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Performance, Caecum Bacterial Count and Ileum Histology of Broilers Fed Different Direct-Fed Microbials

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ABSTRACT

An experiment was designed to evaluate the effect of using three different commercial preparations of Direct-Fed Microbials (DFM) being: A mixture of *Enterococcus faecium* (Protexin®, DFM1), a mixture of *Bacillus subtilis* (Clostat®, DFM2) and *Saccharomyces cerevisiae* yeast cells with its fermentation metabolites (Diamond®, DFM3) on performance, carcass characteristics, caecum bacterial count and changes on ileum histology of broilers. A number of 200 Cobb 500 broiler chicks were fed on 4 dietary treatments from 10-36 days of age: Abasal corn-soybean meal diet served as a control treatment with no supplements or supplemented with the recommended levels of the tested products. No significant differences were detected on Body Weight Gain (BWG) among treatments. Chicks fed the control diet consumed more ($p<0.05$) feed than those fed DFM supplemented diets. No significant differences on Feed Intake (FI) were observed among birds fed the different examined DFM. Birds fed the control diet recorded the worst values ($p<0.01$) of Feed Conversion Ratio (FCR) among the dietary treatments. Addition of DFM to broiler diets improved efficiency of feed utilization (FCR values) by about 6%. Addition of DFM to broiler diets decreased *Escherichia coli* and *Clostridium* spp. count in the ceacum. Also, feeding DFM supplemented diets stimulated histological changes in the ileum villi (height and width along with the number of crypts). These changes reflected a positive effect of the tested products on intestinal efficiency which may explain the improvement in FCR of broilers. It could be concluded that birds fed DFM supplemented diets utilized feed more efficiently than those fed the control diet. The DFM that contain *Enterococcus faecium*, *Bacillus subtilis* or *Saccharomyces cerevisiae* yeast cells with its fermentation metabolites, exert the same effects upon the studied parameters.

Key words: Broiler performance, feed additives, caecum bacteria, intestinal health

INTRODUCTION

Antibiotic feed additives such as Lincomycin, Bacitracin or Virginamycin have been used for decades as growth promoters and to control clostridial infections in poultry. However, such feeding strategy was banned because it develops antibiotic resistance and results in presence of antibiotic residues in poultry products. Therefore, alternative strategies had been introduced as an effective and safe to antibiotics in combating bacterial diseases in poultry particularly clostridial infections and enhance growth performance. Such strategies include using Direct Fed Microbials (DFM) such as probiotics which contain one or more species of useful bacteria or yeasts (Waldroup *et al*., 2003).

Feeding diets supplemented with probiotics may change gastrointestinal tract microflora, improve performance and feed efficiency and might offer great potential both as a feed additive and as a replacement for antibiotics (Mountzouris *et al*., 2007; Samli *et al*., 2007; Vicent *et al*., 2007; El-Husseiny *et al*., 2008).

Using of probiotic containing *Enterococcus faecium* microorganism to broiler diets increased the jejunal villus height (Chichlowski *et al*., 2007) and ileal villus height (Samli *et al*., 2007). Increasing villus height may have positive effect on digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Amat *et al*., 1996). The application of probiotics significantly improved the weight gain of broiler chickens (Mateova *et al*., 2008).

Therefore, the objective of this study was to further evaluate the effects of different Direct Fed Microbial (DFM) as new feeding strategies on performance, carcass characteristics, caecum bacterial count and changes in ileum histology of broilers.

MATERIALS AND METHODS

Birds, diets and housing: A total number of 300 unsexed one day old Cobb broiler chicks were brooded in a warmed fumigated brooder house and fed on a starter diet for 10 days of age. Birds were then individually weighed and 200 chicks with almost the same live body weight were divided into four groups (5 replicates of 10 chicks, each). The average initial live body weight of all replicates was nearly similar. Replicates were randomly allocated a battery that has 20 compartments (5 replicates ×4 dietary treatments). The experiment involved examining of three commercial products of different commercial preparations of Direct-Fed Microbials (DFM) being Protexin® (a mixture of *Enterococcus faecium*, DFM1) produced by Novartis limited, international, UK., Clostat®, (a mixture of *Bacillus subtilis*, DFM2) produced by Kemin Industries, Inc., USA. and Diamond® (*Saccharomyces cerevisiae* yeast cells with its fermentation metabolites, DFM3) produced by Diamond V Mills, Inc.

Basal diets were formulated to cover all the nutrient requirements of Cobb broilers. The formulation and nutrient composition of the starter (1-10 days), grower (11-25 days) and finisher (26-36 days) control diets are shown in Table 1. The control grower and finisher diets were fed with no additives or supplemented with either 0.01% DFM1, 0.06% DFM2 or 0.05% DFM3 as recommended by the producers. Phytase addition to the diets allows reduction of dietary available phosphorus by 0.1% (Sohail and Roland, 1999). Birds were fed the experimental diets for *ad libtum* consumption from 10-36 days of age. Gas heaters were used to keep the required temperature and light was provided 23 h daily during the experimental period. A common vaccination program against AI, ND, IB and IBD was adopted throughout the experimental period.

Performance measurements: After fasting overnight, birds were individually weighed and feed consumption was recorded per replicate at 25 and 36 days of age. Body weight gain and feed conversion ratio were calculated.

Carcass characteristics: At day 36, five representative chicks with body weight close to the group average were selected from each group and slaughtered for carcass characteristics. Chicks were fasted for 12 h then individually weighed, slaughtered, feathered and eviscerated. Weights of carcass, liver, gizzard, heart and abdominal fat were recorded and calculated as percentage of live body weight.

Table 1: Formulation and nutrient composition of the basal diets

Ingredients (%)	Starter diet (1-10)	Grower diet (11-25)	Finisher diet (26-36)
	----------(days)----------		
Yellow corn	56.48	61.47	62.20
Soybean meal (48%)	34.00	29.00	27.50
Corn gluten meal (60%)	3.00	2.50	2.00
Vegetable oil	2.00	3.00	4.50
Dicalcium phosphate	1.70	1.50	1.40
Limestone	1.37	1.25	1.30
Vitamin and mineral mix1	0.30	0.30	0.30
Salt	0.30	0.20	0.25
L-lysine HCl	0.20	0.25	0.15
DL-methionine	0.25	0.20	0.13
Choline HCl	0.15	0.13	0.10
Threionine	0.15	0.10	0.07
Phytase	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated composition[2] (%)			
Crude protein	23.30	21.10	20.00
ME (kcal kg^{-1})	3030.00	3142.00	3237.00
Lysine	1.42	1.31	1.18
Methionine	0.60	0.55	0.48
Met+cystine	1.02	0.92	0.81
Thrionine	0.95	0.85	0.75
Calcium	1.02	0.90	0.86
Nonphytate P	0.45	0.40	0.37

Vitamin-mineral mixture supplied per kg of diet: Vit. A, 12,000 IU, Vit. D3: 2,200 IU, Vit. E, 10 mg, Vit. K3: 2 mg, Vit. B1: 1 mg, Vit. B2: 4 mg, Vit. B6: 1.5 mg, Vit. B12: 10 μg, Niacin, 20 mg, Pantothenic acid: 10 mg, folic acid: 1 mg, Biotin: 50 μg, Ccopper: 10 mg, Iodine: 1 mg, Iron: 30 mg, Manganese: 55 mg, Zinc: 50 mg and Selenium: 0.1 mg, calculated values based on feed composition tables of NRC (1994)

Preparation of competitive exclusion native gut microflora: Caecal contents were immediately collected from the slaughtered birds and tested for the absence of *Clostridium perfringers* and *E. coli*. Half a gram of such material was inoculated into 10 mL Trypticase Soya broth (Weinack *et al*., 1979) and incubated at 37°C for 48 h an aerobically. Using 0.2 mL of the broth culture was then transferred to another 10 mL tube of Trypticase Soya broth and incubated for 48 h an aerobically at 37°C. *Clostridium perfringers* and *E. coli* colonies counting (Collins and Lyne, 1984) caecal content specimens were taken aseptically 1 g of each caecal content and mixed with 9 mL saline for preparation of a tenfold dilution. One ml from each dilution was spread on brilliant green agar plate and incubated at 37°C for 24 h. The total colony count for *Clostridium perfringers* and *E. coli* were calculated. The microbial counts were determined as colony forming units (CFU) g^{-1} of sample.

Histological observations: Representative tissue samples from ileum of the slaughtered birds were taken to study the histological changes associated with the experimental treatments. Samples were fixed in a 10% formalin-saline solution before preparing the histological sections using paraffin method technique. All sections were dehydrated in ascending grades of ethanol, cleared

in xylene and then embedded in paraffin wax. Transverse sections (4-5 microns, thickness) were taken, mounted on glass slides and stained with haemotoxyline and eosin (H and E) stains. All sections were microscopically examined.

Statistical analysis: Data was statistically analyzed for analysis of variance using the General Linear Model of SAS (1990). Significant differences among treatment means were separated by Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance: Performance of chicks fed the different experimental diets is summarized in Table 2. No significant differences were detected on Body Weight Gain (BWG) among treatments during the two feeding intervals (from 10-25 and from 26-36 days of age) and the entire period (from 10-36 days of age). A narrow range on BWG was recorded for the entire period being from 1544 g for birds fed DFM2 and 1580 g for those fed either the control or DFM1 supplemented diets.

Feed Intake (FI) significantly affected by dietary treatments at 25 ($p<0.01$) and 36 ($p<0.05$) days of age. Chicks fed the control diet consumed more ($p<0.05$) feed than those fed DFM supplemented diets. No significant differences on FI were observed among birds fed the different examined DFM. Differences on FI reflected on the calculated values of Feed Conversion Ratio (FCR). Birds fed the control diet recorded the worst values ($p<0.01$) of FCR among the dietary treatments during the different intervals and the entire period. At 36 days of age, birds fed diets containing DFM1, DFM2 or DFM3 recorded FCR values being 1.61, 1.61 and 1.62, respectively, compared to 1.70 for those fed the control diet of no supplement. These results indicated that addition of DFM to broiler diets improve efficiency of feed utilization (FCR values) by about 6%.

Carcass characteristics: Effects of dietary treatments on carcass characteristics of broilers are shown in Table 3. No significant differences were detected on carcass weight, dressing, heart, gizzard and liver (% body weight) among treatments. Although carcass characteristics were not significantly affected by dietary treatments, birds fed DFM1 supplemented diet deposited less abdominal fat compared to the control or those fed DFM2 or DFM3 supplemented diets.

Table 2: Effect of dietary treatments on growth performance of broiler chicks

	Days								
	10-25			26-36			10-36		
Item	BWG (g)	FI (g)	FCR (g/g)	BWG (g)	FI (g)	FCR (g/g)	BWG (g)	FI (g)	FCR (g/g)
Dietary treatments									
Control (no additives)	806	1313^{a}	1.63^{a}	774	1371	1.77^{a}	1580	2684^{a}	1.70^{a}
DFM1	806	1220^{b}	1.51^{b}	774	1320	1.70^{b}	1580	2540^{b}	1.61^{b}
DFM2	771	1177^{b}	1.54^{b}	773	1314	1.70^{b}	1544	2491^{b}	1.61^{b}
DFM3	805	1223^{b}	1.53^{b}	767	1315	1.73^{ab}	1571	2546^{b}	1.62^{b}
Statistics									
SE of means	±6.47	±16.62	±0.02	±7.03	±11.76	±0.01	±8.45	±23.88	±0.01
Significances	NS	*	***	NS	NS	*	NS	**	***

DFM1: A mixture of *Enterococcus faecium*, DFM2: A mixture of *Bacillus subtilis*, DFM3: *Saccharomyces cerevisiae* yeast cells with fermentation metabolites, $^{a-d}$Mean within each column with no common superscript differ significantly ($p<0.05$). *$p<0.05$, **$p<0.01$, ***$p<0.001$, NS: Not significant ($p>0.05$)

Table 3: Effect of dietary treatments on carcass characteristics (% of live body weight) of broiler chicks at 36 days of age

Item	Dressing	Abdominal fat	Liver	Gizzard	Heart
	----------(%)----------				
Dietary treatments					
Control (no additives)	72.01	1.90	2.17	1.57	0.56
DFM1	72.40	1.77	2.36	1.67	0.47
DFM2	72.09	1.97	2.22	1.75	0.49
DFM3	71.59	1.88	2.34	1.74	0.58
Statistics					
SE of means	±0.25	±0.11	±0.15	±0.14	±0.11
Significances	NS	NS	NS	NS	NS

DFM1: A mixture of *Enterococcus faecium*, DFM2: A mixture of *Bacillus subtilis*, DFM3: *Saccharomyces cerevisiae* yeast cells with fermentation metabolites, NS: Not significant ($p>0.05$)

Table 4: Effect of dietary treatments on intestinal bacteria count *of E. coli* and *Clostridium perfringers* of broiler chicks at 36 days of age

Item	*E. coli* ssp. log 10 CFU g^{-1}	*Clostridium perfringers* log 10 CFU g^{-1}
Dietary treatments		
Control (No additives)	7.39^{a}	1
DFM1	7.16^{b}	ND
DFM2	7.22^{ab}	ND
DFM3	7.12^{b}	ND
Statistics		
SE of means	±0.11	
Significances	***	

DFM1: A mixture of *Enterococcus faecium*, DFM2: A mixture of *Bacillus subtilis*, DFM3: *Saccharomyces cerevisiae* yeast cells with fermentation metabolites, ^{a,b}Mean within each column with no common superscript differ significantly ***$p<0.001$, 1: Detectable ND: Non detectable

Caecum bacterial count: The effects of dietary treatments on intestinal microflora (*Escherichia coli* and *Clostridium* spp.) of chicks fed the different dietary treatments are shown in Table 4. The results showed that birds fed the tested DFM supplemented diets had significant ($p<0.001$) less *Escherichia coli* count in the intestine compared to the control birds. *Clostridium* spp. was detected in birds fed the control diet and were not detected on ceacum of birds fed DFM supplemented diets. These results indicated that addition of DFM to broiler diets decreased counts of the harmful bacteria (*Escherichia coli* and *Clostridium* spp.) in the digestive tract of broilers.

Ileum histology: Effects of dietary treatments on ileum histology of broilers are shown in Fig. 1. The results of ileum histology showed some changes related to the feed additives (DFM). The villi height and width along with the number of crypts per the microscopic field were differing between the control and the treated groups. These changes were more obvious in the DFM1 and DFM2 (Fig. 1b and c) followed by the DFM3 (Fig. 1d) group. There were many crypts in these sections compared with control one (Fig. 1a). Also, the epithelial linning of the crypts is the columnar epithelium. These changes may reflect a positive effect of such additives on the digestive tract histology as the major system for nutrients digestion and absorption. Changes in the mucosal architecture in terms of increased ileal villus height to crypts depth were shown in birds fed with DFM supplemented diets.

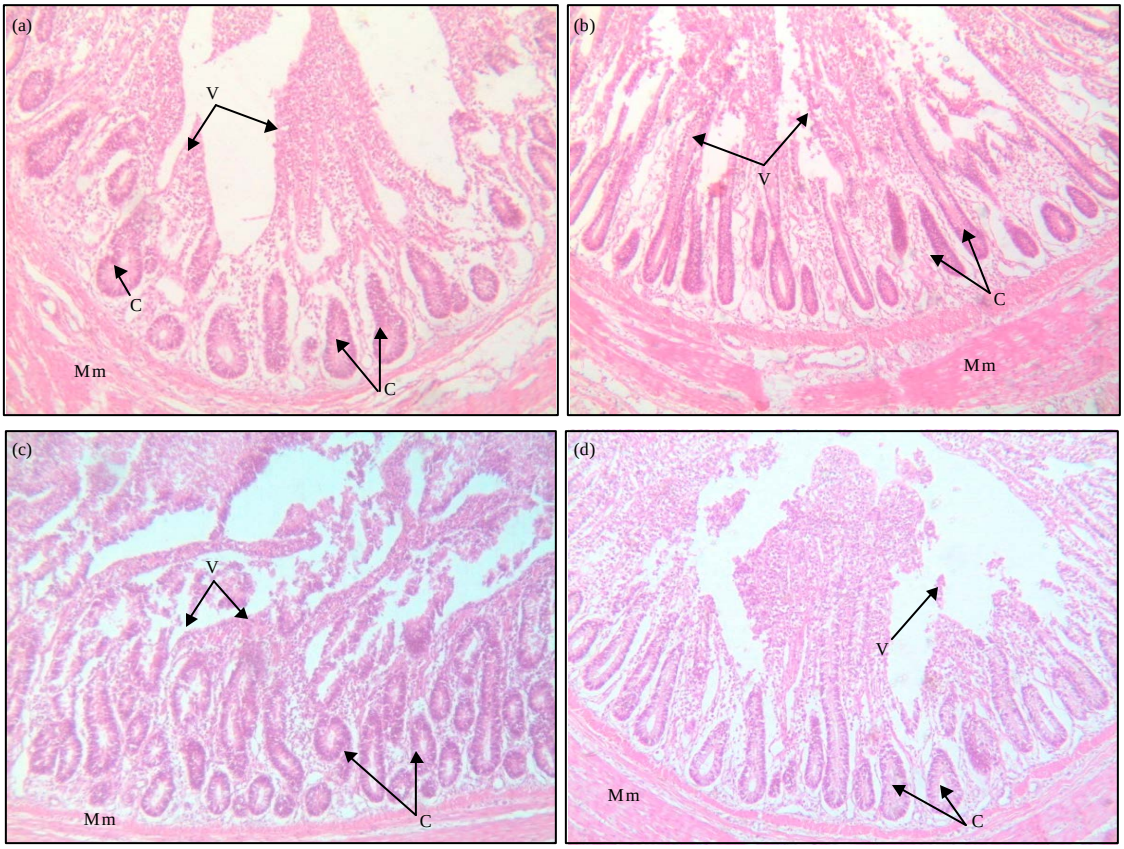

Fig. 1(a-d): Effect of dietary treatments on ileum histology of broiler chicks. (a) T.S. in the ileum from the control group, (b) T.S. in the ileum from the DFM1 group, (c) T.S. in the ileum from the DFM2 group and (d) T.S. in the ileum from the DFM3 group Mm = Muscularis mucosa, C = Crypts of Lieberkuhn, V = Villi

The present results of using DFM in broiler feeding are in agreement with previous results of Waldroup *et al.* (2003) and Midilli *et al.* (2008) who found that body weight gain, carcass weight and carcass yield of broilers were not affected by probiotics, prebiotics or symbiotic supplementation. However, feed conversion ratio was improved significantly in the supplemented treatments compared to the control diet (no additives). Other researchers have demonstrated significant increases in body weight gain in broilers receiving diets supplemented with probiotics (Midilli and Tuncer, 2001). Bozkurt *et al.* (2009) reported that feed additive regimens that include probiotic (DFM) significantly improved growth rate of the birds compared to the control diet. Shareef and Al-Dabbagh (2009) reported that *Saccharomyces cervisiae* supplementation to broilers significantly improved body weight gain, feed consumption and feed conversion ratio.

Addition of DFM led to significant improvement ($p<0.001$) in values of feed conversion ratio (feed/gain) of broilers compared with the control diet. This is in agreement with the findings of Shane (2001) and Pelicia *et al.* (2004) who suggested that such improvement might be due to a better ileal digestibility of nutrients.

Dietary treatments did not cause any significant effect on carcass weight and carcass yield. No significant changes for carcass traits observed in the present study have been reported previously in experiments with probiotics (Khan *et al*., 1992). In the present study, dietary treatments had no significant effect on abdominal fat. Similar results were observed by researchers who studied supplementation of probiotics (Denli *et al*., 2003; Alcicek *et al*., 2004) to broiler diets. Consequently, the carcass characteristics of broilers were not affected by the different feed additives.

The intestinal microbiota plays a vital role in the normal nutritional, physiological, immunological and protective functions of the host animals (Vispo and Karasov, 1997). The composition and metabolic activity of the intestinal microbiota can be influenced by the diet (Netherwood *et al*., 1999). There is a growing interest in the use of a variety of DFM to promote poultry health by altering the intestinal microbial community. Although, a marked proportion of the beneficial effects of DFM so far discussed seem to be attributable to certain epithelial function, there are relatively few experimental data to support this hypothesis.

Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). Ruttanavut and Yamauchi (2010) found that longer villi in the ileum of adult male layer with slight improvement in feed efficiency after dietary addition of Bacillus subtilis var., natto and in broilers after addition *Enteroccocus faecium* or *Eubacterium* sp. (Awad *et al*., 2006).

The present results are in agreement with many previous findings about the beneficial effects of proboitics as growth promoters to broiler diets (Kabir *et al*., 2005; Gunal *et al*., 2006; Sen *et al*., 2011).

Amit-Romach *et al*. (2004) mentioned that the ideal micro-organism for probiotic use must be able to overcome potential hurdles, such as the low pH of the stomach and the presence of bile acids in the intestines. It must also provide competition against the adhesion of gastrointestinal pathogens to the intestinal mucosa while producing organic acids and/or bacteriocin to inhibit pathogen growth which can then establish it and flourish in the intestine.

Abdel-Raheem *et al*. (2012) reported that probiotics (DFM) were shown to alter mucosal architecture in terms of longer villi and improved performance in birds. The intestinal mucosal architecture can reveal useful information on the intestinal function. Taller villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. Also, villus height and crypt depth are a direct representation of the gut function and health. The development of intestinal morphology could reflect the health status of the gastrointestinal tract. Furthermore, new epithelial cells are produced in the intestinal mucosal crypts and migrate along with the villi to the top. The crypt can be regarded as the villus factory. Crypt depths in the ileal mucosa were reduced when the broiler diet was supplemented with DFM3.

The enhanced growth performance of broiler shown in the present study is associated with observed histological changes in villi height, crypts size and depth along with the presence of many goblet cells in the epithelial linning of crypts. This reveals an active hyperplasia in villi epithelium and crypts of LieberKuhn. These changes may reflect a positive effect of such additives on the digestive tract histology as the major system for nutrients digestion and absorption.

CONCLUSION

It could be concluded that using DFM with the supplemental levels in the present study, have stimulated some histological change in the villi histology which may explain the observed improvement in performance and gastrointestinal environment.

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