Effects of the probiotic miacllost (Bacillus subtilis and Entrococus feacium) on growth performance, hematological values and small intestinal morphology of broiler chicks

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Abstract. A total of 264, one-day old chicks were randomly dispensed into 4 groups to assess effects of the probiotic MiaClost (Bacillus subtilis and Entrococcus feacium) on broiler performance, blood values and small intestinal morphology of broilers. Chicks in treatment groups T1, T2, T3 received 0.160 gm, 0.175 gm and 0.190 gm MiaClost per litre of drinking water respectively, whereas the control chicks received additives-free drinking water. Body weight, feed intake, weight gain, and feed conversion ratios (FCR) were recorded on a weekly basis on days 7, 14, 21, 28 and 35. At end of the experiment, blood samples were collected from 12 birds in each group to determine some hematological values including RBCs count, WBCs count, haemoglobin, hematocrit, MCV, MCH and MCHC. In addition, small intestine biopsies were obtained to assess the small intestinal morphology. Significant proportional increase in level of feed intake was apparent in accordance with the increase in level of probiotics supplementation. No significant variation was apparent in the levels of FCR among chicks in different groups. Probiotics supplementation resulted in improvement in weights and weight gain of chickens. No significant variation was seen on hematological values among birds in all groups. A positive MiaClost’s effect was apparent on height & width of the intestinal villi and on depth of mucosal crypts in all treatment groups particularly treatment groups 2 and 3 in comparison with the control group. These results showed that the MiaClost supplementation in drinking water has improved the performance of broiler chicks and has enhanced the lining epithelial cells proliferation and increased the absorptive surface area of the small intestine.

1. Introduction

Antibiotics are widely used in the poultry industry for prophylaxis, treatment & growth promotion and the presence of their residues in poultry meat and eggs represents a critical issue for consumers of these products as they can result in resistance and cross-resistance to antibiotics used for the treatment of bacterial diseases in humans and animals [1;2].
Acknowledgment of this serious problem enforced the prevention of sub-therapeutic antibiotic use in the developed world including Europe & USA and motivated the use of probiotic microorganisms as alternatives to the antibiotics for growth promotion in poultry production [3].

Probiotics are feed supplements or water additives containing living microbial organisms capable to improve the balance of intestinal microflora in the host animals. They can be used to improve growth performance and prevent gastrointestinal infections. A diverse group of microbial species has been used as probiotics in the poultry industry including the Saccharomyces yeast and certain species of the bacterial genera Lactobacillus, Lactococcus, Bacillus, and Bifidobacterium [4].

Upon their consumption, the probiotics don’t leave any residues in animal products and don’t induce microbial resistance; consequently, a lot of researches have been achieved dealing with the use of probiotics as growth promoters [5]. It has been reported that they improve the gastrointestinal microbial balance [6] as well as poultry performance [7].

The MiaClost, a type of probiotics, contains Enterococcus faecium and Bacillus subtilis spores [8]. The Enterococcus faecium, which belongs to the lactic acid bacteria group, are beneficial to improve the balance of intestinal microflora [9] and the Bacillus-containing probiotics are better able to survive in a variety of food products than the other probiotic bacterial species and they can improve the gastrointestinal environment upon their use as feed or water additives [10]. Therefore, this study aimed to explore the effects of MiaClost, as a drinking water additive, on food consumption, feed conversion ratio, weight gain, blood values, and histological morphology of the small intestine as indices for broiler performance.

2. Materials and Methods

2.1. Experimental Design
A total of 264, one-day-old, broiler chicks (Rose 308), obtained from Kosar Company for Agriculture and Poultry were randomly assigned into 4 groups (3 replicates each, 22 chicks/replicate) and housed in separate, temperature, ventilation & light-controlled pens at the College of Agricultural Sciences, University of Sulaimani. All chicks were fed on a starter diet for the first 11 days, grower diet on days 12-25 days and finisher diet on days 26-42. The diets were prepared to fulfil the standard requirements of broiler chicks (with no antibiotics or growth promoter supplementations). The chicks in treatment groups T1, T2, and T3 received MiaClost probiotic (FuTrend, Jordan) in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm/litre respectively, whereas the control group’s chicks were provided with additives-free drinking water.

2.2. Body Weight and Body Weight Gain Measurements
The chicks of each replicate were individually weighed using a manual weight scale at the end of weeks 1, 2, 3, 4 and 5 and subsequently their weight gain was calculated on a weekly basis for the same periods.

2.3. Feed Intake and Feed Conversion Ratio
The feed intake was recorded on a weekly basis at end of the weeks 1, 2, 3, 4 and 5 via weighing the feed amount given to each pen/day and weighing the weekly feed residues in that pen. The weekly feed intake was calculated by discounting the weight of feed residues from the total weight of feed given to that pen in the concerned week. Similarly, the feed conversion ratios were calculated on a weekly basis on days 7, 14, 21, 28 and 35 using the equation formula: FCR = Feed consumed / Weight gain [5].

2.4. Blood tests
On day 42 (at end of the experiment), Fresh blood samples were collected (from the wing vein in heparinised test tubes) from 12 randomly selected birds in each group (4 chicks/replicate) to measure the total RBCs count, WBCs count, Haemoglobin (HGB), Hematocrit (HCT), mean corpuscular...
volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) which were determined in PharmaGene lab at Al Sulaimaniyah Province.

2.5. Histological indices of the small intestine
At end of the experiment on day 42, four birds were randomly selected from each replicate (12 birds/group), sacrificed by cervical dislocation and dissected. Immediately after that, 2 cm long segment was cut from the proximal portion of the jejunum, opened, flushed with normal saline, fixed in 10% neutral buffered formalin solution and undergone a series of histopathological preparations in order to obtain 5 μm thick tissue sections which were subsequently stained by hematoxylin and eosin and examined by different magnifying powers of light microscopy.

2.6. Statistical analysis
Data of body weight means, body gain, feed intake and food conversion ratio were analyzed using the generalized linear model offered by the program XLSTAT version 2016.02.28451. The following model was used: Yij = μ + Ti + Pj + TPij + eij where: Yij is the observation of performance values, μ is overall mean, Ti is a fixed effect of treatments (Control: 0.00, T1: 0.160, T2: 0.175, T3: 0.190 gm of MiaClost per litre of drinking water), Tj is a fixed effect of periods (day 1, 7, 14, 21, 28 and 35), TPij is an interaction between treatments and periods, eij= Random error which was supposed to be equal to zero and the variance is 62e (N~ 0, 62e). Whereas the blood values were analyzed using the SPSS version18. Significant of variance between means of values were determined using Duncan's multiple range test under the probability (p<0.05).

3. Results and Discussions

3.1. Body weight measurements
The body weight (BW) means of the broiler chicks following starting of probiotics (MiaClost) supplementation are shown in table 1. On day 7, no significant variation (P<0.05) was apparent among the BW means in all groups. On day 14, the chicks in T3 group showed the highest BW means (363.60 gm) which was significantly higher (P<0.05) than that of the control group but it was non-significantly higher than that of the T2 and T3 groups. On days 21 and 28, the BW means of groups T1 and T2 was significantly higher (P<0.05) that of the control group and on day 35, the BW means of all treatment groups (T1, T2 and T3) was significantly higher (P<0.05) than that of the control group.

These findings are in agreement with those of [11,12] who stated that probiotics supplementation has improved the chickens' weights; however, they are not in agreement with the finding of [13] who did not establish any positive effect of supplementation of *Saccharomyces cerevisiae* on the chickens' weights.

3.2. Body weight gain
The values of body weight gain means in the broiler chicks following starting of the probiotics (MiaClost) supplementation is shown in table 2. The weight gain means of all treatment groups (T1, **Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively**

| Treatments | 7 day   | 14 day   | 21 day   | 28 day   | 35 day   |
|------------|---------|----------|----------|----------|----------|
| Control    | 117.14 ±1.73 a | 320.66 ±6.55 a | 636.26 ±6.65 a | 1148.46 ±17.57 a | 1594.00 ±31.05 a |
| T1         | 129.87 ±3.54 ab | 357.26 ±15.01 ab | 705.37 ±28.06 b | 1291.66 ±41.27 b | 1841.13 ±56.58 b |
| T2         | 125.42 ±1.16 a | 358.93 ±14.14 a | 703.03 ±29.95 b | 1312.23 ±52.43 b | 1871.33 ±69.51 b |
| T3         | 129.18 ±1.35 a | 363.60 ±8.97 b | 652.27 ±25.03 a | 1237.69 ±50.21 ab | 1846.33 ±71.12 b |

*Within a column, BW means with different alphabetical superscripts differ from each other significantly (P<0.05).

**Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively**
T2 and T3) was significantly higher (P<0.05) than that of the control group on days 7 and 35, whereas on days 14, 21 and 28, no significant variation was evident.

These findings are consistent with the result of [14] who reported similar effects of the probiotics on the weight gain of chickens and with the result of [15] who observed that the probiotic based diets in broilers had resulted in numerical improvement in the chickens’ weight gain than the antibiotic but they are inconsistent with those of [16] who did not observe any positive effect when Streptococcus lactis was incorporated in feed ratio of the chickens. This variability in the response of chickens to the probiotics supplementation in terms of weight gain can be reasonably attributed to the bacterial sensitivity, health and hygiene of birds used in the trials as well as the environmental factors [17].

Table 2. Effect of MiaClost supplementation on weight gain (gram) of the broiler chicks

| Treatments | 7 day       | 14 day       | 21 day       | 28 day       | 35 day       |
|------------|-------------|--------------|--------------|--------------|--------------|
| Control    | 73.00 ±1.73 a | 203.52 ±5.67 a | 315.60 ±8.09 a | 512.20 ±20.10 a | 445.53 ±21.62 a |
| T1         | 85.87 ±3.54 b | 227.38 ±12.71 a | 348.1 ±21.67 a | 586.28 ±30.41 a | 549.46 ±10.29 b |
| T2         | 81.42 ±1.15 b | 233.50 ±13.46 a | 344.09 ±31.33 a | 589.63 ±62.94 a | 578.66 ±33.57 b |
| T3         | 85.18 ±1.35 b | 234.41 ±7.62 a | 288.67 ±14.06 a | 585.39 ±9.48 a | 608.66 ±28.14 b |

*Within a column, weight gain means with different alphabetical superscripts differ from each other significantly (P<0.05).

**Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively.

3.3. Feed intake

The feed intake rates of the broiler chicks following starting of the probiotic (MiaClost) supplementation are shown in table 3. On day 7, no significant variation (P<0.05) was found among the chicks in different groups, whereas on days 14, 21, 28 and 35 the chicks in T1 group (0.160 gm MiaClost /litre of drinking water), T2 group (0.175 gm MiaClost /litter of drinking water) and T3 group (0.190 gm MiaClost /litter of drinking water) showed a significantly higher feed intake (P<0.05) in comparison with chicks of the control group (0.00 gm MiaClost /litre of drinking water).

On day 14, the highest feed intake (312.95 gm) was recorded by the chicks in group T3 whereas on day 21, it was group T2 which showed the highest feed intake (589.51 gm). On days 28 and 35, the highest feed intake was recorded by the chicks in both groups T2 and T3 (900 and 1045.45 gm respectively).

Significant proportional increase in the level of feed intake was apparent in accordance with the increase in the level of MiaClost supplementation starting from day 14 of supplementation till the end of experimental duration. This improvement in the feed intake can be attributed to the effect of MiaClost on increasing the growth of beneficial bacteria naturally found in the intestinal tract resulting in improvement of broiler performance [18] and it is in agreement with the result of [19] who reported similar higher feed intake in broilers supplemented with probiotics. However, it is not compatible with the result of [20;21] who did not find significant differences in levels of feed intake among their groups of chicks that received different levels of probiotics supplementation. This incompatibility can be ascribed to the species of microorganisms found in the tested probiotics, concentration & route of supplementation and nature of the climate where the trial was conducted [22].
Table 3. Effect of MiaClost supplementation on feed intake (gm) of the broiler chicks

| Treatments | 7 day         | 14 day         | 21 day         | 28 day         | 35 day         |
|------------|---------------|---------------|---------------|---------------|---------------|
| Control    | 120 ± 2.11 a  | 244.33 ±3.29 a | 431.66 ±17.14 a | 673 ±33.73 a  | 799 ±36.15 a  |
| T1         | 136.36 ±5.47 a| 302.75 ±9.59 b | 589.51 ±30.58 b | 873.23 ±41.32 b| 1009.24 ±49.79 b |
| T2         | 136.36 ±3.86 a| 289.34 ±8.56 ab| 588.90 ±25.96 b | 882.57 ±38.14 b| 1045.45 ±50.35 c |
| T3         | 136.36 ±7.21 a| 312.95 ±14.4 b | 559.68 ±30.96 b | 900 ±51.1 b   | 1045.45 ±56.42 b |

*Within a column, feed intake means with different alphabetical superscripts differ from each other significantly (P<0.05).

**Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively.

3.4. Feed conversion ratio

No significant variation was apparent in the levels of FCR among chicks of the different treatments groups on days 7, 14, 28 and 35 (Table 4). On day 21, the FCR was significantly higher only in chicks of T3 group compared to the other treatment groups and control group.

Significant increase in the level of FCR was apparent only in chicks of T3 group on day 21 following MiaClost supplementation in comparison with the other treatment groups and control group. This finding is approximately compatible with findings of [23] who didn’t find significant variation in the probiotics fed group in comparison with control one and is also compatible with the results of [24] who stated that the FCR was not statistically different among the treatments and control groups in all of the studied intervals.

Table 4. Effect of MiaClost supplementation on feed conversion ratio of the experimental chicks

| Treatments | 7 day         | 14 day         | 21 day         | 28 day         | 35 day         |
|------------|---------------|---------------|---------------|---------------|---------------|
| Control    | 1.64 ± 0.03 a | 1.20 ± 0.02 a | 1.39 ± 0.05 a | 1.35 ± 0.05 a | 1.81 ± 0.06 a |
| T1         | 1.59 ± 0.06 a | 1.34 ± 0.11 a | 1.69 ± 0.02 a | 1.54 ± 0.08 a | 1.83 ± 0.01 a |
| T2         | 1.67 ± 0.02 a | 1.24 ± 0.08 a | 1.71 ± 0.14 a | 1.52 ± 0.14 a | 1.81 ± 0.12 a |
| T3         | 1.60 ± 0.02 a | 1.33 ± 0.13 a | 1.94 ± 0.07 b | 1.54 ± 0.02 a | 1.72 ± 0.08 a |

*Within a column, mean values with different alphabetical superscripts differ from each other significantly (P<0.05).

**Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively.

3.5. Blood values

On day 42 (at end of the experiment), no significant variations were seen on RBCs count, WBCs count, haemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration among the birds in all groups as shown in table 5.

This result is compatible with the finding of [5] who stated that probiotics additives in birds feed did not induce significant effects on the blood values; however, it is not compatible with that of [25] who reported that the probiotics additives resulted in statistically significant rise in the RBCs count, haemoglobin concentration and haematocrit values of Turkeys. This variation in effects of probiotics on blood values can be ascribed to type and number of bacterial species found in the concerned probiotics [5].
Table 5. Effect of probiotic (MiaClost) supplementation on some blood values of broiler chick

| Test            | Control | T1     | T2     | T3     |
|-----------------|---------|--------|--------|--------|
| RBC (10⁶/μL)    | 2.18 a  | 1.82 a | 2.25 a | 2.28 a |
| WBC (10³/μL)    | 111.82 a| 93.18 a| 95.37 a| 114.16 a|
| HGB (g/dl)      | 13.52 a | 11.27 a| 14.20 a| 14.09 a|
| HCT (%)         | 31.18 a | 31.18 a| 31.98 a| 31.80 a|
| MCV(femtolitres)| 143.16 a| 143.16 a| 142.37 a| 139.51 a|
| MCH (picogram)  | 62.16 a | 62.16 a| 63.28 a| 61.93 a|
| MCHC (g/dl)     | 43.38 a | 43.38 a| 44.40 a| 44.39 a|

*Within a row, blood values with different alphabetical superscripts differ from each other significantly (P<0.05).

**Control chicks were provided with additives-free drinking water whereas T1, T2, and T3 chicks received MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively.

3.6. Histological indices of the small intestine

The microscopic examination of small intestinal tissue sections obtained on day 42 of the chicks age revealed an increase in height and width of the intestinal villi associated with a marked increase in depth of the intestinal crypts in the chicks of all treatment groups particularly treatment groups 2 and 3 in comparison with chicks of the control group which showed normal appearance, height and width of their intestinal villi and normal crypt depth (Figures 1 and 2).

This finding is compatible with the findings of other authors who reported that the villus height has increased in birds as an animal model for investigation of probiotic effects on intestinal morphology and cell proliferation [26;27] and it is also in agreement with the results of [28] who stated that the intestinal crypt depth has been increased in the duodenum, jejenum and ileum of chicks supplemented with probiotics containing Bacillus subtilis spores. However, this result is not compatible with that of [29] who stated that the intestinal crypt depth didn’t change in duodenum and even decreased in ileum of broiler chicks supplemented with probiotics containing Lactobacillus spp. In addition, it has been reported that crypt depth was not changed in broiler chicks supplemented with probiotics containing Saccharomyces cerevisiae yeast [30]. This variation in findings of experimental studies dealt with the effects of probiotics on height and width of intestinal villi and on depth of their crypts in birds can be attributed to the dose, application and species of microorganism found in the concerned probiotic [31].

Figure 1. Microscopic Image of Jejunum section tissue sections obtained from the control chicks (additives-free drinking water). They show approximately normal appearance, height and width of the intestinal villi. H and E (A: X100, B: X200).
Figure 2. Microscopic view of small intestinal tissue sections obtained from chicks of the treatment groups T1, T2 and T3 (MiaClost in drinking water at a concentration of 0.160 gm, 0.175 gm and 0.190 gm per litre respectively). They show an increase in height and width of the intestinal villi particularly in groups T2 and T3. (H and E, X100).

4. Conclusions
The results of the current study revealed that water supplementation of the probiotic MiaClost at a concentration of 0.160, 0.175 gm and 0.190 gm per litre has improved the performance of broiler chicks as indicated by its significant improvement of feed intake, body weight measurements body weight gain, and feed conversion ratio and by its positive effect on the intestinal morphology which was represented by enhancing the lining epithelial cells proliferation and increasing the absorptive surface area of the jejunum. No significant variation was apparent between the supplementation levels 0.175 gm and 0.190 gm per litre indicating that increasing the MiaClost concentration in drinking water for more than 0.175 gm/litre did not induce a better performance.

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