



# Effect of Replacement of Antibiotic with Probiotic on Performance, Carcass Characteristics and Nutrient Retention in Broilers Fed with Meat Cum Bone Meal

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## ABSTRACT

The effect of replacing an antibiotic growth promoter in the diet with a probiotic on growth performance, carcass characteristics and nutrient retention was studied in broilers (Vencobb 430) fed with and without meat cum bone meal (MBM). This experiment which lasted for 42 days consisted of six dietary treatments viz., basal diet (BD), basal diet with MBM (MBM), basal diet with antibiotic (AB), basal diet with probiotic (PB), basal diet with MBM and antibiotic (MBM+AB) and basal diet with MBM and probiotic (MBM+PB). Three-hundred, day-old chicks were randomly allotted in the beginning to these six groups. Body weight gain, feed intake and feed conversion ratio were significantly ( $P < 0.05$ ) affected at 4<sup>th</sup> week of age. Overall, highest body weight gain was recorded in birds fed with AB and PB. Inclusion of probiotic in MBM also showed improved body weight gain when compared to MBM group. Highest feed intake and feed conversion ratio was recorded with AB and PB groups. Birds supplemented with MBM+PB showed improved feed intake and feed efficiency when compared to MBM+AB and MBM diet supplemented groups. Higher dry matter, crude protein, calcium and phosphorus retention ( $P < 0.05$ ) was observed in birds supplemented with PB or AB in diet without MBM when compared to their control. In MBM supplemented groups addition of PB or AB improved crude protein retention. Birds supplemented with PB or AB with or without MBM showed a significant ( $P < 0.05$ ) increase in thigh yield and a non significant reduction in fat yield when compared to their control groups.

**Key words:** Antibiotic, Broilers, Meat cum bone meal, Probiotic.

## INTRODUCTION

Antimicrobial compounds have been long used in poultry diets at sub-therapeutic levels for improving health status and performance in terms of feed conversion and weight gain (Fairchild *et al.*, 2001). As a result, there is an increased accumulation of drug residues in meat and meat products which poses a major public health concern mainly in the form of emergence of drug resistant pathogens. Concerns over development of anti bacterial resistance by food borne bacteria has driven research for alternatives to antibiotics (Owens *et al.*, 2006).

Probiotic, a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989), has been shown to be a promising alternative as growth promoter. Probiotics act through numerous mechanisms like competitive exclusion, neutralization of toxins, increasing digestive enzyme activity *etc.*, which ultimately improve various growth-related parameters.

Meat cum bone meal (MBM), a slaughter by-product rich in phosphorus, calcium and balanced protein (Bozkurt *et al.*, 2004). Incorporation of MBM in diets of birds at 5-10% level could meet the phosphorus requirements (Bozkurt *et al.*, 2004). In Japanese quails, bovine MBM partially replaced soy bean meal in diet as protein source (Pizzolante *et al.*, 2016). However, MBM is often contaminated with microbes which poses threat and also limits its use in the diets (Coradi *et al.*, 2013). Baker's yeast (*Saccharomyces*

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*cerevisiae*), besides being a rich source of crude protein and water-soluble vitamins also secretes digestive enzymes, aiding nutrient digestibility in the host (Paryad and Mahmoudi 2008). Also, bacteria belonging to the genus *Lactobacillus* have the potential to modulate gut microbiota, thereby improving gut health (Jin *et al.*, 1997).

Hence, a probiotic containing *Saccharomyces cerevisiae* and *Lactobacillus sporogenes* along with MBM have been used in the present investigation to test its efficacy as antibiotic replacer in MBM fed birds.

## MATERIALS AND METHODS

Three hundred, day-old broiler chicks, obtained from M/s Venkateswara Hatcheries Pvt. Ltd., Hyderabad were randomly distributed (completely randomized design) into six groups containing ten replicates each. Birds were reared for 42 days in battery brooder cell (6'6" x 4') with an average floor space of 1 sq. feet per bird at Poultry Experimental Station, Instructional Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad in the year 2017. Feed and water were offered *ad libitum*. All birds were maintained under the same managemental conditions and received vaccination as per the schedule. Six experimental diets (Table 1) were formulated using corn and soya. Birds were given pre-starter, starter and finisher rations during weeks 1 and 2, weeks 3 and 4 and weeks 5 and 6, respectively. Experimental diet composition is presented in Table 2.

### Performance parameters

The performance of broiler chicken was evaluated in terms of weekly body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR). Replicate-wise feed intake and body weights were recorded weekly to calculate weekly FCR.

### Metabolic trial

At the end of experiment, a 3-day metabolic trial was conducted using one bird from each replicate. Birds were fed with weighed quantities of respective diets. A pre-weighed plastic sheet was spread over the litter collection tray to collect excreta. The litter was dried at 80°C to estimate dry weight of the excreta. Representative samples offered and left over were collected and dried at 100±5°C for 8-10 hours to estimate the dry matter intake. The 3-day pooled cum dried samples of feed, residue left and excreta samples were analyzed for proximate components as per the procedures described by AOAC (2012).

### Carcass parameters

At the end of the experiment, one bird from each replicate was randomly slaughtered to study the carcass parameters viz., dressing yield, eviscerated yield, individual organ weights (breast, thigh and abdominal fat).

### Statistical analysis

The data were analyzed using one-way analysis of variance (Snedecor and Cochran, 1989) of Statistical Package for the Social Sciences (SPSS), version 16 and significant differences ( $P < 0.05$ ) were compared using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

The results pertaining to the body weight gain are presented in Table 3. The weight gain differed significantly ( $P \leq 0.05$ ) only during the 4<sup>th</sup> week of study with AB and PB groups showing higher body weight gain than other experimental groups.

During 4<sup>th</sup> week, addition of AB to BD improved body weight gain and similar result was obtained with inclusion of PB replacing AB in basal diet. While addition of PB to MBM improved body weight gain when compared to MBM+AB and MBM groups but these results were numerically lower when compared to BD diet groups. Addition of antibiotic to the basal diet containing MBM had no beneficial effect on the body weight gain. Body weights at 6<sup>th</sup> week of age is higher in diet supplemented with PB or AB. However, the values are not statistically significant.

The results of the present study were in agreement with the findings of Latesh *et al.* (2013) who recorded improved body weight gain in broilers upto six weeks of age

**Table 1:** Allotment of test diets.

Treatment	Test Diet
BD	Basal Diet
MBM	Basal Diet + MBM 50g/ton
AB	Basal Diet + Antibiotic* 500g/ton
MBM+AB	Basal Diet + MBM 50g/ton + Antibiotic* 500g/ton
PB	Basal Diet + Probiotic** 500g /ton
MBM+PB	Basal Diet + MBM 50g/ton + Probiotic** 500g/ton

\*Bacitracin Methylene Disalicylate.

\*\**Saccharomyces cerevisiae*: 1.5 x10<sup>8</sup> cfu; *Lactobacillus sporogenes*: 50 million cfu.

**Table 2:** Composition of Test Diets.

Ingredients	Prestarter	Starter	Finisher
Maize	56.87	60.87	64.19
Soy Oil-Veg	1.66	2.69	3.70
Soya DOC 45%	37.69	32.56	28.67
Salt	0.31	0.31	0.31
Sodium bi-carbonate	0.10	0.10	0.10
Dicalcium Phosphate	1.86	1.90	1.63
LSP-Powder	0.69	0.73	0.68
DL-Methionine	0.28	0.25	0.22
L-Lysine HCL	0.18	0.20	0.10
L-Threonine	0.01	0.04	0.05
L-Tryptophan	0.00	0.005	0.00
Choline Chloride,75%	0.10	0.10	0.10
Trace Mineral Mixture	0.10	0.10	0.10
Vitamin Premix	0.05	0.05	0.05
C-Lyte	0.10	0.10	0.10
<b>TOTAL</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
M.E (kcal/kg)	2950	3050	3150
Protien (%)	22.5000	20.5000	18.9000
Dig. Lysine (%)	1.2500	1.1500	0.9600
Dig. Methionine (%)	0.5700	0.5200	0.4800
Calcium (%)	0.9400	0.9400	0.8500
Available Phosphorus (%)	0.4500	0.4500	0.4000
Sodium (%)	0.1600	0.1600	0.1600
Dig. Arginine (%)	1.3853	1.2429	1.1358
Dig. Tryptophan	0.2244	0.2050	0.1823
Dig. Threonine (%)	0.7700	0.7200	0.6800

similarly Alkhalf *et al.* (2010) also reported that addition of probiotics to the diet of broilers improved body weight at 3<sup>rd</sup> week of age and persisted till end of trial. Improved body weight by the supplementation of probiotics in the diet of broilers were also demonstrated by many researchers (Mountzouris *et al.*, 2010 and Nawaz *et al.*, 2016). On the contrary, Smulikowska *et al.* (2005) and Elangovan *et al.* (2011) reported that there was no significant difference in body weight gain of broilers when fed with probiotics.

The data on feed intake (Table 4) revealed that there was no significant ( $P>0.05$ ) difference in feed intake was observed among the experimental groups when compared with their controls. However, during fourth week of age a non significant improvement in feed intake by the birds fed with PB or AB in BD was observed. In MBM challenged birds addition of PB improved feed intake but addition of AB in diet has no effect in feed intake of broilers.

These results of the present study were in agreement with the findings of Banday and Pampori (2006) and Apata (2008) who also reported that the supplementation of dietary probiotics enhanced the level of feed intake by broilers when compared with controls groups. Similarly, Tabidi *et al.* (2013)

and Nawaz *et al.* (2016) also reported an increase in feed consumption of broilers fed with probiotics. Contradict to the present observations Anjum *et al.* (2005), Sarmah *et al.* (2014) reported that supplementation of probiotic has no effect on feed consumption in broilers.

The results pertaining to the feed efficiency of broilers in the present study was presented in the Table 5. The feed conversion ratio during first week and fourth week showed a significantly ( $P<0.05$ ) better feed to weight gain ratio. During 1<sup>st</sup> week AB addition to BD improved feed efficiency however at the same age, MBM challenged groups either addition of AB or PB did not improved feed efficiency. During 4<sup>th</sup> week of age feed efficiency of birds supplemented with BD alone and addition of PB or AB in BD were same indicating that the supplementation of either PB or AB could not improved the feed efficiency. The similar trend was also observed in MBM challenged birds. However, enhanced FCR was seen in birds fed with basal diet without addition of MBM when compared MBM added diets.

Similar results were reported by Zhang *et al.*, (2014) and Gao *et al.*, (2017) who reported better feed efficiency in broilers fed with probiotics in diet. Contrarily, the findings of

**Table 3:** Effect of dietary inclusion of probiotics on weekly and body weight gain (g) in commercial broiler chicken.

Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
BD	132.06	266.86	399.96	482.93 <sup>ab</sup>	600.22	514.65
MBM	133.96	270.34	395.40	462.20 <sup>ab</sup>	596.43	545.68
AB	138.14	274.40	418.56	503.80 <sup>a</sup>	570.38	560.55
MBM+AB	130.20	268.76	397.52	443.84 <sup>b</sup>	586.86	520.50
PB	132.96	271.66	413.18	498.01 <sup>a</sup>	588.39	568.22
MBM+PB	132.24	272.14	394.50	471.26 <sup>ab</sup>	593.81	546.05
SEM	1.1835	0.776	3.633	5.856	11.311	15.664
N	10	10	10	10	10	10
P-value	0.520	0.074	0.263	0.038	0.995	0.916

<sup>abc</sup> Means with different superscript in a column differ significantly:  $P<0.05$ ,  $P<0.01$ , P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean NC: Basal diet; PC: BD with 5% MBM; NC+AGP: NC with 0.05% antibiotic growth promoter; PC+AGP: PC with 0.05% antibiotic growth promoter; NC+PRO: NC with 0.05% probiotic; PC+PRO: PC with 0.05% probiotic.

**Table 4:** Effect of dietary inclusion of probiotics on weekly feed intake (g) in commercial broiler chicken.

Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
BD	143.96	348.68	582.40	773.91	1139.8	1169.2
MBM	148.46	353.22	584.46	768.36	1162.2	1232.4
AB	148.42	351.92	610.38	807.97	1167.3	1287.3
MBM+AB	144.70	349.26	583.24	736.77	1175.1	1202.9
PB	146.80	352.56	602.84	797.30	1146.5	1328.1
MBM+PB	147.40	352.88	588.82	776.38	1174.9	1261.4
SEM	0.985	0.745	4.637	7.386	21.791	31.755
N	10	10	10	10	10	10
P-value	0.702	0.340	0.383	0.087	0.996	0.751

<sup>abc</sup> Means with different superscript in a column differ significantly:  $P<0.05$ ,  $P<0.01$ , P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean NC: Basal diet; PC: BD with 5% MBM; NC+AGP: NC with 0.05% antibiotic growth promoter; PC+AGP: PC with 0.05% antibiotic growth promoter; NC+PRO: NC with 0.05% probiotic; PC+PRO: PC with 0.05% probiotic.

Gunal *et al.* (2006) and Sarmah *et al.* (2014) showed supplementation of probiotics in diet of broilers did not affect feed efficiency.

The results of the present study indicated that inclusion of PB in place of AB could increase the body weight which may be attributed rapid development of beneficial microflora in gut (Edens 2003) and also improved gut health by reducing enteropathogens by several mechanisms which include competitive exclusion, production of anti-microbial substances, antagonistic properties, altering the pH of intestine, competing with pathogens for nutrients and adhesion sites in intestine (Kabir, 2009; Dhama *et al.*, 2011) thereby improving overall performance of broilers. Effectiveness of probiotics on performance might be the final outcome of improved intestinal environment, integrity of intestinal mucosal barrier, improved digestive enzymes activity and improved immune function of intestine (Mountzouris *et al.*, 2010). In the present study higher feed consumption is well compared with higher growth rate and this may be due to increased efficiency of digestion and absorption in the gut of the birds which might have resulted in increased colonization of beneficial bacteria or by the

reduction of enteropathogens by probiotic bacteria in gut. The inclusion of MBM in diets with probiotic or antibiotic supplementation appears to have not much beneficial effect in terms of growth and this might be due to contamination with pathogens and thus might have effected digestion and absorption of nutrients in these groups.

Perusal of the the results on nutrient retention has (Table 6) revealed that there was significant ( $P < 0.05$ ) difference in dry matter, crude protein, calcium and phosphorus retention in broilers fed with different dietary treatments.

Highest dry matter retention was observed in PB fed birds followed by AB group birds. In MBM challenged groups addition of AB improved dry matter retention but PB addition did not showed any difference. Addition of PB in BD improved crude protein retention and was comparable with AB. The same trend was observed in MBM challenged birds. Calcium retention by birds was enhanced by supplementation of PB in the diets which was comparable with AB group birds. No further improvement was observed by the addition of PB or AB to the MBM challenged groups. The similar trend was observed in phosphorus retention. In the present study MBM

**Table 5:** Effect of dietary inclusion of probiotics on weekly feed conversion ratio in commercial broiler chicken.

Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
BD	1.09 <sup>ab</sup>	1.30	1.45	1.60 <sup>c</sup>	1.89	2.27
MBM	1.10 <sup>a</sup>	1.30	1.47	1.66 <sup>a</sup>	1.94	2.25
AB	1.07 <sup>b</sup>	1.28	1.45	1.60 <sup>c</sup>	2.04	2.29
MBM+AB	1.11 <sup>a</sup>	1.29	1.46	1.65 <sup>ab</sup>	2.00	2.31
PB	1.10 <sup>ab</sup>	1.29	1.45	1.60 <sup>c</sup>	1.94	2.33
MBM+PB	1.11 <sup>a</sup>	1.29	1.49	1.64 <sup>ab</sup>	1.97	2.31
SEM	0.004	0.003	0.003	0.006	0.013	0.035
N	10	10	10	10	10	10
P-value	0.048	0.318	0.054	0.003	0.292	0.912

<sup>abc</sup> Means with different superscript in a column differ significantly:  $P < 0.05$ ,  $P < 0.01$ , P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean NC: Basal diet; PC: BD with 5% MBM; NC+AGP: NC with 0.05% antibiotic growth promoter; PC+AGP: PC with 0.05% antibiotic growth promoter; NC+PRO: NC with 0.05% probiotic; PC+PRO: PC with 0.05% probiotic.

**Table 6:** Effect of dietary inclusion of probiotics on nutrient retention (%) in commercial broiler chicken.

Diet	Dry matter	Crude protein	Calcium	Phosphorus
BD	64.61 <sup>c</sup>	63.44 <sup>bc</sup>	56.76 <sup>d</sup>	42.56 <sup>b</sup>
MBM	65.21 <sup>bc</sup>	61.55 <sup>c</sup>	66.66 <sup>b</sup>	52.64 <sup>a</sup>
AB	68.32 <sup>ab</sup>	67.37 <sup>ab</sup>	67.89 <sup>ab</sup>	50.75 <sup>a</sup>
MBM+AB	67.41 <sup>abc</sup>	66.05 <sup>abc</sup>	60.45 <sup>c</sup>	47.34 <sup>ab</sup>
PB	69.27 <sup>a</sup>	70.58 <sup>a</sup>	71.15 <sup>a</sup>	53.44 <sup>a</sup>
MBM+PB	65.58 <sup>bc</sup>	66.86 <sup>abc</sup>	65.58 <sup>b</sup>	46.59 <sup>ab</sup>
SEM	0.490	0.807	0.940	1.118
N	6	6	6	6
P-value	0.019	0.014	0.001	0.029

<sup>abc</sup> Means with different superscript in a column differ significantly:  $P < 0.05$ ,  $P < 0.01$ , P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean NC: Basal diet; PC: BD with 5% MBM; NC+AGP: NC with 0.05% antibiotic growth promoter; PC+AGP: PC with 0.05% antibiotic growth promoter; NC+PRO: NC with 0.05% probiotic; PC+PRO: PC with 0.05% probiotic.

**Table 7:** Effect of dietary inclusion of probiotics on carcass characteristics (%) in commercial broiler chicken.

Diet	RTC (g/kg)	Breast (g/kg)	Liver (g/kg)	Fat (g/kg)	Giblet (g/kg)	Thigh (g/kg)	Muscle pH
BD	701.32	226.23	19.89	12.61	38.49	198.76 <sup>b</sup>	5.72
MBM	699.14	226.43	20.25	12.99	39.80	193.14 <sup>b</sup>	5.73
AB	703.35	229.03	20.32	12.39	40.42	201.68 <sup>ab</sup>	5.67
MBM+AB	702.06	226.83	20.25	12.75	39.91	203.33 <sup>ab</sup>	5.68
PB	714.45	231.85	20.56	8.88	41.97	218.67 <sup>a</sup>	5.74
MBM+PB	705.08	228.47	20.56	11.65	41.26	208.69 <sup>ab</sup>	5.79
SEM	2.177	1.738	0.289	0.489	0.510	2.405	0.013
N	10	10	10	10	10	10	10
P-value	0.414	0.944	0.988	0.138	0.459	0.040	0.168

<sup>abc</sup> Means with different superscript in a column differ significantly:  $P < 0.05$ ,  $P < 0.01$ , P-value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean NC: Basal diet; PC: BD with 5% MBM; NC+AGP: NC with 0.05% antibiotic growth promoter; PC+AGP: PC with 0.05% antibiotic growth promoter; NC+PRO: NC with 0.05% probiotic; PC+PRO: PC with 0.05% probiotics.

challenged group birds showed higher values when compared with BD group birds as it was a good source for dietary calcium and phosphorus.

The results obtained in the present study were in agreement with the findings of Li *et al.* (2008) who also reported a significant improvement in digestibility of dry matter, crude protein, calcium and phosphorus in broilers at 21 and 42 days of age when fed with probiotic alone or in combination with antibiotic. Latesh *et al.* (2013) also reported increased dry matter, nitrogen, calcium and phosphorus retention in birds fed with 0.05% *Saccharomyces cerevisiae*, *Lactobacillus acidophilus* and their combination in diet. Similarly, Lei *et al.* (2015), Nawaz *et al.* (2016) stated that supplementation of probiotic in diet of broilers improved crude protein digestibility. Probiotic enhances the growth of non-pathogenic, anaerobic, facultative, lactic acid producing bacterial population in the gut. Probiotics increase brush-border membrane activity, promote epithelial restitution, prevent epithelial apoptosis, protect tight junctions during inflammation, suppress electrolyte secretion during enteropathogen infection, increase expression of mucin glycoproteins, provide enzymes that may enhance host digestion of dietary nutrients (Marteau *et al.*, 2004; Rioux *et al.*, 2005).

In the present study the enhanced nutrient retention of the birds fed with PB or AB in basal diet without MBM might be due to improved digestion and absorption of nutrients in the gut which was described earlier in the present study. In MBM challenged birds, the inconsistency in the digestibility might be due to the insufficient number, species or strain of microorganisms used in probiotic or dose of probiotic administered to birds. MBM is a good source of protein and minerals like calcium and phosphorus but also major source of bacterial contamination. Addition of probiotic or antibiotic could check the contaminant organisms, thus could improve crude protein retention. However, calcium and phosphorus retention could not be improved probably the acidic environment might have hindered these mineral absorption or contaminant organisms might have interfered with transport pumps of these minerals in intestinal epithelium.

The data pertaining the carcass yields of broilers in the present study was presented in the Table 7. Among the carcass parameters studied, only the thigh yield has differed significantly between the groups ( $P < 0.05$ ), with maximum thigh yield from the birds fed on probiotic alone. Carcass characteristics Ready-to-Cook (RTC), breast, liver giblet yields were numerically high in PB supplemented groups followed by AB supplemented groups. However, the values were not statistically significant. No significant improvement in carcass characteristics with addition of PB or AB with MBM was noticed.

Though no significant difference was observed with fat yield. Numerically low fat yield was noticed in PB supplemented groups when compared to AB supplemented groups.

These findings of our present study were in accordance with those of Islam *et al.* (2004) who reported that there was no significant difference in carcass yield, weight of liver, heart, gizzard and spleen of birds fed with protexin at different graded levels in drinking water. *Lactobacillus sporogenes* supplementation in diet of broilers did not affect the dressing percent, weight of liver, giblet, gizzard and abdominal fat in broilers (Panda *et al.*, 2005). These results were also similar to findings of Paryad and Mahmoudi (2008) who revealed that there was significant increase in leg yield. Fathi *et al.* (2012) reported that supplementation of *Saccharomyces cerevisiae* at 1.25 and 1.5g/kg in diet increased thigh percentage.

The retained protein by probiotic might have diverted for thigh muscle protein synthesis which we have observed in the present study.

## CONCLUSION

In conclusion, supplementation of probiotic with MBM in diet of broilers improved body weight gain, feed intake and feed efficiency which are comparable with antibiotic with MBM diets. Probiotic with MBM also increased crude protein retention, thigh yield in birds. This suggests that probiotic could be used as alternate replacer for antibiotic in MBM fed birds.



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