SAARC J. Agric., 18(1): 209-218 (2020) *DOI: https://doi.org/10.3329/sja.v18i1.48394*

Research Article

EFFECTS OF TURMERIC POWDER ON *CLOSTRIDIUM PERFRINGENS* LOAD IN BROILER CHICKENS

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ABSTRACT

Necrotic enteritis (NE) is a major economic problem in broiler industry globally and is caused by *Clostridium perfringens*. The aim of the study was to know the effects of turmeric on *C. perfringens* in broiler chickens. A total of 3000-day-old Cobb 500 broiler chicks were divided into 6 groups and reared in environment control sheds with similar management. Each group contains 500 chicks and again divided into two subgroups as control and treatment with 250 chicks. In treatment groups added 2gm/kg turmeric powder with basal feeds and reared up to 30 days and follow standard vaccine schedule. Intestinal samples were collected every week from each group to detect *C. perfringens* load. Bodyweight gain, feed conversion ratio (FCR) and mortality rates were also calculated. The findings were loads of *C. perfringens* in treatment groups decreased significantly compared to control groups. At 4th week average count of *C. perfringens* was 4.44±0.12 log CFU/g and 2.68±0.17 log CFU/g in control and treatment groups, respectively. Average chick's mortality decreased significantly in treatment groups. The flock mortality was decreased significantly in treatment groups (1.40%) compared to control groups (2.17%). The FCR become significantly decreased in treatment groups (1.490) compared to control groups (1.571). Therefore, use of turmeric powder in broiler ration can reduce NE by decreasing *C. perfringens* loads and it could be a good source of non-antibiotic growth promoter in poultry towards reduce antibiotic resistance and consumer will get a pathogen free rich protein source.

Keywords: *Clostridium perfringens*, Necrotic enteritis, Broiler, Turmeric, Feed conversion ratio (FCR)

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Received: 05.05.2020 *Accepted: 16.06.2020*

INTRODUCTION

Necrotic enteritis (NE) is a major concern for the broiler industry due to it has significant health and economic impact (Skinner et al., 2010). It can reduce 12.0% body weight and not only that it increases 10.9% feed conversion ratio (FCR) (Wade and Keyburn, 2015). Thus, NE causes about USD878.19 to USD1480.52 financial loss per flock in the USA and USD 2 to 6 billion losses annually in the globe (Wade and Keyburn, 2015; Yang et al., 2019). Maximum digestion, absorption and metabolism of feeds are the key principal of broiler nutrition. NE hampered this physiology by necrosis of intestinal mucosa as well as nutrient absorption become decreased resulting in reduce FCR (Bhuiyan et al., 2019; Yang et al., 2019).

The major cause of NE is *Clostridium perfringens*, rod-shaped, spore-forming, gram-positive, anaerobic bacteria, and ubiquitously harbor in gut (Freedman et al., 2015; Miller et al., 2010). It could become pathogenic and causes NE in poultry when changing the dynamic balance of gut environment by starvation, stress, sudden change of feeds, and/or use of antibiotics as therapeutic purposes (Prescott, 2016; Ali, 2018). So, to prevent NE use of antibiotics as a growth promoter is the easy way of feed manufacturer (Silva et al., 2009). But bacteria become antibiotic resistance due to repeated use of antibiotics with feeds (Diarra et al., 2007; Nhung et al., 2017).

To save lives and microbial biodiversity an alternative use of antibiotics is the prime demand. Turmeric (*Curcuma longa*) is a natural herb under family- Zingiberaceae, extensively used as spice and coloring material in Asian countries. It can act as an important source for an alternative to antibiotics due to its antimicrobial properties. It is less toxic, residue-free and natural compared to synthetic antibiotics or inorganic chemicals (Khan et al., 2012). The active ingredients of turmeric are curcumin, tetrahydrocurcuminoids, demethoxycurcumin and bisdemethoxycutcumin and these are proven against bacteria (Niranjan and Prakash, 2008). Application of turmeric as a supplement in poultry feeds can play an important role in control NE by inhibiting *C. perfringens* and as well as feed efficiency becomes increased (Niranjan and Prakash, 2008; Khan et al., 2012). So, there is a need to explored and elucidate the safe alternatives of antibiotics to control NE. Therefore, the aim of the experimental study was to evaluate the effects of the turmeric powder on *C. perfringens* in intestines to control the NE and use of it instead of antibiotics.

MATERIALS AND METHODS

Turmeric powder trial

Experiment of turmeric powder trial as feed additives, a total 3000-day old Cobb500 unsexed broiler chicks were collected from Nourish Poultry and Hatchery Ltd. Dhaka, Bangladesh with history of same pedigree line. The chicks were divided randomly into 6 groups (replication) as G1, G2, G3, G4, G5, and G6 where 500 chicks present in each group and reared in 6 different sheds. Aga in chicks were

divided into two subgroups within the group by metal wire net as treatment group (n=250) and control group (n=250). Birds were reared in artificial controlled house (Cumberland, USA) maintaining daylight, ventilation, air speed, humidity, temperature etc. In the treatment groups used 2g turmeric powder per kg basal feed and in control groups fed only basal feed. Broiler pre starter (a mash feed) were fed during 0-11 days old age, broiler starter (a mash feed) were fed during 12-21 days age and broiler grower feed (pellet feed) were fed up to 30 days of age as basal feed. These are corn based ready feed manufactured by Nourish Poultry & Hatchery Ltd. Dhaka, Bangladesh with 2950 kcal, 3000 kcal and 3050 kcal energy in broiler pre starter, broiler starter and broiler grower feed, respectively. Artificial brooder was provided during first 10 days and standard management of feeder, waterer and floor space was provided uniform for all birds. Only rice husk used as bedding till 10^{th} days of age and then mixed dried saw dust 50% of total bedding materials. The vaccine schedule was eye drop at day 1 of age by Nobilis® Ma5 + Clone 30 (Intervet International B.V., The Netherlands), at 11^{th} and 18^{th} days of age Nobilis® Gumboro D78 (Intervet International B.V., The Netherlands) in drinking water, and finally used Nobilis® ND Clone 30 (Intervet International B.V., The Netherlands) at 22th day of age in drinking water, there was no use of any antibiotic during experimental period.

Sample collection and processing for *C. perfringens* load analysis

To assess the effects of turmeric powder on *C. perfringens* three healthy birds were selected randomly from each treatment and control group at 0-day, 7^{th} day, 14^{th} day, 21^{th} day and 28^{th} day of age. Euthanized the birds with cervical dislocation by the help of veterinarian and ensure that all muscle contractions have ceased before beginning dissection. Used sterile scissors carefully made an incision through the skin and muscle from the mid abdomen to the breast to expose the abdominal cavity. Then the liver and spleen were separated from the gastrointestinal tract (GI tract). Cut the GI tract from the duodenal loop, just distal of gizzard and just proximal of cloacal vent. Removed the entire GI tract by gently freeing any remaining connective tissue still bound to the body cavity or any other organs and placed the GI tract in a sterile Whirl-Pac bag (Nasco, USA) for further processing. Then GI tract was placed onto a clean and sterile dissecting tray and cut into 10 cm sections from each duodenum, jejunum and ileum. The lumens of GI tract were spread and flush out the digesta by sterile PBS, and kept in sterile stomacher bags. Weight out the 25 g GI tract sections and stomached at 230 rpm for 2 min by adding 250 ml brain heart infusion (BHI) broth (Oxoid, UK) and then 6- fold decimal dilution was done. Then incubated for 24 h at 37^{o}C in anaerobic vented jar with appropriate amount of anaerobic gas generating packs (Mitsubishi Gas Chemical America Inc., New York).

Subsequentially, 100 µl of pre-enriched BHI broth from each dilution was inoculated into plates of tryptose sulphite cycloserine (TSC) agar base (Oxoid, UK) enriched with 5% egg yolk and supplemented with β D-cycloserine (Oxoid, UK). The plates were sealed tightly, incubated for 24 h at 37^{o}C upside down in anaerobic condition. The black colonies on plates were examined and counted for typical colonies of *C. perfringens* and results were recorded as log CFU/ml (Mwangi et al., 2019). Finally, the pure isolates were confirmed by the biochemical (lactose fermentation) and gram staining test.

Body weight gain

At the age of 30th day, total live body weight and total feed consumption of all living birds from treatment and control pans of all 6 replicated were measured. According to the collected parameters the effects of turmeric powder on body weight gain and feed conversion ratio were calculated.

Statistical analysis

The data from sample collection and laboratory results were recorded as coding into Microsoft Excel spreadsheet 2010 (Microsoft Corporation, WA, USA). The *C. perfringens* colony count data were converted into log CFU/ml by Log CFU/mL = Log10 (CFU / (dilution factor*aliquot)). Data regarding total feed intake and body weight gain were recorded daily and feed conversion ratio (FCR) was calculated on weekly basis. The FCR was calculated by dividing the feed intake by weight gain. Then the data of Log CFU/mL and FCR on six treatment and control groups were subjected to one-way ANOVA (analysis of variance) by using GLM (General Linear Model). The mean comparison on treatment and control groups were calculated by using Duncan's multiple range tests. All statistical analysis was done in statistical package SPSS version 25 (2017) (IBM corp. New York, USA). The $P<0.05$ was used to determine the significance.

RESULTS AND DISCUSSION

The *C. perfringens* counts were decreased significantly (p= 0.003) in all six replications of turmeric trial compared to the control groups (Table 1). At day 1 of age in both control and treatment groups and at 1st week of age in treatment groups *C. perfringens* counts was below detection limit (<1.0 log CFU/g) in all six groups. On 2nd week of age average count was 4.24±0.14 log CFU/g in control groups whereas below detection limit (<1.0 log CFU/g) in 4 treatment groups including G2, G4, G5, and G6. During culling at 4th week of age average count declined significantly from 4.44±0.12 log CFU/g to 2.68±0.17 log CFU/g in control groups and treatment groups respectively.

Table 1. The effects of turmeric powder on *C. perfringens* counts of broiler chicken.

Group	Count of *C. perfringens* (log CFU/g)										Mortality (%)	
	DOC		1st week		2nd week		3rd week		4th week			
	C	T	C	T	C	T	C	T	C	T	C	T
G1	<1.0	<1.0	3.47±0.01	<1.0	4.40±0.11	2.47±0.291	4.34±0.61	<1.0	4.25±0.55	2.69±0.21	1.40	0.80
G2	<1.0	<1.0	3.85±0.00	<1.0	4.17±0.21	<1.0	4.25±0.38	1.6±0.44	4.50±0.08	2.84±0.73	1.80	1.20
G3	<1.0	<1.0	4.04±0.06	<1.0	4.30±0.70	2.69±0.17	4.17±0.13	1.77±0.49	4.60±0.71	2.84±0.10	2.00	1.60
G4	<1.0	<1.0	3.95±0.10	<1.0	4.34±0.61	<1.0	4.40±0.19	<1.0	4.47±0.49	2.47±0.26	2.60	2.00
G5	<1.0	<1.0	3.95±0.02	<1.0	4.20±0.22	<1.0	4.40±0.09	1.90±0.64	4.44±0.18	2.77±0.34	2.40	1.80
G6	<1.0	<1.0	3.95±0.03	<1.0	4.00±0.13	<1.0	4.34±0.22	1.60±0.42	4.36±0.06	2.47±0.47	2.80	1.00
Average	<1.0	<1.0	3.95±0.04	<1.0	4.24±0.14	3.58±0.09	4.32±0.14	1.72'±0.12	4.44±0.12	2.68±0.17	2.17	1.40
p	0.003*										0.000*	

*Significant at the 0.05 level; <1.0= not detected; CFU= colony forming unit; DOC= Day old chicks; T= Treatment (2gm turmeric powder per kg basal feed); C= Control (only basal feed)

The chicken mortality rates became significantly (p= 0.000) decreased in treatment group compared to the control groups. Average chicken mortality rates reported 2.17% in control groups and 1.40% in treatment groups (Table 1).

The effects of turmeric powder on FCR of broiler chicken have been demonstrated a significant (p= 0.002) association. The average FCR of control and treatment groups recorded 1.571 and 1.490 respectively (Table 2). About 17.33 g (2.22%) weight gain occurs in treatment groups compared to control groups.

Table 2. The effects of turmeric powder with basal feeds of broiler chickens on feed conversion ratio (FCR).

Data	Group						Average
	G1	G2	G3	G4	G5	G6	
Control	1.533	1.504	1.685	1.583	1.56	1.563	1.571
Treatment	1.501	1.458	1.537	1.566	1.444	1.43	1.49
Differences (g)	16	28	21	9	11	19	17.33 (2.22%)
p	0.002						

*Significant at the 0.05 level

Necrotic enteritis (NE) is a cause of *C. perfringens* that affects on feed utilization by disrupting the intestinal epithelium (Mwangi et al., 2019). The major clinical signs of NE have suddenly increased mortality, reduce weight gain, poor feed conversion, and necroses of intestinal mucosa are major postmortem lesions (Immerseel et al., 2004). The prevalence of *C. perfringens* type A is 64.73% in poultry and well known as a cause of foodborne illness and gas gangrene to humans (Khan et al., 2012; Songer, 1996). The prevalence of NE is demonstrated to be 8% in broiler chickens in Bangladesh (Miah et al., 2011).

Historically herbs and spices are being used as feed additives for farming animals due to its medicinal properties (Frankic et al., 2009). These natural plant products have been proven less toxic, with no residue, and antioxidant and antimicrobial effects so it is now being considered as the ideal feed supplement of livestock worldwide (Khan et al., 2012). Turmeric (*Curcuma longa*) is a natural herb and popular for medicinal properties. The active ingredients of turmeric are tetrahydrocurcuminoids, curcumin, demethoxycurcumin and turmerones that are proven with their anti-inflammatory, anti-carcinogenic, and antibacterial properties (Mitsch et al., 2004). Mitsch et al. (2004) shows the antibiotic property of turmeric on *C. perfringens* and concluded 2gm/kg turmeric in feed supplements can inhibit the growth of *C. perfringens*. There is a rapidly growing interest to produce broiler meat without antibiotics and use of non-antibiotic growth promoters worldwide in order to maintain a bird's health and growth performance (Khan et al., 2012).

The findings of research study included the use of turmeric powder in feed can reduce *C. perfringens* count significantly compared to control group. Researchers identified turmeric as having anti-inflammatory (Lee, 2016), wound healing, and anticancer (Kim et al., 2012) properties. Intestinal health was normal in treatment groups that help to increase absorption of dietary nutrients through smooth intestinal epithelium. As a result, FCR became significantly lower in treatment groups compared to control groups. These results are strongly supported by the experiment of Kafi et al. (2017) they demonstrated that use of turmeric in broiler feed at the rate of 0.75% level as feed additives can improve growth performance as well as increase profitability. Turmeric use in broiler ration can improve growth performance although its concentration, dose, and duration of use varies in different experiments like AL-Kassie et al. (2011) used as 0.75% to 1%, AL-Sultan (2003) used as 0.5 to 1%, and Ahmadi (2010) used as 0.9% in basal feed.

Antimicrobial resistance is an important health issue for both humans and animals globally including Bangladesh (Ahmed et al., 2019; Ali and Hasan, 2018). The use of antibiotics in animal and fish feeds as growth promoter though improve feed efficiency and increase growth but they are modifying intestinal flora and creating a selective pressure to develop antibiotic resistance which favor a resistant bacteria (Collier et al., 2003). So several European countries banned or restricted the use of antibiotic growth promoters in feed (Casewell et al., 2003). The Government of Bangladesh also completely banned antibiotics in animal and fish feeds as a growth promoter by Government of Bangladesh through Fish and Animal Feed Act-2010 (Fish and Animal Feed Act, 2010). The use of anticoccidial drugs and antibiotics in animal feeds to control coccidiosis and NE was the prime focus of animal feed manufacturers (M'Sadeq et al., 2015). Therefore, the application of turmeric in poultry feeds could be a good solution to prevent antimicrobial resistance. Finally, 2% turmeric powder supplements as feed additives in broiler ration could increase feed efficiency, production performance and decrease *C. perfringens* growth in intestine.

CONCLUSIONS

Turmeric (*Curcuma longa*) has been using as traditional herbal medicine and antimicrobial agents. It can be used as a natural growth promoter in broiler diet due to it can reduce *C. perfringens* load in gut, decrease necrotic enteritis as well as increase feed efficiency. However, further study on pharmacological activity, proper dose, and duration of use is required.

ACKNOWLEDGEMENT

The work was supported by grants of the Bangladesh Livestock Research Institute and Nourish Poultry & Hatchery Ltd.

CONFLICTS OF INTEREST

None of the authors have any conflict of interest to declare.

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