Research Article



Probiotic (ROEMIN W2) Improved Growth Performance and Intestinal Histomorphological Structure in Broilers Challenged With E.Coli

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Abstract: Thecurrent study investigated the role of commercial probiotic (ROEMIN W2) on growth performance and in preventing or treating chicks challenged with E.coli.Three hundred one day- old mixed Cobb broiler chicks, divided into 5 groups and reared for 42 days. G1was the control group. G2 received probiotic (ROEMIN W2). G3challenged with E.coli. G4challenged with E.coli after receiving of ROEMIN W2. G5challengedfirstly with E.coli then received ROEMIN W2. Body weight gain, feed consumption, and feed conversion ratio calculated for the complete experimental period. Two birds from each replicate were taken and slaughtered (at the 3rd and 6th weeks) for determination of carcass weight. Duodenum collected for both histomorphological and scanning electron microscope studies.ROEMIN W2 significantly improved performance in G2andG4 in spite of the E. coli infection compared to control group. G5 had the same FCR and carcass weight as the control group in spite of the E. coli infection. There was a significantly decreased growth performance in the E coli infected non ROEMIN W2 supplemented group, G3 compared to all other treatments. The collected data revealed pronounced intestinal villi improvement in groups treated with probiotic, while infected non- treated group showed decrease length of villi and increase depth of the crypts recorded. Scanning Electron microscope of groups treated with probiotics showed normal length long finger-like projection.Crypt area showed a numerous number of proliferating enterocytes having longer microvilli. Whereas the E. coli infected group showed short distorted duodenal villi with massive destruction and loss.

Keywords: Intestine histomorphology,lactic acid bacteria, broilers, growth performance, E.Coli.

INTRODUCTION

In modern intensive poultry production, normal flora is slow in colonizing the intestine of newly hatched chicks (Fuller, 1989). Therefore, antibiotics are used to prevent diseases and improve growth performance. The use of antibiotics in poultry industry led to development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999), and imbalance of normal flora (Andremont, 2000). All of the above led to banning of antibiotic use in poultry diets. One alternative of antibiotics is the use of probiotics.

Probiotics are live microorganisms which, when administered in adequate amount, confer a health benefit on the host (FAO/WHO, 2002) and have beneficial effects on growth performance (Dizaji et al., 2012). Efforts made to develop commercial probiotics in which organisms such as Lactobacillus and Bifidobacterium species are incorporated. These commercial probiotics may modulate gut microbial composition, leading to improved gut health and improved resistance to pathogenic bacteria (Staton et al., 2001).

The objective of the current study was to observe the role of water-solubleprobiotic(ROEMIN W2)on growth performance and in preventing or treating chicks infected with E.coli.

MATERIAL AND METHODS

Experimental Design

Three hundred one day- old, mixed Cobb broiler chicks were used. Chicks were randomly distributed into five groups. Each group had three replicates 20 birds each. Birds reared for 42 days. The first group G1 is the control non-treated non-challenged group. In G2, birds were given the commercial probiotic ROEMIN W2(each g contains Lactobacillus acidophilus 2x108 CFU, Lactobacillus thermophilus 2x108 CFU, Bifidobacterium 1 x108 CFU and Lactose) (China way corporation, Taiwan) at dose of 0.5 g per 1 liter of drinking water daily for 6weeks. G3 wasnon-probiotic treated only challenged with E-coli at 7th day. G4 given ROEMIN W2 from the first day to the 7th day (0.5 g / liter)then challenged with E. coli. G5 challenged with E. coli at 7th day then given ROEMIN W2 after appearance of symptoms of infection for seven days, with double the recommended dose, 1g per 1 liter. Birds housed in floor pens, maintained under continuous lightening program, good ventilation, suitable temperature (begin at 32°C and decreased one °C every two days until 26 °C). Birds had free access to feed and water. Experimental diets formulated according to NRC (1994) Table (1). Birds were vaccinated at the 5th day by Hitchner B1 eye drops (intervet:Holland) for Newcastle disease. Gumboro eye drops vaccine used at 14th and 28th days (BURSA-VAC® Millsboro, Delaware, U.S.A). Lasota was used at 21st and 31st days of age (intervet:Holland) for Newcastle disease. E. coli challenge applied according to Awaad (1972). Birds were inoculated with 0.6 ml saline suspension containing 2x107 C.F.U E. coli strain 078:K80 intra-crop at 7 day old (the bacteria was kindly obtained from Ismailia, animal health research institute).

Ingredients	Starter	Grower-Finisher
	(0-3weeks)	(4-6 weeks)
Ground yellow corn	56.7	66.6
Soya bean meal (44% CP)	29.5	23.53
Fish meal (60.5% CP)	7.0	5.0
Soya bean oil	4.06	2.02
Dicalcium phosphate	0.88	0.6
Limestone	1.26	1.69
DL – Methionine (purity 96%)	0.1	0.06
Iodized sodium chloride	0.25	0.25
Vitamins & mineral premix*	0.25	0.25
Calculated composition		-
Crude protein	22.0	19.0
ME kcal per kg	3060.0	3040.0
Calorie/protein ratio(C/P)	139.0	160.0

Table 1. The composition of the experimental diets.

* Each 2.5 kg contain the following vitamins and minerals:

Vit. A 12 mIU, vit. $D_3 2$ mIU, vit. E 1000mg, vit. $k_3 2000$ mg, vit. $B_1 1000$ mg, vit. $B_2 5000$ mg, vit. $B_6 1600$ mg, vit. $B_{12} 10$ mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 6000mg, zinc 5000mg, iron 3000mg, copper 10000mg, iodine 1000mg, selenium 100mg, cobalt 100mg, carrier(CaCO₃) to 2.5kg. (AGRI-VET. Under technical assistance of HELM Germany)

Evaluation of Growth Performance

Diets offered ad libitum. Water, either supplemented with ROEMIN W2 or not was constantly available. Residual feed collected daily.Body weight was determined weekly on individual basis. Body weight gain, feed consumption and feed conversion ratio (FCR, Feed: Gain)were calculated. The overall Body weight gain, feed consumption, and FCR calculated for the complete experimental period (Brady, 1968).

Two birds from each replicate were taken and slaughtered (at the 3rd and 6th weeks) for determination of carcass weight. Their feather plucked, and their head and feet (shank) cut off.

Histopathological Examination

Specimens from duodenum were collected in 10% buffered formalin for histopathological examination according to (Bancroft et al., 1990).Samples used to evaluate the following parameters: villus height and crypt depth.Quantitative morphometric estimations done using image analyzer (Leica imaging system. Ltd, Cambridge, England).

Scanning Electron Microscope (SEM)

Small pieces 2-3 mm² of the duodenum quickly excised washed in normal saline, fixed in 3% glutaraldehyde for 24 hours at 4°C. Then, the specimens washed in sodium cacodylate buffer (0.1% molarity, pH 7.2) 3-4 times for four hours and post fixation in 1% osmium tetroxide for 24 hoursthen rinsed three times in distilled water. The specimens dehydrated through a graded ascending ethanol series (from 10 to 100%) (30 min each), dried with liquid CO_2 . The specimens mounted on stubs with double sided adhesive taps coated with gold Bancroft et al. (1990). Then examined by a scanning electron microscope (JEOL JXA-840A ELECTRON PROBE MICROANALYZER).In the electron Microscope Unit, of the National center for research, EL Douki, Cairo, Egypt.

Statistical Analysis

Data collected analyzed to compare means, using a statistical software program (SPSS for windows, version 14, USA). Differences among means of different groups carried out using one-way ANOVA with Duncan multiple comparison test.

Results

Growth Performance Parameters

ROEMIN W2 significantly increased final body weight, weight gain, feed consumption and carcass weight in G2 (probiotics only) compared to control group (G1)(Table 2). ROEMIN W2 significantly increased final body weight, weight gain, improved FCR and carcass weight in G4 in spite of the E. coli infection compared to control group, G1. G5 had the same FCR and carcass weight as the control group in spite of the E. coli infection. There was a significantly decreased growth performance in the E coli infected non ROEMIN W2 supplemented group, G3 compared to the control and all other treatments.

Parameters	G1	G2	G3	G4	G5
Initial body weight, g/bird	46.09±0.58	45.42±0.55	46.36±0.60	46.39±0.72	46.36±0.64
Final body weight, kg/bird	2.10±14.76 ^c	2.27±4.38 ^a	1.80±13.22 ^e	2.15±12.83 ^b	2.02±8.82 ^d
Body weight gain, kg/bird	2.05±14.21°	2.23±11.90 ^a	1.80±6.55 ^e	2.10±4.47 ^b	1.96±14.47 ^d
Feed consumption, kg/ bird	3.57±30.50°	3.90±15.04ª	3.66±30.73 ^b	3.53±34.75°	3.37 ± 23.72^{d}
FCR	1.74±0.03 ^b	1.74±0.01 ^b	2.03±0.01 ^a	1.68±0.02°	1.72±0.01 ^{bc}
Carcass weight, kg/bird	1.66±9.03°	1.88±6.16 ^a	1.57±29.04 ^d	1.72±17.35 ^b	1.61±8.56°

Table 2. Effect of different experimental treatments on growth performance (Mean± SE)*

*Means with the different letters (a, b,c...) in the same raw are significantly different $p \le 0.05$.

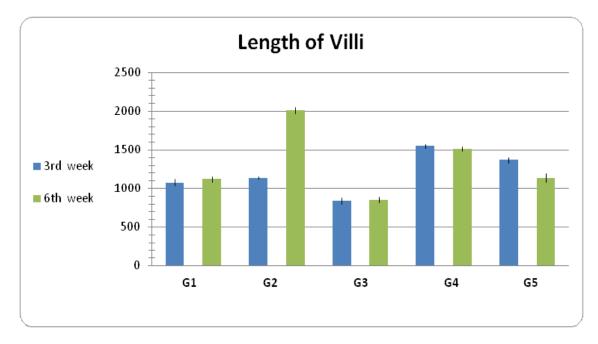
Histomorphological Measurements

Length of intestinal villi

ROEMIN W2improved villi length in G2 (probiotic only) at 3rd week and a significant increase in villi length was seen in G2at 6th week compared with allother groups(Table 3, Chart 1).G4, showed a significant increase in length of villi at 3rd and 6th weeks. ROEMIN W2supplementation kept the villi length in G5 as the control group after E coli infection, whileG3 (infected non-treated group), showed significant decrease in length of villi at 3rd and 6th weeks when compared with other groups.

Table 3. Effect of different experimental treatments on length of intestinal villi (Mean± SE)

Group Time	G1	G2	G3	G4	G5
3 rd week	1076 ^c ±47	1137 ^c ±19	838.5 ^D ±40	1546 ^A ±22	1364 ^B ±37
6 th week	1118.7 ^c ±38	2013 ^A ±43	851.5 ^D ±34	1515 ^в ±16	1137 ^c ±56



Mean within the same raw having different letters (A, B, C...) are highly significant different at p≤0.001

Chart 1. Effect of different experimental treatments on length of intestinal villi

Depth of crypt

G1, at 3rd week showed significant increase in depth of crypt when compared with other groups.At 6th week, showed significant decrease when compared with other groups(Table 4, Chart 2). G3, at 6th week showed a significant decrease in depth of crypt when compared with control group and Probiotics only.G4, at 6th week, showed a significant decrease in depth of crypt when compared with other groups.G5at 6th weeks, showed a significant increase in depth of crypt when compared with other groups.

Table 4. Effect of different experimental treatments on depth of intestinal crypt(Mean±SE)					
Group	G1	G2	G3	G4	G5
Time					
3 rd week	206 ^b ±15	271 ª±10	235 ^{ab} ±22	218 ^b ±18	222 ^b ±17
6 th week	182 °±13	137 ^d ±10	276 ª±15	224 ^b ±10.5	257ª±20

Table 4. Effect of different experimental treatments on depth of intestinal crypt(Mean± SE)
 Image: Comparison of the second secon

Means within the same raw having different letters (a, b, c...) are significant different at p≤0.05

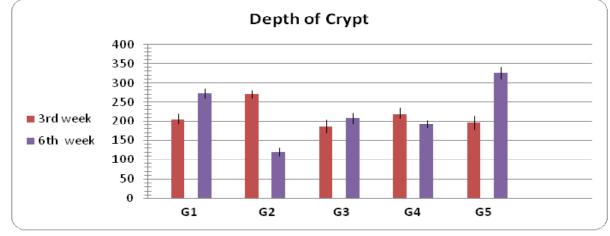


Chart 2. Effect of different experimental treatments on depth of intestinal crypt

Small intestine

G1the control group showed normal long intestinal villi with normal linningepith. (**Fig 1- A**). G2 that fed probiotics only showed normal healthy long intestinal villi (**Fig1-B**). G3 (infected non-treated group) showed severe destruction, necrosis, desquamation, fusion and shortening of duodenal villi (**Fig1-C**). G4 showed normal histological architecture, including long villi with normal epithelial lining and normal intestinal glands (**Fig1-D**). G5 (infected supplemented group) showed mild to moderate destruction, shortening of intestinal villi and hyperplasia of glands (**Fig1-E**).

Scanning Electron Microscope

Reveled the beneficial effect of ROEMIN W2 supplementation. The shape of duodenal villi of control group (G1) showed the normal finger tongue-like projections. Each villus has a wide base triangular on side and has curled tips (Fig 2-a). The lateral side appearance of the villi showed the epithelial cells activity represented by dome -shaped cells with protuberances and epithelial crevice (Fig 2-f). The crypt area showed numerous numbers of proliferating enterocytes with characteristic arrangement as long columnar cells and having long microvilli (Fig 3-a).In G2, the duodenal villi showed normal lengthily long finger-like projection (Fig 2-b). The epithelial cells revealed the normal healthy appearance, dome shaped with longer microvilli and shortening of the depth of crypt area. The crypt area showed a numerous number of proliferating enterocytes that having longer microvilli than that of the control group (Fig 3-b).G3, Showed shortening of intestinal duodenal villi with rough and distorted surface. Massive destruction and loss of several villi were also observed (Fig 2-c) The Intestinal mucosa and epithelial cells of some villi as well as those in crypt areas showed normal lengthy villi (Fig 2-d), crypt area and cells in both villus surfaces was healthy and normal (Fig 3-d). G5, the Intestinal villi and its mucosa showed mild, moderate destruction of the microvilli structure (Fig 2-e). Mild to moderate necrosis and adhesion of epithelial cells with loss of their microvilli. (Fig 3-e).

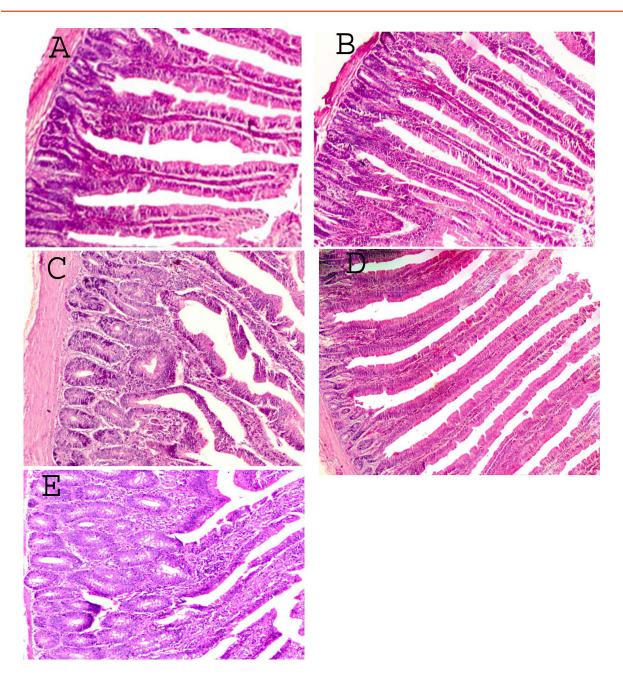


Fig1. Duodenum, H&E (A)& (B) :G1 and G2, 6rd week, showing normal histological integrity of epithelial cell lining the villi. X4. (C): G3, 6th -week showing necrosis, sloughing and desquamation of epithelium, mononuclear cell infiltration, shortening, fusion and atrophy of vill.iX 10. (D): duodenum, G4, 6th week showingnormal histological architecture, including long villi with normal epithelial lining and normal intestinal glands.X10. (E): duodenum, G5, 6th week showing hyperplasia of intestinal glands, mild atrophy and shortening of villi. X4.

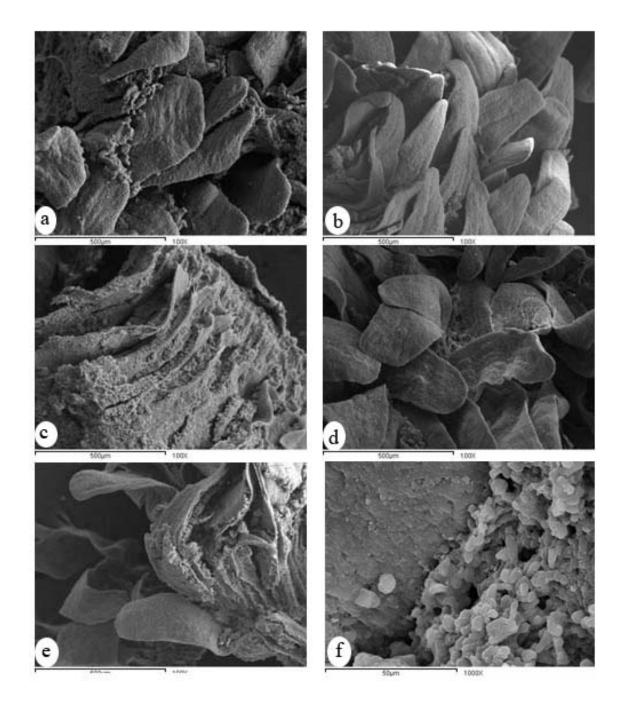


Fig2. Scanning electron micrograph of duodenum showing normal healthy architecture of duodenal villi with smooth surface (a, G2). More healthy lengthy villi (b, G2 LAB). Shortening of intestinal duodenal villi with rough and distorted surface (c, G3). Normal villi length and surface (d, G4). Mild to moderate destruction (e, G5). X 100, Scale bar, 500 μm. Side appearance of villi (f, G1).X 1000. Scale bar, 50 μm

Volume 2

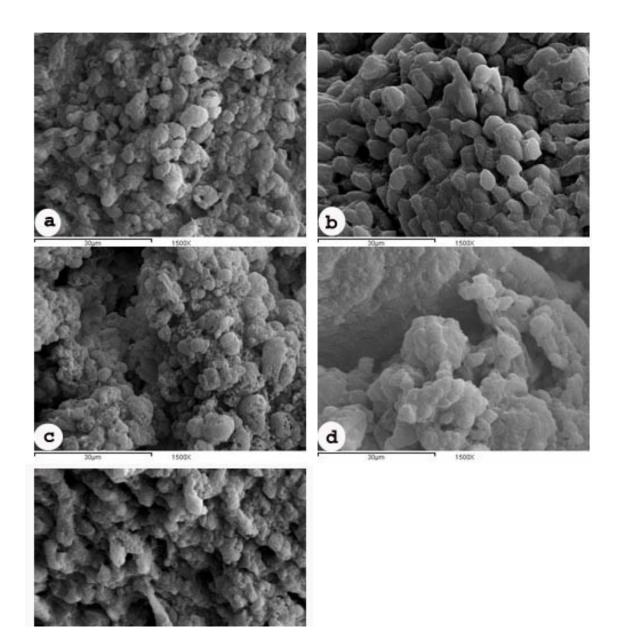


Fig3. Scanning electron micrograph of duodenum crypt area showing numerous numbers of proliferating enterocytes having long microvilli (a, G1). More healthy longer cells with taller microvilli (b, G2). Massive necrosis, adhesion, porous and collapse of epithelial cells with loss of microvilli (c, G3). Normal epithelial cells with long microvilli (d, G4). Mild to moderate necrosis and adhesion of epithelial cells with destruction of their microvilli (e, G5). X 1500, Scale bar, 10µm.

DISCUSSION

The significant improvement in growth performance in the present study is in agreement with previous research done to evaluate the effects of lactobacillus-based probiotic on broiler chicken (Gibson and Fuller, 2000; Zulkifli et al., 2000; Midilli and Tuncer, 2001; Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007; and Hamed Kioumarsi et al., 2012). This improvement is thought to be related to different modes of action, including:(1) maintaining normal intestinal microflora by competitive exclusion and antagonism (Kabiret al., 2005;Kizerwetter-Swida,and Binek, 2009). Probiotic supplementation allowed the rapid establishment of beneficial bacteria in the digestive tract of the bird. Therefore, improved intestinal environment and increased efficiency of digestion and absorption of nutrients (Edens, 2003).(2) Altering metabolism, improving feed intake and digestion by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (Nahanshon et al., 1992; Nahanshon et al., 1993; Dierck1989; Yoon et al., 2004; Awadet al., 2006). Lactobacillus-based probiotic deliver many lactic acid bacteria into the gastrointestinal tract, that modifies the intestinal milieu and deliver enzymes and other beneficial substances into the intestines (Marteau and Rambaud 1993). Supplementation significantly increased the levels of amylase after 40 days of feeding (Jin et al., 2000). Also, L. acidophilus supplementation to chicken increased microvilli height leading to enlargement of the microvilli absorptive surface and enabling optimal utilization of nutrients (Ezema, 2013). (3) Stimulating the immune system (Kabir et al., 2004; Nayebpor et al., 2007; Apata 2008; Brisbin et al., 2008).

In spite of E. coli infection ROEMIN W2 addition lead to an improved performance in G4 and maintained a similar FCR and carcass weight in G5 compared to the control, suggesting an improved intestinal balance of microbial population in probiotic treatments. The addition of probiotic promoted the growth of beneficial bacteria and so provided a healthier intestinal system for better absorption of nutrients (Kelly et al., 1994; Rada and Rychly, 1995; Line et al., 1998; Salminen et al., 1998; and Pascual et al., 1999). While G3 (the non-probiotic supplemented, E coli infected) remained the group with lowest performance. E coli as a pathogen caused disturbances in the normal flora or in the intestinal epithelium that altered the permeability of this natural barrier, facilitating the invasion of pathogens and detrimental substances, modifying the metabolism, the ability to digest and absorb nutrients, and leading to chronic inflammatory processes at the intestinal mucosa (Hofstad, 1972; Podolsky, 1993; Oliveira, 1998). As a result, there was a decrease in the villus, increase in the cell turnover and decrease in the digestive and absorptive activities (Visek, 1978).ROEMIN W2 use counteracted this effect as seen in G4 and G5.Probiotics may produce antimicrobial substances and organic acids that protect the villi and absorptive surfaces against toxins produced by pathogens, as well as stimulate the immune system (Ewing and Cole, 1994; Walker and Duff, 1998; Pelicano et al., 2002).

CONCLUSION

ROEMIN W2 supplementation significantly improved growth performance in broilers. Also displayed a growthpromoting effect in spite of the E. coli infection. This product offers a good antibiotic alternative to improve poultry production. It is our recommendation touse it in commercial farms.

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