

**RESEARCH ARTICLE****The effect of starting age of feeding wetting fermented feed on the intestinal flora, humoral and cellular immunity of broiler chicks****S.A.H. Naji<sup>(1)</sup> J.K.M. Al-Gharawi<sup>(2)</sup>I.F.B. Al-Zamili<sup>(2)</sup>**

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**\*Corresponding Author****S.A.H. Naji****Abstract**

A feeding experiment was conducted to investigate the effect of solid state fermented feed (SSFF) on intestinal microflora and immunity parameters in broiler chicks. 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 and T5 were fed on SSFF from the age of 3, 2 and 1 week of age until the marketing age respectively . Chicks in T6 were fed on the fermented feed from one day old throughout of the experimental period (6 weeks). 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 one kg. of feed. Then the plastic traywas closed ad incubate for 38 h. at  $37\pm2$  °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 cervisia.. 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 ( $P\leq0.05$ ) lower total count for aerobic and coliform bacteria and significantly ( $P\leq0.05$ ) higher logarithmic number of Lactobacilli bacteria in duodenum and cecum control. Bursa weight, bursa relative weight and bursa index were significantly ( $P\leq0.05$ ) improved in treatment groups fed on wetting or fermented feed. Blood serum ELISA titer against NDV, and CMI were significantly ( $P\leq0.05$ ) higher in birds fed on fermented feed.

In conclusion, it can be stated that SSFF seemed to improve the bacterial ecology of the gastrointestinal tract and reduced the level of Enterobacteriaceae in the different parts of gastrointestinal tract and improved immunity parameters in broiler chicks.

## INTRODUCTION

Fermented feed is any sort of regular chicken feed which has been fermented with live culture of bacteria or yeasts. First, the feed is moistened or submerged in water. Then live culture of benefit microbes culture were added to begin fermenting the feed (Afsharmaneshet. al, 2010). You can use the fermented feed after a few hours although many recommend waiting for 24 or 48 or 72 hours to get the full benefits (Naji et. al., 2014). Fermented feed was characterized by high lactic acid concentration (up to 260 mmol/ kg feed) and moderate amounts of acetic acid bacteria (20-30 mmol/ kg feed) and high number of lactic acid bacteria (Log 9-10 cfu/ g feed) and a pH of approximately 4.5 (Aziwoet. al, 2013).

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 (Mathivananet. al., 2006) 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 (Winsenet. al., 2001) and broiler chicks (Hereset. al., 2003). 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 (Yamanet. 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 meatless likely (Donkoret. 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).

At present, the data about the effect of fermented feeds on broiler productivity, physiology, microbiology and immunity are limited. Therefore, the present experiment was conducted to evaluate the effect of starting age of feed SSFF on the intestinal microflora, humoral and CMI of broiler chicks.

## Materials and Methods

### 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 1<sup>st</sup> – February- 2014 to 5<sup>th</sup>- March- 2014 and aimed to evaluate the best age of starting age of start feeding of 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 commercial broiler starter finisher.

**T2:** Fed on wetting feed (1:1, feed: water).

**T3:** Fed on SSFF from 3 week old to marketing age (6 weeks).

**T4:** Fed on SSFF from 2 week old to marketing age.

**T5:** Fed on SSFF from 1 week old to marketing age.

**T6:** Fed on SSFF from 1 day old to marketing age.

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 tears. 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.

**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 were used for the reminder 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 moistened with water (water: feed, 1:1). At the second stage the wetting feed were 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 incubated for 48 h. at  $37\pm2$  °C for complete fermentation.

The IP were purchased from laboratory of poultry technology at agriculture faculty, university of Baghdad. According to the manufacturer information label, each one gram of IP contain at least  $10^9$  cfu of Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium and at least  $10^8$  cfu of Saccharomyces cervisiae. 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 Culture et. al. (2005).

**Sampling procedure and analytical 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 saline) 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 fabricius of 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% CO<sub>2</sub> 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). Saccharomyces cervisiae yeast were enumerated on potato dextrose agar after aerobic incubation at 37 °C for 24 h. bacterial and yeast 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 ( $P\leq0.05$ ) decreased in birds fed fermented feed than those of bird fed unfermented or control day feed. Feeding the fermented feed was significantly ( $P\leq0.05$ ) increased the total number of Lactobacilli in duodenum and cecum of broiler chicks. These findings were in agreement with that reported by Chaing 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 "Fermiotics" 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 (Jinet. 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 starting age of feeding wetting fermented on aerobic, coliform bacteria and Lactobacilli in duodenum and cecum.

Treatment	Duodenum			Cecum		
	aerobic	Coliforms	Lactobacilli	aerobic	Coliforms	Lactobacilli
T <sub>1</sub>	4.07±0.05 a	11.08±0.10 a	5.89 ±0.43e	3.56±0.05 a	8.22±0.15 a	4.77±0.21 e
T <sub>2</sub>	3.85 ±0.04b	10.75±0.09 b	6.33 ±0.51d	3.28±0.04 b	7.96±0.18 b	4.96±0.19 d
T <sub>3</sub>	3.61 ±0.06c	9.67±0.08 c	8.11 ±0.44c	2.78±0.06 c	6.13±0.18 c	5.82±0.23 c
T <sub>4</sub>	3.66 ±0.05 c	9.80±0.12 c	8.20 ±0.50c	2.72±0.05 c	6.07±0.14 c	5.90±0.17 c
T <sub>5</sub>	3.11 ±0.06 d	8.69±0.10 d	9.95 ±0.46b	2.36±0.06 d	5.73±0.12 d	6.53±0.22 b
T <sub>6</sub>	2.83±0.06 e	6.74±0.11 e	10.54 ±0.60a	1.77±0.05 e	5.11 ±0.13 e	7.67±0.20 a

**T1:**Control group fed on dry commercial broiler starter finisher. **T2:** fed on wetting feed (1:1, feed: water). **T3:**Fed on SSFF from 3 week old to marketing age (6 weeks).**T4:**Fed on SSFF from 2 week old to marketing age. **T5:**Fed on SSFF from 1 week old to marketing age. **T6:**Fed on SSFF from 1 day old to marketing age. <sup>a,b</sup> 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.

**Table3.**The effect of starting age of feeding wetting fermented on bursa relative weight and bursa index.

Treatment	relative weight of bursa	bursa index
T <sub>1</sub>	d0.070 ± 0.002	0 f1.000 ±
T <sub>2</sub>	d0.076 ± 0.001	e1.086 ± 0.010
T <sub>3</sub>	c0.089 ± 0.002	d1.271 ± 0.012
T <sub>4</sub>	c0.091 ± 0.002	c1.300 ± 0.011
T <sub>5</sub>	b0.104 ± 0.001	b1.486 ± 0.014
T <sub>6</sub>	a0.126 ± 0.002	a1.800 ± 0.019

T1: Control group fed on dry commercial broiler starter finisher. T2: fed on wetting feed (1:1, feed: water). T3: Fed on SSFF from 3 week old to marketing age (6 weeks). T4: Fed on SSFF from 2 week old to marketing age. T5: Fed on SSFF from 1 week old to marketing age. T6: Fed on SSFF from 1 day old to marketing age. 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 starting age of feeding wetting fermented on Cell mediated immunity (CMI) and Blood serum ELISA titer against NDV.

Treatment	Cell mediated immunity (CMI)	Blood serum ELISA titer against NDV
T <sub>1</sub>	e0.186 ± 0.011	e2886.4 ±294.1
T <sub>2</sub>	d0.214 ± 0.014	d2953.6 ±277.8
T <sub>3</sub>	c0.287 ± 0.014	c3327.3 ± 290.7
T <sub>4</sub>	c0.296 ± 0.021	c3339.8 ±311.3
T <sub>5</sub>	b0.377 ± 0.016	b3511.2 ±296.9
T <sub>6</sub>	a0.523 ± 0.020	a3872.1 ±311.2

T1: Control group fed on dry commercial broiler starter finisher. T2: fed on wetting feed (1:1, feed: water). T3: Fed on SSFF from 3 week old to marketing age (6 weeks). T4: Fed on SSFF from 2 week old to marketing age. T5: Fed on SSFF from 1 week old to marketing age. T6: Fed on SSFF from 1 day old to marketing age. 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; Gaoet. al, 2008, 2009, Huff et.al.2013). This positive effect on the immune system may be partially responsible for the improved broiler performance (Naji et. al., 2014) and turkey live weight at marketing age (Firmanet. 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. Gaoet. 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).

Interestingly, there are fantastic benefits to just feeding a wet feed, even unfermented. One of these is greater nutritional uptake, and therefore better feed utilization and body weight gain (Chen et. al., 2009). SSFF has been reported to be an effective way to reduce anti-nutritional factors in rapeseed meal (Chaing et. al., 2010) copra meal (Hatta and Sundu, 2009) and soybean (Tang et. al., 2012).

Finally, SSFF seemed to play an important role in ecology and microbiology of gastrointestinal tract and immunity parameters of broiler chicks.

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