EFFECTS OF DIETARY INCLUSION OF FERMENTED FEED ON INTESTINAL PARTS AND HISTOMORPHOLOGY IN BROILER

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ABSTRACT

The present experiment was conducted to evaluate the effect of fermented feed on the intestinal morphology and histology of broiler chicks. . A total of 360, one day old, Ross308 broiler chicks were randomly assigned (CRD) into six treatment groups. Chicks in the six treatment groups were fed as follow: (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. Each treatment group was replicated three times with 20 chicks per each replicate. The chicks were raised in a temperature and humidity controlled room with a 24-h. constant light schedule and *ad. libitum* access to water and feed throughout the experimental period which was lasted for six weeks. The data showed that feeding wet and fermented feed were significantly ($p<0.05$) increased the relative weight and length of the small intestine including duodenum, jejunum, ileum, and ceca. A Significant increase ($p<0.05$) in villi height, crypt depth, villi height to crypt depth ratio in duodenum, jejunum and ileum. In conclusion, the results of the current experiment indicated that feeding fermeuted diets could improve the intestinal structure of the broilers, enhance villus height and increased villus height to crypt depth ratio in the duodenum, jejunum, and ileum.

INTRODUCTION

Fermentation is the chemical process that organic substances converted into simpler compounds by the active enzymes, complex organic catalysts, that produced by microorganisms such as bacteria, yeasts, or molds. Enzymes act during hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (more easily digestible) compounds and nutrient (16). The word "fermentation" is derived from the Latin meaning "to boil", from the budding and foaming of early fermenting beverage seemed closely akin to boiling. Although most microbial fermentations are an accomplished in Liquid phase, several advantages occur for solid state fermentations (SSF): (1) Low medium cost, (2) Low water output, (3) Low capital investment, (4) More practical when carried out in the fields (1).

Fermentation was practiced for quite a long time as a means to improve the quality of food. Fermentation process applied to improve the nutritive value of soybean (11) copra meal and tofu waste (14). The fermentation process can create conditions for the growth of microorganisms that break down fiber and anti-nutrients factors. Fermented feed influences the bacterial ecology of the gastrointestinal tract and reduced the level of Enterobacteriaceae in different parts of the gastrointestinal tract in pigs (19) and broiler chicks (8).

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Lactobacilli **and yeast in the kefir which supplemented in drinking water were significantly increased the population of** ***Lactobailli*** **spp. and total aerobic bacteria and decreasing the population of Enterobactciaceae and coliform in the geese intestine (22). Primarily fermented feed causes a reduction of pathogenic bacteria, including** ***Salmonella*** **and** ***Campylobacter*** **in the digestive tract, most particularly in the crop and gizzard. Because the crop often ruptures during slaughter, the decrease level of pathogens in this area in particular makes contamination of meat less likely (6).**

Some antibiotics were used as growth promoters and control of disease in animals. However, the continued use of antibiotics has caused in common problems such as the development of drug resistant bacteria, imbalance of normal microflora and drug residues in animal products (4). Since 2006, antibiotics have been banned for use as feed additives in the European Union. Probiotics have therefore become important as replacement feed additives (17). A probiotics is a live microbial feed supplement that beneficially affects the host animal by improving the intestinal microbial balance. After feeding of probiotics, improvements in growth performance, feed efficiency, immunity parameters and disease resistance have been reported (2). The major probiotic strains include ***Lactobacillus, Saccharomyces, Streptococcus*** **and Aspergillus (18). Presently Bacillus, Lactobacillus and Saccharomyces are the major strains applied in broilers (4, 23).**

Since there have been few investigations on the fermentation of feed with probiotics and was of wet feeding in broiler chicks. The objectives of this study were to examine the effect of wet and fermented on intestinal parts and histo in broiler chicks.

MATERIALS AND METHODS

Preparation of fermented feed

A commercial broiler starter and finisher diet (Table 1) were purchased from local markets. The chicks were fed on a starter diet during the first three weeks, and then transferred to finisher diet that used for the remainderd of the experimental period which was lasted for 6 weeks.

Table 1: Composition of basal diet

Items	Basal Diet	
	1 to 21 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
Metabolizable 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).**

The fermented feed (SSFF) was prepared in two stages; at the first stage the feed were moisted with water (water: feed, 1:1). At the second stage the wetting feed was placed in a plastic tray and inoculated with probiotic (IP) at the rate 10 grams of IP for each one kilogram of feed. Then the plastic trays were closed and incubate for 48 h. at 37±2 ºC for complete fermentation (10).

The IP was purchased from a laboratory of poultry Technology at Agriculture collage, University of Baghdad. According to the manufacture information label, each one gram of IP contains at least 10^9 cfu per of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and at least 10^8 cfu gram of *Saccharomyces cervisia.* Fermented feed was characterized 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.* (5).

Broiler husbandry and experimental design

The experiment was carried out at Poultry Research Farm-Faculty of Agriculture- University of Al-Mothanna, Iraq, during the period from 2nd – November- 2013 to 7th- December- 2013 and aimed to study the appropriate proportion of dry feed replacement with fermented feed. A total of 360 one day old Ross308 broiler chicks were randomly assigned (CRD) chicks in the six experimental groups were fed as follow:

T1: Control group fed on dry feed;
T2: Fed on wetted 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.

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

Sampling procedure and analytic methods

At the end of the feeding trial (day 42), six birds for each treatment were randomly selected and killed by slaughtering. 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; duodenum loop, jejunum the middle sections of the intestine between duodenum and mackles diverticulum, and ileum which located between mackles diverticulum to the ileocecal junction. The two cecum were also separated from the site of ileocecal junction and their relative weight and length were calculated. Samples from intestinal parts were taken for histological study. Samples were fixed in 10% buffered formalin and embedded in paraffin. Three micron thick sections were microtome cut and stained with haematoxylin and eosin. Slides were examined on light microscope to measure crypt depth, villi height and villi height/ crypt depth. Measurements of villus height and crypt depth were taken only from sections were the plane of section ran vertically from the tip of villus to the base of an adjacent crypt.

Values presented are means from 7 samples of villi measured from the tip to the crypt mouth and 7 associated crypts measured from the crypt mouth to the base (20).

Statistical analysis

Data were subjected to statistical analysis using the GLM procedure of SAS (15) statistical software package. When significant differences were noted, mean were compared using Duncan's multiple range test (7).

RESULTS AND DISCUSSION

The relative intestinal parts of the duodenum, jejunum, ileum, and cecum were shown in table 2 and 3. 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. At the percentages of the fermented feed in the diet increased these parameters were increased significantly ($p \leq 0.05$).

Table 2: Effect of fermented feed of the Iraqi probiotic on the relative weight of the small intestine and cecum of broiler.

Treatments	The relative weight (%)				
	The small intestine	Duodenum	jejunum	Ileum	Cecum
T_1	3.73 ±0.031 d	0.57 ± 0.007 d	1.46 ± 0.021 d	1.70 ± 0.018 d	0.45 ± 0.004 c
T_2	4.12 ± 0.026 c	0.69 ± 0.005 c	1.59 ± 0.012 c	1.84 ± 0.022 c	0.50 ± 0.005 c
T_3	4.80 ± 0.022 b	0.84± 0.008 b	1.87 ± 0.021 b	2.09 ± 0.020 b	0.57 ± 0.006 b
T_4	4.90± 0.020 b	0.88 ± 0.007 b	1.89 ± 0.023 b	2.13 ± 0.018 b	0.60 ± 0.005 b
T_5	4.96 ± 0.015 b	0.89 ± 0.016 b	1.91 ± 0.010 b	2.16 ± 0.022 b	0.62 ± 0.007 b
T_6	5.79 ± 0.008 a	1.14± 0.007 a	2.17 ± 0.009 a	2.48 ± 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. [a,b] Means within columns with no letters differ significantly ($p < 0.05$).

Table 3: Effect of fermented feed by the Iraqi probiotic in the relative length of the small intestine and cecum (cm / 100 g body weight) of broiler.

Treatments	The relative length				
	The small intestine	Duodenum	Jejunum	Ileum	Cecum
T_1	9.97 ± 0.14 c	1.66 ± 0.19 d	3.96 ±0.40 c	4.35 ± 0.42 c	1.01 ± 0.10 c
T_2	10.13 ± 0.18 c	1.70 ± 0.21 c	4.02 ± 0.43 c	4.41 ± 0.47 c	1.04 ± 0.11 c
T_3	10.82 ±0.16 b	1.86 ± 0.19 b	4.18 ± 0.47 b	4.78 ± 0.51 b	1.14 ±0.13 b
T_4	10.91 ± 0.21 b	1.89 ± 0.17 b	4.22 ± 0.37 b	4.80 ± 0.43 b	1.15 ±0.11 b
T_5	10.98 ±0.16 b	1.91 ± 0.20 b	4.24 ± 0.50 b	4.83 ± 0.48 b	1.21 ± 0.17 b
T_6	11.33 ± 0.21 a	2.07 ± 0.18 a	4.42 ± 0.22 a	4.96 ± 0.32 a	1.34 ± 0.14a
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. [a,b] Means within columns with no letters differ significantly ($p < 0.05$).

Dietary treatment had a significant ($p \leq 0.05$) effect on villus height, crypt depth, and villus height to crypt depth ratio in the duodenum (table 4.), jejunum (table 5.) and ileum (table 6.). Birds fed the fermented feed had higher ($p \leq 0.05$)

villus heigh and crypt depth in the duodenum, jejunum, and ileum than birds fed entire control dry feed. These birds also had a higher ($p \leq 0.05$) villus high to crypt depth ratio in all these three parts as well.

Table 4: Effect of fermented feed by the Iraqi 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
T_1	117.42 ±1.15 c	15.35 ± 0.18 c	7.65 ± 0.05c
T_2	122.33 ± 2.06 c	15.59 ± 0.21 c	7.85 ± 0.05c
T_3	141.16 ± 1.23 b	17.52 ± 0.19 b	8.06 ± 0.04 b
T_4	146.21 ± 2.33 b	17.72 ±0.18 b	8.25 ± 0.08 b
T_5	150.05 ± 1.17 b	17.97 ± 0.20 b	8.35 ± 0.07b
T_6	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. [a,b] Means within columns with no letters differ significantly ($P < 0.05$).

Table 5: Effect of fermentation feed by the Iraqi 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
T_1	101.12 ±1.07c	14.32 ± 0.15c	7.06 ± 0.09 c
T_2	103.67 ± 1.12 c	14.44 ± 0.12 c	7.18 ± 0.07 c
T_3	113.22 ± 1.61 b	15.18 ± 0.13 b	7.46 ± 0.06 b
T_4	115. 17 ± 1.22 b	15.25 ± 0.21 b	7.55 ± 0.05 b
T_5	117.20 ± 1.16 b	15.37 ± 0.20 b	7.62± 0.06 b
T_6	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. [a,b] Means within columns with no letters differ significantly ($p < 0.05$).

Table 6: Effect of fermentation feed by the Iraqi 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
T_1	41.14 ±0.53 c	8.62 ±0.79 c	4.77 ± 0.05 c
T_2	41.77 ± 0.60 c	8.70 ± 0.68 c	4.80 ± 0.04 c
T_3	50.18 ± 0.54 b	9.81 ± 0.72 b	5.12 ± 0.06 bc
T_4	51.25 ± 0.65 b	9.87 ± 0.71 b	5.19 ± 0.07 b
T_5	53.09 ±0.49 b	10.11 ± 0.81 b	5.25 ± 0.06 ab
T_6	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. [a,b] Means within columns with no letters differ significantly ($p < 0.05$).

In the current study, the morphology and histology parameters of the digestive tracts parts were improved in broiler chicks feed on fermented feed than broilers fed on control diet. These results are in agreement with that of Chiang *et. al.* (3), and Xu *et. al.* (21). The villus height to crypt depth is a very

useful measure to estimate the absorption capacity of the small intestine. Maximum digestion and absorption is believed to occur as villus height to crypt depth ratio increased (3). Changes in the intestinal morphology such as reduced villus height and deeper crypt may also indicate the presence of toxins (20) in the present study, increased villus height and increased villus height to crypt depth ratio were observed in broilers fed on fermented feed compared with unfermented feed. The increased villi height and villus heigh to crypt depth ratio might be associated with the increased number of beneficial bacteria like *Lactobcilli, Bifidobacterium, Bacillus subtilis,* and *Sacchromyces cervisia* (12, 20). Fermented feed was characterized 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 Culter *et. al.* (5). The increased villus height and villus height to crypt depth ratio produced an intestinal structure more oriented to digestion, with improved absorptive and hydrolysis potential, as well as requiring fewer nutrients towards intestinal maintenance. Thus, the intestinal structure of duodenum, jejunum, and ileum is more favorable for the bird and may help to explain the improvement in weight gain and feed conversion (Hu *et. al.*, 2008). The better results obtained of SSF feed might be attributable to the higher production of secondary microbial metabolites during solid fermentation. These metabolites include organic acid (Lactic acid) produced by Lactobacilli, enzymes (amylase and protease) and antimicrobial substances like iturin and surfactin which produced by *Bacillus subtilis* bacteria during solid fermentation.

In conclusion, the results of the current experiment indicated that feeding fermented ration could improve the intestinal structure of the broilers, enhance villus height and increased villus height to crypt depth ratio in the duodenum, jejunum, and ileum.

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تأثير عملية تخمير وترطيب العليقة في بعض الصفات المورفولوجية والنسيجية لأمعاء فروج اللحم

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الملخص

أجريت هذه التجربة بهدف دراسة تأثير تخمير العلف وترطيبه بالماء في بعض الصفات المورفولوجية والنسيجية لأمعاء فروج اللحم. استخدم في البحث **360** فرخاً للحم (**Ross 306**) بعمر يوم واحد وزعت بصورة عشوائية على ست معاملات. غذيت افراخ المعاملة الأولى على عليقة قياسية جافة وعُدَّت معاملة للسيطرة، اما افراخ المعاملة الثانية فقد غذيت على علف مرطب بالماء بنسبة **1:1** (علف الى الماء). غذيت افراخ المعاملات الثالثة، الرابعة، الخامسة والسادسة على العلف المتخمر بالنسب **25**، **50**، **75** و**100**% من استهلاك العلف اليومي طيلة مدة التجربة التي استمرت لمدة ستة اسابيع. قسمت افراخ كل معاملة عشوائيا على ثلاث مكررات وقدم لها العلف والماء بصورة حرة مع الاضاءة المستمرة (**24** ساعة باليوم) طيلة مدة التجربة. إظهرت النتائج بان التغذية على العلف المتخمر والمرطب بالماء قد أدت الى إرتفاع معنوي في الطول والوزن النسبيين لكل من الاثني عشري والصائم واللفائفي وكذلك الاعورين. وأشارت نتائج التحليل الاحصائي أيضاً إن التغذية على العلف المتخمر قد سببت ظهور زيادة معنوية في طول الزغابات وعمق الخبايا ونسبة طول الزغابات الى عمق الخبايا في أجزاء الأمعاء الدقيقة. يستنتج من الدراسة الحالية بان تغذية فروج اللحم على العلف المتخمر والمرطب بالماء لها آثار ايجابية في الصفات المورفولوجية والنسيجية لفروج اللحم.

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