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RESEARCH ARTICLE

Effect of Chlorine Dioxide (Dutrion®) on Growth Performance, gut Histomorphology and Pathogenic Microbial Count of Meat type Birds

A Sultan[1], M Bilal[1], S Khan[1]* and ZU Hassan[2]

[1]Department of Poultry Science; [2]Department of Animal Health, The University of Agriculture, Peshawar, Pakistan
*Corresponding author: dr.zaminaup@gmail.com

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ABSTRACT

The potential benefits of a water acidifying agent chlorine dioxide (Dutrion®) were investigated on the production performance, ileal intestinal microflora and gut histomorphology at day-28. One hundred and sixty, day-old, Cobb-500 male broiler birds were randomly assigned to four treatments i.e. DW-0, DW-0.3, DW-0.4 and DW-0.5 that were replicated (n=4) with 10 birds/replicate. Birds in all groups were reared on floor pens in an open sided house and had *ad libitum* access to feed and water. DW-0 served as control and to DW-0.3, DW-0.4 and DW-0.5 was given 0.3, 0.4 and 0.5 ppm of chlorine dioxide in drinking water, respectively. Lower ileum tissue and digesta samples were collected and performance variables were noted. Chlorine dioxide significantly ($P<0.05$) reduced *E. coli* and *Salmonella* count on day 21st and 28th. It was revealed that gut histomorphology, villus height (920.03 µm) and goblet cell count per unit (80.25) of birds in group DW-0.5 was significantly improved. Feed and water intake was not significantly altered however, body weight gain and FCR significantly improved with increasing level of Dutrion®. Carcass (70.39%) and liver (6.08%) yield showed a significant increase in birds of group DW-0.5. No difference ($P>0.05$) was seen in relative liver and gizzard weight (%) among different treatments. It can be concluded from present findings that addition of chlorine dioxide (Dutrion®) can serve as an effective tool to improve broiler performance by reducing the load of harmful pathogens and improving gut health of birds.

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INTRODUCTION

Water quality is one of the most critical factors limiting broiler performance than feed quality (Abbas *et al*., 2008) and is of great concern to poultry birds. Importance of water is well recognized due to its role in various body physiological functions (Bruno *et al*., 2011). Moreover, as rule of thumb poultry birds drink 1.5 to 2 times more water to feed consumption at optimum temperature (Kellems and Church, 2002). Water plays a key role in enzymatic and chemical reactions, thermoregulation, nutrient transportation and lubrication of joints and organs (Batal *et al*., 2005). Globally water quality is challenged due to increase in environmental pollution that can hamper poultry birds performance negatively (Watkins, 2008). It has been reported that water quality of Peshawar region is contaminated with different pathogens (Rehman and Khan, 2000) lowering quality of drinking water. Furthermore, transmission of bacterial, viral and protozoan diseases could be accelerated due to poor management practices that badly affect water quality (Refregier *et al*., 2001). Increased number of harmful microbes and poor water quality reduces performance of birds by affecting nutrient digestibility and its assimilation (Fronte *et al*., 2013). Due to poor quality of water normal mucosal layer of the intestinal tract is disturbed causing poor litter quality due to water losses and buildup of noxious gases in the poultry house (Cengiz *et al*., 2012; Ahmad *et al*., 2013; Sharaf *et al*., 2013).

Poultry farming in Pakistan is still practiced on conventional standards with less innovations and adoption of modern technology due different reasons. Poultry farmers in majority cannot afford installation of expensive automated drinking system to minimize water contamination.

The use of antibiotics, acidifying agents and sanitizers is commonly practiced to avoid the associated

adverse effects of bad quality water (Binnie *et al*., 2002). Chlorination and acidification of drinking water have been recommended as a potential control measure in broiler production (Philipsen, 2006). Excessive usage of some acidifying agents can sometimes lead to a reduction in the feed and water consumption and depression in growth rate which is probably due to the strong taste of acids. Some sanitizers and chemicals used in poultry water are corrosive and disturb normal gut function with increased mucosa production and sloughing of intestinal wall (Binnie *et al*., 2002; Atapattu and Senevirathne, 2013).

The use of Chlorine dioxide has got a number of advantages over other chemicals and can be used in a wide range of pH to improve water quality (Korn *et al*., 2002). Chlorine dioxide can effectively inactivate viruses by altering capsid proteins of the viruses and other bacteria and maintain a balanced microbiota in the gut (Tian *et al*., 2010). Similarly, it is also highly effective against *E. coli* and makes changes in the physical structure of *Giardia* cyst and its inactivation (Liyanage, 1997). It has also been reported that chlorine dioxide is highly effective against *Salmonella* and other pathogenic microbes (Eryılmaz *et al*., 2013). No or limited research have been conducted to assess the beneficial effects of Chlorine dioxide in drinking water on poultry birds. Present study was therefore planned to examine beneficial impacts of chlorine dioxide (Dutrion®) Duka, Holland) on broiler production, *E. Coli* (CFU) and *Salmonella* load in the lower gastrointestinal tract, gut histomorphology, villus height (µm) and goblet cells count.

MATERIALS AND METHODS

All husbandry practices like handling of live birds, welfare and other lab protocols were pre-approved by the Animal Ethic Committee of the University of Agriculture, Peshawar.

Housing and management: Experimental house was cleaned and fumigated before the commencement of this study in the month of March, 2014. All floor cages (2x5 sq. feet) were cleaned and fitted with round drinkers (23cm diameter) and feeders (20cm diameter). Biosecurity and strict hygienic measures were maintained during the course of trial. Optimum environmental conditions (Temp, 95-75^{o}F; RH, 65-60%) to bird's standard requirements were maintained during the experiment. One hundred and sixty male broiler birds (Cobb-500) were obtained from a local commercial hatchery. These birds were randomly allocated in a completely randomized design to four treatment groups designated as DW-0, DW-0.3, DW-0.4 and DW-0.5 having four replicates (10 birds/ replicate). Birds in group DW-0 were offered fresh drinking water (pH, 7.5; normal color and odour; Total dissolved solids 311mg/I; total bacteria, 300-550 cfu/mL) and Dutrion® (Duka, Holland) was added @ 0.3, 0.4 and 0.5 ppm/lit of water in DW-0.3, DW-0.4 and DW-0.5 treatments having 6.61, 6.11 and 5.75 pH, respectively, for 28 days. During this time all birds were regularly monitored for any untoward behavior and mortality. Sick and dead birds, if any, were removed and unusual observations were recorded.

Feed, water intake and body weight gain was measured and feed conversion ratio (FCR) was calculated. FCR was adjusted for dead birds, if any in different treatments: FCR = (weight of feed consumed)/ (weight gain of survivors + weight gain of mortalities). On day 28, two birds were randomly selected from each replicate. These birds were weighed, Halal slaughtered (Addeen *et al*., 2014) and de-skinned. From killed birds all edible and non-edible parts were removed and dressed carcass was weighed. Carcass yield was expressed in dressing percentage.

Dressing percentage = Dressed weight / Live weight × 100

Liver and heart were removed from these birds. All the adhering tissues were stripped off from these organs, weighed as expressed as proportion to total body weight. Lower ileal digesta samples were collected on day 21 and 28th aseptically, in buffered saline and transported at 4ºC to the microbiology laboratory of the Animal Health Department of The Agriculture University, Peshawar. Serial dilution methods using MacConkey agar (Oxoid, Basingstoke, UK) and Brilliant Green Agar (Oxoid, Basingstoke, UK) for *E.coli* and *Salmonella,* respectively was used. The cultured plates were incubated at 37°C and to enumerate colonies counting chamber was used (Quinn *et al.,* 2002). Total colony counts were expressed as $\log_{10}$ cfu/g of contents determined by multiplying reciprocal of the dilution factor and average numbers of colonies. Confirmation of *E-coli* and *Salmonella* was done with the help of biochemical tests (Khushi *et al*., 2002).

Goblet cells count and villus height: Tissue samples in duplicate (n=64) from the lower ileum of randomly slaughtered birds (n=32) were collected in 10% buffered formalin. The collected samples were fixed in formalin for 7 days. After fixation the samples were shifted to the Laboratory of Histology, Department of Animal Health, The University of Agriculture, Peshawar for further processing. All tissues samples were washed, processed (Tek® Rotary, Japan) and embedded (Tissue-Tek®, Japan). Tissues were sectioned and slides were prepared at 5 µm with microtome (Accu-cut SRM, Japan). All slides were stained with eosin and hematoxylin stain by automatic staining machine. Villus height was measured with the help of micrometer from the top of the villus to the top of the lamina propria. Multiple measures were taken per bird for this variable. For statistical analysis average of these values was used. Goblet cells were counted along the villus length in numerous microscopic fields for each tissue, and average was taken for this variable.

Statistical analysis: All data were statistically analyzed using one way of ANOVA (completely randomized design) and means of different experimental treatments were separated by Duncan's multiple range test using SAS (2004).

RESULTS

Chlorine dioxide had no significant impact on the feed and water intake of birds. However, this had significantly ($P<0.05$) improved body weight gain (8.32 and 9.35%) and FCR (1.86 and 1.82) of birds in group DW-0.4 and DW-0.5, respectively. Difference between

these two groups was however insignificant (Table 1). Chlorine dioxide in drinking water did not make any significant change to the relative weight (%) of liver, heart and gizzard to body weight as indicated in Table 2. It was revealed that Dutrion® had significantly increased carcass yield of broiler birds in the last week of the experiment. Higher carcass percentage yield was observed in group DW-0.5 (70.01%) followed by DW-0.4 (68.50%).

Both *Salmonella* and *E.coli* count were significantly ($P<0.05$) altered at day 21 and 28 in the lower ileum contents as given in Table 3. *Salmonella* count was greatly reduced in the last week of the experiment across all the treated groups however, more pronounced decrease was seen in the values of group DW-0.5 (2.63 and 2.58 log_{10} cfu/g) as compared to low concentration of Dutrion® as shown in Table 3. Significant reduction in *E. coli* count was observed in group DW-0.5 (3.73 and 3.53 log_{10} cfu/g) followed by groups DW-0.4 (3.86 and 3.73 log_{10} cfu/g) at day 21st and 28th, respectively.

Goblet cells count per microscopic field along the sides of villus of lower ileum on day 21 and 28 was significantly higher in groups supplemented with Dutrion® compared to control. DW-0.5 had however, greater number of goblet cells (71.50 and 80.25) among all the treatments on day 21 and 28, respectively (Table 4). Application of Dutrion® had significantly ($P<0.05$) increased villus height of group DW-0.5, 815.01 µm and 920.03 µm on day 21 and 28, respectively, with non-significant difference in group DW-0.4 and DW-0.3 (Table 4; Fig. 1).

Table 1: Effect of Chlorine dioxide (Dutrion®) on feed intake (g), body weight gain (g), feed conversion ratio (FCR) and water intake (mL) of broilers at day 28

Groups	Feed intake (g)	Body weight (g)	FCR	Water Intake (mL)
DW-0	1991.7	997.2^{b}	1.99^{b}	4034.0
DW-0.3	2006.7	1012.0^{b}	1.98^{b}	4013.0
DW-0.4	2013.3	1080.2^{a}	1.86^{a}	4027.0
DW-0.5	1992.7	1090.5^{a}	1.82^{a}	4084.5
Pooled SEM*	35	16	0.12	65
P-Value	0.454	0.03	0.02	0.09

Mean carrying different superscripts are significantly different ($P<0.05$).

Table 2: Effect of chlorine dioxide (Dutrion®) on relative weight of liver, heart and gizzard in percent to body weight and carcass yield (%) of broiler birds at day 28

Groups	Liver	Heart	Gizzard	Carcass yield %
DW-0	2.21	0.68	1.52	62.50^{b}
DW-0.3	2.19	0.71	1.54	63.25^{b}
DW-0.4	2.16	0.61	1.47	68.50^{a}
DW-0.5	2.31	0.70	1.47	70.01^{a}
Pooled SEM	0.09	0.02	0.8	0.63
P-Value	0.12	0.21	0.26	0.02

Mean carrying different superscripts are significantly different ($P<0.05$).

Table 3: Effect of chlorine dioxide (Dutrion®) on *Salmonella* and *E-coli* count (log_{10} cfu/g) count in lower ileum content of broiler birds at day 21 and 28

Groups	*Salmonella* count (log_{10} cfu/g)		*E-coli* count (log_{10} cfu/g)	
	Day-21	Day-28	Day-21	Day-28
DW-0	2.85 ± 0.02^{a}	2.81 ± 0.04^{a}	4.20 ± 0.02^{a}	4.15 ± 0.04^{a}
DW-0.3	2.80 ± 0.01^{ab}	2.77 ± 0.06^{ab}	3.96 ± 0.05^{b}	3.94 ± 0.06^{ab}
DW-0.4	2.77 ± 0.01^{b}	2.74 ± 0.05^{b}	3.86 ± 0.05^{bc}	3.73 ± 0.05^{b}
DW-0.5	2.63 ± 0.03^{c}	2.58 ± 0.04^{c}	3.73 ± 0.07^{c}	3.53 ± 0.04^{b}
P-Value	0.0001	0.0001	0.000	0.029

Mean±SE carrying different superscripts are significantly different ($P<0.05$).

Table 4: Effect of chlorine dioxide (Dutrion®) on goblet cells count and mean villus height (µm) of lower ileum of broiler birds at day 21 and 28

Groups	Goblet cells count		Villus height (µm)	
	Day-21	Day-28	Day-21	Day-28
DW-0	64.25 ± 0.37^{c}	74.00 ± 0.91^{c}	745.0 ± 1.54^{b}	847.5 ± 11.08^{b}
DW-0.3	65.00 ± 0.40^{c}	76.00 ± 0.91^{bc}	797.0 ± 1.88^{a}	900.0 ± 17.79^{a}
DW-0.4	67.00 ± 0.41^{b}	77.50 ± 1.10^{ab}	805.0 ± 1.75^{a}	907.5 ± 13.76^{a}
DW-0.5	71.50 ± 0.64^{a}	80.25 ± 0.75^{a}	815.0 ± 1.93^{a}	920.0 ± 9.12^{a}
p-value	0.000	*0.004*	0.005	0.011

Mean±SE carrying different superscripts are significantly different ($P<0.05$).

DISCUSSION

Chlorine dioxide did not alter water and feed intake of birds though significantly improved body weight gain and efficiency of feed utilization by birds. This could be due to the inhibitory effects of chlorine dioxide on the growth and colonization of pathogenic microflora which competes for the readily available nutrients with the host bird. Açıkgöz *et al.* (2011) reported similar findings when birds were fed some acidifying agents who did not change feed and water intake however, improved body weight gain and FCR. A more balance microbiota with reduced number of pathogens (Dibner and Richards, 2005) and improved gut integrity of the birds as noticed in present study assisted birds in better utilization of feed and improving body weight gain (Cengiz *et al*., 2012). Hence, preventing pathogenic bacteria proliferation and modulation of indigenous bacteria improved health status, immune and performance of the birds. Similarly, findings of (Yang *et al*., 2009; Fronte *et al*., 2013) also support the outcomes of present study. No adverse effect of chlorine dioxide was seen on water intake of birds indicating no or least change in normal taste of water. Chlorine dioxide is however capable to bind with phenols, humic-acid, iron and sulfides which alters the taste and odour of drinking water (Gordon *et al.,* 2000).

There was no alteration in the relative weight (%) of liver, heart and gizzard to body weight with Dutrion® supplementation in drinking water. Islam *et al.* (2008) found a significant increase in visceral organ weight by using different water acidifying agents. Difference with current findings is probably due to the age of birds used in different experiments. Contrary to present findings Cengiz *et al*. (2012) also found better relative weight of gizzard in acidifier supplemented group. Better carcass yield (70.01%) found in present study is the result of higher body weight gain (1090.51g) in Dutrion® supplemented groups is probably due to better nutrient assimilation. Islam *et al*. (2008) noticed that broiler fed on an acidifying agent gained better body weight gain and significantly better carcass yield that coincides with current findings.

Numbers of pathogenic microorganisms (*Salmonella* and *E. coli*) in present study were significantly reduced both at day 21 (2.63 and 3.73 cfu/g, respectively) and 28 (2.58 and 3.53 cfu/g, respectively) at lower ileum probably due to disruption of the permeability of the outer membrane of pathogens by chlorine dioxide (Maćkiewicz and Dziubek, 2005). Dibner and Buttin (2002) reported similar findings of reduced pathogenic load in the intestinal tract of broiler using organic acidifiers and supports findings of present study. Reductions in pathogenic microbes in broiler gut was also reported by

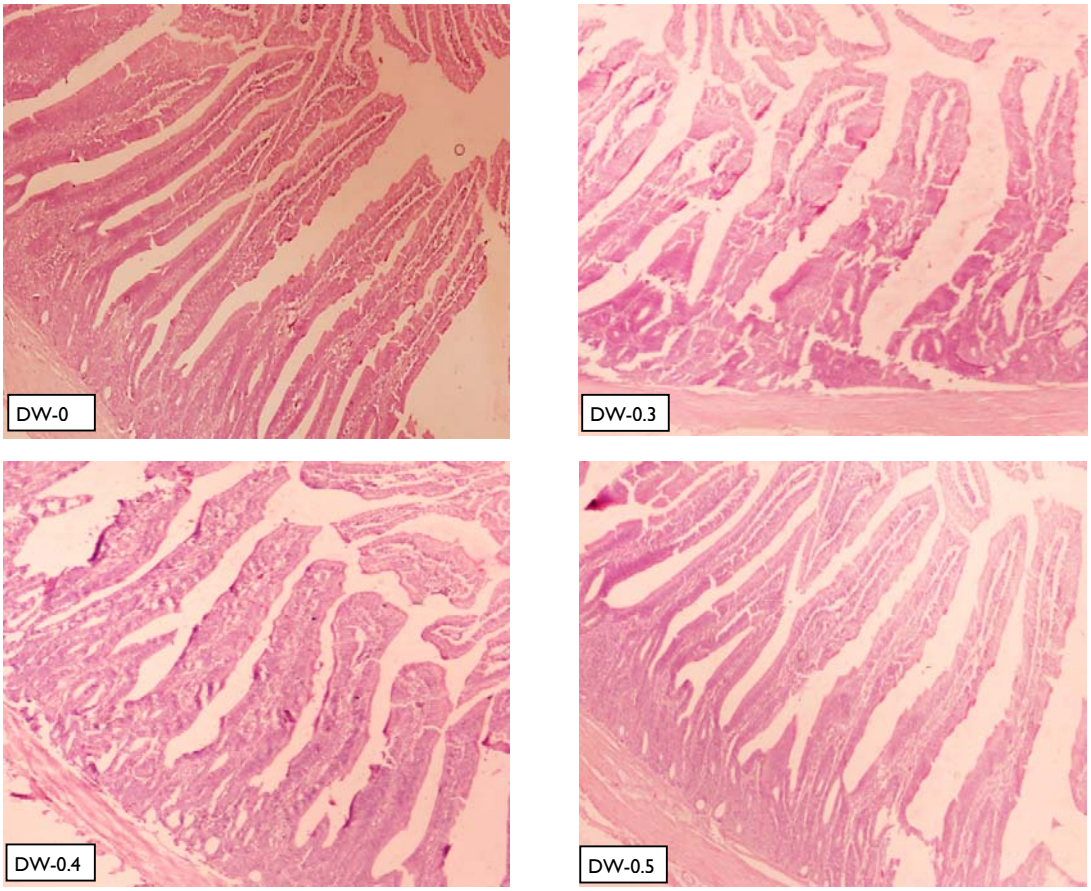

Fig. 1: Photomicrograph of gut of broiler birds supplemented with different levels of chlorine dioxide showing the height villi of at 21st day of age. H&E; X100.

Isabel and Santos (2009) using plant origin essential oils and other acidifying agents and are inline to current study. Khan *et al*. (2013) and Fronte *et al*. (2013) observed similar findings in their studies using acidifying agent in drinking water of poultry birds that significantly reduced *Salmonella* and *E. coli* and support findings of present study

It was observed that Chlorine dioxide did not adversely affect the gut lining however improved its integrity by increasing villus height (920.03µm) and goblet cells count (80.25). This impact could be associated by preventing colonization of pathogenic microbes that cause sloughing of intestinal mucosal lining through pathogenic inflammation deteriorating villus height and function of goblet cells secretion, digestion and absorption of nutrients (Samik *et al*., 2007). Intestinal mucosal layer is highly populated with microbes, both commensals and pathogenic badly affecting intestinal lining. Pathogenic bacteria often lead to infection, disrupting the bird's health resulting necrotic-enteritis, which is responsible for reducing growth rates (Garrido *et al*., 2004) and there proliferation can be prevented by use of quality acidifying agents (Cengiz *et al*., 2012). Figure1 showed that gut lining of Dutrion® supplement is more integrated with improved villus height and goblet cell count. It could be due to reduced number of pathogenic microbes in the lower gut of these birds. Goblets cells are responsible for the secretion of mucus in the form of polymeric mucin glycoprotein. Panda *et al*. (2009) and Cengiz *et al*. (2012) observed similar findings in their research work. They reported that when birds were offered acidifying agents showed remarkable improvement in villus height and gut histomorphology and coincides with the findings of current study. Fronte *et al*. (2013) investigated the influence of an acidifying agent and observed similar finding of improved villus height in treated group supporting outcomes of present study.

Conclusion: It was concluded from present study that Chlorine Dioxide (Dutrion®) possess beneficial impacts to improve broiler performance by reducing the load of harmful pathogens and improving gut health of meat type birds. However, further studies are needed to investigate its effect at different stage of life of broiler birds.

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