The Effect of Probiotics on Broiler Growth and Intestinal Morphology when Used to Prevent \textit{Campylobacter jejuni} Colonization

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Abstract

The aim of this work was to establish the effect of probiotic microorganisms on growth performance and intestinal changes caused by \textit{Campylobacter jejuni} colonization. In this respect, we used four probiotic microorganisms, namely: \textit{Lactobacillus paracasei} JR, \textit{L. rhamnosus} 15b, \textit{Y L. lactis} and \textit{L. lactis} FOA. The administration of probiotic microorganisms in different combinations and in different periods of growth does not significantly influence the bioproductive indices of broilers, that is, the total gain, feed intake and FCR (p>0.05). After studying the intestinal mucosa, it was concluded that the four microorganisms administered in broiler’s food determines changes in the mucosa, inhibiting the development of \textit{Campylobacter jejuni}, by the presence of smaller goblet cells and the presence of reduced leukocyte infiltration in the chorion of the mucosal.

Keywords: bioproductive performances, broiler, probiotics, intestinal mucosa, pathogenic microorganisms

1. Introduction

One of the most important aspects of the nutritionists’ research in poultry is to improve the quality of products (meat, eggs) under security and safety conditions for human consumption. Poultry meat is a type of food widely used worldwide and can represent a source of pathogens in foods such as: \textit{Salmonella enterica} spp., \textit{Escherichia coli}, and \textit{Campylobacter jejuni}. [1, 2]. Therefore, it becomes critical for manufacturers to identify the best methods to reduce possible infections with these poultry meat pathogens [3].

Probiotics are live cultures of microorganisms supplemented in animal nutrition that can beneficially influence the host animal and improve intestinal microbial equilibrium [4]. Studies carried out on broilers indicate a positive response to dietary supplementation with probiotics to reduce pathogenic microbial infestation [5].

Scientific observations have shown a significant improvement in average daily gain and feed consumption of broilers fed with probiotics, correlated with the type of beneficial microorganisms used [6, 7]. Probiotics reduce the level of toxins produced by pathogenic bacteria and determine changes in the morphology of the intestinal wall, reducing the
colonization with pathogenic bacteria, thereby preventing damage to epithelial cells [8]. Another possible mode of action by which probiotics can exert a positive effect lies in the favorable effect on intestinal permeability, which can lead to increased nutrient absorption and thus improve growth performance [9]. The purpose of this study was to establish the effect that some probiotics have on broiler growth performance and the changes induced at intestinal level by *Campylobacter jejuni*.

### 2. Materials and methods

As biological material we used 280 poultry broilers belonging to ROSS308 hybrid, which were randomly divided in 7 experimental variants. Body weight, daily gain and fed intake were monitored at different growth sub-periods: 10, 35 and 42 days. At the end of the experiment, 3 animals were slaughtered for each experimental variant. Samples were collected from intestines (duodenum and cecum) in order to establish the main alterations due to the presence of *Campylobacter jejuni*.

For histopathological examination, fragments from the duodenum and cecum were taken from the individuals of six experimental variants and also from the control group (L0). The fragments of the intestine were fixed in neutral formalin 10% and put in histological paraffin before previously being dehydrated in increasing ethyl alcohol solutions (70°, 80°, 90°, 100°) and clarified in two baths of benzene. The sectioning of the paraffin blocks was carried out using a manually rotary microtome.

| Table 1. Experimental scheme used for determining the effect of probiotic administration in polyculture at a concentration of 10^9 CFU/kg feed |
|---|---|---|---|---|---|---|
| Group L0 | Group L1 | Group L2 | Group L3 | Group L4 | Group L5 | Group L6 |
| Without probiotics (control) | 0-42 days | 0-42 days | 35-42 days | 35-42 days | 0-42 days | 35-42 days |
| Probiotic strains administered | *L. paracasei* CMGB 18 | *L. paracasei* CMGB 18 | *L. paracasei* CMGB 18 | *L. paracasei* CMGB 18 | *L. paracasei* CMGB 18 | *L. paracasei* CMGB 18 |
| | *L. rhamnosus* CMGB 34 | *L. rhamnosus* CMGB 34 | *L. rhamnosus* CMGB 34 | *L. rhamnosus* CMGB 34 | *L. rhamnosus* CMGB 34 | *L. rhamnosus* CMGB 34 |
| | *L. lactis* CMGB 31 | *L. lactis* CMGB 31 | *L. lactis* CMGB 32 | *L. lactis* CMGB 32 | *L. lactis* CMGB 31 | *L. lactis* CMGB 32 |
| Synthetic amino acids added in feed | - | - | - | - | - | L Treonine DL Methionine |

In the structure of compound feed, coccidiostats were not introduced. Chickens were fed with three structures of compound feed according to the recommendations in the growth boom for Ross 308 hybrid, namely: starter, grower and finisher in the following sub-periods: from hatching to 10 days, from 11 days to 35 days and from 36 to 42 days. The light regime was 23 hours light and one hour darkness. The temperature in the hall was 32°C at the beginning of the experiment and was gradually reduced to 21°C at 21 days. The body weight, weight gain and food intake were recorded at 10, 35 and 42 days. At the end of the experiment 3 broilers from each variant were sacrificed and intestine samples were taken to establish the main changes due to the presence of *Campylobacter jejuni*.

### 3. Results and discussion

In order to establish the effect of probiotics on broiler performance parameters the following nutritional and bioproductive indicators were analyzed:

- **evolution of body weight** by weighing at hatching, 10, 24, 35 and 42 days.
- **evolution of total gain** by individual weighing at hatching and the ages of 10, 24 and 35 days.
food intake; expressed in g/head/day was established for each growth sub-period (hatching-10 days, 11-35 days and 36-42 days) and also throughout the experimental period.

Feed consumption ratio was calculated for each growth sub-period (hatching-10 days 11-35 days and 36-42 days) and throughout the experimental period based on data from ingestion of food and related gain for each repetition.

The body weight evolution of broilers belonging to the seven experimental variants can be seen in Figure 1a. Percentage differences arising between groups are shown in Figure 1b.

**Figure 1a.** Body weight of broilers at 42 days

From the graphical representation shown in Figure 6, it is shown that the experimental broiler groups L2 and L3 have the highest mean values of body weight by 3.49% and respectively 2.40% compared to L0. Broilers in groups L1 and L5 show a body mass close to the reference variant (L0), with values only 0.80% and respectively 0.63% higher, and broilers in L4 and L5 presented the end of the experimental period a lower body mass with 1.21% (L4) and 2.46% (L6) with differences that are not statistically significant (p>0.05).

If we refer to L1 group, it can be noticed that broilers from L3 record a body mass of 1.59% higher, and the broilers in L5 group present a 1.16% reduction in body mass, but even this time differences are not significant (p>0.05).

We identified an increase in body mass for the broilers to L2 group compared to L4 variants (of 4.57%) and L6 (of 5.78%) which is statistically insignificant (p>0.05).

The total gain recorded by broilers of the different experimental variants and also statistical indices resulting from processed data are presented in Table 2.

**Total gain in growth in the sub-period from hatching-42 days**

Throughout the growth period, the largest increase in weight was recorded by L2 group (2699.91 g) followed by L3 group (2670.5 g), L1 (2628.63 g), L5 (2622.5 g) L0 (2605.66 g), L4 (2574.88 g) and L6 (2541.5 g). During the entire period of growth, the differences between increases were statistically insignificant (p>0.05).

It can be concluded that the weight gained by broilers is not influenced by the presence of probiotics in the structure of compound feed given to broilers belonging to the different experimental variants.

**Feed consumption during hatching-42 days**

Throughout the growth period, the lowest feed consumption is observed at chickens in L6 group (4700.63 g) followed by L1 (4786.63 g), L3 (4832.00 g), L4 (4837.51 g), L5 (4847.50 g), L2 (4854.39 g) and L0 (4864.58 g).

It can also be observed that during the entire growth period, the differences between consumption are statistically insignificant (p>0.05), and it can thus be concluded that feed consumption is not influenced by the presence of probiotics or of amino acid synthesis above required values in compound feed.

**Feed conversion index (FCR) (kg feed/kg gain in weight)**

Throughout the experimental period (hatching-42 days) the lowest feed conversion index was 1.8 for L2 group and 1.88 for L4 group. The differences between these indices were statistically insignificant (p>0.05).
In contrast with the results obtained in the Fallah experiment and col., 2013 [10], Panda and col., 2005 [10], it can be observed that the administration of probiotics in broiler feed increases final body weight and improves feed conversion ratio (FCR) at 42 days.

Table 2. The effect of probiotics on bioproductive performance in broilers (mean±SD)

<table>
<thead>
<tr>
<th>Item</th>
<th>Gain (kg/bird)</th>
<th>FI² (kg/bird)</th>
<th>F:G³ (kg:kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2603.66±77.91</td>
<td>4864.58±66.47</td>
<td>1.87±0.03</td>
</tr>
<tr>
<td>1</td>
<td>2628.63±18.21</td>
<td>4786.63±34.47</td>
<td>1.82±0.001</td>
</tr>
<tr>
<td>2</td>
<td>2699.91±17.02</td>
<td>4854.39±10.37</td>
<td>1.80±0.01</td>
</tr>
<tr>
<td>3</td>
<td>2670.50±12.02</td>
<td>4832.00±7.92</td>
<td>1.81±0.005</td>
</tr>
<tr>
<td>4</td>
<td>2574.88±51.80</td>
<td>4837.51±38.44</td>
<td>1.88±0.02</td>
</tr>
<tr>
<td>5</td>
<td>2622.50±14.32</td>
<td>4847.50±75.45</td>
<td>1.85±0.01</td>
</tr>
<tr>
<td>6</td>
<td>2541.50±85.38</td>
<td>4700.63±36.70</td>
<td>1.85±0.04</td>
</tr>
<tr>
<td>SEM</td>
<td>16.899</td>
<td>17.176</td>
<td>0.009</td>
</tr>
</tbody>
</table>

¹All means are average of 2 pens per treatment.
²FI=feed intake.
³F:G=feed-to-gain ratio.
⁴Significance level (P≤0.05)

Morphological aspect of the intestine

The histomorphometric study conducted on microscopic sections of the duodenal segment of the small intestine, reveals for broiler individuals in the control group L0, villous structural aspect, slightly basal widened, with a medium height of 2146.40 μm and coated with a prismatic monolayer epithelium. Enterocytes are slightly spaced, they have a height of about 37.15 μm and at the apical pole they present a well developed ribbed plate. Staining with Alcian blue, reveals the presence of goblet cells, with an area of about 37.08 μm² and a perimeter of about 24.10 μm. The cells present lenticular basal nucleus and the cytoplasm has a granular shape, probably associated to the accumulation of mucus. In some areas, goblet cells present a cytoplasm with a spongy appearance, as a consequence of mucus secretion, shown on the villous surface. Intraepithelially, in the basal chorion and over the entire length of the villous chorion, the presence of a moderate leukocyte infiltration can be observed (Figure 2).

Figure 2. Duodenum L0- intestinal mucosa basis. Goblet cells in glandular and villous epithelium. Leukocyte infiltrate (Alcian blue; 400x)

Figure 3. Cecum. L0 Goblet cells and mucus submitted on the mucosal surface (Alcian blue; 400x)
The mucus secreted by goblet cells influences nutrient digestion, the absorption process, the discharge of intestinal contents, and also represents the first barrier of defense against pathogens. The balance between the mucus layer, the intestinal epithelial cells, micro biota and the immune system are essential for maintaining homeostasis of intestinal mucus [11, 12, 13]. Under normal physiologic conditions, the mucus barrier is maintained at the lining surface by a reduced and continuous secretion of mucus, performed by a reduced exocytosis of the goblet cells. However, a number of intraluminal irritants are able to produce a quick "drain" of the goblet cells content, mucus layer, released in such cases, serving to protect the epithelium surface [14, 15]. At the cecal level, by analyzing fixed histological preparations, colored using the Alcian Blue method, lesions of mucous can be observed and also the presence of a thick layer of mucus from mucosal surfaces where PMNN type cell formations and macrophages can be seen (Figure 3). In some areas, infiltrating leukocytes cells aerobe served in the thickness layer of the mucous and capillary ectasia.

In the case of broiler individuals belonging to the experimental group L1, the duodenal mucosa reveals villous lower in height than L0, with an average height of about 1901.55 micrometers, the difference between the two groups being significant (p≤0.05). The enterocytes of the intestinal epithelium present dimensions slightly similar to those in group L0, with values around 36.68 μm. At the apical pole, enterocytes present a uniform ribbed plateau. Although the histomorphometric examination indicates a slight hypertrophy of the goblet cells both in villous and glandular epithelium, substantially of larger dimensions, 41.38 μm², as compared to L0, the differences between the two groups being insignificant. Frequently, the goblet cells have a disorganized aspect, with mucus release. In the chorion of the mucous and intraepithelial, leukocyte infiltration is reduced and the capillary network is slightly hypertrophic.

The cecal mucosa is much thicker than L0, with an average thickness of about 298.65 μm, the difference between the L1 and L0 groups being significant (p<0.001). Intestinal glands are deep and goblet cells present in the epithelial structure are slightly hypertrophic, with intense granular cytoplasm, often with mucus release. Periglandulary, mild edema can be observed. The chorion of the the mucosa shows infiltrating cells and blood capillaries slightly hypertrophic.

Regarding broiler individuals in L2 experimental group, the duodenal mucosa presents villi with similar development observed in group L0, their mean height recorded around 2066.50 μm, the difference between the two groups analyzed not being statistically significant. The intestinal epithelium is uniformly distributed on the surface of the villi and presents a clear ribbed plateau (Figure 4).
It is composed of enterocytes with an average height of about 35.33 μm, similar to groups L0 and L1, and goblet cells, with much smaller sizes compared to previous groups, with an average area of around 19.95 μm². In the case of this histomorphometric parameter, the difference between groups L2, L0 and L1 is significant (p<0.001).

On large territories or areas, goblet cells have an intensely granular cytoplasm, and at glandular level, on smaller areas villous structures can also be observed. The processes of mucus release can be seen (Figure 3). Intraepithelial, but namely in the chorion of the mucosal, the moderate presence of infiltrating leukocyte cells can be observed.

The average thickness of the cecal mucosa is 353.95 μm, the difference between L2 and L0 groups being significant (p<0.001). The epithelium of the mucous membrane, as well as the glandular epithelium is composed mainly of goblet cells. On microscopic sections stained with Alcian Blue, the goblet cells appear homogeneous with granular cytoplasm in the basal portion of the gland (Figure 5). On small areas, goblet cells are of uneven appearance as a result of the mucus release deposited on the surface of the mucosa. In some areas, in the chorion of the mucosa, leukocyte infiltration is reported.

In the case of the experimental group L3, duodenal mucosal villi were recorded to have a size of 2485.60 μm, being greater than those recorded in the experimental groups L0, L1 and L2, with significant difference between groups L3 and L0, and significant (p<0.001) between L3 and L1. The enterocytes of the intestinal epithelium present sizes slightly similar to those in the experimental group L2, values recorded around at the value of 34.32 μm, the difference between the two groups being insignificant, but significant between L3 and L0 groups. At the other apical pole, enterocytes show uniform ribbed plateau.

Goblet cells showed an average value of an area of 28.18 μm² and a perimeter of about 20.58 mm, with significant differences from L0 (p<0.05) and L1 (p<0.001) experimental groups. The cytoplasm of these cells is grainy, but on large villous territories, it presents a clear cytoplasm, forming a thin layer of fine mucus on the mucosal surface.

The cecal mucosa has a similar development to that of the experimental group L2, its average thickness is about 354.73 μm. The statistical analysis of the histomorphometric data reveals that, between cecal mucosal thickness in this group and that of L0 and L1 groups, the difference is significant (p<0.001). On small areas, light epithelial lesions can be observed. Goblet cells present intensely granular cytoplasm and in the crypts, there are abundant deposits of mucus with secretor activity. In the chorion of the mucosal, leukocyte infiltrations and the presence of hypertrophic capillary networks we observed.

In the case of broiler individuals belonging to experimental group L4, the duodenal mucosal villi has an average height of 1936.50 μm, lower than those reported in individuals in group L0, the difference between the two groups being significant (p<0.05). The villous epithelium, presented as a ribbed plate, looks normal, although compared to other groups, the two structures are lower in height, the difference between L4 and L0 groups being significant (p<0.001). The goblet cells are numerous, present all over the villous epithelium and glandular surface, well shaped, with granular cytoplasm and sensitive dimensions similar to those recorded for L0 group, the average area of these cells being about 34.60 μm. The chorion of the mucosal shows a normal leukocyte infiltration, more abundant in certain territories.

The cecal mucosa has an average thickness of 241.55 μm. The differences between this group and groups L0, respectively L2, are significant (p<0.001). Intestinal glands are deep and have glandular epithelium which consists predominantly of goblet cells, with uniform layout in the cecal epithelium and with intense granular cytoplasm. In the chorion of the mucosal, leukocyte infiltration presence is observed, more abundant, relatively on small area.

The broiler individuals in experimental group L5 show duodenal villous with a development similar to other experimental groups, with no significant differences between them. The epithelium of the mucosal appears uniform, consisting of high enterocytes, with average sizes of 37.20 μm, with a clear ribbed plate at their apical pole. The difference between this group and groups L0 and L1 is insignificant regarding epithelium development. Goblet cells have a hypertrophic aspect, similar to the individuals of groups L0 and L1, and their cytoplasm is highly granular. Large territories of the villous epithelium, both at the base of the villi and towards their top, exocytosis
is present, the cytoplasm gaining an vacuolar aspect (Figure 6). The average thickness of the cecal mucosa is 336.35 μm, with similar dimensions between L2 and L3 groups.

The difference between L5 and L0 groups was significant (p<0.001). Goblet cells are hypertrophic and show exocytosis. In the chorion of the mucosal, capillary hypertrophy and leukocyte infiltration are observed (Figure 7).

Regarding the broiler experimental group L6, the intestinal mucosa presents villous with an average height of 2340.55 μm, higher in comparison with L0, L2, and L6, the differences being insignificant between L0 and L2 and significant between L6 and L2 (p<0.05). The villous epithelium appears normal, consisting of average sized enterocytes of 36.45 μm, similar to the other experimental groups and goblet cells of average size of 26.73 μm², the difference between L6 and L0 is significant (p<0.05). Goblet cells have intensely granular cytoplasm. Frequently, both at the base of villi, and also towards the top, goblet cells have an hypertrophic aspect, with eliminated mucus. The loose connective tissue in the mucosal chorion structure presents infiltrating leukocytes cells both basal and villous, abundant in certain territories. Also, such infiltrations can be observed intraepithelial as well. The cecal mucosa has an average thickness of 227.60 μm, with significant differences between L6 and L0, and between L6 and L2 groups (p<0.001). Intraepithelially and in the chorion of the cecal mucosal, as in the duodenal segment, the leukocyte infiltration is abundant, and the capillaries are slightly hypertrophic. Goblet cells have granular cytoplasm, with mucus deposition on the mucosal surface.

Conclusions
- The total gain, feed intake and FCR are not influenced by the presence of probiotics in the forage structure for all studied variants (p>0.05).

References
8. Langout, P., New additives for broiler chickens. Feed Mix, 2000, pp. 24-27