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RESEARCH ARTICLE

Salutary Effects of anti-*Clostridium perfringens* Type A Egg Yolk Antibodies (*IgY*) on Growth Performance and Hemato-Biochemical Parameters in Experimentally Infected Broiler Chicken

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ABSTRACT

Ever since the reduction of antimicrobial growth promoters in animal feed, infectious diseases have re-emerged in certain parts of the world, necrotic enteritis (NE) for one. The current study determines the protective efficacy of egg yolk antibodies (EYAs) in experimentally infected broiler chicken. Eighty (80), day-old broiler chickens were procured and divided into four groups (G_1-G_4). Group G_1 served as a negative control, while G_2 served as positive control viz infected with *C. perfringens* type A (1 x 10^8 cfu/ml) from days 17-19 of the experiment. Groups G_3 and G_4 immunized passively with anti-clostridial *IgY* @ 1 ml per bird between days 21-24 via oral route, while 22nd and 24th days via I/M route, respectively. Two killings were performed (days 26th and 35th) and the birds were observed for growth performance, hematology and serum biochemistry. The study results showed a statistically significant decrease in growth, hematology and serum protein values, while elevation in serum enzyme values of the birds in group G_2 when compared to group G_1. The groups G_3 and G_4 (passively immunized) showed the values less affected and close to the physiological ranges. Hence it was concluded that anti-clostridial EYAs (*IgY*) has ameliorative effects against experimental clostridial infection in broiler chicken.

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INTRODUCTION

The poultry industry in Pakistan is considered one of the growing sub-sectors of the agriculture sector in the national gross domestic product (GDP) and it is growing at a rate of 7.5%, annually. Pakistan is ranked 11th in the total meat producing countries of the world where poultry industry contributed about 35% of the total meat production (Anonymous, 2020-2021). Infectious diseases have always posed a significant economical and health threat to the Pakistan poultry industry. Even some infections have re-emerged due to a gradual shift in husbandry and management practices e.g. shifting towards more mechanized control of the environment and towards organic husbandry due to the ever growing demands in both consumer quality and quantity. Enteric diseases (for example) have re-emerged due to a gradual shift towards antimicrobial feed-growth promoters (AGPs) e.g. probiotics. Hence, economical losses in terms of bird mortality, production losses, contamination of feed, and even zoonoses have increased in some areas of the world (Immerseel *et al.*, 2016).

Necrotic enteritis (NE) is an important enteric disease of poultry caused by an anaerobic Gram-positive bacillus i.e. *Clostridium (C.) perfringens* type A which costs the global poultry industry up to 5-6 billion USD every year (Wade & Keyburn, 2015). This bacterium resides as a physiological microbiome in the intestines of animals and birds (Hafez, 2011). *C. perfringens* is classified into five toxinotypes (A-E) depending upon the type of toxins produced viz. alpha (α), beta (β), epsilon (ε), iota (ι), and enterotoxin (Lacey *et al.*, 2016). Another type- producing both alpha and a pore-forming toxin NetB (also a virulence factor for NE) is reported in birds, known as type G (Rood *et al.*, 2018).

A re-emergence and outbreaks of NE have been reported throughout the world due to the ban on the use of antimicrobials in poultry feed since 2006 (Tamirat *et al.*, 2017). Infected birds show depressed growth with adverse hematological and serum biochemical values (Suryakanth *et al.*, 2019). Egg yolk antibodies (EYAs) the

IgYs are potent protective antibodies in the poultry immune system produced in the egg yolk of the egg (Yegani & Korver, 2007). The EYAs can improve growth performance traits in poultry and can be used for various immunodiagnostic approaches in humans and animals (Cook *et al*., 1999; Iqbal *et al*., 2020). Keeping in view the immuno-modulatory effects of these specific EYAs against enteric pathogens e.g. *Escherichia coli* and *Campylobacter* etc, the present study was designed to evaluate the ameliorative effects of anti-*C. perfringens* type A EYAs against hemato-biochemical parameters in broiler chicken.

MATERIALS AND METHODS

Ethical approval for experimental study: All the experimental research work was done at Animal Care and Research Facility of Department of Pathology, University of Agriculture Faisalabad (UAF), Pakistan, and performed in accordance with guidelines provided and the study approval by the Institutional Biosafety/Bioethics Committee (IBC) of UAF following Punjab Biosafety Rules 2014, Pakistan (vide letter No. 6560/ORIC; dated: 13.09.2017).

Experimental design: A total of 140, one-day-old broiler chicken (Ross-308, Aviagen, Newbridge, UK) were purchased from a local market and provided ad libitum feed and water and reared under standard management for 35 days of the experiment at the Animal Care and Research Facility of the Department of Pathology, Faculty of Veterinary Science, University of Agriculture Faisalabad (UAF), Faisalabad, Pakistan. On day 15th, the birds were divided into four groups (G_1-G_4) comprising twenty birds each.

Production and isolation of anti- *C. perfringens* type A EYAs: The isolated pure colonies (unpublished data) of *C. perfringens* type A from suspected cases of NE were confirmed and maintained at the Department of Pathology, Faculty of Veterinary Science, UAF, Pakistan. Experimental infection in the broiler birds was produced @ 1 x 10^8 colony forming units (CFUs) of the isolate as reported previously (Olkowski *et al*., 2006). Inactivated whole cell antigen (WCA) of the isolate was injected at multiple sites in the breast muscle @ 1 ml per bird with booster doses on days 14 and 28 of the first injection in ten (10) White Leghorn chicken of 40 weeks of age purchased from a local hatchery (Diraviyam *et al*., 2011). The eggs were collected and stored at 4°C and the EYAs were extracted by water dilution method as the water-soluble fraction (WSF) previously described by (Akita & Nakai, 1992). The WSF containing specific anti-clostridial EYAs were assayed against *C. perfringens* by using ELISA (*C. perfringens* whole cells (10 µg/ml) as coated antigen; Rabbit anti-chicken IgG conjugated with horseradish peroxidase as a secondary antibody (Sigma-Aldrich®, USA)) as described previously with some modifications (Sunwoo *et al*., 1996; 2002).

Experimental infection with *C. perfringens* type A: On day 17, group G_2 was experimentally challenged by *C. perfringens* type A @ 1 ml/bird (1x10^8 CFUs/ml) via oral route for three consecutive days (day 17, 18, and 19 of the experiment). Groups G_3 and G_4 were considered as treatment groups. Group G_3 was administered by anti-clostridial EYAs @ 1 ml/bird via an oral route on 21st to 24th days, while group G_4 was given EYAs @ 1 ml/bird via the intramuscular route on 22nd and 24th days of the experiment. G_1 was kept as control (Table 1).

Growth performance parameters: The growth performance traits e.g. live body weight gain (LBW) and feed intake of the birds were recorded at weekly basis.

Blood parameters

Hematology: During the experimental trial, two random slaughtering of birds were performed (five birds from each group) on day 26 and day 35 of the experiment. The blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) coated blood collection tubes (K_3EDTA®, Hamburg, Germany) and processed for hematology, whereas blood samples collected in serum-separating-gel-based blood collection tubes (IMPROVACUTER®, Guangzhou, China) were processed for serum separation and biochemistry analysis. Hematological parameters included total erythrocyte count (TEC) and total leucocyte count (TLC) performed by using a Hemocytometer counting chamber (Marienfield Superior®, Germany), packed cell volume (PCV) measured with micro-hematocrit procedure, erythrocyte indices e.g. mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were also estimated. The values of Hb concertation were measured as previously described method (Hussain *et al*., 2017).

Serum biochemistry: The serum analysis included estimation of serum total protein (STP) (Catalogue # 997180) and albumin (Alb) (Catalogue # 997258), while globulins (Glob) was measured by subtraction of Alb values from STP. The values of creatinine (Creat) (Catalogue # 998891), lactate dehydrogenase (LDH) (Catalogue # 990035), aspartate aminotransferase (AST) (Catalogue # 999500), blood urea nitrogen (BUN) (Catalogue # 996060) and alanine aminotransferase (ALT) (Catalogue # 999200) were also measured by commercially available kits of Quimica Clinica Aplicada (QCA S.A®, Spain), using clinical chemistry analyzer (Microlab 300®, Merck).

Table 1: Layout of experimental trial

Group	Treatment	Route	Dose	Duration
G_1	Control Negative	—	—	—
G_2	*C. perfringens* type A	Per os	1x10^8 cfu/ml	From 17 to 19 days of age
G_3	*C. perfringens* type A	Per os	1x10^8 cfu/ml	From 17 to 19 days of age
	EYAs	Per os	1ml/bird	From 21 to 24 days of age
G_4	*C. perfringens* type A	Per os	1x10^8 cfu/ml	From 17 to 19 days of age
	EYAs	I/M	1ml/bird	At 22nd and 24th days of age

Statistical analysis: The data obtained were statistically analyzed by analysis of variance (ANOVA) and the means values were compared by using Tukey's test using computer SAS University Edition online software SAS stat 15.1 (SAS Institute, Cary, NC, USA) (SAS, 2018).

RESULTS

Growth performance parameters

Live Body weight gain: The live body weight gain (LBW) of birds (weekly) was recorded and presented in Table 2. During the 3rd to 5th weeks, live body weight (LBW) of birds in groups G_2, G_3, and G_4 was significantly ($P \leq 0.05$) lower compared to the control group G_1 and the lowest LBW was recorded in the birds belonged to group G_2, while comparatively higher LBW was recorded in groups G_3 to G_4 compared to group G_2 growth-promoting role of anti-clostridial IgY against *C. perfringens* challenge.

Feed intake: The feed intake of the birds (daily) was recorded and presented in Table 2. During the 3rd to weeks, the feed intake was significantly ($P \leq 0.05$) lower in groups G_2, G_3, and G_4, compared to group G_1 and the lowest feed intake was observed in group G_2, while comparatively higher feed intake was recorded in groups G_3 to G_4 compared to group G_2 indicating protective role of anti-clostridial IgY against *C. perfringens* challenge.

Blood parameters

Hematology: On day 26th, the mean values of the total erythrocyte count (TEC), Hb, PCV, MCH, and MCHC lowered significantly ($P \leq 0.05$), while the values of the total leukocyte count (TLC) and MCV rose significantly ($P \leq 0.05$) in groups G_2, G_3, and G_4 compared to the means of the control group (G_1) (Table 3). On day 35th, the values of TEC, Hb, PCV, and MCHC lowered significantly ($P \leq 0.05$), while the values of TLC, MCH, and MCV rose significantly ($P \leq 0.05$) in groups G_2, G_3, and G_4 when compared to G_1 (Table 4).

Serum biochemistry: On days 26th and 35th, the serum biochemistry showed a significant rise ($P \leq 0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) values among groups G_2, G_3, and G_4 when compared to G_1 (Table 5). Serum proteins estimation showed a significant ($P \leq 0.05$) decline in the values of serum total proteins (STP), albumin and (Alb), and globulins (Glob) among G_2, G_3, and G_4 compared to G_1 (Table 6). The concentrations of serum creatinine (Creat) and blood urea nitrogen (BUN) rose significantly ($P \leq 0.05$) among groups G_2, G_3, and G_4 as compared to G_1 (Table 7).

Table 2: Live body weight gain (g) and feed intake (g) of birds for various groups (Mean ± SD)

Group	Live body weight gain (g/bird/week)			Feed intake (g/bird/day)		
	Week 3	Week 4	Week 5	Week 3	Week 4	Week 5
G_1	626.30±3.30	1024.50±7.18	1565.70±3.22	84.93±0.19	118.64±0.34	150.18±0.30
G_2	541.30±2.26*	774.00±4.99*	1089.39±3.77*	73.23±0.28*	103.44±0.51*	130.82±0.30*
G_3	572.60±3.63*	883.60±3.57*	1292.20±7.61*	76.35±0.29*	101.54±0.39*	138.45±0.33*
G_4	565.70±2.41*	844.10±3.61*	1215.20±5.31*	75.81±0.25*	106.30±0.36*	136.99±0.20*

Significantly different values compared to group G_1 at the level of $P \leq 0.05$ are indicated by (*).

Table 3: Hematological values of birds on day 26 of the experiment (Mean ± SD)

Group	TEC ($x10^6$/µl)	TLC ($x10^3$/µl)	Hb (g/dl)	PCV (%)	MCH (pg)	MCV (fl)	MCHC (g/dl)
G_1	4.35±0.08	13.35±0.08	13.58±0.15	35.73±0.18	30.83±0.20	81.31±0.20	37.80±0.06
G_2	2.44±0.08*	22.42±0.08*	7.44±0.26*	21.83±0.20*	29.59±0.12*	87.57±0.16*	34.10±0.09*
G_3	3.37±0.08*	18.83±0.08*	11.88±0.10*	29.80±0.15*	29.82±0.08*	86.16±0.22*	35.20±0.06*
G_4	2.85±0.08*	19.17±0.08*	11.18±0.18*	27.52±0.17*	29.72±0.08*	86.72±0.18*	34.40±0.08*

Significantly different values compared to group 1 at the level of $P \leq 0.05$ are indicated by (*).

Table 4: Hematological values of birds on day 35 of the experiment (Mean ± SD)

Group	TEC ($x10^6$/µl)	TLC ($x10^3$/µl)	Hb (g/dl)	PCV (%)	MCH (pg)	MCV (fl)	MCHC (g/dl)
G_1	4.45±0.08	13.16±0.11	13.82±0.16	35.73±0.13	30.07±0.18	79.44±0.22	39.10±0.09
G_2	2.48±0.11*	22.46±0.08*	7.66±0.19*	21.94±0.23*	32.52±0.17*	88.02±0.25*	35.20±0.09*
G_3	3.44±0.08*	18.55±0.07*	11.93±0.13*	25.52±0.15*	31.49±0.23*	84.34±0.19*	36.70±0.09*
G_4	2.84±0.08*	19.12±0.10*	11.32±0.18*	23.63±0.20*	31.92±0.15*	85.71±0.19*	36.00±0.07*

Significantly different values compared to group G_1 at the level of $P \leq 0.05$ are indicated by (*).

Table 5: Serum enzymes analysis of birds belonged to various groups (Mean ± SD)

Group	26th day of experiment			35th day of experiment		
	ALT (IU/l)	AST (IU/l)	LDH (IU/l)	ALT (IU/l)	AST (IU/l)	LDH (IU/l)
G_1	23.70±1.64	114.30±1.77	261.90±4.84	34.30±1.34	116.30±1.16	303.00±3.49
G_2	76.50±1.53*	220.40±1.43*	373.40±5.08*	77.80±0.79*	223.50±1.08*	367.20±5.21*
G_3	43.40±0.07*	161.40±0.43*	336.50±4.53*	47.50±1.08*	168.70±1.42*	333.40±4.06*
G_4	51.30±1.57*	179.20±1.55*	335.80±4.87*	56.00±1.49*	181.10±1.37*	332.60±3.44*

Significantly different values compared to group G_1 at the level of $P \leq 0.05$ are indicated by (*).

Table 6: Serum proteins analysis of birds belonged to various groups (Mean ± SD)

Group	26th day of experiment			35th day of experiment		
	STP (g/dl)	Alb (g/dl)	Glob (g/dl)	STP (g/dl)	Alb (g/dl)	Glob (g/dl)
G_1	5.12±0.15	3.95±0.015	1.17±0.19	5.30±0.12	4.17±0.09	1.13±0.14
G_2	3.27±0.13*	1.72±0.08*	1.55±0.14*	3.37±0.09*	1.63±0.11*	1.74±0.08*
G_3	4.00±0.13*	2.55±0.11*	1.65±0.09*	4.03±0.09*	2.52±0.10*	1.57±0.14*
G_4	3.75±0.08*	2.21±0.07*	1.54±0.13*	3.87±0.11*	2.30±0.07*	1.57±0.13*

Significantly different values compared to group G_1 at the level of $P \leq 0.05$ are indicated by (*).

Table 7: Serum analysis of blood urea nitrogen (BUN) and creatinine (Creat) of birds belonged to various groups (Mean ± SD)

Group	26th day of experiment		35th day of experiment	
	BUN (mg/dl)	Creat (mg/dl)	BUN (mg/dl)	Creat (mg/dl)
G_1	14.00±0.49	0.22±0.01	15.30±1.06	0.22±0.01
G_2	40.30±1.25*	0.56±0.01*	42.60±1.26*	0.48±0.01*
G_3	27.30±1.16*	0.42±0.01*	29.40±1.07*	0.44±0.08*
G_4	31.00±0.63*	0.46±0.01*	33.10±1.37*	0.48±0.01*

Significantly different values compared to group G_1 at the level of $p \leq 0.05$ are indicated by (*).

DISCUSSION

NE is an important ailment of the avian alimentary canal caused by *C. perfringens* type A. Recently, *C. perfringens* type A negative for *netB* gene have been isolated from birds during outbreaks investigation in Pakistan (Abadeen *et al.,* 2021). NE affects growth performance and normal hematological and serum biochemical values in broiler birds (Suryakanth *et al.*, 2019). *IgYs* or Egg yolk antibodies (EYAs) are protective poultry antibodies produced in the egg yolk of the egg that can effectively neutralize specific pathogens and may have growth-promoting effect in poultry (Cook *et al.,* 1999; Yegani & Korver, 2007). The present study was designed to evaluate the ameliorative effects of anti-clostridial EYAs against deleterious effects of *C. perfringens* infection on growth performance and hemato-biochemical parameters in broiler birds. For this study, the birds were given *C. perfringens* infection and offered passive immunization by anti-clostridial EYAs and monitored for feed intake (daily) and live body weight gain (weekly). On days 26th and 35th of the experiment, blood samples were collected for hematology and serum biochemistry.

In the present study, the broiler birds in the infected non-treated group (G_2) showed reduced feed intake, poor body condition, and a lower body weight gain compared to the control group (G_1). These findings are in line with previous study of El-Deen *et al.* (2019) who reported poor body condition and higher mortality rates in broiler birds infected orally with *C. perfringens* type A. Elkomy *et al.* (2019) reported decreased body weight gain, loss of body condition and higher FCR values. Suryakanth *et al.* (2019) also investigated higher FCR values, loss of body condition and reduced feed intake in broilers. These changes are speculated due to the toxins produced by *C. perfringens* in the intestines (El-Kady *et al.*, 2012). The birds in passively immunized groups (G_3 and G_4) showed higher feed intake, and improved weight gain compared to group G_2. Tamilzarasan *et al.* (2009) reported that oral administration of 3 ml anti-clostridial *IgY* lowered the morbidity and mortality rates in infected birds without specific disease lesions. The use of EYAs helped to improve growth performance traits by regulating the immune system of birds. The production of interleukin-1 during the inflammatory process resulted in anorexia and muscle waste. Antimicrobials growth promoters specifically target intestinal pathogens but EVAs target specific neuropeptides that help to stimulate the immune system (Cook *et al.*, 1999).

In the current study, hematology indicated a significant ($P \leq 0.05$) reduction in TEC, PCV, and Hb values, whereas a significant ($P \leq 0.05$) increase in the values of TLC in the infected non-treated group (G_2) as compared to the control group (G_1). The serum biochemistry showed elevated values of ALT, AST, BUN, Creat, and Glob, while lower values of STP and Alb in the infected non-treated group (G_2) when compared to the control group (G_1). Similar results were reported in the previous study of Elkomy *et al.* (2019) that broilers challenge with *C. perfringens* type A resulted in a decrease in TEC, Hb, PCV%, STP and Alb values, while an increase in TLC values. Suryakanth *et al.* (2019) observed an increase in ALT and AST, while a decrease in STP levels in *C. perfringens* type A infected broiler birds. The findings of El-Deen *et al.* (2019) showed an increase in TLC, ALT, AST, Creat and Glob values, while a decrease in TEC, STP and Alb values in *C. perfringens* type A challenged birds. In passively immunized groups (G_3 and G_4), the hematology and serum biochemistry values were closer to the normal compared the infected non-treated group (G_2). This effect could be utilized to ameliorate clostridial infection. Higher values of STP are associated with damage of endoplasmic reticulum present in hepatic cells due to biding of tRNA with clostridial metabolites and cause inhibition of protein synthesis (Shane *et al.,* 1985).

The current study can be concluded as the anti-clostridial *IgY* isolated from egg yolk of the immunized hens effectively ameliorated the effects of *C. perfringens* type A infection on growth performance, hematology, and serum biochemistry of broiler chicken. The studies including the combination of *IgY* with other alternatives e.g. enzymes and probiotics to investigate the growth-promoting and therapeutic roles with an ultimate goal of antibiotic residue-free poultry and their products remain overdue.

Author's contribution: Conceptualization, ZUA and MTJ methodology and investigation, ZUA data curation and analysis, ZUA and MTJ writing-original draft preparation, ZUA writing-review and editing, MTJ supervision MTJ, FR and SUR.

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