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The Effects of Fermented Feed on Broiler Production and Intestinal Morphology

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ABSTRACT

The present experiment was conducted to evaluate the effects of wet feed and fermented feed on the intestinal morphology and histology of broiler chicks. A total of 360 one day old Ross 308 broiler chicks were randomly assigned (CRD) into six treatment groups. Chicks were fed: (T1) Control group of dry feed; (T2) Fed on wet feed (1:1, feed: water); (T3) 25% fermented feed + 75% dry feed; (T4) 50% fermented feed + 50% dry feed; (T5) 75% fermented feed + 25% dry feed and (T6) 100%, fermented feed throughout the experimental period. Each treatment group was replicated three times using 20 chicks per replicate. The chicks were raised at a temperature and in humidity controlled room with a 24-h. constant light and had ad. libitum access to water and feed throughout the experimental period which lasted for six weeks. The results showed that all diets containing fermented feed, especially 100%, had dependent effects on the evaluated production characteristics. The feeds had significantly ($P < 0.05$) increased the relative weight and length of the small intestine. Likewise, it raised the villi height, crypt depth and the percentage of the villi height to crypt depth in duodenum, jejunum and ileum increased. In conclusion, the results of the current experiment indicated that fermented feed with probiotic would be beneficial economically since the broiler feed conversion ratio had been improved (2.4% in T2; 4.1% in T3; 5.3% in T4; 5.3% in T5 and 7.7% in T6) as a consequence to enhance their intestinal morphology.

Keywords: Fermented feed, broiler, intestinal morphology, histology

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INTRODUCTION

The main concept of fermenting feed with probiotic is to improve the quality of feed. That application was practiced by Lokman et al. (2015) when one and two grams of prepared probiotic was fermented with the daily feed of local Malaysian chicken (Akar Putra). The authors reported that noticeable enhancement in the production parameters especially when 2 grams of probiotic was used. Fermentation process could improve the nutritive value of the soybean (Mathivanan et al., 2006), copra meals (Hatta & Sundu, 2009) and tofu waste (Rasud, 2009). Fermented feed influenced the bacterial ecology of gastrointestinal tract and reduced the level of *Enterobacteriaceae* in the different parts of gastrointestinal tract in pigs (Winser et al., 2001) and broiler chicks (Heres et al., 2003). *Lactobacilli* and yeast in the kefir, which supplemented in drinking water, significantly increased the population of *Lactobailli* spp. and total aerobic bacteria, thus decreasing the population of *Enterobactciaceae* and coliform in the goose intestine (Yaman et al., 2006). Primarily fermented feed caused a reduction of pathogenic bacteria, including *Salmonella* and *Campylobacter* in the digestive tract, most particularly in the crop and gizzard. Since the crop often ruptures during slaughter, the decrease level of pathogens in this area, in particular, makes contamination of meat less likely (Donkor et al., 2006).

Antibiotic has been used as feed additive to improve growth performance and control disease in animals. However, the continued use of antibiotics has resulted in common problems such as the development of drug-resistant bacteria, imbalance of normal microflora and drug residues in animal products (Chen et al., 2009). Since 2006, antibiotics have been banned for use as feed additives in the European Union. Probiotics has therefore become important as the replacement of feed additives (Steiner, 2006). It is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. After feeding of probiotics, improvements in growth performance, feed efficiency, immunity parameters and disease resistance have been reported (Al-Gharawi, 2012). The major probiotic strains include *Lactobacillus*, *Saccharomyces*, *Streptococcus* and *Aspergillus* (Tannock et al., 2001). At present, *Bacillus*, *Lactobacillus* and *Saccharomyces* are the major strains applied in broilers (Zhang et al., 2005; Chen et al., 2009).

Since very few investigations on the fermentation of broiler feed with probiotic have been done, therefore, the present study was undertaken to evaluate the effects of fermented feed and fermented feed with probiotic on the broiler production performance, as well as the intestinal morphology and histology of broiler chicken.

MATERIALS AND METHODS

Preparation of the Fermented Feed

A commercial broiler starter and finisher diet (Table 1) was purchased from local markets. The chicks were fed the starter diet during the first three weeks, followed by the finisher diet until 6 weeks.

Whole starter and finisher feed were fermented with and without the addition of probiotic. The feed was moistened with water (1 liter water: 1 kg feed), placed in a plastic tray and inoculated with prepared probiotic (PP) at the rate 10 grams of PP for each one kilogram of feed. Then, the plastic trays were kept and incubated in a small room for 48 h. at 37±2ºC.

The PP was purchased from laboratory of poultry technology at the Agriculture Faculty, University of Baghdad. According to the manufacture information label, each gram of PP contained at least 10^9 cfu of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and at least 10^8 cfu of *Saccharomyces cervisia.* Fermented feed was characterised by high lactic acid concentration (up to 260 mmol/ kg feed) and moderate amounts of acetic acid (20-30 mmol/ kg feed), high number of lactic acid bacteria (Log 9-10 cfu/ g. feed) and pH of approximately 4.5-5.0, as described by Cutlure et al. (2005).

Broiler Husbandry and Experimental Design

The experiment was carried out at the poultry research farm in the Faculty of Agriculture, University of Al-Mothanna. A total of 360 one-day-old Ross308 broiler chicks were randomly assigned (CRD) into six experimental groups, which were daily fed, as follows:

T1: Control group fed on dry feed.
T2: Fed on wet feed (1:1, feed: water).
T3: 25% fermented feed + 75% dry feed.
T4: 50% fermented feed + 50% dry feed.
T5: 75% fermented feed + 25% dry feed.
T6: 100% fermented feed.

Each treatment group was replicated three times with 20 chicks per replicate. The chicks were reared in battery cages (1.5 × 1.0 m) with four tiers. The chicks were raised in a temperature and humidity controlled room with a 24-h. constant light and had *ad. libitum* access to water and feed throughout the experiment.

Sampling Procedure and Analytic Methods

Production parameters, which included final body weight and weekly feed conversion ratio, were recorded. At the end of the feeding trial, six birds per treatment were selected at random and slaughtered for sampling. The intestinal parts' weight and length and relative parts; weight and length were calculated according to live body weight.

The intestinal parts were separated carefully; duodenal loop, jejunum the middle sections of the intestine between the duodenum and mackles diverticulum, and ileum, which are located between mackles diverticulum to the ileocecal junction. The cecum was also separated from the site of ileocecal junction and their relative weight and length were calculated. Samples of the intestinal parts were taken for histological study. The samples were fixed at 10%

Table 1
Composition of basal diet.

Items	Basal Diet	
	1 to 22 d	23 to 35 d
Corn	44.9	53.10
Wheat	18.0	15
Soybean meal (45%)	33	27
Mineral and vitamin premix	1	1
Oil	2	3
Limestone	0.8	0.6
Dicalcium phosphate	0.3	0.3
Total	100 %	100 %
Calculated analysis		
Crude protein (%)	21.92	19.70
Metabolism energy (kilo calorie per kg. Diet)	2990	3100
Calcium (%)	0.93	0.85
Phosphorus (%)	0.48	0.45
Methionine (%)	0.55	0.50
Lysine (%)	1.35	1.25
Methionine + Cysteine (%)	0.85	0.91
Folic acid	1.1	1.2

Produced by Ghadeer Babylon, calculated analysis according to NRC (1994).

buffered formalin and embedded in paraffin. Three micron sections were microtome cut and stained with haematoxylin and eosin. Slides were measured by light microscopy to measure crypt depth, villi height and villi height/ crypt depth. Measurements of villus height and crypt depth were taken only from the sections where the plane of section ran vertically from the top of villus to the base of an adjacent crypt. Values presented are means from the seven samples of villi measured from the tip to the crypt mouth and seven associated crypts measured from the crypt mouth to the base (Xu et al., 2003).

Statistical Analysis

Data generated from the present experiment were subjected to statistical analysis using the GLM procedure of SAS (SAS, 2001) statistical software package. When significant differences were noted, means were compared using Duncan's multiple range tests (Duncan, 1955).

RESULTS

There was noticeable significant interaction for using fermented feeds with probiotic compared to the control group. The results revealed that using 25%, 50%, 75% and 100% fermented feed with prepared probiotic (PP) in the daily feed had dependent effects on the evaluated characteristics. Chicken in

T6 achieved higher ($P < 0.01$) body weight (2092.87 g), followed by T5 (1971.44 g), T4 (1901.99 g), T3 (1862.97 g), T2 (1799.88 g) and the control (1700.30 g). Meanwhile, chicken received fermented feed with PP exhibited lower ($P < 0.05$) feed conversion ratio than the control group at the end of the experiment (Table 2).

Morphological measurements of the duodenum, jejunum, ileum and cecum are shown in Table 3 and Table 4. Birds fed on fermented feed had higher ($P \leq 0.05$) relative weight and relative length of duodenum, jejunum, ileum and cecum when compared with birds fed on the control dry feed. As the percentages of the fermented feed in the diet increased, these parameters were significantly ($P \leq 0.05$) increased.

Dietary treatment had a significant ($P \leq 0.05$) effect on villus height, crypt depth, and villus height to the crypt depth ratio in the duodenum (Table 5), jejunum (Table 6) and ileum (Table 7). Birds fed on the fermented feed had higher ($P \leq 0.05$) villi height and crypt depth in the duodenum, jejunum, and ileum than birds fed control dry feed. These birds also had a higher ($P \leq 0.05$) villi high to the crypt depth ratio in all these three parts.

TABLE 2
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on the feed conversion ratio FCR (g food/ g weight gain) of broiler.

Treatments	Week					Mean FCR
	1	2	3	4	5	
T1	1.57± 0.01[a]	1.60± 0.03[a]	1.66± 0.03[a]	1.73± 0.02[a]	1.87± 0.03[a]	1.69± 0.04[a]
T2	1.54±0.02[ab]	1.57± 0.02[ab]	1.63± 0.03[ab]	1.70± 0.04[ab]	1.80± 0.02[b]	1.65± 0.02[b]
T3	1.52± 0.03[ab]	1.55± 0..01[b]	1.61± 0.01[b]	1.67± 0.01[bc]	1.75± 0.03[c]	1.62± 0.03[b]
T4	1.51± 0.01[b]	1.54± 0.03[b]	1.60± 0.03[bc]	1.65± 0.03[c]	1.72± 0.02[c]	1.60± 0.02[b]
T5	1.51± 0.02[b]	1.54± 0.02[b]	1.59± 0.01[bc]	1.64± 0..01[cd]	1.70± 0.01[c]	1.60± 0.03[b]
T6	1.49± 0.02[b]	1.52± 0.03[b]	1.56± 0.02[c]	1.60± 0.02[d]	1.65± 0.03[d]	1.56± 0.01[c]
Significant level	*	*	*	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts ([a,d]) in columns differ significantly ($P < 0.05$).

Table 3
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on the relative weight of the small intestine and cecum (%) of broiler.

Treatments	The relative weight				
	The small intestine	Duodenum	jejunum	Ileum	Cecum
T1	3.7±0.031[d]	0.5± 0.007[d]	1.46± 0.021[d]	1.7± 0.018[d]	0.45± 0.004[c]
T2	4.1± 0.026[c]	0.69 ± 0.005[c]	1.5± 0.012[c]	1.84± 0.022[c]	0.50± 0.005[c]
T3	4.80± 0.022[b]	0.84± 0.008[b]	1.8± 0.021[b]	2.0± 0.020[b]	0.57± 0.006[b]
T4	4.90± 0.020[b]	0.8± 0.007[b]	1.8± 0.023[b]	2.13± 0.018[b]	0.6± 0.005[b]
T5	4.96 ± 0.015[a]	0.8 ± 0.016[b]	1.9± 0.010[b]	2.16± 0.022[b]	0.6± 0.007[b]
T6	5.7± 0.008[a]	1.14± 0.007[a]	2.17± 0.009[a]	2.4± 0.011[a]	0.72± 0.009[a]
Significant level	*	*	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts ([a,d]) in columns differ significantly ($P \leq 0.05$).

Table 4
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on the relative length of the small intestine and cecum (cm/ 100 g body weight) of broiler.

Treatments	The relative weight				
	The small intestine	Duodenum	jejunum	Ileum	Cecum
T1	9.97± 0.14[c]	1.66± 0.19[d]	3.96±0.40[c]	4.35 ± 0.42[c]	1.01 ± 0.1[c]
T2	10.1± 0.18[c]	1.70 ± 0.21[c]	4.02± 0.43[c]	4.41 ± 0.47[c]	1.04 ± 0.11[c]
T3	10.8±0.16[b]	1.86± 0.19[b]	4.18± 0.47[b]	4.78± 0.51[b]	1.14 ±0.13[b]
T4	10.9± 0.21[b]	1.89± 0.17[b]	4.22± 0.37[b]	4.80± 0.43[b]	1.15 ±0.11[b]
T5	10.9±0.16[b]	1.91± 0.2[b]	4.24± 0.5[b]	4.83± 0.48[b]	1.21 ± 0.17[b]
T6	11.3± 0.21[a]	2.07 ± 0.18[a]	4.42 ± 0.22[a]	4.96 ± 0.32[a]	1.34 ± 0.14[a]
Significant level	*	*	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts ([a,d]) in columns differ significantly ($P \leq 0.05$).

Table 5
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on villus height, crypt depth (μm) and the ratio of villus height to crypt depth in the duodenal of broiler.

Treatments	Villus height (μm)	Crypt depth (μm)	The ratio of villus height to crypt depth
T1	117.42 ±1.15[c]	15.35 ± 0.18[c]	7.65 ± 0.05[c]
T2	122.33 ± 2.06[c]	15.59 ± 0.21[c]	7.85 ± 0.05[c]
T3	141.16 ± 1.23[b]	17.52 ± 0.19[b]	8.06 ± 0.04[b]
T4	146.21 ± 2.33[b]	17.72 ±0.18[b]	8.25 ± 0.08[b]
T5	150.05 ± 1.17[b]	17.97 ± 0.2[b]	8.35 ± 0.07[b]
T6	166.36 ± 1.42[a]	18.82 ± 0.18[ab]	8.84 ± 0.05[a]
Significant level	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts ([a,c]) in columns differ significantly ($P \leq 0.05$).

Table 6
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on villus height, crypt depth (μm) and the ratio of villus height to crypt depth in the jejunum of broiler.

Treatments	Villus height (μm)	Crypt depth (μm)	The ratio of villus height to crypt depth
T1	101.12 ±1.07[c]	14.32 ± 0.15[c]	7.06 ± 0.09[c]
T2	103.67 ± 1.12[c]	14.44 ± 0.12[c]	7.18 ± 0.07[c]
T3	113.22 ± 1.61[b]	15.18 ± 0.13[b]	7.46 ± 0.06[b]
T4	115. 17 ± 1.22[b]	15.25 ± 0.21[b]	7.55 ± 0.05[b]
T5	117.20 ± 1.16[b]	15.37 ± 0.2[b]	7.62± 0.06[b]
T6	132. 18 ± 1.36[a]	16.41 ± 0.19[a]	8.05 ± 0.08[a]
Significant level	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts ([a,c]) in columns differ significantly ($P \leq 0.05$).

Table 7
The effect of 25%, 50%, 75% and 100% fermented feed with probiotic on villus height, crypt depth (μm) and the ratio of villus height to crypt depth in the ileum of broiler.

Treatments	Villus height (μm)	Crypt depth (μm)	The ratio of villus height to crypt depth
T1	41.14 ±0.53^{c}	8.62 ±0.79^{c}	4.77 ± 0.05^{c}
T2	41.77 ± 0.6^{c}	8.70 ± 0.68^{c}	4.80 ± 0.04^{c}
T3	50.18 ± 0.54^{b}	9.81 ± 0.72^{b}	5.12 ± 0.06b^{c}
T4	51.25 ± 0.65^{b}	9.87 ± 0.71^{b}	5.19 ± 0.07^{b}
T5	53.09 ±0.49^{b}	10.11 ± 0.81^{b}	5.25 ± 0.06^{ab}
T6	61.55 ± 0.56^{a}	11.04 ± 0.64^{a}	5.58 ± 0.05^{a}
Significant level	*	*	*

T1: Control group fed on dry feed. **T2:** fed on wetting feed (1:1, feed: water). **T3:** 25% fermented feed + 75% dry feed. **T4:** 50% fermented feed + 50% dry feed. **T5:** 75% fermented feed + 25% dry feed. **T6:** 100% fermented feed throughout the experimental period. Mean values with different superscripts (^{a,c}) in columns differ significantly ($P \leq 0.05$).

DISCUSSION

Dependent effects on production parameters were achieved by using 25%, 50%, 75% and 100% fermented feed with prepared probiotic (PP) in the daily broiler feed. The positive effects of probiotics to the Akar Putra chicken also used *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and *Saccharomyces cervisia* with dry fermented feed (Lokman et al., 2015) and wet fermented feed (Jawad et al., 2016). This result could be strongly supported when the worst feed conversion ratio was reported for the control group compared to the groups of layer and turkeys fed probiotics based on *Lactobacillus* sp. and *Saccharomyces cerevisiae* in the diet (Besnard et al., 2000; Ayanwale et al., 2006; Lokman et al., 2015). Adversely, Mutus et al. (2006) did not find any influence of probiotic to feed conversion ratio. Furthermore, Ahmad (2004), and Yousefi and Karkoodi (2007) reported that broiler feed intake and feed conversion ratio was not affected by the dietary probiotic and yeast supplementation. Some researchers reported that when chicks were housed in a clean environment, probiotic did not affect performance (Anderson et al., 1999).

In the current study, the morphology and histology parameters of the digestive tract parts were improved in the broiler chicks feeding on fermented feed than the broilers feeding on control diet. These results are similar to those reported by Chaing et al. (2010) and Xu et al. (2012).

The villus height to crypt depth is a very useful measure to estimate the absorption capacity of the small intestine. Maximum digestion and absorption are believed to occur as villus height to the crypt depth ratio increased (Chaing et al., 2010). Changes in the intestinal morphology such as reduced villus height and deeper crypt may also indicate the presence of toxins (Xu et al., 2003). In the present study, increased villus height and increased villus height to the crypt depth ratio were observed in the broilers feeding on fermented feed compared with unfermented feed. The increased villi height and villus height to the crypt depth ratio might be associated with the increased number of beneficial bacteria like *Lactobcilli*, *Bifidobacterium*, *Bacillus subtilis* and *Sacchromyces cervisia* (Xu et al., 2003; Naji & Al-Mosawi, 2014). Fermented feed was characterised by a high number of lactic acid bacteria (Log 9-10 cfu/ g feed) and pH of approximately 4.5-5.0, as described by Cutlure et al. (2005). The increased villus height and villus height to the crypt depth ratio produced an intestinal structure more oriented to digestion, with improved absorptive and hydrolysis potential, as well as fewer nutrients requirement for intestinal maintenance. Thus, the intestinal structures of duodenum, jejunum and ileum are more favourable for the bird and may help to explain the improvements in weight gain and feed conversion (Feng et al., 2007a). The good results obtained from the solid state fermentation feed might be attributed to the higher production of secondary microbial metabolites during fermentation. These metabolites include organic acid (lactic acid) produced by Lactobacilli, enzymes (amylase and protease) and antimicrobial substances like iturin and surfactin produced by *Bacillus subtilis* bacteria during solid fermentation (Feng et al., 2007b).

Conclusively, the results of this study indicated that using fermented feeds with probiotic caused significant improvement in the intestinal structure of the broilers, including villus height and villus height to the crypt depth ratio, as well as higher body weight and improvement in the feed conversion ratio.

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