



IJBSM December 2024, 15(12): 01-09

Article AR5751

Stress Management DOI: HTTPS://DOI.ORG/10.23910/1.2024.5751

# Research Article

# Effect of *Bekang*, Naturally Fermented Soyabean Food, as Alternative to Antibiotic Growth Promoter on Feed Efficiency, Blood Biochemical and Gut Health Parameters of Broiler Birds

Rajat Buragohain<sup>1×10</sup> and Suraj Das<sup>2</sup>

Dept. of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram (796 015), India

<sup>2</sup>Dept. of Animal Nutrition College of Veterinary Sciences & Animal Husbandry, Selesih, Aizawl, Mizoram (796 015), India



**Corresponding** ≥ drrajat57@gmail.com



<u>🕑</u> 0000-0001-8140-6480

#### **ABSTRACT**

he present study was conducted during the month of October-November, 2021 to assess effect of Bekang, naturally L fermented soyabean food of Mizoram, on feed efficiency and gut health parameters of broilers as alternative to AGP. Total 240 day-old Vencobb-400 broiler chicks were assigned six dietary treatments dividing into six homogenous groups - standard rations (T<sub>1</sub>), standard rations with AGP (Bacitracin Methylene Disalicylate) @ 0.5 g kg<sup>-1</sup> (T<sub>2</sub>), standard ration with fermented feed by Lactobacillus acidophilus (106–108 CFU g<sup>-1</sup>) @ 100 g kg<sup>-1</sup> (T<sub>2</sub>), and standard rations with Bekang @ 50 g kg<sup>-1</sup> (T<sub>4</sub>), 75 g kg<sup>-1</sup> ( $T_s$ ) and 100 g kg<sup>-1</sup> ( $T_c$ ), respectively. Findings revealed significant effect of *Bekang* on body weight gain at  $42^{nd}$  day of age, feed conversion ratio and economic return score of broilers. No significant effect was observed on blood biochemical parameters. Histomorphological studies revealed significantly ( $\rho$ <0.05) increased villi length, width, and decreased crypt depth in all prats of small intestine for Bekang supplementation. Caecal bacterial counts revealed increasing trend of lactic acid bacteria and decreased Salmonella and E. coli counts in T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> compared to T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. An increasing trend of antibody titre against NDV on 35th day of age indicated improvement of immune status in Bekang supplemented birds. From the findings, it was concluded that Bekang could be a potential alternative to AGP and could be recommended at a level of 50 g to 100 g kg<sup>-1</sup> in rations of broiler birds.

KEYWORDS: Bekang, additive, AGP, feed efficiency, gut health, broilers

Citation (VANCOUVER): Buragohain and Das, Effect of Bekang, Naturally Fermented Soyabean Food, as Alternative to Antibiotic Growth Promoter on Feed Efficiency, Blood Biochemical and Gut Health Parameters of Broiler Birds. International Journal of Bio-resource and Stress Management, 2024; 15(12), 01-09. HTTPS://DOI.ORG/10.23910/1.2024.5751.

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

#### 1. INTRODUCTION

【 】 Ith 8−10% annual growth rate, poultry farming business is emerging as one of India's fastestgrowing business in agricultural sectors (Borah and Halim, 2014). Broiler production accounts nearly 90% of total output (USD 28.18 billion) from poultry sector in India (Soundararajan, 2023). Producing 8.8 million tons of meat per annum, India is 8th in world chicken meat production (Anonymous, 2022–23). With growing consumer demands and importance for high-quality protein for healthy life (Anonymous, 2009; Mottet and Tempio, 2017), need for increasing the production efficiency has become the paramount important for poultry sector (Zampiga et al., 2021). The wide gap between demand and supply of feed resources has compelled the poultry sector to search for feed resources and feeding strategies which can increase feed efficiency of poultry (Rinttila and Apajalahti, 2013).

Contributing nearly 2/3<sup>rd</sup> of total expenditure, feeding cost and profitability are negatively correlated and feed efficiency is the principal contributor to profitability of broiler production (Aljebory and Naji, 2021). Farmers supplement AGPs to improve feed efficiency in broilers as they improve gut health and prevent sub-clinical infections favoring nutrient digestibility and growth performance. AGPs favorably modify gut microbial population eliminating or inhibiting pathogenic organisms reducing morbidity and mortality to diseases (Salaheen et al., 2017; Paul et al., 2022). However, during the recent decades, it has been realized that AGPs have many negative effects including the development of antimicrobial resistance and health hazards to consumers for residues in the meat and meat products. Considering disadvantages, European Union had banned AGPs in the rations of livestock and poultry since 1st January 2006 and thereafter, researches were trying to find out suitable alternatives throughout the world (Yadav et al., 2016; Mehdi et al., 2018; Attia et al., 2023). Ban has negatively affected broiler production for increased disease incidence and mortality. Fermented feed is one of the promising alternatives to AGPs for its multifaceted beneficial effects. The lactic acid bacteria and organic acids (Wang et al., 2018 and Jazi et al., 2018) of fermented feed promote gut health by preventing the growth and multiplication of pathogenic bacteria and thereby enhancing nutrient utilization and growth performance (Shabani et al., 2019) of broiler birds. Soumeh et al. (2019), recorded improved performance in broilers for feeding fermented soyabean. Similar observations were also reported by Tang et al. (2012), Kim et al. (2016) and Premathilaka et al. (2020) in broilers. Nutrient digestibility enhanced for feeding fermented feed (Drazbo et al., 2018, Ruei et al., 2018) for improved gut health (Chiang et al., 2010, Zhang et al., 2016, Emami et al., 2020) without affecting blood biochemical parameters (Sugiharto et al., 2016, Chachaj et al., 2019) in broilers.

In NE states of India, indigenous people prepare grainbased fermented foods for day-to-day consumption and marketing. The fermented foods contain beneficial LAB and organic acids with inherent food components having potentiality to be exploited as alternative to AGPs. Bekang is one of such soyabean-based indigenous fermented food product prepared by ethnic communities of NE states. It is a naturally fermented soyabean food which is prepared from small-sized, dry seeds of soybean (Tamang, 2010). As reported Bekang contained 7 different species of Bacillus (8.4 log CFU g<sup>-1</sup>) and LAB (4.0 log CFU g<sup>-1</sup>) along with yeast (Tamang, 2015) for which Bekang may be considered as a good source of probiotics bacteria. In the present study, therefore, an attempt was made to utilize Bekang as probiotics source alternative to AGP i.e. Bacitracin Methylene Disalicylate (BMD) in the rations of broiler birds to study the effects on feed efficiency and gut health parameters.

#### 2. MATERIALS AND METHODS

# 2.1. Study location, duration and IAEC approval

The experiment was carried out at the Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram for a period of 42 days. The study was approved by Institutional Animal Ethics Committee (IAEC) registered under CPCSEA via approval reference number CVSC/CAU/IAEC/20-21/P-26.

### 2.2. Design of the experiment

Two hundred forty-one-day old Venncob 400 broiler chicks were distributed randomly into six groups following completely randomized block design ( $T_1$  to  $T_6$ ). They were assigned the following dietary treatments:

- $T_1$ : Standard rations formulated as per Anonymous (2007).  $T_2$ : Standard rations with antibiotics growth promoter (Bacitracin Methylene Disalicylate) @ 0.5 g kg<sup>-1</sup> ration.  $T_3$ : Standard rations with fermented feed by *Lactobacillus acidophilus* ( $10^6-10^8$  CFU g<sup>-1</sup>) @ 100 g kg<sup>-1</sup> ration.  $T_4$ : Standard rations with *Bekang* @ 50 g kg<sup>-1</sup> ration.  $T_5$ : Standard rations with *Bekang* @ 75 g kg<sup>-1</sup> ration.  $T_6$ : Standard rations with *Bekang* @ 100 g kg<sup>-1</sup> ration.
- 2.3. Ingredient and nutritional composition of standard rations, preparation of fermented feed and composition of Bekang

The ingredient and nutritional composition of the standard rations are presented in table 1.

The 'Bekang' was precured from Aizawl city market, Mizoram. The average dry matter, crude protein, ether

Table 1: Ingredient and nutritional composition (%) in standard rations (Anonymous, 2007).

Items	Pre-starter	Starter	Finisher
Ingredient %			
Yellow Maize	55	55	59
Rice polish	0	0	0
Soyabean meal	31	28	24
Ground nut cake (SE)	10	11	10
Vegetable oil	1	3	4
Dicalcium phosphate	1	1	1
Lime stone powder	1.1	1.1	1.15
Common salt	0.3	0.3	0.3
L- Lysine	0.25	0.25	0.2
DL- Methionine	0.2	0.2	0.2
Coccidiostat	0.04	0.04	0.04
Toxin binder	0.03	0.03	0.03
Trace mineral-vitamin mixture*	0.07	0.07	0.07
Anti-oxidant	0.01	0.01	0.01
Nutrient composition			
Crude protein (%)	23.10	22.17	20.31
ME(Kcal kg <sup>-1</sup> )*	3013	3128	3236
Lysine (%)*	1.43	1.17	1.02
Methionine (%)*	0.51	0.49	0.44
Calcium (%)	1.02	1.05	1.03
Phosphorous (%)	0.73	0.71	0.73

<sup>\*</sup> Vitamin A- 7,00,000 IU, Vitamin D3- 70,000 IU, Vitamin E- 250 mg, Zinc- 9600 mg, Magnesium- 6000 mg, Manganese- 1500 mg, Iron- 1500 mg, Copper- 1200 mg, Iodine- 325 mg, DL-Methionine- 1000 mg, Cobalt- 150 mg, Potassium- 100 mg, Sodium- 5.9 mg, Calcium- 25.5%, Phosphorus- 12.75%, Sulphur- 0.72% (per kg)

extract, crude fibre, total ash and nitrogen free extract (%) of *Bekang* were 28.50, 36.75, 27.04, 5.85, 4.37 and 25.99, respectively (on dry matter basis).

For preparation of fermented feed, *Lactobacillus acidophilus* freeze-dried culture (Hi-media, India) was procured and revived following standard procedure and was sub-cultured in De Man Rogosa Sharpe (MRS) broth. 100 ml of 24 hrs. old culture of *Lactobacillus acidophilus* was inoculated in 1 kg of moist standard ration (Ration and water, 1:1 w/w) and was incubated at 37°C for 24 hours. The fermented feed was dried under the sun till moisture (%) came down to about 12%. Presence of viable bacteria in the fermented dried feed was ascertained by counting the bacteria and

was found to be in the range 106 to 109 cfu g-1 of feed.

#### 2.4. Housing and feeding management

The chicks were housed on deep litter system. For the first 10 days, the chicks were housed in well-ventilated battery brooder and from 8<sup>th</sup> day onwards, they were housed in deep litter house partitioning into 24 pens providing floor space of 1.5×1.5 ft² per bird. Rice husk and saw dust were used as litter materials (1:1). Plastic wire nets were used for making the pens for different replicates. Feeds and water were offered *ad libitum* keeping daily feed intake record. Vaccination and deworming were done as per standard schedules. Twenty-four hours light was provided. Strict biosecurity was maintained throughout the feeding period.

# 2.5. Calculation of feed efficiency

Feed efficiency was calculated as feed conversion ratio (FCR) and economic return score (ERS). Feed efficiency was calculated at weekly interval by dividing the average total feed intake by the average body weight gain in a particular week.

The ERS was calculated by the following formula:

ERS=(Livability %×Average final weight (kg))/(Age of birds ×FCR×Cost of feed (₹ kg<sup>-1</sup>))×100

### 2.6. Studies on blood biochemical parameters

Blood was collected on 21<sup>th</sup> and 42<sup>nd</sup> day of trial from five birds of each treatment. Around 2 ml of blood was drawn from the wing vein in a vacutainer containing clot activator and the serum was separated by centrifugation at 3000rpm for 5 minutes and stored at -20°C until further analysis. The biochemical parameters namely, serum glucose, serum total protein, albumin, globulin, serum total cholesterol, HDH and LDL cholesterol, serum AST and ALT, total urea and serum calcium were estimated using commercial analytical kits of Coral Clinical Systems, India.

#### 2.7. Studies on gut health parameters

### 2.7.1. Histo-morphological studies of small intestine

Five birds from each treatment were slaughtered on 42<sup>nd</sup> day of age. About 2 to 3 cm of duodenum, jejunum and ileum were removed and washed with 0.9% saline to remove the contents. Gut segments were fixed in 10% neutral-buffered formalin. The samples were dehydrated, cleared, and paraffin embedded and stained with Delafield's Hematoxylin and Eosin, the tissue slides were stained, and mounted on distend plasticiser xylene based on protocol described by Incharoen et al. (2009). All measurements were taken in Advanced Olympus trinocular research microscope to measure the villus height, villus width and crypt depth.

#### 2.7.2. Caecal bacterial counts

For caecal bacterial counts, caecal samples were enumerated for lactic acid bacteria (LAB), Escherichia coli and Salmonella by pour plate method. Caecal contents were collected aseptically on 42<sup>nd</sup> day of age from 5 birds of each treatment at the time of slaughtering the birds for histo-morphological studies. The caecal contents were kept at 4°C on the day of collection until processing for bacteriological count. About 10 g fresh caecal content was mixed with sterile normal saline (1:10 w/v), vortexed for 3-4 minutes and the supernatant was used for microbial counting. The filtrate was serially diluted to 10<sup>10</sup> with normal saline. For LAB, E. coli, and Salmonella, MRS (Hi-Media), EMB (Hi-Media) and SS agar (Hi-Media) were used, respectively. The relevant agar plates were incubated at 37°C for 24h and the colonies were counted as colony forming unit (cfu  $g^{-1}$  caecal content) and expressed as  $\log_{10}$ cfu g<sup>-1</sup> of caecal content.

### 2.7.3. Studies on immunity

Immunity of the birds was assessed by haemagglutination inhibition (HI) test at 35<sup>th</sup> day of age against new castle disease vaccine. Blood samples were collected from 5 birds of each treatment at 35<sup>th</sup> day of age and haemagglutination inhibition test (HI) was performed following OIE protocol (Anonymous, 2012).

#### 2.8. Statistical analysis

The data were analysed by one-way ANOVA as per the procedure described by Snedecor and Cochran (1994). Probability values  $p \le 0.05$  were declared as significant and the values of 0.05 were declared as trend. When treatment effect was found to be significant, the differences among the treatment means were identified using Duncan's test.

# 3. RESULTS AND DISCUSSION

#### 3.1. Growth and feed efficiency of broiler birds

There was significant (p<0.01) effect of Bekang on

growth performance of broilers indicated by significantly improved average daily gain in body weight and weight at  $42^{\text{nd}}$  day of age, and also significantly better (p<0.01) feed conversion ratio (FCR) and economic return score (ERS) at 42<sup>nd</sup> day of age (Table 2). The body weight at 42<sup>nd</sup> day of age, FCR and ERS were significantly better (p<0.01) in all Bekang supplemented groups than AGP supplemented group. FCR was non-significant between fermented feed and Bekang supplemented groups, but body weight at 42<sup>nd</sup> day of age and FRS was significantly higher (p<0.01) in the later. The significantly favourable effect of Bekang on growth and feed efficiency might be for probiotics and anti-oxidative properties as Bekang contained numerous Bacillus (8.4 log CFU g<sup>-1</sup>), Lactic acid bacteria (4.0 log CFU g<sup>-1</sup>), and yeast, and had DPPH scavenging (477.2 g ml<sup>-1</sup>) and ABTS radical scavenging activity (158.9 g ml<sup>-1</sup>) (Tamang et al., 2015).

### 3.2. Effect of Bekang on blood biochemical parameters

Analysis of blood serum on 21<sup>st</sup> and 42<sup>nd</sup> days of age for biochemical parameters, namely the serum glucose, total protein, albumin, globulin, serum total cholesterol, LDL cholesterol, HDL cholesterol, serum urea, calcium, AST and ALT revealed no significant differences (*p*>0.05) between the groups indicating that supplementation of *Bekang* did not have any adverse effects on health of the broilers (Table 3). Similar non-significant effects of fermented feeds were also reported by Apata et al. (2011), Teng et al. (2017), Ruei et al. (2018) and Drazbo et al. (2018) in broilers. Serum LDL cholesterol level showed decreasing trend in the *Bekang* supplemented groups compared to control which might be an indication of positive effect of supplementing *Bekang* in broilers.

# 3.3. Effect of Bekang on gut histomorphology of broiler birds

The small intestine of birds plays significant role in nutrient digestion and assimilation. Intestinal development can be evaluated through measurement of the crypt depth (CD), villus length (VL) and width (VW), and surface area.

Table 2: Performance parameters of broilers supplemented with Bekang							
Parameters	$T_{_1}$	$T_2$	$T_3$	$\mathrm{T_{_4}}$	$T_{5}$	$T_{_6}$	SEm/p-value
BW at day <sup>-1</sup> (g)	39.62± 0.03	39.60± 0.04	39.28± 0.12	39.61± 0.33	39.62± 0.20	39.63± 0.22	$0.06/0.71^{NS}$
BW at 42 <sup>nd</sup> day (g)	1798.10 <sup>a</sup> ± 2.27	1898.70 <sup>b</sup> ± 3.29	2189.10 <sup>c</sup> ± 4.10	2195.00°± 16.70	2234.20 <sup>d</sup> ± 3.79	2243.40 <sup>d</sup> ± 7.16	36.91/<0.01**
FCR	1.93°± 0.01	1.92± 0.01	1.73± 0.01	1.71b± 0.01	$1.69^{b} \pm 0.01$	1.70 <sup>b</sup> ± 0.01	0.04/<0.01**
ERS	4.84 <sup>a</sup> ± 0.01	5.03± 0.01	$7.11^{cd} \pm 0.02$	$7.09^{\circ} \pm 0.05$	$7.19^{de} \pm 0.01$	7.21°± 0.02	0.22/<0.01**

BW: Body weight, FCR: Feed conversion ratio, ERS: Economic return score, Means bearing different superscripts (a, b, c) in a row differs significantly [NS: Non-significant, \*means (p<0.05), \*\* means (p<0.01)]

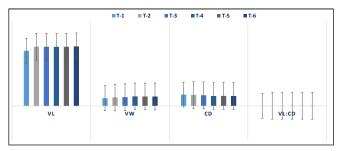
	3: Effect of <i>Bekan</i> $T_1$	<u> </u>			т	Т	1/CE
Day		$T_2$	$T_3$	$T_4$	$T_5$	$T_6$	p-value/SEm
21	glucose (mg dl <sup>-1</sup> ) 225.66±5.70	-	225 72 . 7 27	236.73±13.07	216.75±3.24	220 20 2 62	2 62/0 E4NS
		225.45±7.64	225.73±7.37			239.30±3.62	3.62/0.54 <sup>NS</sup>
42	222.15±1.67	225.84±6.32	220.87±2.53	237.08±15.15	231.60±13.49	231.87±16.75	4.06/0.89 <sup>NS</sup>
21	total protein(mg 3.49±0.02	3.74±0.15	3.65±0.26	3.66±0.08	3.77±0.33	3.20±0.13	0.08/0.39 <sup>NS</sup>
42	3.49±0.02 3.81±0.33	3.74±0.13 3.75±0.19	3.65±0.26 3.51±0.09	3.70±0.60	3.77±0.33 3.59±0.26	3.20±0.13 3.56±0.20	0.08/0.39
	3.01±0.33 albumin (mg dl-1		3.31±0.09	3.70±0.00	3.39±0.20	3.30±0.20	0.76/0.36
21	1.12±0.14	1.12±.0.04	1.21±0.14	1.30±0.14	1.18±0.11	1.10±0.04	0.04/0.83 <sup>NS</sup>
42	1.12±0.14 1.45±0.27	1.12±.0.04 1.16±0.12	1.21±0.14 1.19±0.06	1.30±0.14 1.22±0.07	1.15±0.11 1.35±0.75	1.10±0.04 1.30±0.17	$0.04/0.83$ $0.06/0.78^{NS}$
	1.43±0.27 globulin (mg dl-1		1.19±0.00	1.22±0.07	1.33±0.73	1.30±0.17	0.06/0.78
21	2.37±0.12	2.61±0.18	2.44±0.12	2.36±0.16	2.59±0.29	2.31±0.12	$0.06/0.76^{NS}$
42	2.45±0.15	2.39±0.24	2.31±0.05	2.48±0.20	$2.39\pm0.29$ $2.28\pm0.14$	2.31±0.12 2.27±0.70	$0.63/0.16^{NS}$
	total cholesterol		2.31±0.03	2.40±0.20	2.20±0.14	2.27 ±0.70	0.03/0.10
21	110.10±1.74	109.78±2.59	109.10±1.18	108.88±3.71	117.74±3.31	110.00±3.78	1.24/0.30 <sup>NS</sup>
42	108.17±4.23	107.80±2.65	109.52±3.81	113.80±3.85	114.90±4.29	114.25±4.16	1.51/0.62 <sup>NS</sup>
	LDL cholesterol		107.32_0.01	110.0020.03	11 1.702 1.27	11 1.23 = 1.10	1.51/ 0.02
21	16.37±1.80	13.97±2.01	12.80±2.38	13.51±1.99	14.72±3.87	14.34±0.33	1.51/0.91 <sup>NS</sup>
42	18.60±2.33	18.79±1.17	17.05±1.81	18.44±2.30	12.97±6.39	17.00±2.98	1.23/0.81 <sup>NS</sup>
	HDL cholestero						
21	83.72±0.3	84.68±2.96	88.58±3.88	88.98±2.94	89.68±2.94	88.16±3.15	$1.09/0.56^{ m NS}$
42	83.73±0.39	82.18±1.60	83.71±1.45	90.59±1.91	89.23±3.33	90.69±2.93	1.13/0.64 <sup>NS</sup>
Urea (	mg dl <sup>-1</sup> )						
21	6.91±0.05	7.13±0.21	7.18±0.26	6.93±0.04	7.08±0.95	6.97±0.15	$0.05/0.63^{NS}$
42	7.10±0.10	7.01±0.18	7.05±0.03	7.19±0.19	6.95±0.18	7.09a±0.06	$0.52/0.88^{\rm NS}$
Serum	calcium (mg dl <sup>-1</sup> )	)					
21	8.87±0.86	8.31±0.61	8.72±1.16	9.32±0.71	9.34±0.87	9.51±0.95	$0.31/0.92^{\rm NS}$
42	9.55±1.10	10.89±0.89	10.34±1.31	10.88±0.72	10.29±1.31	10.27±1.31	$0.40/0.96^{\rm NS}$
AST (	I U <sup>-1</sup> )						
21	159.91±1.52	158.22±0.62	154.1 0±5.13	157.79±0.97	148.60±1.13	148.77±4.25	$1.46/0.58^{NS}$
42	161.97±1.03	158.28±1.79	156.66±3.81	153.93±5.66	151.55±3.66	153.43±4.33	$1.52/0.54^{\rm NS}$
ALT (	I U <sup>-1</sup> )						
21	5.01±0.14	5.07±0.13	5.67±0.35	5.25±0.25	4.89±0.50	4.73±0.31	$0.12/0.52^{\rm NS}$
42	4.45±0.29	$3.75 \pm 0.31$	3.75±0.31	3.99±0.22	4.00±0.19	4.05±0.23	$0.10/0.51^{\rm NS}$

Means bearing different superscripts (a, b, c) in a row differs significantly [NS: Non-significant, \*means (p<0.05), \*\* means (p<0.01)]

Significantly (p<0.01) higher villi length of duodenum was recorded in  $T_6$  followed by  $T_2$ ,  $T_3$ ,  $T_5$ ,  $T_4$  and  $T_1$  (Figure 1). There was no significant difference (p<0.05) in villi width between  $T_4$ ,  $T_5$  and  $T_6$ , but was significantly higher than  $T_1$ ,  $T_2$  and  $T_3$ . No significant difference (p>0.05) was

recorded between treatments for crypt depth and villi length: crypt depth ratio. But, compared to  $T_1$ , crypt depth was less in other treatments. Crypt dept was also less in  $T_4$ ,  $T_5$ , and  $T_6$  than  $T_1$ ,  $T_2$  and  $T_3$ . In the jejunum and ileum also, villi length was significantly ( $\rho$ <0.01) more in

Bekang supplemented groups ( $T_4$ ,  $T_5$  and  $T_6$ ) than  $T_1$  and  $T_2$  (Figure 2 and 3). The villi width was comparatively more in Bekang supplemented groups. There was no significant difference (p>0.05) in crypt depth among the groups, but was observed to be comparatively less in Bekang supplemented groups.



Figuew 1: Histomorphology of dedunum (μm)

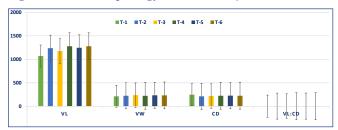


Figure 2: Histomorphology of jejunum (μm)

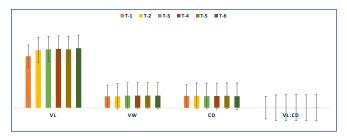


Figure 3: Histomorphology of ileum (μm)

Better villi length contributes to more absorptive ability of small intestine (Sayan et al., 2018). Positive effect of *Bekang* might be for LAB which could produce short chain fatty acids to stimulate epithelial cells and enterocytes and increased the villi length. Xu et al. (2012), reported that soybean meal fermented with *Bacillus licheniformis* enhanced (*p*<0.05) villus length and villus length to crypt depth ratio in the duodenum and jejunum of small

intestine. Similar findings were also reported by Saad et al. (2016) in birds fed fermented feed and Teng et al. (2017) in broilers fed fermented wheat bran with *Bacillus amyloliquefaciens*. The villus height to crypt depth ratio is an effective measure of small intestine's absorption capacity. When villus height to crypt depth ratio increases maximal digestion and absorption occurs. Increased villi height and villus height to crypt depth ratio in the present study might be associated with increased beneficial LAB changing intestinal morphology in favourable way.

### 3.4. Immune status of the broilers

The immune status evaluated by assessing the antibody titre against Newcastle Disease Virus (HI titre Log<sub>10</sub>) of  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$  and  $T_6$  were estimated as 0.85±0.02,  $0.87 \pm 0.08$ ,  $0.90 \pm 0.02$ ,  $0.91 \pm 0.02$ ,  $0.92 \pm 0.02$  and  $0.93 \pm 0.02$ , respectively. There was no significant difference (p>0.05) among treatments, but an increasing trend was observed in the *Bekang* supplemented groups. Feeding of fermented meal was known to boost the antibody concentration and T-cells of birds (Xi-Jie et al., 2007). LAB, particularly Lactobacilli, stimulates production of Th2 cytokines including IL-4 and IL-10 which increase B-cell growth and Ig isotype switching, both of which are necessary for antibody production. Beside antibody-mediate immune response in chickens, giving fermented diets had been shown to boost cell-mediated immune responses (Gao et al., 2009 and Xi-Jie et al., 2007) which is due to increased LAB concentration in fermented feeds. Lactobacillus acidophilus had a direct impact on Th1-cell immunity by modifying the numbers and activity of antigen-specific T-lymphocytes (Parvinder and Aruna, 2012) and/or could have an indirect influence on T-lymphocyte activity by stimulating other cell types, such as phagocytes.

# 3.5. Caecal microbial count

No significant difference (p>0.05) was observed in caecal Lactic acid bacteria (LAB), Escherichia coli and Salmonella count at  $42^{nd}$  day of age (Table 4). LAB count was comparatively higher in Bekang supplemented groups with the highest value recorded in  $T_5$  (7.12±0.28  $\log_{10}$  CFU  $g^{-1}$ ). In  $T_5$  and  $T_6$ , Salmonella counts were lesser than other groups. Lowest E. coli count was recorded  $T_3$  followed by

Table 4: Caecal microbial counts (log <sub>10</sub> CFU g <sup>-1</sup> ) of broilers at 42 <sup>nd</sup> days of age								
Bacteria	$T_{_1}$	$T_2$	$T_3$	$T_{_4}$	$\mathrm{T}_{\scriptscriptstyle{5}}$	$T_6$	SEm/p-value	
LAB	6.74±0.16	6.66±0.20	6.91±0.18	6.82±0.32	7.12±0.28	6.8±0.29	$0.92/0.83^{NS}$	
Salmonella	6.62±0.31	6.63±0.01	6.64±0.22	6.66±0.34	6.54±0.23	6.59±0.20	$0.85/0.99^{NS}$	
E. coli	6.91±0.28	6.64±0.54	6.44±0.32	6.58±0.34	6.94±0.24	6.63±0.25	$0.10/0.75^{NS}$	

Means bearing different superscripts (a, b, c) in a row differs significantly [NS: Non-significant, \*means (p<0.05), \*\* means (p<0.01)]

 ${\rm T_4}$  and  ${\rm T_6}$ , but were comparable with other groups without any significant difference. Higher concentrations of lactic acid and low pH in fermented and *Bekang* supplemented feeds might have suppressed the development of *Salmonella* and *Escherichia coli* (Murry et al., 2004 and Niba et al., 2009).

### 4. CONCLUSION

Behang could be exploited as alternatives to antibiotics growth promoters in the rations of broiler birds and could be recommended at a level of 50 g to 100 g kg<sup>-1</sup> of ration.

#### 5. ACKNOWLEDGEMENT

The authors are thankful to Vice-Chancellor, Central Agricultural University (Imphal) for financial support and facilities to carry out the study successfully.

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