Dietary Effects of *Lactobacillus acidophilus* Probiotic on the Haematological Parameters, Total Serum Proteins and Lipids of Lahore Pigeon (*Columbia livia*)

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Abstract: The probiotic *Lactobacillus acidophilus* was given to Lahore pigeons to study the changes in the haematological parameters, total serum proteins and lipids in their blood. This probiotic has increased the PCV, RBCs, haemoglobin, TLCs, basophils, eosinophils, monocytes, lymphocytes, total serum proteins and serum lipids but has decreased the heterophils count in the pigeons. Production of more number of leukocytes is the direct indication of the enhancement of innate immunity that is the first hand mechanism to protect pigeons from various pathogens. This probiotic improved the level of serum protein and lipids that are good indicators of layer quality. So, it will be a good nutritional supplement to pigeons.

Keywords: Lahore pigeons, *Lactobacillus acidophilus*, probiotic, Haematological parameters, serum proteins, lipids

1. Introduction

Probiotics are standardized pure or mixed cultures of harmless bacteria or yeasts that give marked performance result in animals when they are used as feed supplements; they modify the natural microflora of intestine in such a way as to enhance the feed utilization ratio of birds (Fuller, 1989). They are potential alternatives to conventional antibiotics for preventing the intestinal colonization of pathogenic bacteria (Stuart, 1984) and they have growth promoting capability for which they have been recommended as direct fed microbes in animal feeds (Anadan et al., 2006). Recently probiotics containing Lactobacilli have been recommended as dietary supplements to aquatic animals, fishes, poultry, turkey, duck, cattle and humans for their health benefits.

Since Lactobacilli are the components of the normal microflora of the intestine of most animals and birds and since they produce lactic acid that is a component of glucose metabolism in the intestinal cells, they are found to be superior to the other species that have been currently used as probiotics (Guerra et al., 2007). At present *Lactobacillus plantarum*, *L. casei*, *L. acidophilus* and *L. bulgaricus* have widely been used as a potential probiotic to tone up the immune response and growth attributes in fishes (Chelladurai et al., 1912), shrimps (Moriarity, 1999), humans (Szajewska et al., 2001), mouse (Alak et al., 1997), chicken (Dalloul et al., 2003), and cattle (Casas and Dobrogosz, 2000; Marie-Agnès Travers et al., 2011). They have been included in several formulations being recommended as tonics for domesticated birds, veterinary animals and man.

Although many species of Lactobacilli are living in the gut of young chicks, only a few of them survive in the gut of 6-8 weeks old chicks since most of them are eliminated by the fluctuations in the pH of intestinal fluids (Kim et al., 1996). As soon as the beneficial microbes are eliminated, some dreadful microbes come into colonize the gut surface, which results in intestinal problems in the birds and reduction in the growth and reproductive attributes of the birds (Fuller, 1986). *Lactobacillus acidophilus* is one of the species of gut microflora gradually being depleted from the intestine of fowls and pigeons (Ng et al., 2009). This probiotic can tolerate pH as low as 2.5 for 4 hours (Jacobsen et al., 1999), tolerate 0.3% of bile salts (Liong and Shah, 2005), inhabit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella enteric* (Gauri Dixit et al., 2013), and can adhere to the intestinal cells at the rate of 123-158/100 epithelial cells (Sarem et al., 1996). Probiotic use of this bacterium cures antibiotic-induced diarrhoea (Pochapin, 2000), prevents colon cancer (Wollowski et al., 2001) and stimulates the innate immunity of hosts (Isolauri et al., 2001). In germ-free chickens, *L. acidophilus* has elevated the levels of total serum protein and hemoglobin concentration (Pollmann et al., 1980), which is also true with broiler chickens (Abdul-Rahman et al., 1994) and Japanese quail (Abd El-Azeem et al., 2001). Abdul Rahim et al (1996) have shown that use of *Lactobacillus acidophilus* to chickens has increased the layer-quality of the chickens and lowered the cholesterol content in plasma and egg yolk. Tollba and Mahmood (2009) have shown that there is a significant increase in counts of erythrocytes (RBC’s), leukocyte (WBC’s), lymphocytes, eosinophils and basophils, while heterophil count is low when chicken are fed with Lactobacilli at normal temperature (23ºC). Lilhehoj and Chung (1992) had reported elevated lymphocytes count in the intestinal propria and blood of chicken receiving Lactobacilli probiotic.

There has hardly been any scientific work on the probiotic use of *L. acidophilus* to pigeons. This present study aims at investigating the changes in the haematological parameters, serum proteins and lipids when Lahore pigeon are fed with *L. acidophilus*.  

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2. Materials and Methods

2.1. Birds and Experimental Design

One-year old Lahore pigeons (Columba livia domestica; family: Columbidae; order: Columbiformes) were chosen as the experimental birds for this study. 50 pairs of pigeons were divided into five groups each with 10 pairs and every group was grown in a separate loft of 5’ x 7’ x 3’ size. The lofts were constructed with wooden frame, steel plated roof and wire mesh floor and lateral sides. These lofts were kept at a height of 2.5’ from the ground level for reducing dampness facilitating the rapid spreading of pathogenic germs. Feed mixture (Table-1) was given at the rate of 120 ml per pair/day. Vitamins required for the birds were provided along with the drinking water at the rate of 5ml of Vimeral® (vitamin mix)/ 1 liter water. This feed composition was maintained throughout the study period for feed uniformity in the experimental pigeon groups.

Lactobacil - the trade name of probiotics being manufactured by Infar (India) Limited - containing not less than 10 million lyophilized cells of Lactobacillus acidophilus was dissolved in distilled water so as to have the concentrations of 10^1, 10^3, 10^5 and 10^7 cells / ml respectively. 10 ml of suitable dilution of Lactobacilli was added into the drinking water bowel kept in the respective lofts for providing the required number of lactobacilli cells to each and every bird in the lofts.

Group I: Normal feed only (control)
Group II: Normal feed + 10^1 cells of Lactobacilli / day
Group III: Normal feed + 10^3 cells of Lactobacilli / day
Group IV: Normal feed + 10^5 cells of Lactobacilli / day
Group V: Normal feed + 10^7 cells of Lactobacilli / day

2.2. Collection of Blood

The wing surface at the elbow joint was sterilized by wiping with cotton soaked with surgical spirit and blood sample was taken from the jugular vein through vein puncture using 23 G sterile hypodermic needle of Dispovan Insulin syringe. About 2 ml of blood was taken in from a pigeon, as done by Oladele et al. (2008), on the day of experiment and the samples taken from a pair of birds were pooled together as one sample (4 ml) for investigation. Of this, 2 ml is stored in labeled Bijou bottles containing ethylene diamine tetra acetic acid (EDTA) at the concentration of 2mg/ml as anti-coagulant for the study of haematological parameters and the remaining 2 ml blood was stored in yet other labeled bottle without any anti-coagulant for the preparation of serum. Thus samples were taken from one pair in each group at the regular interval of 7 days.

2.3. Haematological Parameters

Hematological parameters like packed cell volume (PCV), red blood cells (RBC) count, haemoglobin (Hb) concentration, total leukocytic count (TLC) and differential count for heterophilis, basophilis, eosinophilis, monocytes and lymphocytes were determined using standard techniques described by Rehman et al. (2003).

2.4. Preparation of Serum

2 ml of each blood sample was taken in a test tube and its mouth was closed with a cotton plug. The test tube was kept undisturbed at 37°C for one hour and then the blood was centrifuged at 2000g for 10 minutes. Serum in the fluid was carefully poured into a screw-cap tube and stored at -20°C for the further study.

2.5. Protein Estimation

The total serum protein content was estimated with the VetScan® Blood Analyzer (Abaxis, Inc.) using the standard protocol described in the VetScan System Operator’s manual, 2000. The results were expressed in grams per deciliter (dL).

2.6. Lipids Estimation

Total serum lipid content (total cholesterol + triglycerides) was estimated with the automatic blood Analyzer Prietest Easylab 2.1 (Robonick Company, USA) using the operator’s User manual and the results were expressed in mg/ml.

3. Results

3.1. Packed Cell Volume (PCV)

The PCV of pigeons fed with Lactobacilli had increased significantly (p<0.05) over the PCV of control group pigeons (Table 2). Oral administration of 10^1, 10^3, 10^5 and 10^7 Lactobacilli per day increased the PCV (from 49.7±1.41% - 0.61 x 10^7) to 46.9±1.42, 48.7±1.39, 49.8±1.71, 49.7±1.41% respectively. In all the experimental groups, except control, there was a notable increase in the PCV from the 7th day to 35th day. The PCV was high on the 21st day at the dietary administration of 10^5 and 10^7 cells / day.

3.2. Red Blood Corpuscles (RBC) Count

Table 3 shows that the RBC count of pigeons fed with Lactobacilli had increased significantly (p<0.05) compared to that of control group pigeons. Dietary supplementation of 10^1, 10^3, 10^5 and 10^7 Lactobacilli per day increased the RBC count of pigeons fed with Lactobacilli at the concentrations of 10^1, 10^3, 10^5 and 10^7 cells / ml respectively instead of 2.8±0.29 - 0.61 x 10^6/dL in control. Lactobacilli treatment had enhanced the RBC count of experimental groups in due course from the 7th day to 35th day, but the highest RBC count was recorded on the 35th day at the dietary administration of 10^5 and 10^7 cells / day.

3.3. Haemoglobin Content

Data in the table 4 reveals that the haemoglobin level of pigeons fed with Lactobacilli had increased significantly (p<0.05) compared to that of control group pigeons, Lactobacilli at the concentrations of 10^1, 10^3, 10^5 and 10^7 cells / day increased the haemoglobin content from...
9.83±0.72 g/dL (control) to 9.93±0.73, 11.11±0.33, 12.13±0.28 and 12.13±0.48 g/dL. Lactobacilli had slightly increased the haemoglobin content of pigeons starting from 7th day to 35th day, but the peak was observed on the 28th and 35th days at the higher doses.

3.4. Total Leucocytes Count (TLC)

Results in the Table 5 make out a clear point that the TLC of pigeons fed with Lactobacilli significantly (p<0.05) differed from the control group. The dietary supply of 10^1, 10^1, 10^3 and 10^7 Lactobacilli/day increased the TLC to 25.34±1.10, 26.22±1.28, 28.28±1.28, 28.78±1.18 x 10^9/L respectively instead of 24.54±1.13 x 10^9/L in control. All the experimental groups, except control, showed a rise in the TLC from the 7th day to 35th day. The highest TLC value was noted on the 35th day at the highest dosage of administration.

**Table 1:** Composition of normal feed.

| Ingredients       | Percentage |
|-------------------|------------|
| Wheat grains      | 35%        |
| Finger millet     | 15%        |
| Pearl millet      | 15%        |
| Green pea         | 30%        |
| Grid*             | 4.97%      |
| Vimeral **        | 0.5 ml/pair|

*Grid: 1 kg contains 100 g charcoal, 100g egg shell, 75g limestone, 150g table salt and 575g brick powder; **Vimeral ®: 1ml contains vitamin A -12,000 IU; Vitamin B12 – 20 mcg; vitamin D2 -6,000 IU; and vitamin E -40mg.

Table 2: Change in the PCV of pigeon’s blood due to the dietary supply of Lactobacilli. (%)

| Time interval | Normal feed (Control) | Normal feed + 10^1 Lactobacilli / day | Normal feed + 10^3 Lactobacilli / day | Normal feed + 10^5 Lactobacilli / day | Normal feed + 10^7 Lactobacilli / day |
|---------------|-----------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 7th day       | 46.4±1.65* a          | 46.9±1.24* a                           | 47.4±1.39* a                           | 47.7±1.45* a                           | 47.9±1.42* a                           |
| 14th day      | 46.4±1.66* a          | 46.9±1.29* a                           | 48.2±1.62* a                           | 49.8±1.26* a                           | 49.8±1.19* a                           |
| 21st day      | 46.4±1.67* a          | 46.9±1.32* a                           | 48.3±1.59* a                           | 49.9±1.29* a                           | 49.8±1.21* a                           |
| 28th day      | 46.4±1.83* a          | 46.9±1.43* a                           | 48.4±1.53* a                           | 49.4±1.72* a                           | 49.4±1.31* a                           |
| 35th day      | 46.4±1.71* a          | 46.9±1.42* a                           | 48.7±1.39* a                           | 49.8±1.71* a                           | 49.7±1.41* a                           |

Figure after ± represents standard deviation; n =10 pairs; * not significant; ** (p<0.05); *** (p<0.01).

Table 3: Change in the RBC count of pigeon’s blood due to Lactobacilli probiotic (N x 10^9/L).

| Time interval | Normal feed (Control) | Normal feed + 10^1 Lactobacilli / day | Normal feed + 10^3 Lactobacilli / day | Normal feed + 10^5 Lactobacilli / day | Normal feed + 10^7 Lactobacilli / day |
|---------------|-----------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 7th day       | 2.8±0.39* a           | 2.9±0.28* a                           | 3.3±0.27* a                           | 4.0±0.62* a                           | 4.1±0.42* a                           |
| 14th day      | 2.8±0.52* a           | 2.9±0.42* a                           | 3.3±0.36* a                           | 3.4±0.57* a                           | 3.4±0.36* a                           |
| 21st day      | 2.8±0.29* a           | 2.9±0.39* a                           | 3.3±0.21* a                           | 3.2±0.31* a                           | 3.3±0.41* a                           |
| 28th day      | 2.8±0.34* a           | 2.9±0.44* a                           | 3.3±0.41* a                           | 3.4±0.73* a                           | 3.4±0.78* a                           |

Figure after ± represents standard deviation; n =10 pairs; * not significant; ** (p<0.05); *** (p<0.01).

Table 4: Change in the Haemoglobin level of pigeons due to Lactobacilli probiotic (g/dl)

| Time interval | Normal feed (Control) | Normal feed + 10^1 Lactobacilli / day | Normal feed + 10^3 Lactobacilli / day | Normal feed + 10^5 Lactobacilli / day | Normal feed + 10^7 Lactobacilli / day |
|---------------|-----------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 7th day       | 9.83±0.69* a          | 9.91±0.69* a                           | 10.32±0.16* a                          | 11.29±0.46 a                          | 11.30±0.21 a                          |
| 14th day      | 9.83±0.70* a          | 9.93±0.70* a                           | 10.22±0.34* a                          | 12.12±0.54 a                          | 12.13±0.14 a                          |
| 21st day      | 9.83±0.69* a          | 9.93±0.71* a                           | 11.10±0.60 b                          | 12.13±0.90 b                          | 12.14±0.40 b                          |
| 28th day      | 9.83±0.68* a          | 9.93±0.72* a                           | 11.11±0.33 b                          | 12.12±0.73 a                          | 12.13±0.33 b                          |
| 35th day      | 9.83±0.72* a          | 9.93±0.73* a                           | 11.11±0.33 b                          | 12.13±0.28 b                          | 12.13±0.48 a                          |

Figure after ± represents standard deviation; n =10 pairs; * not significant; ** (p<0.05); *** (p<0.01).

Table 5: Total Leucocytes Count (TLC) of pigeons fed with Lactobacilli. (N x 10^3/µl)

| Time interval | Normal feed (Control) | Normal feed + 10^1 Lactobacilli / day | Normal feed + 10^3 Lactobacilli / day | Normal feed + 10^5 Lactobacilli / day | Normal feed + 10^7 Lactobacilli / day |
|---------------|-----------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 7th day       | 24.54±1.12* a         | 24.74±1.16* a                          | 25.26±1.12 a                          | 26.67±2.2 a                           | 26.84±1.12 b                          |
| 14th day      | 24.54±1.12* a         | 25.23±1.13* a                          | 26.20±2.20 a                          | 28.28±2.22 a                          | 28.43±2.23 b                          |
| 21st day      | 24.54±1.13* a         | 25.34±1.13* a                          | 26.18±1.22 b                          | 28.28±1.28 b                          | 28.58±1.22 b                          |
| 28th day      | 24.54±1.12* a         | 25.34±1.10* a                          | 26.22±1.28 a                          | 28.28±1.28 b                          | 28.72±1.18 b                          |
| 35th day      | 24.54±1.12* a         | 25.34±1.13* a                          | 26.22±1.17 a                          | 28.28±1.21 b                          | 28.68±1.20 b                          |

Figure after ± represents standard deviation; n =10 pairs; * not significant; ** (p<0.05); *** (p<0.01).
3.5. Heterophils Count

Dietary administration of Lactobacilli had significantly (p<0.05) reduced the heterophils count compared to that of the control group. Daily supply of $10^1$, $10^3$, $10^5$ and $10^7$ Lactobacilli notably enhanced the basophils count to 0.47±0.9, 0.64±0.11, 0.64±0.11 and 0.64±0.11/µl respectively. However, this modulating effect was found to be the maximum at high doses (300mg and 400mg /day) on the 35th day.

3.6. Basophils Count

Table 7 shows that basophils count in pigeons fed with Lactobacilli was significantly different from the control group. Daily supply of $10^1$, $10^3$, $10^5$ and $10^7$ Lactobacilli had lowered the heterophils count to 0.47±0.9, 0.64±0.11, 0.64±0.11 and 0.64±0.11/µl (Table 8).

Figure after ± represents standard deviation; n =10 pairs; * not significant; a = (p<0.05); b = (p<0.01).

| Time interval | Normal feed (Control) | Normal feed + $10^1$ Lactobacilli / day | Normal feed + $10^3$ Lactobacilli / day | Normal feed + $10^5$ Lactobacilli / day | Normal feed + $10^7$ Lactobacilli / day |
|---------------|-----------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| 7th day       | 0.45±0.9             | 0.46±0.8      | 0.48±0.9       | 0.51±0.9       | 0.51±0.8       |
| 14th day      | 0.45±0.11            | 0.47±0.7     | 0.50±0.10      | 0.61±0.12      | 0.62±0.6       |
| 21st day      | 0.45±0.9             | 0.47±0.9     | 0.51±0.8       | 0.64±0.11      | 0.64±0.12      |
| 28th day      | 0.44±0.83            | 0.47±0.7     | 0.51±0.8       | 0.64±0.11      | 0.63±0.11      |
| 35th day      | 0.46±0.9             | 0.47±0.9     | 0.51±0.8       | 0.64±0.11      | 0.64±0.9       |

Figures after ± represent standard deviation; n =3 birds; * not significant; a = (p<0.05); b = (p<0.01).

Table 8: Basophils count of pigeons fed with Lactobacilli probiotic. (N x 10^3/µl)

| Time interval | Normal feed (Control) | Normal feed + $10^1$ Lactobacilli / day | Normal feed + $10^3$ Lactobacilli / day | Normal feed + $10^5$ Lactobacilli / day | Normal feed + $10^7$ Lactobacilli / day |
|---------------|-----------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| 7th day       | 0.38±0.9             | 0.38±0.8      | 0.38±0.9       | 0.39±0.9       | 0.39±0.9       |
| 14th day      | 0.38±0.10            | 0.39±0.13     | 0.39±0.14      | 0.40±0.7       | 0.40±0.8       |
| 21st day      | 0.38±0.12            | 0.39±0.15     | 0.39±0.17      | 0.40±0.9       | 0.40±0.9       |
| 28th day      | 0.38±0.9             | 0.39±0.16     | 0.39±0.18      | 0.40±0.10      | 0.40±0.11      |
| 35th day      | 0.38±0.9             | 0.39±0.18     | 0.39±0.18      | 0.40±0.11      | 0.40±0.12      |

Figures after ± represent standard deviation; n =10 birds; * not significant; a = (p<0.05); b = (p<0.01).

Table 9: Monocytes count in the blood of pigeons supplied with Lactobacilli. (N x 10^3/µl)

| Time interval | Normal feed (Control) | Normal feed + $10^1$ Lactobacilli / day | Normal feed + $10^3$ Lactobacilli / day | Normal feed + $10^5$ Lactobacilli / day | Normal feed + $10^7$ Lactobacilli / day |
|---------------|-----------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| 7th day       | 1.21±0.8             | 1.23±0.6      | 1.30±0.9      | 1.35±0.10     | 1.36±0.9      |
| 14th day      | 1.22±0.6             | 1.30±0.7     | 1.41±0.9      | 1.50±0.9      | 1.51±0.8      |
| 21st day      | 1.22±0.4             | 1.33±0.6     | 1.46±0.8      | 1.51±0.8      | 1.52±0.4      |
| 28th day      | 1.21±0.5             | 1.35±0.3     | 1.46±0.3      | 1.50±0.3      | 1.50±0.11     |
| 35th day      | 1.23±0.4             | 1.36±0.4     | 1.46±0.8      | 1.51±0.10     | 1.51±0.11     |

Figures after ± represent standard deviation; n =3 birds; * not significant; a = (p<0.05); b = (p<0.01).
count of experimental groups compared to the control. The highest modulating effect was observed at high doses (300mg and 400mg /day) on the final day of the experiment.

3.8. Monocytes Count

Dietary supply of $10^1$, $10^3$, $10^5$ and $10^7$ Lactobacilli per day increased the monocytes count from $1.21\pm0.8$ to $1.36\pm0.4$, $1.46\pm0.8$, $1.51\pm0.10$, $1.51\pm0.11 \times 10^6$/µl respectively (Table 9). Monocytes count of pigeons receiving Lactobacilli was increased significantly ($p<0.01$) compared to the control. The monocytes count was increasing slightly but surely from the 7th day to 35th day.

3.10. Total Serum Proteins

Data in the table (11) gives a clear idea that Lactobacilli had significantly ($p<0.05$) increased the total serum protein of pigeons compared to the control. When pigeons were fed with Lactobacilli at the concentration of $10^3$, $10^5$ and $10^7$ Lactobacilli/day increased the total serum protein was estimated at the dietary administration of $10^5$ and $10^7$ cells /day on the very last day.

3.11. Serum Lipids

Results in the table (12) shows that the serum lipids (total cholesterol + triglycerides) content of pigeons fed with Lactobacilli was significantly ($p<0.01$) different from the control. Lactobacillus acidophilus at the concentration of $10^3$, $10^5$ and $10^7$ cells/day increased the serum lipids level (from 4.9±0.6 mg/ml in control) to 263.29±10.1,
Investigation, which shows that Lactobacilli probiotic but Tollba and Mahmood (2009) have shown that the normal PCV of Columba livia is around 28-30%. Results of the present study agrees with Fudge (2000) and are much higher than the values estimated by Saleem et al (2008) who had investigated the blood parameters of pigeons growing in natural habitat. The PCV is determined by the size and number of various blood cells in the blood samples (Oladele et al., 2008), so that it is believed that Lactobacillus probiotic has stimulated the synthesis of various blood cells in pigeons. Tollba and Mahmoud (2009) have also come to the similar conclusion when they attempted to investigate the effect of Lactobacillus casei probiotic on haematological parameters of chicken. Further, it was also found to be true with Japanese quail (Abd El-Azeem, 2001).

Ritchie et al (1994) have stated that the normal range of RBC count in feral pigeons is 3.1 – 4.5 x 10^6/DL. However, Mubarak and Rizvi (2002) had reported that RBC level of healthy pigeon is 2.5 x 10^6/DL. Results of present study coincide with the reports of Mubarak and Rizvi (2002) and Ritchie et al (1994). Dietary supplementation of Lactobacilli to chicken has significantly increased the RBC count (Tollba and Mahmoud, 2009), which indicates that certain components in the Lactobacilli stimulate the intestinal cells to release interleukins taking part in the production of red blood cells. Haemoglobin level is nearly 1/3 of the hematocrit value of the blood (Ramnik Sood, 1994), and hence the haemoglobin level would be positively correlated with the RBC count. Since Lactobacilli have increased the RBC count, the haemoglobin count has also increased simultaneously.

According to Fudge (2000), the total leukocytes count (TLC) of pigeon is within the range of 9-13 x 10^3/µl, but Saleem et al (2008) had observed the TLC value as high as 27.15 x 10^3/µl in healthy pigeons. Results of present study agreed with the TLC value reported by Saleem et al (2008) and Mubarak and Rizvi (2002). The TLC value was here much higher than the values prescribed by Fudge (2000), because Lactobacilli have increased the amount of lymphocytes, which are components of leukocytes, to a large extent in pigeons.

Regarding the heterophils count, the present observation coincided with Fudge (2000). Farnmel et al, (2006) found that in chicken lactobacilli slightly stimulate heterophils but Tollba and Mahmood (2009) have shown that there was a significant decrease in heterophil count when chicken were fed with Lactobacilli. Results of the present investigation, which shows that Lactobacilli probiotic reduces the heterophils production in pigeon, were in the same line of reports made by Tollba and Mahmood (2009).

Fudge (2000) stated that the basophils count in healthy Columba livia is less than one cell/µl. Higher proportions of basophils in the blood of pigeons show that the birds have already been infected with some kinds of mild pathogens (Saleem et al., 2008; Vazquez et al., 2010). There was only a slight rise in the basophils count of pigeons in response to Lactobacilli probiotic because certain components in the bacterial cells up regulate some cytokines taking part in the basophils production, which agrees with the reports of Tollba and Mahmoud (2009).

In healthy pigeons, eosinophils count is zero (Fudge, 2000) but there is rise in the eosinophils count in cases where there is worm infestation or infections with pathogenic germs (Coles, 1980; Saleem, et al., 2008). Even though L. acidophilus is not at all a pathogen, some of its cellular components, during digestive cleavage, up regulate the production of cytokines that take part in the eosinophils production. Hence, there is a slight increase in the eosinophils count while feeding the pigeons with this probiotic.

Monocytes, which are necessary for phagocytosis (Carlos Janueira et al., 1992), were more in infected pigeons (Saleem et al, 2008) but almost zero in the blood of disease-free pigeons (Fudge, 2000). The rise in the monocytes count of pigeons in response to this probiotic might be due to the up regulation of certain cytokines taking part in the monocytes production as reported by Tollba and Mahmood (2009). The standard value of lymphocyte count is 5.7 x 10^3/µl (Fudge,2000), while it is much higher in pigeons infected pathogens (Ritchie et al, 1994; Saleem et al, 2008; Oladele et al., 2008). Lactobacilli might have increased the number of lymphocytes by up regulating the expression of cytokines necessary for the proliferation of lymphocytes as suggested by Lillehøj and Chung (1992).

Bone marrow which is the site of haematopoiesis contains all the cytokines required for the proliferation and differentiation of haemopoetic cells via positive and negative regulation of various cytokines, cytokine receptors and other regulatory peptides. A combination of more than one cytokine in small concentrations may up regulate or down regulate the different lineages of haemopoetic precursors to produce characteristic cell types (Kittler et al., 1992). Bagby and Heinrich (2000) clearly reviewed that in humans IL-3, IL-9, IL-11 and GM-CSF are required for the production of erythrocytes from myeloid progenitors, IL-3, GM-CSF, M-CSF and G-CSF are necessary for the production of neutrophils, IL-3, IL-5 and GM-CSF are inevitable for the production of eosinophils, IL-3 and TGF promote the production of basophils, IL-1, IL-6,TNF and GS-CSF are required for the production of monocytes, and IL-2, IL-7, IL-4, IL-10, IL-12, IL-13, IL-14 and IL-16 required for the formation and proliferation of lymphocytes from lymphocytes progenitors. Further, IL-1 and TNF act synergistically to stimulate the myeloid progenitors to produce red blood cells (Kittler et al., 1992). Lactobacilli supplementation...
has increased RBCs, basophils, eosinophils, monocytes and lymphocytes but decreased the heterophils count in pigeons. Therefore, it is assumed that Lactobacilli might have up regulated the expression of IL-1, IL-2, IL-3, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, GM-CSF, TGF and TNF while down regulated the expression of IL-5, M-CSF and G-CSF in pigeons. However, it needs further confirmation by RT-PCR with known probes. In the same line of invention, Choi et al (1999) had already proved that Lactobacilli up regulate the expression of IFN-γ, IL-1, IL-12, IL10 and IGF-β in domestic fowls. The presence of IL-1, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL -18, TGF and INF in pigeons was already demonstrated by Philipp Olias et al. (2013).

Probiotic use of L. acidophilus to germ-free chicken has elevated the levels of total serum protein (Pollmann et al. 1980), broiler chickens (Abdul-Rahman et al. 1994) and Japanese quail (Abd El-Azeem et al. 2001). Abdul Rahim et al (1996) have shown that use of Lactobacillus acidophilus to chickens has increased the layer-quality of the chickens due to the production of more amounts of serum proteins. In support of the above results, the present study reveals that there is a considerable increase in the total serum protein content in pigeons fed with this probiotic compared to the control. Successful colonization of Lactobacilli on the intestinal walls results in more nutrients mobilization into the body tissues and fluids for increasing the protein synthesis (Pulverer et al. 1990); the higher rate of protein synthesis might be the reason for high total serum proteins in the blood.

Abdul Rahim et al (1996) have shown that use of Lactobacillus acidophilus to chickens has lowered the cholesterol content in the blood but increased the triglycerides, so that there is a slight elevation in the level of serum lipids in chickens. This is also proved to be true in pigeons while feeding them with this probiotic.

5. Conclusion

Dietary supplementation of Lactobacillus acidophilus has increased the PCV, RBCs, haemoglobin, TLCs, basophils, eosinophils, monocytes, lymphocytes, total serum proteins and serum lipids but has decreased the heterophils count in Lahore pigeons. Production of more number of leukocytes is considered as the direct indication of the enhancement of innate immunity that is the first hand mechanism to protect pigeons from various pathogens. Further, it reveals that L. acidophilus has immunomodulatory capabilities in pigeons. Higher level of serum protein and lipids are good indicators of layer quality of the poultry, which implies that this probiotic will be a good nutritional supplement to meat-type pigeons.

References

[1] Abd El-Azeem, F.; Faten, A. A. Ibrahim; and Nematallah, G. M. Ali, (2001), Growth performance and some blood parameters of growing Japanese quail as influenced by dietary different protein levels and microbial probiotics supplementation. Egypt. Poult. Sci., 21: 465–489

[2] Abdulrahim, S.M.; Haddadim, M.S.Y.; Hashlamoun, E.A.R & Robinson, R.K. (1996). The influence of Lactobacillus acidophilus and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. British of Poultry Science, Vol.37, No.2, pp. 341-346

[3] Abdul-Rahman, S. A., Abou Ashour, A.M; and Zeweil, H. S. (1994). Effect of probiotic and virginiamycin supplementation on performance of broiler chicks. Menofiya J. Agri. Res. 19: 241 – 256.

[4] Alak, J. I. B., B. W. Wolf, E. G. Mduvwva, G. E. Pimentel-Smith, and O. Adeyemo, “Effect of Lactobacillus reuteri on intestinal resistance to Cryptosporidium parvum infection in a murine model of acquired immunodeficiency syndrome,” The Journal of Infectious Diseases, vol. 175, no. 1, pp. 218–221, 1997.

[5] Anadon, A., Martinez-Larranaga, M. R., & Aranza Martínez, M. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regulatory Toxicology and Pharmacology: RTP, 45(1), 91–95.

[6] Casas, I.A. and W. J. Dobrogosz, (2000)“Validation of the probiotic concept: Lactobacillus reuteri confers broad-spectrum protection against disease in humans and animals,” Microbial Ecology in Health and Disease, vol. 12, no. 4, pp. 247–285, 2000.

[7] Choi, K. D., H. S. Lillehoj, and D. S. Zalenga. 1999. Changes in local IFN-γ and TGF-β4 mRNA expression and intraepithelial lymphocytes following Eimeria acervulina infection. Vet. Immunol. Immunopathol. 71:263–275.

[8] Coles, E. H., 1980. Veterinary Clinical Pathology. 3rd. Ed., W. B., Sounder’s Company, Philadelphia USA.

[9] Dalloul, R.A., H. S. Lillehoj, T. A. Shellem, and J. A. Doerr, (2003) “Intestinal immunomodulation by vitamin A deficiency and lactobacillus-based probiotic in Eimeria acervulina-infected broiler chickens,” Avian Diseases, vol. 47, no. 4, pp. 1313–1320.

[10] Dixit G, Samarth D, Tale V, Bhadekar R (2013) Comparative studies on potential probiotic characteristics of Lactobacillus acidophilus strains. Eurasia J Biosci 7: 1-9. http://dx.doi.org/10.5053/ejobios.2013.7.0.1

[11] Fuller, R. (1989). Probiotics in man and animals. Journal of Applied Bacteriology, Vol.66, pp. 365-378.

[12] Bagby, G.C. and Heinrich, M.C. (2000) Growth Factors, Cytokines, and the Control of Hematopoiesis. In: Hoffman: Hematology: Basic Principles and Practice, 3rd ed., 2000 Churchill Livingstone, Inc.

[13] Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001) Probiotics: effects of immunity. American Journal of Clinical Nutrition, 73 (2 Suppl): S444-S450.

[14] Jacobsen CN, Nielsen VR, Hayford AE, Moller PL, Michaelsen KF, Parregaard A, Sandstrom B, Tvede M, Jakobsen M (1999) Screening of probiotic activities of forty-seven strains of Lactobacillus sp. by in-vitro techniques and evaluation of colonization ability of five selected strains in human. Applied and Environmental Microbiology 65: 4949-4956.
[15] Kittler EL, McGrath H, Temeles D et al: Biologic significance of constitutive and subliminal growth factor production by bone marrow stroma. Blood 79:3168, 1992

[16] Lillehoj HS, Chung KS. 1992. Postnatal development of T-lymphocyte subpopulations in the intestinal intraepithelium and lamina propria in chickens. Veterinary Immunology and Immunopathology, 31:347-360.

[17] Liong MT, Shah NP (2005) Acid and Bile Tolerance and Cholesterol Removal Ability of Lactobacilli Strains. Journal of Dairy Science, 88(1): 55-66.

[18] Ng SC, Hart AL, Stagg A J, Knight SC (2009) Mechanisms of action of probiotics: recent advances. Inflammatory Bowel Diseases, 15: 300-310. http://dx.doi.org/10.1002/ibd.20602

[19] Philipp Olias, Anne Meyer, Robert Klopfleisch, Michael Lierz, Bernd Gencer and Achim D Gruber (2013) Modulation of the host Th1 immune response in pigeon protozoal encephalitis caused by Sarcozystis calchasi. Veterinary Research, 44:10.

[20] Pochapin M (2000) The effect of probiotics on Clostridium difficile diarrhoea. American Journal of Gastroenterology, 95(suppl.): S11-S13.

[21] Pollmann, D.S.; Danielson, D.M. and Peo, E.R. (1980). Effects of microbial feed additives on performance of starter and growing-finishing pigs. J. of Animal Science, 51: 577-581.

[22] Pulverer G, Ko HL, Roszkowski W, Beuth J, Yassin A, Jelaszewicz J. 1990. Digestive tract microflora liberates low molecular weight peptides with immuno-triggering activity. Zentralblatt fur Bakteriologie, 272:318-27.

[23] Ramnik Sood (1994) Medical Laboratory Technology, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi.

[24] Ritchie BW, Harrison GJ, Harrison LR: Avian Medicine: Principles and application Lake Worth, Florida: Wingers Publishing, Inc; 1994.

[25] S. H. Kim, S.Y. Park, S. J. Lee1, and K. S. Ryu, 1996. An Isolation of Lactobacillus spp. and its feeding Influence on Performance of Broiler Chicks. National Livestock Research Institute, Daejeon 305-365, Dept. of Animal Sci., Chonbuk National University, Chonju, 561-756 Korea.

[26] Sareml F, Sarem-Damerdjil LO, Nicoias JP (1996) Comparison of the adherence of three Lactobacillus strains to Caco-2 and Int-407 human intestinal cell lines. Letters in Applied Microbiology, 22(6): 439-442. http://dx.doi.org/10.1111/j.1472-765X.1996.tb01198.x

[27] Szajewska, H., M. Kotowska, J. Z. Mrukowicz, M. Arm`anska, and W. Mikolajczyk, “Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants,” Journal of Pediatrics, vol. 138, no. 3, pp. 361–365, 2001.

[28] Tollba, A. A. H and R. M. Mahmoud (2009) How to Control the Broiler Pathogenetic Intestinal Flora Under Normal or Heat Stress Conditions: 1. Medicinal Plants, Probiotics- Sand as Litter, Egypt. Poult. Sci. Vol (29) (II): (565-587)

[29] Wollowski I, Rechksiemi G, Pool-Zobel BL (2001) Protective role of probiotics and prebiotics in colon cancer. American Journal of Clinical Nutrition, 73(2 Suppl): 451S-455S.

[30] Rehman, H., S. Abbas and N. Jiajahet (2003). Laboratory Manual of Physiology, Vol. 1. Society of Veterinary Physiology, Lahore, Pakistan.

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