

## Original Research Article

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## Effect of Solid State Fermented Dried Feed with Avian Specific *Lactobacillus species* and *Bacillus subtilis* on Haematological, Blood Biochemical and Anti-oxidant Profile of Broiler Birds

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### ABSTRACT

The present experiment was conducted to study the effect of feeding fermented dried feed with avian specific *Lactobacillus species* and *Bacillus subtilis* in broiler birds. Two hundred and fifty one day old mixed sex commercial (Venn Cobb) broiler chicks were randomly distributed into five groups (Gr 1, Gr 2, Gr 3, Gr 4 and Gr 5) of 50 birds each by following completely randomized block design. Each group, comprised of five replicated pens (n=5) and each replicate contained ten broiler chickens. The Group 1 was fed the basal diet as per BIS, 2007, without any supplementation and was served as control (C). Birds in Group 2 (AGP) were fed basal diet supplemented with the Antibiotic Growth Promoters i.e. Bacitracin methylene disalicylate @ 0.5 g/kg of feed. Birds in Group 3 (FBS) were fed basal diet fermented with *Bacillus subtilis*. Birds in Group 4 (FLB) had received the basal diet fermented with avian specific *Lactobacillus species*, isolated and characterized from broiler bird itself and birds in Group 5 (FBSLB) had received the basal diet fermented with *Bacillus subtilis* & avian specific *Lactobacillus species*. Duration of experiment was 42 days. Blood was collected on day 28 and day 42 for checking haematological parameters like RBC, WBC, PCV and Hb, blood biochemical values and anti-oxidant profile. It was observed that RBC, WBC, PCV and Haemoglobin values at day 42 was found similar (P>0.05) between different treatment groups. Total protein was found to be significantly higher (P<0.05) in FBSLB at day 28 and it was significantly higher (P<0.01) in FLB group at day 42 as compared to other treatment groups. Average glucose concentration in serum was significantly higher (p<.01) in AGP & FLB group as compared with control, FBS & FBSLB group. Albumin and globulin value also showed significant difference (P<0.05) between experimental groups. Average values SGPT and SGOT at day 28 and 42 was found similar (P>0.05) in comparison to control group. Triglyceride and cholesterol level were not affected (P>0.05) due to treatment imposed in this study. Though HDL and LDL cholesterol concentrations in serum were not affected by the treatments imposed in this study, HDL cholesterol was tended (p=.07) to be increased in broilers fed fermented dried feed as compared to control group. Anti-oxidant status in blood serum was found significantly higher (P<0.01) for FBSLB in comparison to other treatment groups. It could be concluded that fermented dried feed showed better blood biochemical profiles and improved anti-oxidant status in broiler birds.

#### Keywords

Fermented dried feed, Biochemical parameters, Haematology, Anti-oxidant profile, Broilers

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## Introduction

A number of feed additives including antibiotics have been widely used in the poultry industry for several decades. Antibiotics used in this way will get accumulated in the tissues of birds leading to antibiotic resistance in human through food chain ultimately ending up in therapeutic failure (Levy and Marshall, 2004). To prevent the risk of developing pathogens resistant to antibiotics and also to satisfy consumer demand for a food chain free of drugs, the use of antibiotics in feed in the European Union was banned in January 2006 (Griggs and Jacob, 2005) and this policy is being considered in other parts of the world including India. Consequently, the poultry industry seeks an alternative for antibiotics as growth promoters like probiotics, prebiotics, phytobiotics, organic acids, immune stimulants etc. As an alternative to antibiotic growth promoter, probiotics or direct-fed microbes (DFM) and fermented feed have been used to improve growth performance and enhance the health status of poultry (Teng *et al.*, 2017). Because of unstable effects of supplemental probiotics in broiler diets, fermented feed with probiotic bacteria have played a promising alternative to antibiotic growth promoter to improve the growth performance (Xie *et al.*, 2016) and gastro intestinal health of broilers (Missotten *et al.*, 2013).

Fermentation is a dynamic process involving microorganisms, substrates and environmental conditions to convert complex substrates into simpler compounds (Nibaet *et al.*, 2009). Many micro-organisms have been used to ferment the feed. *Bacillus subtilis* can secrete protease, amylase, and lipase which can improve growth performance in broilers (Santoso *et al.*, 2001). Reports suggested that complex carbohydrates are poorly metabolised by *Lactobacillus* (Dworkin *et al.*, 2006).

However, if complex carbohydrates degraded to low-molecular weight carbohydrates by solid-state fermentation, *Lactobacillus* could ferment these low-molecular weight carbohydrates to lactic acid (Chen *et al.*, 2013). Therefore, two stage solid state fermentation i.e. at first stage fermentation of feed with *Bacillus subtilis* and second stage by avian specific *Lactobacillus Spp* might have better result in terms of performance of broiler birds.

Compared to feeding of fermented feed as such in pigs, feeding of fermented wet feeding broilers may decrease the feed intake during starter and grower phases. Main reasons for this decreased feed intake are, moist feed may be too bulky for small birds and the diet may lose its attractiveness after fermentation (Missotten *et al.*, 2013). Moreover, wet fermented feed is very difficult to apply in poultry feed industry. Because of this reason solid state fermented dried feed with *Bacillus subtilis* and *Lactobacillus* might be better substitute for antibiotic growth promoter. The literatures on effect of fermented dried feed on blood biochemistry and antioxidant status are very scanty. Therefore, the present study was aimed to study the effect of fermented dried feed on haematological, blood biochemical anti oxidant status of broiler chicken

## Materials and Methods

### Experimental design and diet

The study was carried out at ILFC, College of veterinary science and animal husbandry, central agricultural university, Aizwal, Mizoram. Two hundred and fifty, one day old mixed sex commercial (Venn Cobb 400) broiler chicks almost similar body weight from a single hatch were procured from local market. The chicks were randomly distributed into five groups (Gr 1, Gr 2, Gr 3, Gr 4 and Gr 5) with 50 birds each by following Completely

Randomized Block Design. Each group, comprised of five replicated pens (n=5) and each replicate contained ten broiler chickens. Three types of standard broiler diets have been prepared i.e. broiler pre-starter (1-7 days of age), broiler starter (8-21 days of age) and broiler finisher (22-42 days of age) as per BIS (2007) specification. The Group 1 was fed the basal diet without any supplementation and was served as control (C). Birds in Group 2 (AGP) were fed basal diet supplemented with the antibiotic growth promoters i.e. Bacitracin methylene disalicylate @ 0.5 g/kg of feed. Birds in Group 3 (FBS) were fed basal diet fermented with *Bacillus subtilis*. Birds in Group 4 (FLB) had received the basal diet fermented with avian specific *Lactobacillus species* which was isolated from broiler bird itself and birds in Group 5 (FBSLB) had received the basal diet fermented with *Bacillus subtilis* and avian specific *Lactobacillus species*.

#### **Preparation of fermented dried feed by solid state fermentation**

Starter cultures were prepared by taking 0.5 kg of wet compounded broiler feed having the feed:water ratio of 1:1.5 and adding 1 ml of nutrient broth culture of *Bacillus subtilis* (ATCC® 19659™) and 1 ml of MRS broth culture of *Lactobacillus spp.* in two glass jars separately and was incubated at 37 °C for 24 hrs. *Lactobacillus spp.* used in this study was isolated, screened and identified by PCR.

After 24 hrs of incubation *Bacillus subtilis* starter culture was added separately to 12.5 kg of feed having feed:water ratio of 1:1.5 and kept at room temperature for 24 hrs for proper fermentation and *Lactobacillus* starter culture was added separately to 12.5 kg of feed having feed:water ratio of 1:1.5 and kept at room temperature for 24 hrs for proper fermentation. pH of the feed was monitored to check the quality of fermented feed. pH in the

range 4 to 4.2 indicated proper fermentation (Table 1).

For making two stage solid state fermented feed with *Bacillus subtilis* and *Lactobacillus spp.*, to 12.5 kg of feed having feed:water ratio of 1:1.5, 0.5 kg of *Bacillus subtilis* starter culture was added and kept at room temperature for 24 hrs for proper fermentation. To the same feed 0.5 kg of *Lactobacillus* starter culture was added and again kept at room temperature for 24 hrs. pH in the range 4 to 4.2 indicates proper fermentation.

After fermentation, feed was dried under the sun till the moisture comes down to 12%. Viable bacteria used for fermentation was tested and found out that they are present on the feed in the range  $10^6$  to  $10^9$  cfu/g of feed.

#### **Feeding management and vaccination**

Birds were raised under deep litter system of management. They were subjected to standard management and health practices. The experimental birds were offered ad libitum quantity of feed daily in the clean feeders as per above feeding plan. Every day morning, the left over feeds (residual feeds) was collected from the feeders and measured. Drinkers were washed daily and fresh water was served daily ad libitum during experimental period lasting for six weeks. The birds were vaccinated against New castle disease (NDV) and Infectious Bursal Disease at the 7th and 14th day respectively, booster dose vaccine against NDV was also done at 21st day.

#### **Blood collection and analysis**

Blood was collected in EDTA vial for haematological analysis and on clot activator vial for blood biochemical and anti-oxidant profile at 28 and 42 day of age from brachial

vein by using 2ml syringe. Haematological parameters like RBC, WBC, PCV and haemoglobin were found out by using Semi Automated Blood Analyser. Blood biochemical parameters like glucose, total protein, albumin, globulin, triglyceride, cholesterol, HDLC, LDL and enzymes like SGPT and SGOT were analysed by using Fugifilm clinical chemistry analyser. Antioxidant profile of serum was measured at day 28 and 42 by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996).

### Statistical analysis

The data was analysed by one way analysis of variance (ANOVA) using SPSS (1997) in a completely randomized design. The test was employed for identifying the significant differences amongst the different treatments. Probability values less than 0.05 is considered to be statistically significant and the values  $P \leq 0.01$  was declared as trend.

## Results and Discussion

### Hematological parameter

The count of RBC and WBC, PCV (%) and Haemoglobin (g/dl) values in experimental birds were found non-significant ( $P > 0.05$ ) among treatment groups fed fermented feed with *Bacillus subtilis* and avian specific *Lactobacillus spp.* (Table 2). Concomitant to the current findings, Sugiharto *et al.*, (2016) found o that feeding of fermented dried cassava in broilers did not affect ( $P > 0.05$ ) the RBC and WBC values in comparison with control group.

On the contrary, Muhammad and Oloyede (2009) observed that RBC, WBC, PCV and hemoglobin value of broiler birds fed *Aspergillus niger* fermented *Terminalia catappa* seed meal-based diet was found

significantly lower ( $P < 0.05$ ) compared to soybean based control diet. But compared to raw *Terminalia catappa* seed meal-based diet fed broilers, the values observed in case of fermented *Terminalia catappa* seed meal-based diet was found significantly higher ( $P > 0.05$ ), which showed that fermentation of feed can alleviate some negative effect of non-conventional feed stuffs.

In the current experiment we could observe that haematological parameters were within normal range and feeding fermented feed to broiler did not have any adverse effect on haematological parameters.

### Blood biochemical parameter

Table 3 presents Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on serum Glucose (mg/dl), Total protein (g/dl), Albumin (g/dl), Globulin (g/dl), SGPT (U/I) and SGOT (U/I) in broiler chickens. From the perusal of the table 3, it was observed that glucose concentration at day 28 was significantly ( $P < 0.05$ ) higher for AGP and average value of glucose at day 28 and 42 was also found significantly ( $P < 0.01$ ) higher for AGP & FLB group as compared to control. Concomitant with our findings, Yongna *et al.*, (2016) found significantly higher ( $P < 0.01$ ) glucose value at day 21 in fermented rapeseed meal fed group with *Bacillus subtilis*, *Candida utilis* and *Enterococcus faecalis* together fed groups in comparison to non-fermented rapeseed meal fed group. On contrary to our findings, Apata (2011) found out that serum glucose value was not significantly ( $P > 0.05$ ) altered in broilers fed fermented *Terminalia catappa* fruit meal with *Aspergillus niger* as replacement of maize up to 80%, compared to control group.

Total protein concentration of serum was found significantly higher ( $P < 0.05$ ) at day 28 in FBSLB group among different treatment

groups and on day 42 significantly higher ( $P < 0.01$ ) value for total protein was shown by FLB as compared to control group. Albumin concentration in serum was significantly higher ( $P < 0.01$ ) in FLB group at day 42 in comparison to control group. This finding is in accordance with findings of Yongna *et al.*, (2016) who observed that broilers fed fermented rapeseed meal with *Bacillus subtilis*, *Candida utilis* and *Enterococcus faecalis* together showed significantly higher ( $P < 0.05$ ) total protein value at day 21 and at day 42 ( $P < 0.01$ ) and albumin value at day 21 compared to non-fermented rapeseed meal fed group. Yongwei *et al.*, (2017) also found out

that broilers fed fermented cotton seed meal with *Bacillus subtilis* ST-141 and *Saccharomyces* N5 showed significantly higher ( $P < 0.05$ ) total protein and albumin on day 21 and 42 compared with bird fed unfermented cotton seed meal. However, Tang *et al.*, (2012) reported that on contrast to corn-soybean meal based control diet, experimental diets replacing soybean meal with 4 %, 8 % and 12% of fermented cottonseed meal with *Bacillus subtilis* BJ-1, didn't showed any significant difference ( $P > 0.05$ ) on serum total protein and albumin concentrations on day 21 and 42.

**Table.1** Ingredients composition of basal diet for Pre-starter, Starter and Finisher diet

Ingredients	Pre-starter	Starter	Finisher
Maize	59.14	61	64.8
Soyabean meal	33.6	30.71	26.2
Fish Meal	3.8	4.3	3.5
Rice bran oil	0	0.8	2.21
Dicalcium phosphate	1.24	0.90	0.90
Sodium Chloride	0.3	0.3	0.3
Limestone powder	1.12	1.25	1.21
Methionine	0.27	0.27	0.27
Lysine	0.22	0.11	0.12
Threonine	0.045	0.045	0.045
Cocciostat	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05
Trace mineral mixture	0.05	0.05	0.05
Vitamin premix	0.015	0.015	0.015
Choline chloride	0.05	0.05	0.05
Antioxidant	0.01	0.01	0.01
	100	100	100

**Table.2** Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on RBC ( $\times 10^6/\mu\text{l}$ ), WBC ( $\times 10^3/\mu\text{l}$ ), PCV (%) and hemoglobin (g/dl) changes in experimental birds

	Group- 1 (C) <sup>^</sup>	Group- 2 (AGP)	Group- 3 (FBS)	Group- 4 (FLB)	Group- 5 (FBSLB)	P value
<b>RBC</b>	2.33±0.12	2.35±0.20	1.92±0.13	2.23±0.11	2.36±0.05	0.13 <sup>NS</sup>
<b>WBC</b>	6.03±0.37	6.33±0.71	6.13±0.46	6.55±0.11	6.07±0.24	0.98 <sup>NS</sup>
<b>PCV</b>	35.97±1.86	36.30±3.12	33.53±1.41	34.64±0.91	35.60±0.56	0.82 <sup>NS</sup>
<b>Hb</b>	14.40±0.79	14.10±1.26	13.33±0.45	14.04±0.43	13.73±0.35	0.87 <sup>NS</sup>

NS=Non-significant; abc means with different superscript in the same row differ significantly; C=Control; AGP=Antibiotic growth promoter; FBS=Fermented feed with *Bacillus subtilis*; FLB=Fermented feed with *Lactobacillus spp.*; FBSLB=Fermented feed with both *Bacillus subtilis* and *Lactobacillus spp.*

**Table.3** Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on serum Glucose (mg/dl), Total protein (g/dl), Albumin (g/dl), Globulin (g/dl), SGPT (U/I) and SGOT (U/I) in broiler chickens

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (FBS)	Group- 4 (FLB)	Group- 5 (FBSLB)	
<b>GLUCOSE</b>						
<b>d 28</b>	228.00±7.54 <sup>b</sup>	323.2±22.89 <sup>a</sup>	229.40±5.83 <sup>b</sup>	278.2±19.8 <sup>ab</sup>	253.2±30.25 <sup>b</sup>	0.01 <sup>**</sup>
<b>d 42</b>	2.43±10.59	2.97±16.26	2.60±16.42	2.96±29.00	2.62±10.28	0.17 <sup>NS</sup>
<b>Average</b>	235.4±6.61 <sup>c</sup>	310.1±13.9 <sup>a</sup>	244.90±9.70 <sup>c</sup>	287.0±16.8 <sup>ab</sup>	257.8±15.1 <sup>bc</sup>	0.01 <sup>**</sup>
<b>TOTAL PROTEIN</b>						
<b>d 28</b>	3.18±0.14 <sup>ab</sup>	3.02±0.07 <sup>b</sup>	3.20±0.04 <sup>ab</sup>	3.00±0.07 <sup>b</sup>	3.34±0.05 <sup>a</sup>	0.046 <sup>*</sup>
<b>d 42</b>	3.04±0.07 <sup>c</sup>	3.46±0.02 <sup>b</sup>	3.52±0.08 <sup>b</sup>	3.96±0.16 <sup>a</sup>	3.52±0.15 <sup>b</sup>	<0.01 <sup>**</sup>
<b>Average</b>	3.11±0.08	3.24±0.08	3.36±0.07	3.48±0.18	3.43±0.08	0.11 <sup>NS</sup>
<b>ALBUMIN</b>						
<b>d 28</b>	1.10±0.05 <sup>a</sup>	1.00±0.03 <sup>bc</sup>	1.00±0 <sup>bc</sup>	0.98±0.02 <sup>c</sup>	1.08±0.02 <sup>ab</sup>	0.041 <sup>*</sup>
<b>d 42</b>	0.90±0.03 <sup>c</sup>	1.02±0.02 <sup>bc</sup>	1.00±0 <sup>bc</sup>	1.32±0.13 <sup>a</sup>	1.14±0.05 <sup>ab</sup>	0.002 <sup>**</sup>
<b>Average</b>	1.00±0.04	1.01±0.02	1.00±0	1.15±0.08	1.11±0.03	0.06 <sup>NS</sup>
<b>GLOBULIN</b>						
<b>d 28</b>	2.08±0.10	2.02±0.06	2.20±0.04	2.02±0.07	2.26± 0.06	0.07 <sup>NS</sup>
<b>d 42</b>	2.14±0.09 <sup>b</sup>	2.44±0.02 <sup>a</sup>	2.52±0.08 <sup>a</sup>	2.66±0.12 <sup>a</sup>	2.38±0.11 <sup>ab</sup>	0.01 <sup>**</sup>
<b>Average</b>	2.11±0.06	2.23±0.08	2.36±0.07	2.34±0.12	2.32±0.06	0.20 <sup>NS</sup>
<b>SGPT</b>						
<b>d 28</b>	6.00±00	4.80±0.20	5.60±0.24	5.60±0.40	4.80±0.58	0.08 <sup>NS</sup>
<b>d 42</b>	4.60±0.24	5.20±0.37	4.20±0.37	5.60±0.40	5.00±0.30	0.08 <sup>NS</sup>
<b>Average</b>	5.30±0.26	5.00±0.21	4.90±0.31	5.80±0.29	5.00±0.37	0.20 <sup>NS</sup>
<b>SGOT</b>						
<b>d 28</b>	167.6±10.7 <sup>b</sup>	203.0±8.11 <sup>a</sup>	162.4±15.1 <sup>b</sup>	144.2±7.94 <sup>b</sup>	170.8±8.8 <sup>b</sup>	0.013 <sup>*</sup>
<b>d 42</b>	171.8±6.36	196.2±14.7	187.4±15.9	206.6±29.6	156.6±11	0.31 <sup>NS</sup>
<b>Average</b>	169.7±5.92	199.6±8.03	174.9±11.2	175.4±17.8	163.7±7.1	0.20 <sup>NS</sup>

NS=Non-significant; abc means with different superscript in the same row differ significantly; C=Control; AGP=Antibiotic growth promoter; FBS=Fermented feed with *Bacillus subtilis*; FLB=Fermented feed with *Lactobacillus spp.*; FBSLB=Fermented feed with both *Bacillus subtilis* and *Lactobacillus spp.*; d=day; \* means (P<0.05), \*\* means (P<0.01)

**Table.4** Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on Triglyceride (mg/dl), Cholesterol (mg/dl), LDL (mg/dl) and HDL (mg/dl) in broiler chicken

Attributes	Treatment					P value
	Group-1 (C)	Group- 2 (AGP)	Group- 3 (FBS)	Group- 4 (FLB)	Group- 5 (FBSLB)	
<b>TRIGLYCERIDE</b>						
<b>d 28</b>	56.00±8.17	73.80±4.26	57.80±8.68	65.80±5.88	50.80±6.62	0.188 <sup>NS</sup>
<b>d 42</b>	37.2± 6.82	52.00± 10.73	34.00±4.04	57.80±11.12	62.00±1.38	0.071 <sup>NS</sup>
<b>Average</b>	46.60±5.92	62.90±6.54	45.90±6.01	61.80±6.08	56.40±3.69	0.11 <sup>NS</sup>
<b>TOTAL CHOLESTEROL</b>						
<b>d 28</b>	114.60±5.18	110.0±3.30	115.80±1.32	120.2±2.08	114.8±4.00	0.38 <sup>NS</sup>
<b>d 42</b>	109.20±4.52	110.20±6.22	113.60±3.26	115.20±4.20	118.00±3.26	0.624 <sup>NS</sup>
<b>Average</b>	111.90±3.36	110.1±3.32	114.70±1.7	117.7±2.36	116.4±2.49	0.27 <sup>NS</sup>
<b>HDL</b>						
<b>d 28</b>	83.00±3.67	90.80±1.74	96.00±2.88	94.80±3.01	96.00±5.18	0.074 <sup>NS</sup>
<b>d 42</b>	84.80±3.25	76.80±4.22	81.00±5.54	74.40±5.30	85.20±3.14	0.349 <sup>NS</sup>
<b>Average</b>	83.90±2.33	83.80±3.18	88.50±3.86	84.60±4.45	90.60±3.37	0.547 <sup>NS</sup>
<b>LDL</b>						
<b>d 28</b>	14.80±3.85	11.24±3.07	8.24±1.82	10.46±1.21	13.96±1.76	0.38 <sup>NS</sup>
<b>d 42</b>	20.20±2.44	22.40±2.45	22.32±2.11	17.00±3.00	25.44±0.95	0.16 <sup>NS</sup>
<b>Average</b>	17.50±2.33	16.82±2.63	15.28±2.69	13.73±1.87	19.70±2.13	0.45 <sup>NS</sup>

NS=Non-significant; abc means with different superscript in the same row differ significantly; C=Control; AGP=Antibiotic growth promoter; FBS=Fermented feed with *Bacillus subtilis*; FLB=Fermented feed with *Lactobacillus spp.*; FBSLB=Fermented feed with both *Bacillus subtilis* and *Lactobacillus*

**Table.5** Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on FRAP (10micro g TE/10 micro litre) value in blood serum

Day	Group- 1 (C)	Group- 2 (AGP)	Group- 3 (FBS)	Group- 4 (FLB)	Group- 5 (FBSLB)	P value
<b>28 d</b>	1.38±0.08 <sup>b</sup>	1.74±0.09 <sup>b</sup>	1.80±0.17 <sup>b</sup>	1.39±0.10 <sup>b</sup>	2.96±0.41 <sup>a</sup>	<0.01
<b>42 d</b>	1.31±0.07 <sup>b</sup>	1.63±0.09 <sup>b</sup>	1.70±0.17 <sup>b</sup>	1.35±0.07 <sup>b</sup>	2.74±0.43 <sup>a</sup>	0.001*
<b>Avg</b>	1.34±0.05 <sup>b</sup>	1.68±0.06 <sup>b</sup>	1.75±0.11 <sup>b</sup>	1.37±0.06 <sup>b</sup>	2.85±0.28 <sup>a</sup>	<0.01

NS=Non significant; abc means with different superscript in the same row differ significantly; C=Control; AGP=Antibiotic growth promoter; FBS=Fermented feed with *Bacillus subtilis*; FLB=Fermented feed with *Lactobacillus spp.*; FBSLB=Fermented feed

Globulin concentration was significantly higher (P<0.01) for FBS, FLB and AGP on day 42 compared to control. On contrary to this Apata (2011) found out that compared to control group significantly decreased (P <.05)

serum concentrations for globulin was observed when replacement of maize up to 80% with fermented *Terminalia catappa* fruit meal with *Aspergillus niger* was done on broilers.

Average values of both SGPT and SGOT were found non-significant ( $P>0.05$ ) among the treatment groups. In accordance to our findings, Rwei *et al.*, (2018) found out that *Bacillus subtilis* var. natto N21 + *Bacillus coagulans* L12 fermented feed showed non-significant ( $P>0.05$ ) effect with regards to SGOT level on fermented and control groups.

Stahly *et al.*, (1994) reported that body protein anabolism was relevant to the content of serum total protein and albumin, which showed a positive correlation and reflected on improvement in deposition of protein in the tissues. Significantly higher value for total protein in FLB and FBSLB in our study may be attributed to increased body protein anabolism and subsequent deposition of protein in the tissues of these two groups.

### **Serum lipid profile**

Table 4 presents Effect of fermented feed with avian specific *Lactobacillus spp.* and *Bacillus subtilis* on Triglyceride (mg/dl), Cholesterol (mg/dl), LDL (mg/dl) and HDL(mg/dl) in broiler chicken. It was observed that triglyceride concentration was not affected ( $P>0.05$ ) by the treatments imposed in this study. This finding is similar to the findings of Santoso *et al.*, (2001) who found out that fermented feed produced by using *Bacillus subtilis* did not influence the concentrations of triglycerides in broiler birds. But at the same time the present study did not agree with Yongna *et al.*, (2015) and Yongwei *et al.*, (2017). Yongna *et al.*, (2016) who observed that broilers fed fermented rapeseed meal with *Bacillus subtilis*, *Candida utilis* and *Enterococcus faecalis* together showed significantly lower ( $P<0.01$ ) triglyceride value on day 21 compared to non-fermented rapeseed meal fed group. Yongwei *et al.*, (2017) found out that broilers fed fermented cotton seed meal with *Bacillus subtilis* ST-141 and *Saccharomyces* N5 showed significantly lower ( $P<0.05$ )

triglyceride on day 42 compared with bird fed unfermented cotton seed meal. William and Fuller (1971) reported that certain bacteria can split conjugated bile acids i.e. taurocholic acid into taurine or choline, which may lead to impairment in fat absorption. Furthermore, Santoso *et al.*, (1995) observed that fermented product from *Bacillus subtilis* reduced hepatic acetyl- coA carboxylase enzyme activity leading to lower hepatic triglyceride synthesis. In the present study, however we couldn't find any changes in triglyceride concentration in serum.

It was observed that cholesterol concentration was similar ( $P>0.05$ ) among the treatment groups. Similar to this finding, Santoso *et al.*, (2001) found that fermented feed produced by using *Bacillus subtilis* did not influence the concentrations of cholesterol in broiler birds. Apata (2011) also reported that compared to control group serum cholesterol value was not significantly ( $P >.05$ ) altered when replacement of maize up to 80% with fermented *Terminalia catappa* fruit meal with *Aspergillus niger* was done in the diet of broilers. Contradictory to this findings Teng *et al.*, (2017) found out that fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* had a tendency to reduce serum cholesterol compared to the control group ( $p=.054$ ). Pereira and Gibson (2002) reported that cholesterol in gastro intestinal tract of animals could be taken into the cellular membrane of the *Lactobacillus*. Though HDL and LDL cholesterol concentrations in serum were not not affected by the treatments imposed in this study, HDL cholesterol was tended ( $p=.07$ ) to be increased in broilers fed fermented dried feed as compared to control group.

### **Serum anti-oxidant status**

FRAP value in blood serum was found significantly higher ( $P<0.01$ ) in FBSLB group at day 28 and at day 42 in comparison to

control group. This finding is in agreement with studies conducted by Yongwei *et al.*, (2017). Yongwei *et al.*, (2017) who found that broilers fed fermented cotton seed meal with *Bacillus subtilis* ST-141 and *Saccharomyces* N5 showed higher serum and liver anti-oxidative abilities compared with bird fed unfermented cotton seed meal (Table 5).

Fermented feed might alleviate the tissue lipid peroxidation, decrease oxidative damage and might enhance antioxidant capacity (Hu *et al.*, 2015). After fermentation and drying of feed since the feed is retaining the live microbes used for fermentation like *Bacillus subtilis* and *Lactobacillus* in this case the increased antioxidant effect might be due probiotic activity of the feed. Wang *et al.*, (2009) reported that *Lactobacillus acidophilus* ATCC 4356 inhibited linoleic acid peroxidation and scavenged free radicals. *Lactobacillus fermentum* I5007 also demonstrated the ability to scavenge free radicals in vitro. Lactic acid bacteria provides defense by inducing anti-inflammatory cytokines and reducing pro-inflammatory cytokines from intestinal epithelial cells Walsh *et al.*, (2008) and some lactic acid bacteria will enhance the gut inflammatory immune response also (Matsuguchi *et al.*, 2003).

It could be concluded that fermented dried feed showed better blood biochemical profiles and improved anti-oxidant status in broiler birds.

## References

Apata, D.F. 2011. Effect of *Terminalia catappa* fruit meal fermented by *Aspergillus niger* replacement of maize on growth performance, nutrient digestibility, and serum biochemical profile of broiler chickens. *Biotechnol. Res. Int.*, 2011: 1-6.

- Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal. Biochem.*, 239: 70-76.
- BIS (2007). Poultry Feeds Specification, 5<sup>th</sup> rev. edn. New Delhi, IS 1374.
- Chen, W., Zhu, X.Z., Wang, J.P., Wang, Z.X. and Huang, Y.Q. 2013. Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* fermented liquid feed on growth performance, relative organ weight, intestinal microflora, and organ antioxidant status in Landes geese. *J. Anim. Sci.*, 91: 978–985.
- Dubernet, S., Nathalie, D. and Micheline, G. 2002. A PCR-based method for identification of *lactobacilli* at the genus level. *FEMS Microbiol. Lett.*, 214: 271-275.
- Dworkin, M., Falkow, S., Schleifer, K.H., Rosenberg, E. and Stackebrandt, E. 2006. The genera *Lactobacillus* and *Carnobacterium*. *The prokaryotes*. Vol. 4, 3rd ed. New york, Springer Verlag. pp. 320–403.
- Griggs, J.P. and Jacob, J.P. 2005. Alternatives to antibiotics for organic poultry production. *J. Appl. Poult. Res.*, 14: 750–756.
- Hu, Y., Wang, Y., Li, A., Wang, Z., Zhang, X., Yun, T. and Yin, Y. 2015. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food Agric. Immunol.*, 27: 1–12.
- Levy, S.B. and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.*, 10: 122–29.
- Matsuguchi, T., Takagi, A., Matsuzaki, T., Nagaoka, M., Ishikawa, K., Yokokura, T. and Yoshikai, Y. 2003. Lipoteichoic acids from *Lactobacillus* strains elicit

- strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. *Clin.Diagn. Lab. Immunol*, 10: 259–266.
- Missotten, J.A., Michiels, J., Dierick, N., Owyn, A., Akbarian, A. and De Smet, S. 2013. Effect of fermented moist feed on performance, gut bacteria and gut histomorphology in broilers. *Br. Poult. Sci*, 54(5): 627-634.
- Niba, A.T., Beal, J.D., Kudi, A.C., Brooks, P.H. 2009. Potential of bacterial fermentation as a biosafe method of improving feeds for pigs and poultry. *Afr. J. Biotechnol*, 8: 1758-1767.
- Pereira, D.I and Gibson, G.R. 2002. Cholesterol assimilation by lactic acid bacteria and *Bifidobacteria* isolated from the human gut. *Appl. Environ Microbiol*. 68: 4689-4693.
- Ruei, H.Y., Chia, W.H. and Kuo, L.C. 2018. Screening lactic acid bacteria to manufacture two-stage fermented feed and pelleting to investigate the feeding effect on broilers. *Poult. Sci*, 97: 236–246.
- Santoso, U., Tanaka, K. and Ohtania, S. 1995. Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. *Br. J. Nutr*, 74: 523 – 529.
- Santoso, U., Tanaka, K., Ohaniand, S. and Saksida, M. 2001. Effect of fermented product from *Bacillus subtilis* on feed efficiency, lipid accumulation and ammonia production in broiler chicks. *Asian-australas. J. Anim. Sci*, 14: 333 – 337.
- Stahly, T. S., Williams, N. H. and Zimmerman, D. R. 1994. Impact of carbide on rate, efficiency and composition of growth in pigs with a low and high level of immune system activation. *J. Anim. Sci*, 72: 165–174.
- Sugiharto, S., Turrini, Y. and Isroli, I. 2016. Performances and haematological profile of broilers fed fermented dried cassava (*Manihotes culenta* Crantz). *Trop Anim Health Pro*, 48:1337–1341.
- Tang, J.W., Sun, H., Yao, X.H., Wu, Y.F., Wang, X. and Feng, J. 2012. Effects of replacement of soybean meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow-feathered broilers. *Asian-australas. J. Anim. Sci*, 25: 393-400.
- Teng, P.Y., Che, L.C., Chung, M.H., Shen, C.C. and Tzu, T.L. 2017. Effects of solid-state fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on growth performance and intestinal microbiota in broiler chickens. *Ital. J. Anim. Sci*, 16(4): 552–562.
- Walsh, M.C., Gardiner, G.E., Hart, O.M., Lawlor, P.G., Daly, M., Lynch, B., Richert, B.T., Radcliffe, S., Giblin, L. and Hill, C. 2008. Predominance of a bacteriocin-producing *Lactobacillus salivarius* component of a five-strain probiotic in the porcine ileum and effects on host immune phenotype. *FEMS Microbiol. Ecol*, 64: 317–327.
- Wang, A.N., Yi, X.W., Yu, H.F., Dong, B. and Qiao, S.Y. 2009. Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing-finishing pigs. *J. Appl. Microbiol*. 107: 1140–1148.
- Williams, D.J.J. and Fuller, R. 1971. The influence of the intestinal microflora on nutrition. In; physiology and biochemistry of the domestic fowl (Ed. D. J. Bell and B.M. Freeman). Vol. 3. Academic press, London, England, pp. 73-92.
- Xie, P.J., Huang, L.X., Zhang, C.H. and Zhang, Y.L. (2016). Nutrient assessment of olive leaf residues

processed by solid-state fermentation as an innovative feedstuff additive. *J. Appl. Microbiol.*, 121: 28-40.

Yongna, H., Yongwei, W., Aike, L., Zhisheng, W., Xiaolin, Z., Tingting, Y., Liangwei, Q. and Yunhao, Y. 2016. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food Agric. Immunol*, 27(2): 182–193.

Yongwei, W., Qingqing, D., Dan, S., Weiwei, W., Hang, Z., Li, W. and Aike, Li. 2017. Effects of fermented cottonseed meal on growth performance, serum biochemical parameters, immune functions, antioxidative abilities, and cecal microflora in broilers. *Food Agric. Immunol*, 28 (4): 725–738.

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