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# Effect of *Lactobacillus* in Broiler Chicken Fed Diets Enriched with ω-3 Fatty Acids

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## ABSTRACT

A research was conducted to study the effect of *Lactobacillus* supplementation in broiler chicken fed diet enriched with ω-3 fatty acids. Two species of *Lactobacilli* were isolated from chicken gut and identified by *in vitro* sugar fermentation tests and by the gene sequencing of 16S RNA-23S RNA inter spacer region. The acid tolerance, bile tolerance and antioxidant activity test were done to determine the maximum probiotic potency by using probiotic score. *L. salivarius* with probiotic score of 170.94 compared to *L. agilis* with score of 129.14 was chosen for the feeding trial in broiler chicken. The freeze-dried *L. salivarius* exhibited 50% viability post freezing. In the feeding trial, the effect of *L. salivarius* at different level of supplementation *viz.,* $T_1$-without *L. salivarius* supplementation, $T_2$- supplemented with *L. salivarius* at $10^6$cfu/kg, $T_3$- supplemented with *L. salivarius* at $10^9$cfu/kg and $T_4$- supplemented with *L. salivarius* at $10^{12}$cfu/kg in broiler chicken fed diet enriched with ω-3 fatty acid was studied. Group $T_4$ showed significant ($P<0.05$) increase in weight gain and improvement in feed conversion ratio (FCR). There was no significant ($P>0.05$) difference in the meat quality parameters among treatments. The $T_1$ showed significantly ($P<0.05$) increased level of linolenic acid and ω-3 fatty acids (0.60 ± 0.1 and 6.96 ± 0.99 g/100 g total fatty acid (FA), respectively). There was no significant ($P>0.05$) increase in linoleic acid, eicosa-pentaenoic acid (EPA), docosahexaenoic acid (DHA) and ω-6 fatty acids among the treatments. But the $T_4$ showed numerical increase in EPA level (1.52 g/100 g total FA). It is concluded that $T_4$ with dietary ω-3 to ω-6 fatty acid ratio of 1:3 and *L. salivarius* inclusion of $10^{12}$cfu/kg feed had produced broiler meat with ω-3 to ω-6 fatty acid ratio of 1:2.17 and EPA level of 1.52 g/100 g Total FA.

**Key words:** *Lactobacillus*, Broiler chicken, ω-3 fatty acids, Eicosapentaenoic acid, Docosahexaenoic acid

## INTRODUCTION

Chicken meat is fast becoming a solution to increasing meat demand because of its high protein content as well as lower cholesterol and fat content. The deficiency of ω-3 fatty acids as well as imbalance of ω-3: ω-6 ratio in the human diet is considered to be an important reason for the prevalence of certain degenerative diseases. A hen living in nature freely will have a good balance between ω-3 and ω-6 fatty acids as it selects its own feed. But the poultry feed used by the farmers contain higher amount of ω-6 fatty acids compared to ω-3 fatty acids. This will result in a high concentration of the ω-6 fatty acids and less EPA and DHA in chicken meat.

Probiotics, classified as zootechnical feed additives (European Commission, 2003), improve the gastro intestinal health and intestinal function through the live beneficial microorganisms (Fuller, 1989). In order to obtain the numerous health benefits of probiotics, a minimum recommended level of viable microorganisms ($10^6$cfu/g) is necessary during consumption (Samona and Robinson, 1994). Bacteria belonging to different lactic acid bacteria (LAB) are available as probiotic supplements, but *lactobacilli* are the most commonly used.

Salma *et al.* (2007) reported that dietary supplementation of probiotic bacteria could improve fatty acid profile in broiler chicken. Ringo *et al.* (1998) demonstrated that dietary lipids had an effect on gastrointestinal flora, in particular, on the level of lactic acid bacteria. Kankaanpaa *et al.* (2001) showed that poly unsaturated fatty acids (PUFA) altered bacterial adhesion sites on Caco-2 cells. It is suggested that dietary PUFA affect mucosal adhesion sites for gastrointestinal bacteria by modifying the composition of the intestinal wall. Thus, probiotic bacteria and PUFA

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mutually benefit each other. So, in this experiment are studied the effect of *Lactobacillus* on the growth performance and meat quality parameters of broiler chicken fed diet enriched with ω-3 fatty acids.

## MATERIALS AND METHODS

Two strains of *Lactobacilli* were isolated from the mucosa of the gut of healthy 35 days old chicken and they were identified by sugar fermentation test (Lan *et al.,*2003) and confirmed by gene sequencing of 16S RNA-23S RNA inter spacer region. DNA was isolated from the culture as per the procedure of Pospiech and Neumann (1995). The Polymerase Chain Reaction (PCR) on synthesized DNA was carried out according to Seal *et al.* (1995). The 16S RNA-23S RNA interspacer region of the DNA was analyzed using gel extraction kit (Bio basic) as per the protocol given in the kit. Then the 16S RNA -23S RNA inter spacer region gene sequencing was done (Eurofin Pvt Ltd, Bangalore) on the eluted DNA and the sequences were compared with nonredundant nucleotides in the GenBank database using BLAST.

'Probiotics score' was considered as yardstick to identify the best species specific probiotics among probiotics organisms. 'Probiotics score' for chicken, among the isolated *Lactobacilli* species, was formed by considering the acid tolerance at pH 2, bile tolerance at 0.3 % bile acid in the MRS medium, and antioxidant ability of *Lactobacilli*.

The method of Khalil *et al.* (2007) was followed for acid tolerance test and bile tolerance test. Antioxidant activity was calculated by the reaction to thiobarbituric acid according to the methodology of Ohkawa *et al.* (1979). The experiments were conducted in triplicate.

The probiotics score was measured by assessing the cumulative points generated by each species for acid tolerance, bile tolerance at third hour of incubation and antioxidant ability of *Lactobacilli*. The cumulative points

**Table 1. Sugar fermentation pattern of *Lactobacillus* species from the isolates of the chicken gut**

| Sugars | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 | Isolate 5 | Isolate 6 |
|---|---|---|---|---|---|---|
| Lactose | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + |
| Fructose | + | + | + | + | + | + |
| Salicin | - | - | - | - | + | + |
| Arabinose | - | - | - | - | - | - |
| Sorbitol | $+_{w*}$ | + | + | $+_{w}$ | - | - |
| Xylose | - | - | - | - | - | - |
| Maltose | + | + | + | + | + | + |
| Melibiose | - | + | + | + | + | + |
| Mannose | + | + | + | + | + | + |
| Rhamnose | - | + | + | + | + | + |
| Raffinose | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + |
| Trehalose | - | - | - | - | + | + |
| Mannitol | - | - | - | - | + | + |
| Amygdalin | + | + | + | + | + | + |
| Esculin | - | - | - | - | + | + |
| Gluconate | - | - | - | - | - | - |
| Melezitose | - | - | - | - | - | - |

*$+_{w\ \text{Weak reaction}}$ + positive reaction – negative reaction

were calculated by summing up the per cent value of individual species for each test and this per cent value for each test was calculated by dividing the result of the respective test for individual species by total of results obtained for all the species for that particular test and then multiplied by 100. Whichever organism resulted in highest cumulative points was considered to have highest probiotics score and hence the best species specific probiotics (SSP).

The identified best species specific probiotics was preserved through freeze drying until further use by following the procedure adopted to freeze dry species specific probiotics (Pascaul *et al.,* 1999). The species specific probiotics in powder form in the vial was sealed with hand operated aluminium capper and stored at 4ºC until use. The freeze dried product containing species specific probiotics was evaluated for viable cell count by pour plate technique prior to usage.

Sugar fermentation test (Lan *et al.,* 2003) with 1 % peptone water was performed to identify the species of the isolated pure culture of the lactic acid bacteria. The experiment was conducted in triplicate. The results of sugar fermentation test were compared with Bergey's table (Kandler and Weiss, 1986) to identify the *Lactobacilli* species.

To assess the effect of *L. salivarius* on broiler chicken fed diet enriched with ω-3 fatty acids, a feeding trial was conducted for 35 days. The feeding trial had four experimental groups including control group ($T_1$) (without *L. salivarius*) and three treatment groups with $10^6$cfu/kg feed ($T_2$), $10^9$cfu/kg feed ($T_3$) and$10^{12}$cfu/kg feed ($T_4$) of *L. salivarius*. The composition of broiler starter (0-21 days) diet is shown in Table 4. Similarly, ingredient composition of broiler finisher (22-35 days) diet is shown in Table 5. The experimental diet was formulated as per BIS (2007) specifications.

In the *in vivo* trial, 120 numbers of day-old broiler chicks (COBB-400) belonging to a single hatch were purchased from a commercial farm. Three replicates of 10 birds were maintained for each treatment. The management practices adopted were as per the standards and were uniform for all the treatment groups. The birds were fed *ad libitum* quantity of their respective experimental diet in separate feed troughs. Clean drinking water was provided *ad libitum*. The birds were weighed individually once in a week in a calibrated balance to document their weight gains. The body weight was gender corrected. The feed conversion ratio of individual birds was calculated at 35 days by dividing the total feed consumed (kg) per bird with its respective

**Table 2. Acid tolerance test, bile tolerance test and antioxidant capacity of *Lactobacillus* species (Mean* ± SE)**

| TEST | HOURS | *Lactobacillus salivarius* | | *Lactobacillus agilis* | |
|---|---|---|---|---|---|
| | | Pre- freeze drying | Post- freeze drying | Pre- freeze drying | Post- freeze drying |
| Acid tolerance Test | 1st | 0.155$^{b}$±0.002 | 0.154$^{b}$±0.001 | 0.132$^{a}$±0.001 | 0.130$^{a}$±0.002 |
| (Optical density of | 2nd | 0.156$^{b}$±0.003 | 0.156$^{b}$±0.004 | 0.135$^{a}$±0.002 | 0.132$^{a}$±0.012 |
| MRS medium at | 3rd | 0.186$^{b}$±0.004 | 0.186$^{b}$±0.013 | 0.152$^{a}$±0.002 | 0.150$^{a}$±0.011 |
| hourly interval) | 4th | 0.193$^{b}$±0.006 | 0.191$^{b}$±0.004 | 0.164$^{a}$±0.001 | 0.157$^{a}$±0.001 |
| Bile tolerance test | 1st | 0.212$^{b}$±0.011 | 0.202$^{b}$±0.001 | 0.201$^{a}$±0.002 | 0.171$^{a}$±0.004 |
| (Optical density of | 2nd | 0.226$^{b}$±0.003 | 0.212$^{b}$±0.014 | 0.101$^{a}$±0.002 | 0.101$^{a}$±0.011 |
| MRS medium at | 3rd | 0.188$^{b}$±0.004 | 0.153$^{b}$±0.006 | 0.100$^{a}$±0.012 | 0.094$^{a}$±0.014 |
| hourly interval) | 4th | 0.152$^{b}$±0.007 | 0.126$^{b}$±0.012 | 0.089$^{a}$±0.002 | 0.071$^{a}$±0.006 |
| Antioxidant activity Malondialdehyde concentration (nmol/ml) | | 0.352$^{a}$±0.03 | 0.453$^{a}$±0.03 | 0.410$^{b}$±0.04 | 0.501$^{b}$±0.04 |

$^{a,b}$Means with different superscripts in a column differ (P≤0.05) *Mean of 3 observations

**Table 3. Percent viability and concentration of *L. salivarius* before and after lyophilization**

| Characteristics | Concentrations of *L. salivarius* (cfu)/ml | | |
|---|---|---|---|
| | $10^6$ | $10^9$ | $10^{12}$ |
| Viability before lyophilisation (cfu/ml) | $100 \times 10^6$ | $32 \times 10^9$ | $8.3 \times 10^{12}$ |
| Viability after lyophilisation (cfu/ml) | $50 \times 10^6$ | $17.33 \times 10^9$ | $4.33 \times 10^{12}$ |
| % Viability | 50.00 | 51.00 | 50.00 |
| Product yield after lyophilisation (g/ml) | 0.67 | 0.60 | 0.50 |
| Concentration of *L. salivarius* in lyophilized powder (cfu/g) | $75 \times 10^6$ | $29 \times 10^9$ | $9 \times 10^{12}$ |

cumulative body weight gain (kg).

The pH, water holding capacity (WHC), tyrosine value and thiobarbituric acid conent of breast meat sample from 6 birds randomly selected from each treatment were done on the day of slaughter, as well as on 1st, 3rd and 5th day after slaughter. The pH of the meat sample was measured using a digital pH meter (Digisun Electronic System, Model: 2001) by following the procedure of Trout *et al.* (1992). The water holding capacity of the meat was calculated by following the protocol outlined by Grau and Hamm (1957). The Tyrosine value of the meat was calculated by a modified method of Pearson (1968) as described by Strange *et al.* (1977). The Thiobarbituric acid content of the meat sample was calculated by a method of Strange *et al.,* (1977). Shear force value of meat sample was recorded by using Warner Bratzler Shear Press as per Bratzler (1954). At the time of slaughter, 1 cm

**Table 4. Ingredient composition (g/kg) of broiler starter (0-21 days) diet**

| Ingredients | Treatment groups | | | |
|---|---|---|---|---|
| | $T_1$ (Control) | $T_2$ ($10^6$cfu/kg feed) | $T_3$ ($10^9$cfu/kg feed) | $T_4$ ($10^{12}$ cfu/kg feed) |
| Maize | 531.5 | 531.5 | 531.5 | 531.5 |
| Soya bean meal | 390.0 | 390.0 | 390.0 | 390.0 |
| Palm oil | 30.0 | 30.0 | 30.0 | 30.0 |
| Linseed oil | 5.0 | 5.0 | 5.0 | 5.0 |
| Sardine oil | 5.0 | 5.0 | 5.0 | 5.0 |
| Dicalcium phosphate | 18.0 | 18.0 | 18.0 | 18.0 |
| Calcite | 11.5 | 11.5 | 11.5 | 11.5 |
| Vitamin-Mineral mixture* | 2.0 | 2.0 | 2.0 | 2.0 |
| L-Lysine | 0.3 | 0.3 | 0.3 | 0.3 |
| DL-Methionine | 1.7 | 1.7 | 1.7 | 1.7 |
| Common Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| **Calculated Values** | | | | |
| ME (kcal/kg) | 3102 | 3102 | 3102 | 3102 |
| Crude protein (%) | 22 | 22 | 22 | 22 |
| Lysine (%) | 1.2 | 1.2 | 1.2 | 1.2 |
| Methionine (%) | 0.5 | 0.5 | 0.5 | 0.5 |

*Provided per kilogram of diet: Vitamin A 10000 IU, Vitamin $D_3$ 3000 IU, Vitamin E 40 IU, Vitamin $K_3$ 1.5 mg Vitamin $B_{12}$ 0.01 mg, Biotin 0.15 mg, Choline 500 mg, Folic acid 1 mg, Niacin 40 mg, Pantothenic acid 15 mg, Pyridoxine 5.5 mg, Riboflavin 6.5 mg, Thiamine 2.5 mg, Copper 12 mg, Iodine 1.6 mg, Iron 80 mg, Manganese 100 mg, Zinc 80 mg.

thickness breast muscle was collected from 30 broiler carcasses with 2 birds selected randomly from each replicate contributing 6 birds per treatment and kept at – 20°C till measurement. Sensory evaluation was assessed by subjecting six meat samples from each treatment to a sensory scores of colour, odour and overall acceptability by trained and semi trained panel drawn from the Department of Livestock Products Technology (Meat Science), Madras Veterinary College, on a 9-point Hedonic scale of a standard score card. Data obtained were subjected to analysis of variance (ANOVA) using the package of SPSS (version 17.0) When significant differences (P<0.05) was detected, multiple range test was used to separate the mean values (Duncan, 1955).

## RESULTS AND DISCUSSION

The results of *in vitro* sugar fermentation test to identify *Lactobacillus* species from the isolates derived from chicken gut are presented in Table 1.The results revealed that sorbitol was not fermented by isolates 5 and 6 alone. Further, trehalose was fermented by isolates 5 and 6 alone similarly isolates 5 and 6 fermented mannitol. These fermentation features of isolates 5 and 6 differentiated them from isolates 1,2,3 and 4. The isolates were subjected to 16S-23S RNA interspacer region and further confirmed at *Lactobacillus* species level by sequencing. The isolates 1,2,3 and 4 were identified as *L. salivarius* and isolates 5 and 6 were *L. agilis*. The PCR products were observed in 1.5 % agarose gel electrophoresis and presented in plate 1.

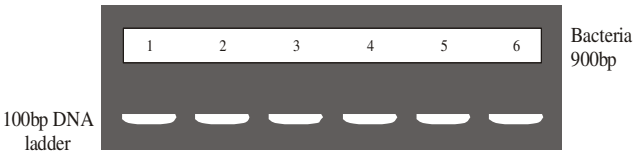


**Plate 1. Agarose gel electrophoresis of the isolates obtained from the chicken gut**

The gene sequence obtained from the isolates were then analysed with GenBank sequences using

**Table 5. Ingredient composition (g/kg) of broiler finisher (22-35 days) diet**

| Ingredients | Treatment groups | | | |
|---|---|---|---|---|
| | $T_1$ (Control) | $T_2$ ($10^6$cfu/kg feed) | $T_3$ ($10^9$cfu/kg feed) | $T_4$ ($10^{12}$ cfu/kg feed) |
| Maize | 580.5 | 580.5 | 580.5 | 580.5 |
| Soya bean meal | 331.7 | 331.7 | 331.7 | 331.7 |
| Palm oil | 38.0 | 38.0 | 38.0 | 38.0 |
| Linseed oil | 7.0 | 7.0 | 7.0 | 7.0 |
| Sardine oil | 5.0 | 5.0 | 5.0 | 5.0 |
| Dicalcium phosphate | 18.0 | 18.0 | 18.0 | 18.0 |
| Calcite | 11.5 | 11.5 | 11.5 | 11.5 |
| Vitamin-mineral mixture* | 2.0 | 2.0 | 2.0 | 2.0 |
| L-Lysine | 0 | 0 | 0 | 0 |
| DL-Methionine | 1.3 | 1.3 | 1.3 | 1.3 |
| Common Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| **Calculated Values** | | | | |
| ME (kcal/kg) | 3208 | 3208 | 3208 | 3208 |
| Crude protein (%) | 20 | 20 | 20 | 20 |
| Lysine (%) | 1.0 | 1.0 | 1.0 | 1.0 |
| Methionine (%) | 0.45 | 0.45 | 0.45 | 0.45 |

*Provided per kilogram of diet: Vitamin A 10000 IU, Vitamin $D_3$ 3000 IU, Vitamin E 40 IU, Vitamin $K_3$ 1.5 mg Vitamin $B_{12}$ 0.01 mg, Biotin 0.15 mg, Choline 500 mg, Folic acid 1 mg, Niacin 40 mg, Pantothenic acid 15 mg, Pyridoxine 5.5 mg, Riboflavin 6.5 mg, Thiamine 2.5 mg, Copper 12 mg, Iodine 1.6 mg, Iron 80 mg, Manganese 100 mg, Zinc 80 mg.

BLAST to check for homology. Based on sequencing data, *L. salivarius* and *L. agilis* were identified. The BLAST analysis revealed 99 % homology with respective reference strain available in GenBank.

The results of the acid tolerance test, bile tolerance test and antioxidant activity of the *Lactobacilli* is presented in the Table 2. *L. salivarius* exhibited highest (P<0.05) acid tolerance and bile tolerance and significantly (P<0.05) lower malondialdehyde concentration when compared to the *L. agilis,* both pre-freezing and post- freezing. The *Lactobacillus* species, *L. salivarius* showed maximum probiotic score of 170.95, when compared to *L. agilis* with probiotic score of 129.14. Hence *L. salivarius* was chosen as the best probiotic and freeze dried to be used for the *in vivo* feeding trial. Gunasekaran (2008) showed that out of four species of Lactobacillus obtained from the chicken gut (*L. salivarius*, *L. acidophilus*, *L. crispatus*, *L. fermentum*). *L. salivarius* with 93.4/300 was having the maximum probiotic score. Ever since, Mitsuoka (1969) isolated *L. acidophilus*, *L. fermentum* and *L. salivarius* from the chicken gastrointestinal tract, many workers identified *L. salivarius*, *L. acidophilus* and *L. fermentum* as predominating *Lactobacilli* species in the chicken gut.

Probiotics specificity differs from species to species because of their biotypes (Mitsuoka, 1969). If probiotic bacteria of the species are exchanged to another species, they do not colonize the gut mutually (Tannock *et al.,* 1982). Colonization of the gut is best achieved if the probiotic organism is derived from the same species of animal (Gibson and Fuller, 2000). Hence, in this study, probiotic bacterial isolates were derived from the gut of broiler chicken to evolve species specific probiotics.

Data on the viability percentage of freeze dried *L. salivarius* before and after freeze drying, for different inclusion levels to be used for *in vivo* feeding trial are presented in Table 3. After freeze drying the *L. salivarius* was evaluated for viability percentage which was found to be 50 to 51 %. Palmfeldt and Hahn-Hagerdal (2000) showed that *Lactobacillus reuteri* cultivated at pH 6 showed viability of 50 % irrespective of harvest time once it entered the stationary phase.

The viability percentage of a freeze dried bacteria depends upon many factors including the protectant used as well as pH. Palmfeldt and Hahn-Hagerdal (2000) showed that at pH 5 the culture of *L. reuteri* showed 80 % survivability at 2.5 hours after entering the stationary phase. Zayed and Roos (2004) showed that when trehalose and sucrose were added in addition to skim milk it gave a survival rate of 83–85 % immediately after freeze-drying, and enhanced stability during subsequent storage. Jalali *et al.* (2012) showed that maximum survival rate of 72-76 % for *L. paracasei* subsp. *tolerance* and *L. delbrueckii* subsp. *bulgaricus* with media containing 6 % skim milk, 8 %

**Table 6. Effect of *L. salivarius*on weekly weight gain and cumulative FCR in chicken fed ω- 3 enriched diets (Mean* ± SE)**

| Treatment (ω-3 : ω-6) 1 : 3+ *L. salivarius*(cfu/ kg feed) | Weekly weight gain (g) NS | | | | | Cumulative | |
|---|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | WG | FCR NS |
| $T_1$ (Control) | 108.70±0.88 | 302.9±1.8 | 350.6$^{a}$±1.02 | 402.3$^{a}$±1.12 | 421$^{a}$±2.10 | 1585.5$^{a}$±6.1 | 1.874$^{b}$±0.02 |
| $T_2$ (*L. salivarius* $10^6$) | 110.70±0.9 | 302.6±2.02 | 351.6$^{a}$±2.07 | 401.4$^{a}$±1.03 | 424.9$^{a}$±2.13 | 1591.2$^{a}$±7.3 | 1.864$^{b}$±0.04 |
| $T_3$ (*L. salivarius* $10^9$) | 113.2±1.5 | 304.2±1.9 | 354.5$^{a}$±1.01 | 405$^{a}$±1.05 | 427.2$^{a}$±1.19 | 1604.1$^{a}$±5.7 | 1.844$^{b}$±0.05 |
| $T_4$ (*L. salivarius* $10^{12}$) | 115.2±1.2 | 309.1±2.08 | 379.2$^{b}$±1.09 | 458.8$^{b}$±1.19 | 511.2$^{b}$±1.87 | 1773.5$^{b}$±5.8 | 1.748$^{a}$±0.11 |

*Mean of 30 observations; $^{a, b}$ means with different superscripts in a column differ (P≤0.05); NS – Not significant (P>0.05); WG - weight gain; FCR - feed conversion ratio

trehalose and 4 % sodium ascorbate.

The freeze dried *L. salivarius* had count of $75\times10^6$cfu/g, $29\times10^9$cfu/g and $9\times10^{12}$/g as assessed by viable cell count by pour plate technique. In order to obtain $10^6$, $10^9$ and $10^{12}$cfu/kg feed in broiler feed, the freeze dried product were included at the rate of 0.013g, 0.034g and 0.11g/kg feed, respectively. Gunasekaran (2008) produced freeze dried *L. salivarius* with $10^{13}$cfu/g.

Data pertaining to the effect of *L. salivarius* on weekly weight gain and feed conversion ratio is presented in Table 6. The treatment $T_4$ with *L. salivarius* inclusion at $10^{12}$ cfu/kg feed had significantly ($P<0.05$) higher weekly weight gain at third, fourth and fifth week (379.2 ± 1.09, 458.8 ± 1.19 and 511.2 ± 1.87 g, respectively) and had significant ($P<0.05$) improved feed efficiency (1.748± 0.11). The $T_4$ group also showed significant ($P<0.05$) increase in cumulative weight gain (1773.5 ± 5.8). The improvements in body weight and feed to gain ratio of broilers fed *Lactobacillus* supplement were probably due to the *Lactobacillus* spp. used in the supplement. It has been suggested that to obtain the best effects from *Lactobacillus* as a growth promotant, the bacteria used must be able to survive the gastrointestinal tract so that their beneficial functions could be performed (Jin *et al.,* 1996). The *Lactobacillus* spp. used in the present study are resistant to the bile and acidic conditions as shown by the results of acid tolerance test and bile tolerance test. This may be the reason for their positive influence on the weight gain and feed conversion ratio. Peng *et al.*(2016) showed that when the basal diet was mixed with *L. plantarum* at the dose rate of $2x10^9$cfu/kg the average daily weight gain and feed conversion ratio improved ($P<0.05$) in broiler chicken.

Data pertaining to the effect of *L. salivarius* on

**Table 7. Effect of *L. salivarius* on the fatty acid (FA) concentrations (g/100 g total FA) of broiler chicken breast meat (Mean* ± SE)**

| Fatty acid | Dietary concentration of *L. salivarius* (cfu/kg feed) | | | |
|---|---|---|---|---|
| | $T_1$ (Control) | $T_2$ ($10^6$) | $T_3$ ($10^9$) | $T_4$ ($10^{12}$) |
| Myristic acid (C14:0) NS | 0.60±0.03 | 0.56±0.07 | 0.60±0.04 | 0.47±0.16 |
| Palmitic acid (C16:0) | $23.95^a$±0.27 | $23.47^a$±0.43 | $25.51^b$±0.35 | $23.77^a$±0.51 |
| Stearic acid (C18:0) NS | 12.01±0.37 | 12.12±0.78 | 11.30±0.40 | 12.05±0.23 |
| Arachidic acid (C20:0) | $0.6^a$±0.03 | $0.69^{ab}$±0.06 | $0.77^b$±0.06 | $0.67^{ab}$±0.05 |
| Behenic acid (C22:0) NS | 6.99±0.44 | 7.35±0.42 | 6.28±1.1 | 7.92±0.46 |
| Oleic acid (C18:1 ω -9) NS | 23.66±1.06 | 24.88±1.06 | 23.94±0.42 | 23.57±0.87 |
| Palmitoleic acid (C16:1 ω -7) NS | 1.05±0.22 | 1.46±0.26 | 1.09±0.07 | 1.03±0.18 |
| Linoleic acid (C18:2 ω -6) NS | 14.92±0.73 | 16.05±0.25 | 15.56±0.33 | 14.84±0.39 |
| Linolenic acid (C18:3 ω -3) | $0.60^b$±0.1 | $0.50^{ab}$±0.05 | $0.51^{ab}$±0.03 | $0.38^a$±0.04 |
| EPA (C20:5 ω -3) NS | 1.43±0.05 | 1.50±0.11 | 1.36±0.06 | 1.52±0.08 |
| DHA (C22:6 ω -3) NS | 4.91±0.16 | 4.33±0.16 | 4.7±0.15 | 4.91±0.20 |
| Others | $9.26^b$±0.61 | $7.10^a$±0.68 | $8.38^{ab}$±0.18 | $8.87^b$±0.35 |
| SFA NS | 43.15±1.01 | 43.19±0.81 | 44.46±0.48 | 4.88±0.54 |
| MUFA | $24.71^a$±0.83 | $26.34^b$±0.72 | $25.03^{ab}$±0.61 | $24.6^a$±0.72 |
| ω 3 | $6.96^b$±0.19 | $6.32^a$±0.13 | $6.40^a$±0.13 | $6.8^{ab}$±0.15 |
| ω 6 NS | 14.92±0.73 | 16.05±0.25 | 15.73±0.33 | 14.84±0.39 |
| PUFA NS | 21.88±0.14 | 22.37±0.44 | 22.13±0.53 | 21.64±0.13 |
| ω -3 to ω -6 ratio | 1:2.1 | 1:2.56 | 1:2.44 | 1:2.17 |

*Mean of 6 observations ; $^{a,b,c,d}$means with different superscripts in a column differ ($P\leq0.05$) ; NS – Not significant ($P>0.05$)

the fatty acid profile of broiler chicken breast meat is presented in Table 7. There was no significant (P>0.05) difference in linoleic acid, EPA, DHA and ω-6 fatty acids content among the groups. The $T_1$ groups showed significantly (P<0.05) higher level of linolenic acid and ω-3 FA (fatty acids) (0.60 ± 0.1 and 6.96 ± 0.99g/100 g of total FA, respectively). The short chain fatty acids did not differ significantly among treatments which ranged from 43.15±1.01 to 44.88± 0.54 g/100 g of total FA.

The $T_2$ group showed significantly (P<0.05) higher MUFA percentage (26.34 g/100 g of total FA) as compared to other groups. Present study did not result in any significant increase in EPA and DHA level among groups, however, *L. salivarius* supplementation at $10^{12}$cfu resulted in numerical increase in EPA level (1.52 g/100 g of total FA) as compared to the control (1.43 g/100 g of total FA) which is 6.29 % more than that of control. Similarly, dose rate of *L. salivarius* did not influence the EPA and DHA level among *L. salivarius* supplemented treatments.

There was also no significant difference in ω-3 to ω-6 ratio between $T_1$ and $T_4$. The ω-3 to ω-6 ratio was significantly increased at L. *salivarius* supplementation at $10^6$ and $10^9$ cfu/kg feed when compared to control and *L. salivarius* supplementation

**Table 8. Effect of *L.salivarius* on the meat quality parameters of broiler chicken fed diet enriched with ω 3 fatty acids (Mean* ± SE)**

| Meat quality parameters | Days | Treatments | | | |
|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| pH | 0 NS | 6.15±0.03 | 6.15±0.02 | 6.12±0.01 | 6.15±0.02 |
| | 1 NS | 5.78±0.02 | 5.77±0.01 | 5.78±0.01 | 5.77±0.01 |
| | 3 NS | 6.08±0.01 | 6.08±0.01 | 6.07±0.01 | 6.07±0.01 |
| | 5 NS | 6.18±0.01 | 6.18±0.02 | 6.17±0.01 | 6.17±0.01 |
| WHC % | 0 NS | 66.62±1.9 | 66.67±1.4 | 67.26±2.4 | 67.24±1.8 |
| | 1 NS | 62.89±1.0 | 62.14±1.1 | 62.88±2.1 | 62.61±1.4 |
| | 3 NS | 60.57±2.9 | 60.63±1.4 | 59.92±2.5 | 59.95±2.8 |
| | 5 NS | 58.18±1.5 | 58.81±1.2 | 57.78±1.1 | 57.86±1.1 |
| Tyrosine value(mg/ | 0 NS | 13.49±1.04 | 13.33±0.95 | 12.88±1.02 | 13.57±1.02 |
| 100 g meat) | 1 NS | 16.49±0.59 | 16.31±0.89 | 16.34±1.11 | 16.52±0.71 |
| | 3 NS | 18.27±0.85 | 18.46±1.17 | 17.98±0.95 | 18.04±0.84 |
| | 5 NS | 19.16±0.44 | 19.47±0.55 | 18.85±0.22 | 19.13±0.62 |
| Thiobarbituric acid | 0 NS | 0.051±0.001 | 0.050±0.001 | 0.048±0.001 | 0.047±0.001 |
| (Malonaldehyde,mg/kg) | 1 NS | 0.070±0.001 | 0.068±0.003 | 0.069±0.003 | 0.070±0.003 |
| | 3 NS | 0.109±0.002 | 0.109±0.003 | 0.109±0.002 | 0.107±0.002 |
| | 5 NS | 0.127±0.002 | 0.127±0.00 | 0.126±0.003 | 0.127±0.003 |
| Shear force value ($kg/cm^2$)NS | | 0.91±0.07 | 0.91±0.03 | 0.92±0.05 | 0.91±0.04 |
| Sensory Characters | AppearanceNS | 6.60±0.37 | 7.00±0.21 | 7.33±0.23 | 7.33±0.45 |
| | FlavourNS | 6.00±0.61 | 7.00±0.21 | 7.00±0.26 | 7.33±0.37 |
| | JuicinessNS | 5.83±0.62 | 7.17±0.31 | 6.67±0.37 | 6.83±0.61 |
| | TendernessNS | 6.33±0.54 | 7.33±0.33 | 7.33±0.37 | 7.17±0.60 |
| | Overall acceptabilityNS | 6.67±0.86 | 6.67±0.56 | 7.00±0.60 | 7.00±0.31 |

*Mean of 6 observations; NS – Not significant (P>0.05)

at $10^{12}$cfu/kg.

The *L. salivarius* supplementation showed non-significant difference (P>0.05) in the pH, WHC, tyrosine value, thiobarbituric acid value, shear force value and sensory characterstics of chicken meat between the treatments as shown in Table 8. Yang and Chen (1993) reported that pH value was 6.23 and increased by extended storage which is similar to the present study. Meng *et al.* (2010) observed increase in pH values in growing pigs when fed with combination of *Bacillus* and *Clostridium* in diets with high energy and crude protein content. Si *et al.* (2006) suggested that the diet is one of the main factors that modified the antimicrobial effect *in vitro*. They suggested that the different percentage of crude protein and energy levels will alter the influence of probiotics on meat quality by influencing their actions in GI tract.

## CONCLUSION

Supplementation of *Lactobacillus salivarius* at the dose rate of $10^{12}$cfu/kg to ω-3 fatty acids enriched diet will produce broiler chicken meat with ω-3: ω-6 ratio of 1:2.17, and increase weight gain and feed conversion ratio in broiler chicken.

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