*Indian J. Anim. Nutr.* 2021. 38 (1): 55-60
doi: 10.5958/2231-6744.2021.00008.6

Indian Journal of Animal Nutrition

# Supplementation of Lauric Acid, Probiotic and their Combination on Performance and Immune Response 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 performance 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 $T_1$- control diet, $T_2$- 0.05% antibiotic (AB), $T_3$- 0.05% lauric acid (LA), $T_4$- 0.1% probiotic in the form of *Bacillus subtilis* PB6 (BS), and $T_5$-0.05% organic acid +0.1% probiotic. The results revealed significantly (P<0.05) higher body weight gain, feed consumption and better feed conversion in LA+BS combination group followed by LA and BS alone compared to antibiotic (AB) and control diet at 42 d. The percent breast yield and thymus weights were increased (P<0.05) in LA and BS as compared to control diets. Humoral immune response (NDV titers) was higher (P<0.05) in LA+BS group followed by BS compared to control and AB. Further, no significant ((P>0.05) effect was observed on cutaneous basophilic hypersensitivity response among different treatments. Therefore, it can be concluded that supplementation of lauric acid (0.05%) + probiotic (0.1%) combination could be safely included as an alternative to antibiotic growth promoter in broilers.

**Key words:** Antibiotic, Broilers, Lauric acid, Probiotic, Performance, Immunity

## INTRODUCTION

Conventionally, antibiotic growth promoters (AGP) are being utilized to stabilize the intestinal microbial flora to improve the gut health and overall performances of chickens. However, lately, because of negative human health issue of antibiotic resistance, feed manufacturers and the animal growers have been effectively looking to a viable alternative to AGP. Probiotics are viable micro-organisms that provide beneficial effects on host by modifying the intestinal ecosystem (Fatufe and Matanmi, 2011), which improves absorption of nutrients (Awad *et al.,* 2006) and also positively modulate the immune system (Apata, 2008). *Bacillus subtilis* (BS) PB6 strain (BS PB6) is capable of stimulating growth of beneficial intestinal bacteria, such as *Lactobacillus* species and improved weight gain and feed efficiency of broilers (Teo and Tan, 2006). Lauric acid is a fatty acid with a chain length of 12 carbon atoms, which have antibacterial function similar to short chain fatty acids (Dibner and Buttin, 2002; Skrivanova *et al.,* 2006; Nava *et al.,* 2009). Even though the beneficial effects of both BSPB6 and lauric acid have been documented, there is a scarcity of data on the efficacy of combining lauric acids and BSPB6. Therefore, this study was designed to evaluate the effect of lauric acid and BSPB6 either alone or in combination on growth performance 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 7$^{th}$ (primary) and 28$^{th}$ (booster) days of age, and infectious bursal disease (intermediate – Georgia strain) vaccine on 14$^{th}$ (primary) and 21$^{st}$ (booster) days of age. Five experimental diets were formulated: Control diet ($T_1$), control diet+0.05% antibiotic ($T_2$), control diet+0.05%

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

| Ingredient | Pre-starter | Starter | Finisher |
|---|---|---|---|
| Maize | 54.0 | 55.5 | 59.1 |
| Oil | 3.00 | 5.30 | 5.90 |
| Soybean meal | 38.5 | 34.8 | 30.95 |
| Shell grit | 1.36 | 1.40 | 1.60 |
| Dicalcium phosphate | 1.80 | 1.70 | 1.90 |
| Salt | 0.40 | 0.40 | 0.40 |
| DL-Methionine | 0.25 | 0.29 | 0.20 |
| L-Lysine HCl | 0.25 | 0.34 | 0.18 |
| Additives* | 0.13 | 0.13 | 0.13 |
| **Nutrient composition(calculated)** | | | |
| ME (kcal/kg) | 2912 | 3080 | 3166 |
| Crude protein (%) | 22.5 | 21.0 | 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.

lauric acid (LA) ($T_3$), control diet+0.1% *Bacillus subtilis* PB6 (BS PB6) ($T_4$), and control diet+0.05% LA+0.1% BSPB6 ($T_5$). 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).

Body weight of individual birds were recorded at the beginning of the trial and thereafter on weekly basis up to 42 days of age. Feed consumption was recorded at weekly intervals up to 42 days of age. One bird from each replicate was randomly selected, starved over night with free access to water, weighed and sacrificed by cervical dislocation on the next day to study the carcass parameters.

The humoral immunity was estimated in birds by measuring antibody titre to Newcastle disease (ND) vaccine (antibody production against ND virus), and the antibody titers ($log_2$) were measured following the standard procedure (Wegmann and Smithies, 1966). The cell mediated immune (CMI) response was tested using a standard procedure (Corrier and DeLoach, 1990) to measure cutaneous basophilic hypersensitivity (CBH) to phytohaemagglutinin phosphate (PHA-P).

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 Tukey's test using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at $P<0.05$.

## RESULTS AND DISCUSSION

Cumulative body weight gain was significantly ($P<0.05$) higher in birds fed LA, BSPB6 and LA+BSPB6 during the 42 days trial period, as well as during starter (15-28 d), and finisher (29-42 d) phase of the trial compared to AB and control diet (Table 2). These results are in agreement with Fatufe and Matanmi (2011) and Rout *et al.* (2016) who reported that supplementation of probiotic+ acidifier resulted in higher body weight gain. No significant ($P>0.05$) difference was observed in feed consumption during pre-starter phase (Table 3). In starter phase, feed consumption was significantly ($P<0.05$) higher with the inclusion of LA,

**Table 2. Effect of lauric acid and probiotic on body weight gain (BWG) (g/bird) in commercial broiler chicken**

| Diets# | Pre-starter (0-14 d) | Starter (15-28 d) | Finisher (29-42 d) | Cumulative BWG (0-42 d) |
|---|---|---|---|---|
| Control | 269.09 | 557.07$^{c}$ | 694.07$^{c}$ | 1520.4$^{c}$ |
| AB | 271.76 | 602.52$^{b}$ | 750.66$^{b}$ | 1624.9$^{b}$ |
| LA | 258.78 | 731.06$^{a}$ | 818.24$^{a}$ | 1808.0$^{a}$ |
| BSPB6 | 268.61 | 707.10$^{a}$ | 823.50$^{a}$ | 1799.4$^{a}$ |
| LA+BSPB6 | 265.76 | 704.40$^{a}$ | 836.94$^{a}$ | 1823.0$^{a}$ |
| SEM | 2.601 | 11.239 | 11.119 | 19.966 |
| P- value | 0.589 | 0.001 | 0.001 | 0.001 |

$^{a,b,c}$Means bearing different superscripts within a column are significantly (P<0.05) different; #AB, 0.05% antibiotics; LA, 0.05% lauric acid; BSPB6, 0.1% *Bacillus subtilis* PB6; LA+BSPB6, 0.05% lauric acid + 0.1% *Bacillus subtilis* PB6

BSPB6 and LA+BS compared with AB and control diets. During finisher phase (29-42d), feed intake was significantly (P<0.05) higher in birds fed BSPB6 compared to birds fed AB and control diet, and was intermediate in birds fed LA and LA+BSPB6. Cumulative feed consumption (0-42 d) was significantly (P<0.05) higher among the groups fed diet contained LA, BSPB6 and LA+BSPB as compared to diets containing AB and control diets. Similar results were shown by Paul *et al.* (2007) and Rout *et al.* (2016) in chickens fed diet supplemented with probiotics and organic acids. Improved body weight and feed consumption might be due to the beneficial effects of probiotics and organic acid on the gut microflora (Anjum *et al.,* 2005), resulting in improved palatability (Haque *et al.,* 2009) and nutrient digestibility (Jeong and Kim, 2014).

Inclusion of LA and BSPB6 either alone or in combination resulted in significantly (P<0.05) better FCR than control birds during starter phase (Table 3). During finisher phase (29-42d), significantly (P<0.05) better FCR was observed in birds supplemented with LA, BSPB6, AB and LA+BSPB6, and FCR was poor in control group. Cumulative FCR was significantly (P<0.05) better in birds fed with LA+BSPB6 followed by LA supplementation and was poor in birds fed with AB and control diet. Several researchers reported better FCR due to feeding of LA (Venkatasubramani *et al.,* 2014) and BS (Patel *et al.,* 2015) in the diets of broiler chickens. The complimentary effect of organic acid and probiotic on feed conversion ratio observed in this present study might be due to high antibacterial activity of

**Table 3. Effect of lauric acid and probiotic on feed intake (g/bird) and feed conversion ratio (FCR)**

| Diets# | Pre-starter | | Starter | | Finisher | | Cumulative | |
|---|---|---|---|---|---|---|---|---|
| | Feed intake | FCR | Feed intake | FCR | Feed intake | FCR | Feed intake | FCR |
| Control | 323.24 | 1.20$^{b}$ | 1027.96$^{c}$ | 1.85$^{a}$ | 1603.00$^{b}$ | 2.31$^{a}$ | 2954.10$^{b}$ | 1.94$^{a}$ |
| AB | 336.72 | 1.24$^{a}$ | 1096.76$^{b}$ | 1.82$^{a}$ | 1604.10$^{b}$ | 2.14$^{b}$ | 3037.50$^{b}$ | 1.87$^{b}$ |
| LA | 320.80 | 1.24$^{a}$ | 1217.94$^{a}$ | 1.67$^{b}$ | 1707.80$^{ab}$ | 2.09$^{b}$ | 3246.60$^{a}$ | 1.79$^{c}$ |
| BS | 329.96 | 1.23$^{ab}$ | 1192.54$^{a}$ | 1.68$^{b}$ | 1773.30$^{a}$ | 2.16$^{b}$ | 3295.80$^{a}$ | 1.83$^{cb}$ |
| LA+BS | 331.68 | 1.25$^{a}$ | 1177.71$^{a}$ | 1.67$^{b}$ | 1723.20$^{ab}$ | 2.02$^{b}$ | 3232.80$^{a}$ | 1.77$^{c}$ |
| SEM | 3.312 | 0.005 | 12.665 | 0.014 | 19.837 | 0.023 | 27.669 | 0.013 |
| P- value | 0.567 | 0.027 | 0.001 | 0.001 | 0.014 | 0.001 | 0.001 | 0.001 |

$^{a,b,c}$Means bearing different superscripts within a column are significantly (P<0.05) different; #AB, 0.05% antibiotics; LA, 0.05% lauric acid; BSPB6, 0.1% *Bacillus subtilis* PB6; LA+BSPB6, 0.05% lauric acid + 0.1% *Bacillus subtilis* PB6

**Table 4. Effect of lauric acid and probiotic on carcass characteristics**

| Diets# | Carcass Yield | Breast yield | Abdominal fat | Liver | Heart | Gizzard | Bursa | Spleen | Thymus |
|---|---|---|---|---|---|---|---|---|---|
| Control | 65.84 | 15.80$^{b}$ | 0.97 | 2.56 | 0.63 | 1.94 | 0.066 | 0.132 | 0.477$^{b}$ |
| AB | 66.84 | 15.40$^{b}$ | 1.22 | 2.32 | 0.56 | 1.94 | 0.068 | 0.142 | 0.637$^{ab}$ |
| LA | 67.27 | 17.60$^{a}$ | 1.26 | 2.29 | 0.59 | 1.98 | 0.080 | 0.134 | 0.658$^{ab}$ |
| BS | 67.09 | 17.90$^{a}$ | 1.24 | 2.08 | 0.62 | 1.93 | 0.067 | 0.118 | 0.739$^{a}$ |
| LA+BS | 67.57 | 18.70$^{a}$ | 1.15 | 2.14 | 0.62 | 1.91 | 0.075 | 0.152 | 0.787$^{a}$ |
| Control | 65.84 | 15.80$^{b}$ | 0.97 | 2.56 | 0.63 | 1.94 | 0.066 | 0.132 | 0.477$^{b}$ |
| SEM | 0.296 | 0.301 | 0.036 | 0.057 | 0.016 | 0.030 | 0.002 | 0.007 | 0.031 |
| P- Value | 0.413 | 0.001 | 0.064 | 0.062 | 0.652 | 0.947 | 0.290 | 0.623 | 0.014 |

$^{a,b,}$Means bearing different superscripts within a column are significantly (P<0.05) different; #AB, 0.05% antibiotics; LA, 0.05% lauric acid; BSPB6, 0.1% *Bacillus subtilis* PB6; LA+BSPB6, 0.05% lauric acid + 0.1% *Bacillus subtilis* PB6

organic acids reducing the enteropathogens, and supporting the proliferation of probiotic organisms and other beneficial acid-tolerant bacteria in the gut (Nava *et al.,* 2009). The probiotics produce short chain acids like lactic, acetic and other organic acids, which are responsible for a reduction in the intestinal pH (Varalakshmi *et al.,* 2013) that facilitates the growth of beneficial microbiota in the gut.

No significant (P>0.005) difference was recorded for different carcass parameters, except for breast yield and thymus weight which were higher (P<0.05) in LA+BSPB6 groups as compared to control (Table 4). Abudabos *et al.* (2013) reported similar results where no significant difference was observed in different slaughter variables. Whereas, Zeitz *et al.* (2015) and Malik *et al.* (2016) reported increase (P<0.05) in breast yield by the supplementation of organic acids and probiotics. The increasing effects of dietary LA on breast yield could be related to an increase in insulin action and carbohydrate metabolism. Release of insulin enhances glucose absorption by breast muscle and the energy gained from glucose oxidation is utilized for protein synthesis (Shokrollahi *et al.,* 2014).

NDV titers were significantly (P<0.05) higher in birds of group LA+BSPB6 followed by BSPB6, and was lower in control and AB groups indicating better immune status of birds fed combination of LA and BSPB6. However, no significant difference was

**Table 5. Effect of lauric acid and probiotic on NDV titers ($log_2$) and skin thickness (mm) with response to cutaneous injection to PHA-P**

| Diets# | NDV titers ($log_2$) | Cutaneous basophilic hypersensitivity (mm) |
|---|---|---|
| Control | 4.00$^{c}$ | 144.69 |
| AB | 3.87$^{c}$ | 146.51 |
| LA | 4.50$^{bc}$ | 155.19 |
| BS | 4.75$^{b}$ | 152.67 |
| LA+BS | 6.62$^{a}$ | 150.34 |
| SEM | 0.182 | 3.579 |
| P-Value | 0.001 | 0.896 |

$^{a,b,c}$Means bearing different superscripts within a column are significantly (P<0.05) different; #AB, 0.05% antibiotics; LA, 0.05% lauric acid; BSPB6, 0.1% *Bacillus subtilis* PB6; LA+BSPB6, 0.05% lauric acid + 0.1% *Bacillus subtilis* PB6 observed for cell mediated immune response among the various groups (Table 5). It has been reported by Rout *et al.* (2015) that the supplementation of LA and BS in the diet of chickens alone or in combination increased the humoral and cell mediated immune response. The better immune response could be linked to an increased ($P<0.05$) weight of thymus as observed in the present study. No mortality was recorded during the entire experimental period.

## CONCLUSION

It can be concluded that supplementation of lauric acid (0.05%) in combination with probiotic (0.1% *Bacillus subtilis* PB6) improved the overall performance and immunity of broilers. Thus, lauric acid along with probiotic could be safely included as an alternative to antibiotic growth promoter in broiler diets.

## ACKNOWLEDGEMENTS

The authors are thankful to the Department of poultry science, Rajendranagar, Hyderabad and ICAR- DPR, Hyderabad for providing the required support.

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Received on 03-07-2021 and accepted on 27-07-2021