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Effect of supplementation of Lauric Acid, Probiotic and their Combination on Nutrient retention, Total Bacterial Count, Gut pH and Cost Economics of Commercial Broiler Chicken

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ABSTRACT

The objective of the present study was to determine the synergistic effect of lauric acid and probiotic on the nutrient retention, total bacterial count, gut pH and cost economics of broiler chicken. A total of 250-day-old male broiler chicks were randomly distributed into five dietary treatment groups each having ten replicates with five chicks in each and were raised for a period of 42 days. Diets contained T1- control diet, T2- 0.05% antibiotic (AB), T3- 0.05% lauric acid (LA), T4- 0.1% probiotic in the form of *Bacillus subtilis* PB6 (BS), and T5-0.05% lauric acid +0.1% probiotic. The results revealed no significant effect on dry matter (DM) and crude protein (CP) retention but energy retention was significantly ($P<0.05$) higher with LA, BS and AB compared to control. The total bacterial count was significantly ($P<0.05$) reduced in LA followed by LA+BS compared to control, AB and BS. The pH in proventriculus and gizzard was significantly ($P<0.05$) reduced in LA and BS compared to AB and control. The pH in duodenum was reduced in LA compared to other treatments with no significant effect in the ileal pH. LA in combination with BS or alone in broiler diets was economically better ($P<0.05$) compared to AB and control birds. Therefore, it can be concluded that supplementation of lauric acid (0.05%) + probiotic (0.1%) combination has resulted in gut acidification, reduced total bacterial load, improved nutrient absorption and is economically better. Thus, could be safely included as an alternative to antibiotic growth promoter in broilers.

KEY WORDS: Antibiotic, Broilers, Cost economics, Lauric acid, Nutrient retention, Probiotic

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INTRODUCTION

Poultry birds are vulnerable to potentially pathogenic micro-organisms such as *Escherichia coli*, *Salmonella spp*. and *Clostridium perfringens*, with pathogenic microflora being established in the small intestine, thereby competing with the host for nutrients and depressing the growth performance and increasing the incidence of diseases. To stabilize the intestinal microbial flora antibiotic growth promoters were used in poultry feed. But due to its prohibition in animal diets, alternative to antibiotic growth promoters like organic acids, enzymes, probiotics, prebiotics and medicinal herbs are being used as feed additives in poultry production (Adil et al., 2011).

Bacillus subtilis PB6 strain is a probiotic which is capable of producing beneficial intestinal microorganisms, such as *Lactobacillus* species and improve the growth performance by secreting protease, amylase and lipase (Zamanzad et al., 2011). Lauric acid is medium chain fatty acid (MCFA) with chain length of 12 carbon atoms having antibacterial function similar to short chain fatty acids (Skrivanova et al., 2006). Even though the beneficial effects of *Bacillus subtilis* and lauric acid are documented, there is a paucity of information on the effectiveness of combining lauric acid and *Bacillus subtilis* PB6 strain on nutrient retention and gut health in broilers. Hence, this study was designed to evaluate the synergistic effect of lauric acid and *Bacillus subtilis*

PB6 strain on nutrient retention, gut health and cost economics of broiler chicken.

MATERIALS AND METHODS

To conduct the above study, 250-day-old male broiler chicks (cobb 400) were randomly distributed into five dietary treatment groups each having 10 replicates with 5 chicks each. The chicks were housed in battery brooder cell (2'×2') with an average floor space of 82 square inches per bird. Birds were immunized against Newcastle disease (ND) with lasota vaccine on 7th (primary) and 28th (booster) days of age, and infectious bursal disease (intermediate – Georgia strain) vaccine on 14th (primary) and 21st (booster) days of age. Five experimental diets were formulated: T1- Control i.e, basal diet (BD), T2- BD + 0.05% antibiotic (Bacitracin Methylene Disalicylate) (AB), T3- BD + 0.05% lauric acid (LA), T4- BD + 0.1% *Bacillus subtilis* PB6 (BS) and T5- BD + 0.05% LA + 0.1% BS. Feed was offered *ad lib* to the birds during pre-starter (0-14 d), starter (15-28 d) and finisher (29-42 d) period (Table 1) to meet the nutrient requirements (BIS, 2007).

Table 1. Ingredient composition of basal diet (kg/100 kg)

Ingredient	Pre-starter (0-14d)	Starter (15-28d)	Finisher (29-42d)
Maize	54.0	55.5	59.1
Oil	3.0	5.3	5.9
Soyabean meal	38.5	34.8	30.95
Shell grit	1.36	1.4	1.6
Dicalcium phosphate	1.8	1.7	1.9
Salt	0.4	0.4	0.4
DL-Methionine	0.25	0.29	0.2
L-Lysine HCl	0.25	0.34	0.18
Additives*	0.13	0.13	0.13
Nutrient composition			
ME (kcal/kg)	2912	3080	3166
Crude protein (%)	22.5	21	19.5
Lysine (%)	1.26	1.24	1.03
Methionine (%)	0.56	0.58	0.47
Calcium (%)	0.93	0.92	1.00
Available phosphorous (%)	0.45	0.43	0.45

*Supplied per kg of diet: retinol acetate 2.75 mg, cholecalciferol 0.03 mg, á tocopherol 10 mg, pantothenic acid 10 mg, riboflavin 10 mg, biotin 0.08 mg, menadione 2 mg, choline 650 mg, copper 8 mg, boron 45 mg, manganese 80 mg, zinc 60 mg, selenium 0.18 mg, monensin sodium 50 mg and hydrated sodium calcium alumino silicates 800 mg.

One bird from each replicate was randomly selected, starved over night with free access to water, weighed and sacrificed by cervical dislocation on 42nd day to study the gut pH and total bacterial count. Gut (proventriculus, gizzard, duodenum and ileum) pH was recorded using digital pH meter immediately after collection of gastro-intestinal contents from respective part of gut (Corrier et al., 1990). To study the total viable colony counts, intestinal content was collected in test tubes and each sample was serially diluted and plated on Nutrient agar by surface spread method. Visible microbial colonies were counted and expressed as log_{10} value (Ahmed and Yang, 2017). At the end (42 days) of the experiment, a metabolic trial of 3 days duration was conducted. one bird from each replicate was randomly selected and separated in individual cages to determine the nutrient retention (DM, CP, GE). The analysis of feed and dried excreta was done according to A.O.A.C (2005). The economics of using probiotics and organic acids in diets was calculated in terms of returns over feed cost.

Data obtained were analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989). Comparison of means were done using Duncan's multiple range test (Duncan, 1955) using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at P<0.05.

RESULTS AND DISCUSSION

No significant (P<0.05) difference was observed in DM and CP retention between the treatments (Table 2). The energy retention was significantly (P<0.05) higher in LA+BS, AB, BS and LA and was lowest in control. The results are in agreement with authors who observed no significant difference in DM and CP with supplementation of organic acid (Venkatasubramani et al., 2014), probiotic (Patel et al., 2016) or their combination (Rodjan et al., 2017). Similarly, Jeong and Kim (2014) also reported an improvement in gross energy digestibility by supplementation of *Bacillus subtilis* spores in broiler diet.

The total viable count was significantly (P<0.05) reduced due to the inclusion of LA followed by LA+BS compared to control, AB and BS (Table 3). Similar results were observed with the supplementation of organic acid alone (Ramarao et al., 2004) and in combination with probiotic (Gunal et al., 2006). In contrast, some authors reported that addition of probiotics (Sen et al., 2012) and organic acids either alone or in combination had no significant difference on total bacterial count (Agboola et al., 2015).

Inclusion of LA, BS and LA+BS in diets significantly (P<0.05) lowered the pH in proventriculus and gizzard compared to AB and control (Table 4). Duodenal pH was significantly (P<0.05) lowered in LA compared to other treatments. However, no significant (P>0.05) difference was observed in ileal pH. The results are in agreement with Ramigani et al. (2015), Fattah et al. (2008) and Ghazalah et al. (2011). In contrast, Denli et al. (2003) and Kral et al. (2011) reported no significant (P>0.05) effect in the intestinal pH with the supplementation of probiotic and organic acids either alone or in combination in broiler diets.

Table 2. Effect of lauric acid and *Bacillus subtilis* PB6 on nutrient retention (%) of broiler chicken (42d of age)

Treatment	DM %	Energy%	Crude protein%
Control	64.9	67.3^{b}	80.0
AB	68.9	73.8^{a}	81.9
LA	68.2	71.4^{a}	82.4
BS	68.5	72.4^{a}	84.3
LA+BS	69.6	73.9^{a}	85.1
SEM	0.679	0.711	0.982
P value	0.242	0.033	0.429

Means bearing different superscripts within a column are significantly (P<0.05) different. AB = 0.05% BMD, LA = 0.05% Lauric acid, BS = 0.1% *Bacillus subtilis* PB6, LA+BS = 0.05% Lauric acid + 0.1% *Bacillus subtilis* PB6

Net profit obtained by the sale of birds were significantly (P<0.05) higher in LA+BS and LA compared to control in which profit was lowest (Table 5). Whereas the net profit was intermediate in AB and BS. The results are in agreement with Venkatasubramani et al. (2014) and Patel et al. (2015) who observed reduced cost of production by acidifier and probiotic supplementation respectively. The percent additional profit of LA, BS and LA+BS was 3.7%, 2.6% and 4.1% over control and was 2.1%, 1% and 2.5% over AB respectively.

Table 3. Effect of lauric acid and *Bacillus subtilis* PB6 on total bacterial count (log_{10} cfu/g) of broiler chicken

Treatment	Total bacterial count (log_{10} cfu/g)
Control	6.28^{a}
AB	6.05^{a}
LA	5.24^{c}
BS	6.18^{a}
LA+BS	5.61^{b}
SEM	0.087
P value	0.001

Means bearing different superscripts within a column are significantly (P<0.05) different. AB = 0.05% BMD, LA = 0.05% Lauric acid, BS = 0.1% *Bacillus subtilis* PB6, LA+BS = 0.05% Lauric acid + 0.1% *Bacillus subtilis* PB6

Table 4. Effect of lauric acid and *Bacillus subtilis* PB6 on gut pH of broiler chicken at 42d of age

Treatment	Proventriculus	Gizzard	Duodenum	Ileum
Control	4.41^{a}	3.90^{a}	5.80^{a}	7.15
AB	3.63^{b}	3.65^{a}	5.78^{a}	7.10
LA	3.07^{c}	3.52^{b}	5.21^{b}	7.06
BS	3.17^{c}	3.53^{b}	5.66^{a}	7.13
LA+BS	3.04^{c}	3.43^{b}	5.57^{a}	7.07
SEM	0.088	0.054	0.045	0.014
P value	0.001	0.048	0.001	0.208

Means bearing different superscripts within a column are significantly (p<0.05) different. AB = 0.05% BMD, LA = 0.05% Lauric acid, BS = 0.1% *Bacillus subtilis* PB6, LA+BS = 0.05% Lauric acid + 0.1% *Bacillus subtilis* PB6

Table 5. Effect of lauric acid and *Bacillus subtilis* PB6 on cost economics (profit/kg live weight) of broiler chicken upto 42d of age

Treatment	Total Feed Intake (kg/bird)	Cost of Feed Consumption (Rs/bird)	Final Body Weight (g/bird)	Sale amount (Rs/bird)	Return Over Feed Cost (Rs/bird)	Profit/kg live weight (Rs/kg)
Control	2954	86.0	1564	133	46.9	30.0^{c}
AB	3037	89.1	1699	144	55.3	31.6^{bc}
LA	3246	94.8	1852	157	62.6	33.7^{a}
BS	3295	96.6	1843	156	60.0	32.6^{ab}
LA+BS	3232	94.9	1866	158	63.7	34.1^{a}
SEM	-	-	-	-	-	0.342
P value	-	-	-	-	-	0.001

Means bearing different superscripts within a column are significantly (P<0.05) different. AB = 0.05% BMD, LA = 0.05% Lauric acid, BS = 0.1% *Bacillus subtilis* PB6, LA+BS = 0.05% Lauric acid + 0.1% *Bacillus subtilis* PB6. Sale price per bird was taken as 85 Rs/kg live weight. Cost of lauric acid – 120 Rs/kg, cost of probiotic (*Bacillus subtilis* PB6) – 170 Rs/kg, cost of antibiotic – 450 Rs/kg.

CONCLUSION

It can be concluded that supplementation of 0.05% lauric acid in combination with 0.1% *Bacillus subtilis* PB6 improved the absorption of nutrients from the gut and further resulted in gut acidification, reduced total bacterial load and thereby reducing the cost of production.

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