



RESEARCH ARTICLE

The effect of feed wetting and fermentation on the intestinal flora, humoral and cellular immunity of broiler chicks

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Abstract

During recent years solid state fermented feed (SSFF) has been introduced with great success in poultry nutrition, where it has shown to have some beneficial properties in particular considering animal health. The present experiment was conducted to evaluate the effect of feed wetting and fermented feed on the intestinal flora and immunity response parameters of broiler chicks. The SSFF were prepared in two stages; at the first stage the commercial broiler feed were moistened with tap water at the rate 1:1 (1 liter water for each one kg. feed). At the second stage the wetting feed were placed in a plastic tray and inoculated with Iraqi probiotic (IP) at the rate 10 grams of IP for each one kg. of feed. Then the plastic tray was closed and incubate for 48 h. at 37 ± 2 °C for complete fermentation.

The IP was purchased from laboratory of poultry technology at agriculture faculty, university of Baghdad. Each one gram of IP contains at least 10^9 cfu of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and at least 10^8 cfu of *Saccharomyces cerevisia*.

A total of 360 one day old Ross308 broiler chicks were randomly assigned to the six experimental diets in a completely randomized design (CRD). Each treatment group was replicated three times with 20 chicks per replicate. Chicks in T1 group were fed on dry feed throughout of the experimental period which was lasted for six weeks and used as control. Chicks in T2 group were fed with wetting feed. Chicks in T3, T4, T5 a T6 were fed on SSFF at the proportion of 25, 50, 75 and 100% from total daily feeding respectively in order to determine the appropriate proportion of dry feed replacement. Experimental parameters measured included: total plate count for aerobic, Lactobaclli, and coliform bacteria in the duodenum and cecum content, bursa weight, bursa index, blood serum titer against Newcastle disease virus (NDV) and T-delate hypersensitivity test were used to measure the cell mediated immunity (CMI).

The data showed that birds fed on fermented feed had significantly ($P \leq 0.05$) lower total count for aerobic and coliform bacteria and significantly ($P \leq 0.05$) higher logarithmic number of Lactobacilli bacteria in duodenum and cecum when comperd with control. Bursa weight, bursa relative weight and bursa index were significantly ($P \leq 0.05$) improved in treatment groups fed on wetting or fermented feed. Blood serum ELISA titer against NDV, and CMI were significantly ($P \leq 0.05$) higher in birds fed on fermented feed.

In conclusion, it can be stated that feed fermentation generally improves bacterial ecology of the gastrointestinal tract and immunity response in broiler chicks, therefore be a new handle on future strategy to control chicken disease.

INTRODUCTION

Fermentation is the chemical transformation of organic substances into simpler compounds by the active enzymes, complex organic catalysts, which produced by microorganisms such as bacteria, yeasts, or molds. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (more easily digestible) compounds and nutrient (Shurtleff and Aoyagi, 2007). 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 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 (Adams *et al.*, 2002).

Fermentation has been practiced for quite a long time as a means to improve the quality of food. Fermentation process has been applied to improve the nutritive value of soybean (Mathivanan *et al.*, 2006) and copra meal (Hatta and Sunda, 2009). The fermentation process can create conditions for the growth of microorganisms that break down fiber and anti-nutrients. 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 (Winsen *et al.*, 2001) and broiler chicks (Heres *et al.*, 2003). Lactobacilli and yeast in the kefir which supplemented in drinking water were significantly increased the population of Lactobacilli spp. and total aerobic bacteria and decreasing the population of Enterobacteriaceae and coliform in the geese intestine (Yaman *et al.*, 2006).

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 (Donkor *et al.*, 2006, Chokboonmongkol *et al.* 2013, Kilonzo *et al.* 2013).

Antibiotic have been used as feed additives 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 have therefore become important as replacement feed additives (Steiner, 2006). A probiotics is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (Zang *et al.* 2014). Afterfeeding of probiotics, improvements in growth performance, feed efficiency, immunity parameters and disease resistance have been reported (Al-Gharawi, 2012, Bai *et al.* 2013).

The major probiotic strains include Lactobacillus, Saccharomyces, Streptococcus and Aspergillus (Tannock, 2001). Presently Bacillus, Lactobacillus and Saccharomyces are the major strains applied in broilers (Zhang *et al.*, 2005; Chen *et al.*, 2009, Shanmugasundaram 2013).

Since there have been few investigations of the fermentation of feed with probiotics and used wet feeding in broiler chicks. The objectives of this study were to clarify the effect of wet and fermented feed on intestinal microflora populations and immunity in broiler chicks.

Materials and Methods

Preparation of fermented feed:

A commercial broiler starter and finisher diet (Table 1) were purchased from local market. Chicks were fed on starter diet during the first three weeks, and then transferred to finisher diet for the remainder of the experimental period which was lasted for 6 weeks.

The fermented feed (SSFF) were prepared at two stages; at the first stage the feed were moisted with water (water: feed, 1:1). At the second stage the wetting feed were placed in a plastic tray and inoculated with Iraqi 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 °C for complete fermentation.

The IP were purchased from laboratory of poultry technology at agriculture faculty, university of Baghdad. According to the manufacture information label, each one gram of IP contain at least 10⁹ cfu of *Lactobacillus acidophilus*, *Bacillus subtilis*, Bifidobacterium and at least 10⁸ cfu of *Saccharomyces cerevisia*. 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.* (2005).

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 chicks into six experimental groups and 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 replicate. Chicks were reared in battery cages (1.5 × 1.0 m) with four tiers. 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 experimental period, two birds per replicate were slaughtered and blood collected in heparinized tube and centrifuged at 2000 round per minute for 15 min. serum samples were then isolated and stored at 20 °C until use for analysis. Serum titer against NDV were determined by using an indirect enzyme-linked immunosorbent assay (ELISA) as described by Feng *et. al.* (2009). Toe web swelling test was utilized as an indicator of CMI response. At 30 d of age 'chicks of T2, T3, T4, T5, and T6 received intradermal injection of NDV solution (1000 doze vial of ND dissolved with 3cc of normal slain) in the toe web (Corrier and Deloach, 1990). Skin thickness at the spot of injection was measured by a thickness gauge before injection (time 0) and at 48h post injection (time 2). The ratio of increase in skin thickness (r) was calculated by dividing the thickness at 48h post injection over the thickness at time 0. Data on the ratio of the increase of skin thickness were used in the statistical analysis.

Additionally, the bursa of fabricus for these chickens were removed and weighted. Relative bursa weight to body weight and bursa index were calculated. One gram from duodenum and cecum contents were diluted 10-fold with buffered peptone water, and then one hundred microliters of supernatant was pour on to macConkey agar and Lactobacilli MRS agar and incubated at 37 °C with 13% CO₂ for 48 h. in order to plate count for coliform and Lactobacilli respectively. Total plate count for aerobic bacteria on nutrient agar was also performed by using pour plate count procedure according to APHA (1978). Bacterial colonies were counted and expressed as colony forming units per gram (cfu/ g).

Statistical analysis:

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

Results and discussion:

The effect of feeding wetting and fermented feed on microbial counts is shown in table 2. The total number of aerobic and coliform bacteria in duodenum and cecum were significantly ($P \leq 0.05$) decreased in birds fed fermented feed than those of bird fed unfermented or control dry feed. Feeding the fermented feed were significantly ($P \leq 0.05$) increased the total number of Lactobacilli in duodenum and cecum of broiler chicks. These findings were in agreement with that reported by Chiang *et. al.* (2010); Uchewa and Onu (2012) and Firman *et. al.* (2013). Fermented feed led to a much healthier gastrointestinal tract. One study suggested that fermented feed should be called "Fermbiotics" because it provides the same benefits as probiotics in the human diet (Niba *et. al.*, 2009). Primarily fermented feed cause a reduction of pathogenic bacteria including Salmonella and Campylobacter in the digestive tract. The lactic and acetic acid produced by the bacteria in the fermented feed creates an acidic environment with a PH about 4. At this level of acidity, molecules of acid can enter the bacteria through their cell membranes, and the increased acidity within the cells interferes with enzymatic processes, killing the bacteria (Heres *et. al.*, 2003). Fermented feed is somewhat more effective against Salmonella and Campylobacter because Lactobacillus also outcompetes the Salmonella for nutrients in the feed itself (Heres *et. al.*, 2002).

Furthermore, Lactobacilli strains have been found to be inhibit the growth of three serotypes of *E. coli* in vitro (Jin *et. al.*, 1996, Cao *et. al.* 2013).

Table1. 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).

Table2. The effect of wetting and fermented feed on aerobic, coliform bacteria and Lactobacilli in duodenum and cecum.

Treatment	Duodenum			Cecum		
	aerobic	Coliforms	Lactobacilli	aerobic	Coliforms	Lactobacilli
T ₁	5.33 ± 0.05 a	12.12 ± 0.09 a	4.86 ± 0.05 c	4.75 ± 0.08 a	9.14 ± 0.18 a	3.16 ± 0.20 c
T ₂	5.32 ± 0.07 a	12.05 ± 0.06 a	4.97 ± 0.11 c	4.69 ± 0.05 a	9.01 ± 0.22 a	3.32 ± 0.20 c
T ₃	4.97 ± 0.05 b	10.96 ± 0.07 b	6.66 ± 0.08 a	3.97 ± 0.07 b	8.04 ± 0.19 b	4.43 ± 0.21 a
T ₄	4.88 ± 0.05 b	10.85 ± 0.08 b	6.80 ± 0.07 b	3.82 ± 0.06 b	7.93 ± 0.24 b	4.59 ± 0.27 b
T ₅	4.79 ± 0.06 b	10.65 ± 0.08 b	6.95 ± 0.06 b	3.76 ± 0.08 b	7.66 ± 0.21 b	4.72 ± 0.19 b
T ₆	3.96 ± 0.06 c	9.16 ± 0.10 c	8.04 ± 0.06 a	2.44 ± 0.09 c	6.52 ± 0.23 c	5.98 ± 0.20 a

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 common superscript differ significantly ($P < 0.05$).

Bursa of fabricius weight, bursa relative weight and bursa index data are shown in table 3. Birds fed on wetting and fermented feed had higher ($P \leq 0.05$) bursa weight, bursa relative weight and bursa index compared with those of birds fed control dry feed. Measurement of immune organ weight is a common method for evaluation of immune status. Abul *et. al.* (2012) and Ao *et. al.* (2011) demonstrated that bird fed diet containing fermented ingredients could influence the immune organ weights.

Table 3. The effect of wetting and fermented feed on bursa relative weight and bursa index.

Treatment	relative weight of bursa	bursa index
T ₁	0.075 ± 0.001c	1.000 ± 0c
T ₂	0.079 ± 0.002c	1.053 ± 0.012c
T ₃	0.098 ± 0.002b	1.307 ± 0.015b
T ₄	0.101 ± 0.001b	1.347 ± 0.019b
T ₅	0.104 ± 0.003b	1.387 ± 0.021b
T ₆	0.131 ± 0.002a	1.747 ± 0.020a

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 common superscript differ significantly ($P < 0.05$).

The effect of feeding wetting and fermented feed on both humeral and CMI are shown in table 4. Blood serum ELISA titer against NDV and CMI were significantly ($P \leq 0.05$) higher in birds fed on fermented feed.

These results are in agreement with that of Tang *et. al.* (2012) who showed that a significant increase in serum immunoglobulin M, immunoglobulin G and complement C4 level when compared with broiler fed control soy-corn diet. Feeding fermented soybean meal (FSBM) were also significantly increased the level of IgA and IgG than birds fed entire soybean (Xu *et. al.*, 2012; Feng *et. al.*, 2007). Cao *et. al.* 2013 showed that feeding diet supplemented with probiotic were significantly increased the immunity against *Escherichia coli*.

Table 4. The effect of wetting and fermented feed on Cell mediated immunity (CMI) and Blood serum ELISA titer against NDV.

Treatment	Cell mediated immunity (CMI)	Blood serum ELISA titer against NDV
T ₁	c0.199 ± 0.013	c2906.7 ± 222.2
T ₂	c0.205 ± 0.016	c2975.4 ± 232.6
T ₃	b0.514 ± 0.034	b3688.1 ± 323.6
T ₄	b0.525 ± 0.041	b3704.2 ± 320.2
T ₅	b0.542 ± 0.041	b3743.8 ± 310.17
T ₆	a0.617 ± 0.059	a4087.9 ± 239.7

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 common superscript differ significantly ($P < 0.05$).

These results suggest that the fermented feed have an immune modulating impact on broiler chicks both in standard production situations and during challenge (El-Husseiny *et. al.*, 2008; Gao *et. al.*, 2008, 2009, Huff *et. al.* 2013). This positive effect on the immune system may be partially responsible for the improved broiler performance (Bai *et. al.*, 2014) and turkey live weight at marketing age (Firman *et. al.*, 2013, Huff *et. al.* 2013).

Previous studies indicated that the fermentation can increase the content of small size peptides, which may improve the immune function of animal (Feng *et. al.*, 2007; Chen *et. al.*, 2009). Wang *et. al.* (2003) stated that piglets increased the concentration of immunoglobulin by adding 3 g/kg small peptides in the basal diets. Gao *et. al.* (2009) isolated anti-oxidant peptides from cottonseed protein hydrolysates which may improve the immune function

as well. Thus, the increase in the serum ELISA titer against NDV may be attributed to small peptides formed during the fermentation process (Feng *et al.*, 2007). On the other hand, the live microbes in the fermented feed may also act as probiotic to enhance the humoral immune response (Apata, 2011).

Taking together, solid state fermentation of broiler feed with viable benefit microbes of Iraqi probiotic seemed to improve the intestinal microflora balance and immunity in broiler chicks.

Further investigation on the practical application of fermented feed considering feeding management, microbiology and immunity parameters in broiler and layer chicks should be carried out in the future.

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