Dietary Effects of *Lactobacillus acidophilus* Probiotic on the Haematological Parameters, Total Serum Proteins and Lipids of Lahore Pigeon (*Columba 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, haemoglobins, 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 (Anadon *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-Agn`es 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), inhibit 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 quil (Abd El-Azeem et al. 2001). Abdhul 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), leukocytic (WBC's), lymphocytes, eosinophils and basophils, while heterophil count is low when chicken are fed with Lactobacilli at normal temperature (23ºC). Lillehoj 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*.

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 90 grams per pair of pigeons per day and drinking water was provided 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, basophils, eosinophils, 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 (Roboniek 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 46.4±1.66%) 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/ day increased the RBC count to 2.9±0.44, 3.3±0.41, 3.4±0.73, 3.4±0.78 x 10^6/dL 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, 10^3, 10^5 and 10^7 Lactobacilli/ day increased the TLC to 25.34±1.10, 26.22±1.28, 28.28±1.28, 28.78±1.18x 10^6/dL respectively instead of 24.54±1.13 x 10^6/dL 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.

Table1: Composition of normal feed.

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

*Grid: I 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 B_{12} – 20 mcg; vitamin D_2 -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*	46.9±1.24 a	47.4±1.39 a	47.7±1.45 a	47.9±1.42 b
14th day	46.4±1.66 *	46.9±1.29 a	48.2±1.62 a	49.8±1.26 a	48.9±1.19 a
21st day	46.4±1.67 a	46.9±1.32 *	48.3±1.59 a	49.9±1.29 b	49.8±1.21 b
28th day	46.4±1.83 *	46.9±1.43 a	48.4±1.53 b	49.4±1.72 a	49.4±1.31 a
35th day	46.4±1.71*	46.9±1.42 b	48.7±1.39 a	49.8±1.71 b	49.7±1.41 b

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

Table 3: Change in the RBC count of pigeon's blood due to *Lactobacilli* probiotic (N x 10^6/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	2.8±0.61 a	2.9±0.23 a	3.2±0.41 a	3.4±0.61 b	3.5±0.23 a
14th day	2.8±0.39 *	2.9±0.28 a	3.3±0.22 a	4.0±0.62 a	4.1±0.42 b
21st day	2.8±0.52 *	2.9±0.42 a	3.3±0.36 b	3.4±0.57 b	3.4±0.36 b
28th day	2.8±0.29 a	2.9±0.39 a	3.3±0.21 a	3.2±0.31 b	3.3±0.41 a
35th day	2.8±0.34 *	2.9±0.44 a	3.3±0.41 a	3.4±0.73 b	3.4±0.78 b

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

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 *	9.91±0.69 *	10.32±0.16 a	11.29±0.46 a	11.30±0.21 b
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 *	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 *	9.93±0.73 a	11.11±0.33b	12.13±0.28 b	12.13±0.48 a

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

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 *	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 *	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 *	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; a = ($p<0.05$); b = ($p<0.01$).

Table 6: Heterophils 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	5.42± 0.45 *	5.38± 0.35 a	5.28± 0.53 a	5.14± 0.36 a	5.10± 0.27 a
14th day	5.42± 0.63 *	5.32± 0.23 b	5.12±0.39 b	5.2±0.34 b	4.90±0.24 b
21st day	5.42± 0.61 *	5.23± 0.21 a	5.9±0.23 b	4.81±0.24 b	4.72±0.44 b
28th day	5.42± 0.60 *	5.19± 0.42 *	5.2±0.27 a	4.62±0.42 a	4.51±0.37 b
35th day	5.42± 0.60 *	5.11± 0.21 a	4.89±0.38 a	4.43±0.37 a	4.36±0.18 a

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

Table 7: 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	0.45±0.9 a	0.46±0.8 a	0.48±0.9 a	0.51±0.9 a	0.51±0.8 b
14th day	0.45±0.11 *	0.47±0.7 a	0.50±0.10 b	0.61±0.12 b	0.62±0.6 b
21st day	0.45±0.9 a	0.47±0.9 a	0.51±0.8 a	0.64±0.11 a	0.64±0.12 b
28th day	0.44±0.8 *	0.47±0.7 *	0.51±0.8 *	0.64±0.11 b	0.63±0.11 b
35th day	0.46±0.9 a	0.47±0.9 *	0.51±0.8 a	0.64±0.11 b	0.64±0.9 b

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

Table 8: Eosinophils count of pigeons fed with the 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 a	0.39±0.9 a	0.39±0. 9b
14th day	0.38±0.10 a	0.39±0.13 b	0.39±0.14 b	0.40±0.7 b	0.40±0.8 b
21st day	0.38±0.12 *	0.39±0.15 a	0.39±0.17 b	0.40±0.9 b	0.40±0.9 b
28th day	0.38±0.9 a	0.39±0.16 a	0.39± 0.18 a	0.40± 0.10 b	0.40± 0.11b
35th day	0.38±0.9 *	0.39±0.18 *	0.39±0.18 a	0.40±0.11 a	0.40±0.12b

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 a	1.23±0.6 a	1.30±0.9 a	1.35±0.10b	1.36±0.9 b
14th day	1.22±0.6 *	1.30±0.7 b	1.41±0.9 b	1.50±0.9 b	1.51±0.8 b
21st day	1.22±0.4 a	1.33±0.6 b	1.46±0.8 b	1.51±0.8 a	1.52±0.4b
28th day	1.21±0.5 *	1.35±0.3 a	1.46±0.3 a	1.50±0.3 b	1.50±0.11b
35th day	1.23±0.4 *	1.36±0.4 *	1.46±0.8 a	1.51±0.10 a	1.51±0.11 b

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

3.5. Heterophils Count

Dietary administration of Lactobacilli had significantly ($p<0.05$) reduced the heterophils count compared to that of control (Table 6). Lactobacilli at the doses of 10^1, 10^3, 10^5 and 10^7 cells/ day had lowered the heterophils count to 5.11± 0.21, 4.89±0.38, 4.43±0.37, 4.36±0.18 x 10^3/µl respectively. The maximum reduction in heterophils count was observed on the 35th day.

3.6. Basophils Count

Table 7 shows that basophils count in pigeons fed with Lactobacilli was significantly ($p<0.05$) different from 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, 0.51±0.8, 0.64±0.11, 0.64 ±0.9 x 10^3/µl respectively. However, this modulating effect was found to be the maximum at high doses (300mg and 400mg /day) on the 35th day.

3.7. Eosinophils Count

Feeding the pigeons with 10^1, 10^3, 10^5 and 10^7 Lactobacilli/day had slightly increased the eosinophils count to 0.39±0.18, 0.39±0.18, 0.40±0.11, 0.40±0.12 x 10^3/µl respectively instead of 0.38±0.12 x 10^3/µl (Table 8). There was a significant ($p<0.05$) rise in the eosinophils 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±0.8 to 1.36±0.4, 1.46±0.8, 1.51±0.10, 1.51±0.11 x 10^3/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.

3.9. Lymphocytes Count

Table 10 depicts that in pigeons fed with Lactobacilli the lymphocytes count was significantly ($p<0.05$) increased compared to the control. Oral administration of 10^1, 10^3, 10^5 and 10^7 Lactobacilli/ day increased the lymphocytes count to 12.86±1.12, 14.33±1.13, 14.69±1.12 and 14.73±1.16 x 10^6/dL respectively compared to the control (12.46±1.13 x 10^6/dL). Lactobacilli supplementation increased the lymphocytes count day by day and its highest level was recorded at the dietary administration of 10^5 and 10^7 cells / day on the final day of the experiment.

Table 10: Lymphocytes count in 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	12.46±1.13 ^{a}	12.61±0.73 ^{a}	13.04±1.14 *	13.13±1.14 ^{a}	13.14±1.10 ^{b}
14th day	12.46±1.12 *	12.83±1.12 ^{b}	14.23±1.9 ^{b}	14.69±1.12 ^{b}	14.73±1.11 ^{b}
21st day	12.46±1.13 ^{a}	12.87±1.10 ^{b}	14.33±1.11^b	14.73±2.21 ^{b}	14.74±2.19^b
28th day	12.46±1.12 *	12.84±0.72 ^{a}	14.33±1.19 ^{a}	14.73±1.20 ^{a}	14.75±1.18 ^{b}
35th day	12.46±1.12 *	12.86±1.12 ^{a}	14.33±1.13 *	14.69±1.12 ^{a}	14.73±1.16^b

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

Table 11: Total serum protein (g/dL) of pigeons fed with 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	4.9±0.5 ^{a}	5.0±0.3 ^{a}	5.1±0.2 ^{a}	5.2±0.2 ^{a}	5.2±0.3 ^{b}
14th day	4.9±0.6 ^{a}	5.1±0.2 ^{b}	5.6±0.3 ^{b}	5.8±0.4^b	5.9±0.2^b
21st day	4.9±0.3 ^{a}	5.2±0.2 ^{b}	5.7±0.2 ^{b}	5.9±0.3 ^{b}	5.7±0.4 ^{b}
28th day	4.9±0.3 ^{a}	5.2±0.1 ^{a}	5.7±0.2 ^{a}	5.8±0.2 ^{a}	5.8±0.2 ^{b}
35th day	4.9±0.3 *	5.2±0.2 *	5.7±0.3*	5.9±0.2 *	5.9±0.3 ^{a}

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

Table 12: Serum lipids (mg/ml) of pigeons fed with Lactobacilli probiotic.

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	242.38±8.3 ^{a}	248.25±10.1 ^{a}	257.24±11.2 ^{b}	260.32±1.3 ^{a}	263.18±9.4 ^{b}
14th day	242.41±8.4 ^{a}	252.31±10.3 ^{b}	254.39±10.2 ^{a}	262.20±10.1 ^{b}	264.27±9.2 ^{b}
21st day	242.39±8.5 ^{a}	259.28±9.7 ^{a}	260.28±11.3 ^{b}	263.19±11.4^b	266.33±9.4 ^{a}
28th day	242.38±8.1 *	262.29±10.4 ^{b}	263.19±10.4 ^{a}	264.17±10.2 ^{a}	268.18±10.3 ^{a}
35th day	242.40±8.4 ^{a}	263.29±10.1 ^{a}	264.27±9.2 ^{a}	266.23±9.7 ^{b}	269.22±10.1 ^{b}

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

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 10^1, 10^3, 10^5 and 10^7 cells/ day, serum protein level increased from 4.9±0.6 g/dl (control) to 5.2±0.2, 5.7±0.3, 5.9 ±0.2 and 5.9±0.3 g/dL. Lactobacilli had augmented the serum protein level in all the experimental groups of pigeons starting from 7th day to 35th day. The highest level of 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 10^1, 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, 264.27±9.2, 266.23±9.7 and 269.22±10.1 mg/ml respectively. During this treatment, serum lipids content was found to be increased day-by-day during the course of this experiment.

4. Discussion

The probiotic *Lactobacillus acidophilus* has changed the haematological parameters, total serum proteins and lipids content in the blood of pigeons considerably. According to Fudge (2000), the normal range of the PCV of healthy pigeons is 42 -50% but Saleem *et al* (2008) found 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). Farnnel *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 Lactobacilii 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 basophills count of pigeons in response to Lactobacilli probiotic because certain components in the bacterial cells up regulate some cytokines taking part in the basophills production, which agrees with the reports of Tollba and Mahmood (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 in a slight increase in the eosinophils count while feeding the pigeons with this probiotic.

Monocytes, which are necessary for phagocytosis (Carlos Junueira *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 Lillehoj 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 quil (Abd El-Azeem *et al*. 2001). Abdhul 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.

Abdhul 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, haemoglobins, 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 immumomudulatory 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.

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