to its normal level. This restoration of TBARS to the normal level can increase antioxidant enzymatic activities like SOD, CAT, GPx, etc. Increased antioxidant enzymatic activities lead to the restoration of liver injuries to normal architecture and brought all the physiological processes of the liver up to normal (Patel *et al.*, 2012).

CONCLUSION

Based on the results of the present study it is concluded that the medicinal plant Coriander seed extract significantly improved the overall performance of the broiler chicks during induced hepato-toxicity. It improved the antioxidant host body system in terms of SOD and CAT to reduce the oxidation processes and normalized the liver biomarkers AST and ALT which indicated normal health of the liver after induced toxicity with a marked reduction in the mortality due to induced toxicity as compared to the positive group. The commercial liver tonics are expensive as compared to the medicinal plants used in the current

study and dose optimization study is required in the different strains of the poultry.

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IBR approval

The experimental work was approved by the Board of Study (BOS) before the start of the experimental trial (No: 5258-A/UAP, Dated: 22/11/2020).

Ethics approval

The experimental procedures used in the study were according to the guidelines of the Ethical Review Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar.

Statement of conflict of interest

The authors have declared no conflict of interest.

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