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Effects of dietary supplementation of *Bekang* as probiotics source on growth, nutrient utilization, carcass traits and production efficiency of broilers

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ABSTRACT

The *Bekang* is a rich source of probiotics and may be a suitable alternative to fermented feed and AGPs for broilers. One-day old 240 broiler chicks were assigned to 6 dietary treatments: T_1 (basal ration), T_2 (basal ration + Bacitracin methylene disalicylate @ 0.5 g/kg basal ration), T_3 (basal ration + fermented feed by *Lactobacillus acidophilus* @ 100 g/kg), T_4 (basal ration + *Bekang* @ 50 g/kg), T_5 (basal ration + *Bekang* @ 75 g/kg) and T_6 (basal ration + *Bekang* @ 100 g/kg). The broiler chicks were housed under deep litter system with vaccination and deworming as per schedule. Feeding and watering were done *ad lib*. A metabolic trial and study on carcass traits were conducted at 6th week and FCR and ERS were calculated. Feed intakes were significantly higher in T_3, T_4, T_5 and T_6 than T_1 and T_2; whereas body weight gain and final body weight were significantly high in T_5 and T_6. No significant effects on nutrient utilization and carcass traits were recorded between groups. Significantly better FCR was recorded in T_3, T_4, T_5 and $T_6$and highest ERS was recorded in T_6. The study revealed that *Bekang* could be a substitute of fermented feed and AGP and dietary supplementation as low as 50 g/kg ration may be recommended to broilers.

Keywords: *Bekang*, Broiler, Carcass traits, Feed efficiency, Growth, Nutrient digestibility

Negative impacts of antibiotics growth promoters (AGPs) and their ban by the European Union as feed additives since 1st of January 2006 (Gregori *et al*. 2008) have accelerated the pace of search for cost-effective alternatives around the globe. In this regard, fermented foods have been emerging as promising alternatives of AGPs for their manifold positive effects. In Mizoram, India locally harvested soybean seeds are fermented by the ethnic communities to prepare a delicious food item known as *Bekang,* which is very popular throughout the state. Traditionally, it is prepared from dry small-sized soybean immersing in water for 10-12 hours and then boiling for 2-3 hours until soybean seeds become soft and then allowing to ferment for 3-4 days wrapped with fresh *Calliparpa aroria* leaves or leaves of *Phrynium* species before consumption as *Bekang* (Tamang *et al*. 2012).

Bekang is reported to have 7 different species of *Bacillus* (8.4 log CFU/g) and many lactic acid bacteria (4.0 log CFU/g), namely *Bacillus subtilis, B. licheniformis, B. pumilus, B. sphaericus, B. coagulans, B. brevis, Enterococcus faecium, B. circulans, E. durans, E. hirae, E.cecorum, E. raffinossus* along with yeast namely, *Saccharomyces cerevisiae, Debaryomyces hansenii and Pichia burtonii,* which has established *Bekang* as a good source of probiotics bacteria and yeast. *Bekang* was also reported to have DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity of 477.2 g/ml and ABTS [2,2-azino-bis (3-ethylbenzothiazoline-6sulfonic acid) radical scavenging activity of 158.9 g/ml (Tamang 2015). Considering the above properties, *Bekang* may be a potential candidate for utilization as the alternative to fermented feed with lactic acid bacteria (LAB) and the AGPs. Therefore, in our experiment, *Bekang* was utilised as 'probiotics source' at different levels to assess its effects against basal feed fermented with *Lactobacillus acidophilus* and AGP, i.e. bacitracin methylene disalicylate (BMD) on growth performance, nutrient utilization, carcass traits and feed utilization efficiency of broilers.

MATERIALS AND METHODS

The study was carried out in the year 2021 at Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram.

Experimental birds and design of experiment: Two hundred and forty one-day old broiler chicks (Cobb 400) were equally divided into 6 homogenous groups of 40 chicks in each group. All experimental groups were further divided into 4 equal replicates in a completely randomised block design.

The dietary treatments were: T-1: Birds were fed basal ration (control group); T-2: Birds were fed basal ration + Bacitracin Methylene Disalicylate @ 0.5 g/kg basal ration;

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T-3: Birds were fed basal ration + fermented feed with *Lactobacillus acidophilus* (10^6–10^8 CFU/g) @ 100 g/kg; T-4: Birds were fed basal ration + *Bekang* @ 50 g/kg; T-5: Birds were fed basal ration + Bekang @ 75 g/kg; T-6: Birds were fed basal ration + Bekang @ 100 g/kg.

For the initial 7 days, birds were housed in well-ventilated battery brooder and then in deep litter house partitioning into 24 pens providing floor space of 1.5 ft. × 1.5 ft per bird. Rice husk and saw dust (1:1, w/w) were used as litter materials. Plastic wire nets were used for making the pens for different replicates. Continuous light was provided. Deworming and vaccination were done as per standard schedules.

Approval of Institutional Animal Ethics Committee (IAEC): The study was approved by Institutional Animal Ethics Committee (registered under CPCSEA) of College of Veterinary Sciences & Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram (approval reference number CVSC/CAU/IAEC/20-21/P-26).

Preparation of experimental rations: The basal experimental rations were formulated following BIS (2007) standards. The *Bekang* was procured from the local markets of Aizawl city, Mizoram. Two types of *Bekang* are popular among the people of Mizoram, i.e. the dry and wet *bekang* and the wet *Bekang* was used in the present study.

For preparation of fermented feed with *Lactobacillus acidophilus*, freeze-dried culture (ATCC) was procured (Hi-media, India) and revived following standard procedure and then sub-cultured in MRS (De Man, Rogosa and Sharpe) broth. 100 ml of 24 h. old culture of *Lactobacillus acidophilus* in MRS broth was inoculated in 1 kg of moist basal ration (1:1, w/w feed and water) and incubated at 37°C for 24 h. The fermented feed was dried under the sun till moisture came down to nearly 12% before feeding to the birds.

Table 1. Feed ingredient composition (%) in the basal experimental rations

Ingredient	Broiler Pre-starter	Broiler Starter	Broiler Finisher
Yellow Maize	55	55	59
Soybean Meal	31	28	24
Ground nut cake (SE)	10	11	10
Vegetable Oil	1	3	4
Di-calcium 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 mixture*	0.05	0.05	0.05
Vitamin premix**	0.02	0.02	0.02
Anti-oxidant	0.01	0.01	0.01
Total	100	100	100

*Manganese sulphate – 120 g, Zinc sulphate – 120 g, Ferrous sulphate – 20 g, Copper sulphate – 4 g, Cobalt chloride – 200 mg, Potassium iodide – 200 mg, Sodium selenite – 40 mg (1 kg). **Vit.A- 125 lac IU, Vit.D3- 25 lac IU, Vit.B2- 5 g, Vit.E- 9000 IU, Vitamin K3- 1.2 g, Vit.B1- 900 mg, Vit.B6- 1.8 g, Vit.B12- 2500 mcg, Niacin-1.4 g, Folic acid–100 mg, Biotin–10000 mcg (500 g).

Metabolic trial: A metabolic trial was conducted during the 6th week of age. From each group, 5 birds were selected for the metabolic trial. After one day adaptation, the actual collection period was started in the metabolic cages. Total feed provided and residue left were recorded daily during the metabolic trial. Wholesome drinking water was provided *ad lib.* to the broiler birds. Faeces voided within 24 h was collected and weighed for each bird under metabolic trial. About 1/20th part of the faeces was mixed thoroughly and was kept in petri dish and then kept in hot air oven at 100°C for overnight for determination of dry matter. Another sample of about 1/30th part of faeces was weighed and was preserved in 1:4 H_2SO_4 for estimation of nitrogen content. After 3 days, the pooled samples of faeces were mixed thoroughly and a suitable amount of undried preserved sample was weighed in duplicate for estimation of nitrogen. The dried sample after grinding was utilised for analysis of other proximate principles. The proximate principles were analysed as per the methods of AOAC (2012).

Statistical analysis: The data were analyzed with one-way ANOVA using SPSS in a completely randomized design as per the procedure described by Snedecor and Cochran (1994). Probability values ≤0.05 were declared as significant and the values of 0.05 - 0.10 were considered as trend. For significant treatment effect, the differences between the treatment means were identified using Duncan's test.

RESULTS AND DISCUSSION

Nutritional composition of basal rations and Bekang: The nutrient compositions of the basal rations are presented in Table 2. The dry matter (%) of *Bekang* was 28.50% and the crude protein, crude fibre, ether extract, total ash and nitrogen free extract percentage (on DM basis) were 36.75, 27.04, 5.85, 4.37 and 25.99%, respectively. Tamang *et al.* (2012) also reported moisture percentage of *Bekang* of Mizoram as 63.50% and the DPPH scavenging and ABTS radical scavenging activity as 477.2 g/ml and 158.9 g/ml, respectively. Slightly higher moisture percentage in *Bekang* utilized in our present study might be related to differences in sources from which it was purchased and also might be related to variability of water content that remained after boiling the seeds of soybean before keeping for fermentation. Similar results were also observed by Jazi *et al*. (2018). Yuan *et al*. (2013) reported the crude protein, dry matter, and total ash contents of fermented soybean as 50.86, 92.63, and 6.73%, respectively.

Feed intake: The average feed intake of birds between the groups was not significant (P>0.05) in the 1st week. However, during the 2nd to 6th weeks, feed intakes in T-2, T-3

Table 2. Nutritional composition (on DM basis) of basal experimental rations

Attribute	Broiler Pre-starter	Broiler Starter	Broiler Finisher
Dry matter (%)	85.74	83.99	83.07
Crude protein (%)	23.10	22.17	20.31
Crude fibre (%)	4.56	4.49	4.18
Ether extract (%)	2.44	3.32	2.54
Total ash (%)	5.53	5.62	5.60
Nitrogen free extract (%)	64.37	64.41	67.37
Calcium (%)	1.02	1.05	1.03
Phosphorous (%)	0.73	0.71	0.73
Lysine (%)*	1.43	1.17	1.02
Methionine (%)*	0.51	0.49	0.44
ME(Kcal/kg)*	3013	3128	3236

*Calculated value, DM, Dry matter; CP, crude protein; CF, Crude fibre; EE, Ether extract; TA, Total Ash; NFE, Nitrogen Free Extract; Ca, Calcium; P, Phosphorous; ME, Metabolizable energy.

and *Bekang* supplemented groups were significantly higher than T-1. During 8-21 days of rearing, feed consumption increased significantly (P<0.01) in *Bekang* supplemented groups compared to T-1, T-2 and T-3 and similar findings were also recorded during 22-42 days. The findings might be the indications of positive effects of *Bekang* in feed intakes and might be for incorporation of fermented *Bekang* and its positive impacts on palatability, as also reported by Lokman *et al*. (2015) in local Malaysian chicken and Feng *et al*. (2007) in broilers.

Growth performance: Positive effects on body weight gain for supplementation of *Bekang* was observed from 2nd week onwards (Table 3). Although growth rate was significantly higher for AGP supplementation than the control broilers, but was significantly (P<0.01) lesser than *Bekang* supplemented groups (50-100 g/kg feed). Among the *Bekang* supplemented groups, rate of gain was directly proportional to the rate of inclusion of *Bekang* in the feed. Highest body weight gain was recorded in T-6 (100 g/kg *Bekang*) at 42nd day of age, but statistically was not significant between T-5 and T-6. The findings might be the indications that *Bekang* might have positive effects on utilization of feed for probiotics effects as fermentation is considered as a dynamic process of converting complex substrates into simpler molecules by incorporating microbes, substrates, and ambient variables (Niba *et al*. 2009). Fermentation also results higher concentration of organic acids and low *p*H for the lactic acid bacteria (LAB) (Canibe and Jensen 2012) which can prevent feed from pathogens before to feeding to the birds (Niba *et al*. 2009) and thereby improving gut health (Sun *et al.* 2013) and hence growth rate of broilers (Xie *et al.* 2016). Generation of secondary microbial metabolites during fermentation might also be attributable to improved body weight gain of broiler chicks fed *Bekang*. Organic acids and enzymes generated by LAB, and antibacterial compounds such as iturin and surfactin produced by *Bacillus subtilis* also have positive impacts on gut health (Feng *et al*. 2007) for decreased gastric *p*H and pathogenic microbes. Increased production of short chain fatty acids might also be responsible for better feed digestibility and improved growth performance (Mathivanan *et al*. 2006) of broilers. Similar findings were also reported by Olnood *et al*. (2015), Peng *et al*. (2016) and Yun *et al*. (2017).

Feed conversion efficiency: Feed conversion efficiency (FCR) was significantly higher in *Bekang* supplemented groups than the other groups. During the 22-42 days and overall FCR at 42nd day of age were significantly higher (P<0.01) in T-3, T-4, T-5 and T-6 than in T-1 and T-2 (Table 3). Improved FCR might be related to better digestibility (Zhang *et al*. 2016) and favourable effects of multi-species probiotics bacteria of *Bekang* resulting in improved feed intake and gut health favouring growth performance of the broilers.

Nutrient utilization: The apparent digestibility of

Table 3. Effect of dietary supplementation of *Bekang* on productive performance of broiler birds

Age (Day)	T-1	T-2	T-3	T-4	T-5	T-6	SEM	p-value
Average feed intake (g/birds)								
1-7	124.64	123.61	123.51	124.14	123.96	125.43	0.32	0.57^{NS}
8-21	662.31^{a}	741.56^{b}	821.78^{c}	798.52^{c}	802.41^{c}	801.25^{c}	11.98	$<0.01^{**}$
22-42	2613.20^{a}	2707.10^{b}	2779.30^{c}	2772.90^{c}	2802.30^{cd}	2829.10^{d}	15.97	$<0.01^{**}$
1-42	3404.30^{a}	3576.40^{b}	3728.10^{cd}	3699.20^{c}	3732.30^{cd}	3759.40^{d}	26.72	$<0.01^{**}$
Average body weight gain (g/birds)								
1-7	84.24	84.78	84.54	84.42	84.71	84.52	0.13	0.90^{NS}
8-21	396.83^{a}	409.03^{b}	486.72^{c}	486.36^{c}	491.62^{c}	493.69^{c}	8.67	$<0.01^{**}$
22-42	1274.20^{a}	1365.30^{b}	1578.50^{c}	1584.60^{c}	1618.30^{d}	1625.60^{d}	28.45	$<0.01^{**}$
1-42	1758.50^{a}	1859.10^{b}	2149.80^{c}	2155.40^{c}	2194.60^{d}	2203.80^{d}	36.92	$<0.01^{**}$
Feed Conversion Ratio (FCR)								
1-7	1.47	1.45	1.46	1.47	1.46	1.48	0.01	0.43^{NS}
8-21	1.66^{a}	1.81^{b}	1.68^{b}	1.64^{b}	1.63^{b}	1.62^{b}	0.02	$<0.01^{**}$
22-42	2.05^{a}	1.98^{b}	1.76^{c}	1.75^{c}	1.73^{c}	1.74^{c}	0.27	$<0.01^{**}$
1-42	1.93^{a}	1.92^{a}	1.73^{b}	1.71^{b}	1.70^{b}	1.70^{b}	0.02	$<0.01^{**}$

Means bearing different superscripts (a,b,c,d) in a row differ significantly.

nutrients was comparable in all the groups (Supplementary Table 1). However, DM digestibility was improved in *Bekang*-fed broilers than the others. Similar findings were also reported by Yun *et al*. (2017) and Mountzouris *et al.* (2010) in broilers at 10^8 CFU/g probiotics/kg of ration supplementation and might be attributed to increased beneficial intestinal microflora. Similar trends were recorded for other nutrients also. Improved digestibility of crude protein, crude fibre, ether extract and nitrogen free extract in *Bekang* supplemented groups than the control might be the indication of positive effects of feeding *Bekang* to the broilers. Similar observations were also reported by Zhang *et al*. (2014).

Carcass traits of broilers: All the carcass traits (dressing percentage, prime cuts and edible offals) were statistically insignificant among the experimental groups indicating that feeding of *Bekang* might not have any positive or negative impacts (Supplementary Table 2). Kayode *et al*. (2012) observed that supplementation of fermented mango kernel cake with *Aspergillus niger* and *Penicillium chrysogenum* (60% of diet) showed no effect on breast meat yields or abdominal fat deposition in broilers. Similar insignificant observations were also reported by Santoso *et al*. (1995), Skrede *et al*. (2003), Suthattin *et al*. (2016) and Zhang *et al.* (2016).

Economic return score: The ERS was higher in fermented feed and *Bekang* supplemented groups than the AGP supplemented and control group. Higher ERS in the *Bekang* supplemented groups might be the indication of better liveability, FCR, and growth performance as compared to the others and the control group (Supplementary Table 3).

From the findings of the study, it could be concluded that wet *Bekang* could be exploited as 'probiotics source' as dietary additive alternative to the feed fermented with lactic acid bacteria and antibiotic growth promoters in the rations of broiler birds. Keeping in view the performance of the broiler birds in terms of growth performance, liveability, feed conversion efficiency and economic return score, supplementation of wet *Bekang* as low as 50 g/kg ration might be recommended in the rations of broilers.

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