Effect of Different Growth Promoters on the Cecal Microflora and Performance of Broiler Chickens

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Abstract


The objective of this study was an evaluation of the effects of different growth promoters: probiotic Lacto-Sacc® (Alltech, Inc. USA), nutritive premix Pharmastim and nutritive antibiotic Avilamycin on the cecal microflora and performance of broiler chickens. The experiment was conducted using four feeding groups of one-d-old broiler chicken: Group I: Basal diet without supplement (Untreated control); Groups II, III and IV: Basal diet supplemented with probiotic Lacto-Sacc®, (1.0 kg/t); nutritive premix Pharmastim, (0.3 kg/t); and nutritive antibiotic Avilamycin (5.0 mg/kg), respectively. The addition of Lacto-Sacc® to the basal diet significantly increased the count of lactobacilli and enterococci in the cecum of the broiler chickens and depressed the count of coliforms by the low pH of cecal content. There was no significant difference in the count of lactobacilli, enterococci, and coliforms present in the cecum of the untreated control, Pharmastim and Avilamycin-fed groups. Lacto-Sacc® enhanced growth of beneficial microorganisms, regulated the microbial environment in the cecum. Lacto-Sacc® significantly increased the body weight of broiler chickens (P<0.001), compared to the untreated control and the groups fed Pharmastim and Avilamycin. The Lacto-Sacc®, Pharmastim and Avilamycin supplements improved feed conversion ratio by 8.8, 1.1 and 1.9% (P<0.001), compared to the untreated control, respectively.

These results suggested that feed additives based on microorganisms provide an alternative to antimicrobial substances in broiler nutrition. Probiotics, and especially Lacto-Sacc® can reduce or replace antibiotics used for growth promotion in broiler chickens.

Key words: broilers, nutrition, cecal microflora, growth promoters, probiotics, antibiotics

Introduction

The microbial populations that inhabit the gastrointestinal tract (GIT) of poultry have been extensively studied in recent years (Barrow, 1992; Chiang and Hsien, 1995; Jin et al., 1996; Farran et al., 2004). Many workers have studied the cecal microbial...
flora of the poultry. Several investigations on indigenous microflora of chickens, laying hens, turkeys, pheasants, and ducks have shown that the cecum contains the largest number of resident obligate and facultative anaerobic bacteria (Smith, 1965; Timms, 1968; Barnes and Impey, 1970, 1972; Barnes et al., 1972; Salanitro et al., 1974a, 1974b) that perform a number of beneficial functions. The mechanisms whereby these bacteria are maintained in the cecum are poorly understood (Barrow, 1992). Although many studies have been carried out in the intestinal flora of the bird and in particular the chicken, one is still far from understanding the factors that govern the presence of particular groups of microorganisms in various parts of the intestine and the role they may play in the nutrition and physiology of the bird.

Intensive rearing conditions for livestock are believed to contribute to a delay or disturbance in the development of intestinal microflora. Chickens reared in commercial hatcheries are never exposed to bacteria from the mother hen and therefore the development of their intestinal microflora is delayed. At hatching, the intestinal tract is essentially sterile, but it becomes rapidly colonized by microorganisms from the environment (Stavric, 1994). Several weeks are required to establish an adult-type microflora in the cecum (Smith, 1965). Nurmi and Rantala (1973) recognized that the susceptibility of young chickens to colonization with pathogens was due to the delayed establishment of their microflora.

The cecal microflora of the alimentary tract has a significant effect on the health and performance of poultry. There is considerable evidence that the cecal microflora exert protection against the establishment of microbial pathogens such as Salmonella (Nurmi and Rantala, 1973) and Campylobacter (Barrow, 1992), which preferentially colonize the cecum.

It is well established that the manipulation of the gastrointestinal microflora (GIM) of poultry has a significant effect on the growth rate and efficiency of feed utilization. However, the influences of antimicrobial agents on microbial population of the cecum are less understood.

Antibiotics disturb the microbial balance in the cecum. The sensitivity of chickens to Salmonella infection increases after antibiotic treatment (Stavric, 1994). Although antibiotics have been used as therapeutic agents and growth promoters since the 1950s, there has been concern that their usage as growth promoters could result in the development of resistant populations of bacteria, making subsequent therapy with antibiotics difficult (Fuller, 1989; Skinner, 2003). Furthermore, consumers' concern regarding antibiotic residues in meat has also been growing.

Because of the growing concern over the transmission and the proliferation of resistant zoonotic bacteria via the food chain, the European Union (EU) decided in 1999 to ban 4 commonly used antimicrobial growth promoters (AGP) (virginiamycin, spiramycin, tylosin, and zinc bacitracin). During 2003, the EU also signalled its intention to remove the other AGP by January 2006 (flavomycin and avilamycin/monensin and salinomycin).

Currently, there are a number of non-antibiotic alternatives. Some of these alternatives may include significant changes in husbandry practices or the strategic use of probiotics, prebiotics, acidifiers, enzymes, herbal products, microflora enhancers, and immuno-modulators (Ferket, 2004).
Modulation of the intestinal bacteria towards a "healthy" community by feeding live bacteria (so called "probiotics") or speciality carbohydrates supporting beneficial bacteria (so called "prebiotics") is currently under active research (Apajalahti et al., 2004.). The target of such nutraceutical products is to improve gastrointestinal health by selecting for beneficial microflora and suppressing known intestinal and food-borne pathogens.

The interest in the use of probiotics as substitute is not surprising, especially in view of some claims that they offer a similar growth promoter benefit (Simon, 2003). The main benefits attributed to probiotics as natural feed additives are: restoration and maintenance of the proper balance of the microflora in the intestinal tract during times of stress, diseases and following antibiotic therapy. Probiotics act as natural growth promoters, and improve the general health and performance of poultry (Dawson, 2001). Many reports and reviews have shown that the oral administration of probiotics improve growth rate, feed conversion (Jernigan et al., 1985; Hussein and Ashry, 1991; Cho et al., 1992; Baidya et al., 1993; Va Holourek, 1993; Alvares et al., 1994; Manieckan et al., 1994; Chiang and Hsieh, 1995; Denev, 1996, 1997; Georgieva et al., 2000; Simon, 2003; Skiner, 2003; Edens, 2003) and enhance immune response and health (Perdigon et al., 1995; Maasen et al., 2000; Sotirov et al., 2000, 2001; Vitini et al., 2001 Denev, 2000, 2003). For the above reasons, there is a wide interest in replacing AGP with more natural feed additives - probiotics.

Although the cecum is an area of high microbial activity in the intestine of chickens, the effects of such factors as a diet on the cecal microflora is still not properly understood, because a number of predominant anaerobic bacteria present have not yet been isolated and characterized. There is also little information available describing the predominant species of bacteria occurring in the GIT of broiler chickens fed different growth promoters, especially nutritive antibiotics as well as probiotics.

The objective of the current study was to examine the effect of probiotic Lactosac®, a nutritive premix Pharmastim and Avilamycin supplementation on the cecal microflora and growth performance of broiler chickens.

**Materials and Methods**

**Chickens, Diet and Husbandry**

Two hundred and forty-one-d-old female broiler chickens (Arbor Acres) supplied by a commercial broiler hatchery were used in the current experiment. An environmentally controlled research building with floor pens was used to house 60 birds per pen (0.16 m² per bird). Litter was new pine wood shavings. Birds were randomly assigned to treatment pens with empty barrier pens between treatment pens. An initial brooding temperature of 35°C was maintained for the newly hatched chickens and reduced in weekly increments to a constant temperature of 25°C at 3 weeks of age. After that, environmental temperature and ventilation were regulated according to standard management practices for bird age and external weather. Lighting was continuous and feed and water were provided *ad libitum*.

The chickens were divided into four groups of 60 chickens each. During the starter (0 to 28 d) and finisher (29 to 49 d) periods, all groups were fed the basal starter or finisher diets. The diet composition is presented in Table 1. We compared four feeding groups: Group I: Basal diet
without supplement (Untreated control); Group II: Basal diet supplemented with Lacto-Sacc® (Alltech Inc., USA) (1.0 kg/t), contained per kg: Lactobacillus acidophilus (NLC 84), minimum 110 billion (CFU); Streptococcus faecium (NCS 97), minimum 77 billion CFU; Yeast culture - Saccharomyces cerevisiae, minimum. 1 980 billion cells, and enzymes; Group III: Basal diet supplemented with Pharmastim (Biovet, Bg), (0.3 kg/t), contained antibiotic Flavophospholipolum (Flavomycine) - 2,0 and Excipients ad 100.0), Group IV: Basal diet supplemented with Avilamycin (Elanco, USA) (5.0 mg/kg active substance).

**Sampling procedures**

At the end of the experimental period, (49 d of age) five birds from each group were randomly selected and killed by CO₂ asphyxiation for collection of cecal contents. The intestinal tract was removed and placed in a container under a flow of oxygen-free CO₂. By using aseptic techniques, both cecum with contents were ligated, removed from the dissected body cavity, and the contents were squeezed into a beaker being flushed with CO₂. The cecal contents were collected aseptically, mixed, while being flushed with CO₂, before the dilutions were made.

A pH-meter with a microelectrode measured the pH of cecal contents from each group. The test method was that used by Barnes et al. (1979).

**Microbiological analyses**

**Media.** The count of viable microbes in the cecal contents were determined using various media: 1) The Anaerobic dilution solution (ADS) used in this work was a modification of the dilution blank of Holdeman and Moore (1977) containing (g/l): K₂HPO₄ x 3H₂O, 4.105; KH₂PO₄, 1.636; cysteine hydrochloride, 0.5; gelatin, 2.0; and resazurin, 1.0. The solution was pre-reduced before use; 2) VLMH Medium (Barnes and Impey, 1970), containing (g/l): tryptone, 10.0; NaCl, 5.0; beef extract, 3.0; yeast extract, 5.0; cysteine hydrochloride, 0.4; glucose, 2.5 (pH 7.2±0.2) enriched with menadione (0.5 µg/ml) and hemin (5.0 µg/ml) and solidified with 1.5% agar (Schneitz et al., 1981) was used to enumerate the total anaerobic and aerobic counts. The Anaerobic dilution solution and VLMH Medium were prepared from individual ingredients (Merck, Germany); 3) Rogosa Agar (Difco Laboratories, USA) was used for enumeration of lactobacilli; 4) Slanetz and Bartley Medium (Oxoid UK, CM377) was used for enumeration of enterococci; 5) MacConkey Agar (Difco Laboratories, USA) for coliforms bacteria; 6) Bacto Brilliant Green agar (Difco Laboratories, USA) for Salmonella spp.

**Enumeration Methods.** Serial 10-fold dilutions were prepared with sterile pre-reduced ADS. All dilution steps and inoculation of media were carried out under anaerobic conditions according to procedures described by Moore and Holdeman (1974). The serially diluted cecal contents were used for the enumeration of the anaerobic and facultative anaerobic organisms. All the plates used for the enumeration of the cecal microflora were freshly prepared and poured a day before use, pre-dried at 60°C for 15 minutes and stored in the anaerobic conditions. The diluted cecal contents were incorporated into plates of VLMH Medium, Rogosa Agar, Slanetz and Bartley Medium and MacConkey's Agar, and Bacto Brilliant Green agar to enumerate the total anaerobic and facultative anaerobic organisms,
lactobacilli, enterococci, coliforms, and *Salmonella* counts respectively. The plates were incubated anaerobically in Gas Pak® anaerobic system (BBL Microbiology Systems, Cockeysville, MD. USA.) at 37°C for 5 days (Total anaerobic count); at 37°C for 48 h (lactobacilli and enterococci), and aerobically at 37°C for 24 h (facultative aerobic count, coliforms and *Salmonella*). The total colony counts per g of undiluted cecal content from each medium were obtained as the weighed mean from two or three highest duplicate dilutions that showed growth.

**Performance data**

During the 7 weeks experimental period, the following performance criteria were studied: a) Live body weight (LBW) was recorded individually before feeding to the nearest 0.1 g at the beginning of the trial (after hatching) and then to the nearest 0.01 kg at 1, 2, 3, 4, 5, 6, and 7 weeks of age; b) Daily weight gain (DWG), kg; c) Feed conversion ratio (FCR) was calculated on the basis of kg of feed/kg weight gain.

**Statistical analysis**

All bacterial populations estimates were calculated and reported on a per g (wet weight) basis of cecal content. The mean and standard deviation of the bacterial counts (CFU/g) were calculated using logarithmic values. Student's t-test, conducted using Microsoft Excel was used to determine the significance of the differences between the experimental groups.

**Results and Discussion**

The composition of the cecal microflora of broiler chickens is shown in Table 1. The total viable count of obligate and facultative anaerobes in the cecal contents was high in all experimental groups. These bacteria formed the major part of the cecal flora. There was no significant difference in the total count of bacteria present in the cecum of the control and experimental groups. Many other investigations have shown that the cecum contains the largest number of bacteria, most of which are obligate and facultative anaerobes (Barnes and Impey, 1970; Barnes et al., 1972; Salanitro et al., 1978; Sofos et al., 1985).

The results of the present study on the bacterial counts of obligate and facultative anaerobes in cecal contents of broiler chickens indicate also that Pharmastim and Avilamycin have no inhibitory effect on cecal microflora. According to Bare and Wiseman (1964) the mechanisms that have been proposed to explain the growth promoting effect of antibiotics usually involve inhibition or stimulation of microorganisms in the intestinal tract. Ilieva et al. (1997) reported that the nutritive effect of Avilamycin is a result of its favorable effect on the intestinal microflora.

Lactic acid bacteria, particularly lactobacilli, constitute an important group of microorganisms colonizing the GIT of chickens (Smith, 1965; Barnes, 1979). Mead and Adams (1975) have shown that the number of lactobacilli in the cecal tract of young chickens increases during the first 14d from 510 to 910/g feces. *Lactobacillus acidophilus, Lactobacillus salivarius* and *Lactobacillus reuteri* have been shown to be the most common species in the chicken gut by Sara et al. (1985). The colonization of the GIT by lactobacilli has long been believed to promote health and productivity.

The experimental data obtained by the microbiological analysis indicated that the...
Lactobacillus population increased predominantly when broiler chickens consumed a diet supplemented with probiotic. The results presented in Table 2 show clearly that the lactobacilli were the most numerous microorganisms present in the cecum of broiler chicks receiving a basal diet with the microbial supplement Lacto-Sacc®. Lacto-Sacc® significantly increased the total number of lactobacilli in the cecal content of broiler chicks (P<0.001). Bonomi et al. (1995), and Maruta et al. (1996) observed similar effects of Lacto-Sacc® (Paik et al., 1990) and other directly fed microbials (Edens, 2003).

The counts of lactobacilli were significantly lower (P<0.001) in the cecal contents of the untreated control, Pharmastim and Avilamycin-fed chickens. However, no significant difference was observed in the count of lactobacilli present in the cecum

### Table 1
Composition of basal starter and finisher diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (To 28/d)</th>
<th>Finisher (29 to 49/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>52.99</td>
<td>61.89</td>
</tr>
<tr>
<td>Soybean meal (41% CP*)</td>
<td>31.15</td>
<td>24.35</td>
</tr>
<tr>
<td>Sunflower meal (38% CP*)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>5.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Animal Fat</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Vitamin mineral premix*</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.40</td>
<td>1.30</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Total:</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Calculated nutrient content:**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter (ME, MJ/kg)</th>
<th>Finisher (ME, MJ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>22.20</td>
<td>18.82</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.28</td>
<td>1.01</td>
</tr>
<tr>
<td>Methionine + cystine, %</td>
<td>0.90</td>
<td>0.75</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.99</td>
<td>0.90</td>
</tr>
<tr>
<td>Phosphorus (available), %</td>
<td>0.45</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* Vitamin-mineral premix supplied the following vitamins and minerals per kg of diet: vitamin A, 200 000 IU; vitamin D₃, 500 000 IU; vitamin E, 4 200 mg; vitamin K₃, 400 mg; vitamin B₁₂, 200 mg; vitamin B₆, 1000 mg; vitamin B₉, 400 mg; vitamin B₂, 1000 mg; vitamin B₃, 400 mg; vitamin B₅, 5 000 mg; Calcium D-pantoten acid, 2 000 mg; Folic acid, 100 mg; Cholin chloride, 80 000 mg; Fe, 5 462 mg; Mn, 10 342 mg; Cu, 926 mg; Zn, 12 727 mg; Co, 54,5 mg; I, 137 mg, and Se, 18,3 mg.
Effect of Different Growth Promoters on the Cecal Microflora and Performance...

of the control, Pharmastim and Avilamycin-fed groups. These results indicated that the above nutritive antibiotics do not inhibit the population of Lactobacilli in the cecal content of broiler chickens. Dut and Devriese (1981) also reported that lactobacilli isolated from cecum of poultry are resistant to some growth promoting agents, particularly bacitracin, nitrovin, carbadox, flavomycin, lincomycin, oleandomycin, spiramycin, and tylosin.

Enterococci form a significant part of the intestinal flora of the bird throughout life (Barnes et al., 1978). The results of the present study (Table 2) indicate that Lacto-Sacc® increases the number of enterococci population in the cecum of broiler chickens. In the control, Pharmastim and Avilamycin-treated groups, the number of cecal Enterococci was significantly lower in comparison with the Lacto-Sacc®-fed chickens P <0.001). However, no observable significant difference in the counts of Enterococci between the control, and Pharmastim and Avilamycin-fed broiler chickens were found.

One of the most important functions of probiotics is the control or elimination of enteropathogens (Fuller, 1989; Gibson et al., 1997; Denev, 1996, 1977, 2000, 2003; Edens, 2003). The mechanism by which this occurs is still not well elucidated. Among the suggestions that have been considered are competition for limited carbon sources, and the production of antibacterial compounds (organic acids, hydrogen peroxide, bacteriocins).

The present study has shown that the probiotic Lacto-Sacc® significantly decreased the number of coliforms in the cecal content of broiler chicks compared to the control, Pharmastim and Avilamycin-fed chicks (P<0.001). Chapman (1989), Paik et al. (1990) and Bonomi et al. (1995) have also reported that the addition of probiotics to the diet increased the development and activity of intestinal microflora, by increasing the number of LAB, including lactobacilli and by decreasing the number of coliforms, particularly E. coli. Many other investigators have studied the potentials of probiotics, which

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### Table 2

<table>
<thead>
<tr>
<th>Bacterial counts**</th>
<th>Untreated Control</th>
<th>Lacto-Sacc®</th>
<th>Pharmastim</th>
<th>Avilamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total anaerobes</td>
<td>11.663±0.018a</td>
<td>11.533±0.250a</td>
<td>11.468±0.180a</td>
<td>11.762±0.150a</td>
</tr>
<tr>
<td>Facultative</td>
<td>9.595±0.005a</td>
<td>9.485±0.015a</td>
<td>9.433±0.016a</td>
<td>9.689±0.006a</td>
</tr>
<tr>
<td>Anaerobes Lactobacilli</td>
<td>6.878±0.003a</td>
<td>8.467±0.015b</td>
<td>6.764±0.069a</td>
<td>6.608±0.013a</td>
</tr>
<tr>
<td>Enterococci</td>
<td>6.422±0.024a</td>
<td>7.039±0.035b</td>
<td>6.307±0.066a</td>
<td>6.460±0.176a</td>
</tr>
<tr>
<td>Coliforms</td>
<td>5.826±0.006a</td>
<td>3.969±0.020b</td>
<td>5.656±0.012a</td>
<td>5.666±0.008a</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean of five chickens ± SE of the mean.
** Log10/g (wet weight) of cecal contents
a – d: Different letters indicate significant differences (P < 0.001)
exert *in vitro* inhibitory effects toward enteric microorganisms (Gilliland and Speck, 1979; Edens, 2003) and *in vivo* growth competition with *E. coli* in gnotobiotic chickens (Watkins et al., 1982; Watkins and Miller, 1983).

No cecal Salmonella was found from birds fed the different supplements at 49 d of age (Table 2).

The inclusion of Lacto-Sacc® in the diet of broiler chickens decreased significantly (P<0.001) the pH of the cecal content in comparison with the control, Pharmastim, and Avilamycin-fed chickens (Figure 1). The low pH produced in the cecum by the probiotic microorganisms was partly responsible for the suppression of coliforms in the cecum. The feeding of Lacto-Sacc® may have greater significance in birds with enteric disease caused by pathogenic *E. coli* (Kovacz-Zomborszky et al., 1994). Kumprecht et al. (1994) and Rada et al. (1995) also reported that including probiotics in the poultry diet decreases pH in the cecum. Similar results were observed with the nutritive premix Pharmastim compared to the control (P 0.05). There was no significant effect of Avilamycin on the pH of the cecal contents in broiler chickens.

The effects of Lacto-Sacc®, Pharmastim and Avilamycin on broiler performance are presented in Table 3. These data indicate that at the end of the experimental period the probiotic Lacto-Sacc® had a beneficial effect on growth performance. It significantly increased the LBW and DWG of broiler chickens (P<0.001). The LBW of Lacto-Sacc® fed chicks were 7.5, 3.4, and 3.6 % higher in comparison with the untreated control, Pharmastim and Avilamycin fed chicks respectively. A similar beneficial effect of Lacto-Sacc® and other probiotics on poultry LBW and DWG was obtained by Gous (1990); Wiseman (1990); McGinnis (1992), Gippert et al. (1992), Gippert and Bodrogil (1992); Samanta and Biswas (1995), Wambeke et al. (1995); Vladimirova and Sourjitska (1996); Edens (2003).

The LBW and DWG of Pharmastim, and Avilamycin fed broiler chickens were low and unaffected by treatment. The difference between groups with and without nutritive antibiotics (untreated control) was not significant. The results concerning the effect of the nutritive antibiotic Avilamycin that we obtained is different from the ones reported by Jambos et al. (1995). Chapman (1989) obtained similar results as the results achieved by us with other nutritive antibiotics in comparison with probiotics. Ayed et al. (2004) reported that Avilamycin decreases chicken growth performance and has undesirable side effect on health.

The supplementation of probiotic Lac-
to-Sacc® to the basal diet improved the
efficiency of feed conversion in compari-
son with the untreated control, Pharmastim
and Avilamycin fed broiler chickens (Table
3). During the experimental period, the
best feed conversion was appeared chick-
ens fed Lacto-Sacc® (2.077 kg) which
needed 8.8% less feed for 1.0 kg weight
gain, in comparison with the untreated
control (2.277 kg). The differences among
the untreated control and other experimen-
tal groups were less - 1.1% (2.251 kg
Pharmastim supplemented) and 1.9%
(2.233 kg Avilamycin supplemented).

The better results in body growth and
feed efficiency obtained in the Lacto-
Sacc® fed chickens are most probably due
to the beneficial effect of this probiotic
on cecal microflora particularly on the Lac-
tobacilli and the inhibition of enteropatho-
genic, and harmful bacteria. On the other
hand, Lacto-Sacc® contains some en-
zymes - amylase, protease, and cellulase
that improve digestibility and feed conver-
sion of the diet.

Mortality was not affected by any of
the dietary treatment during the trial, which
is in agreement with Farran et al. (2004)
who used prebiotics, Avilamycin, Flavo-
mycin, and Parks et al. (2001), who used
mannanoligosaccharides, Bambermy-cin
and Virginiamycin. Zulkifli et al. (2000)
when feeding broiler diets supplemented
with Lactobacillus spp. cultures or ox-
ytetracycline reported similar lack of ef-
f ect on mortality. According to Wenk
(1990) and Edens (2003) feed additives
based on microorganisms provide an al-
ternative to antimicrobial growth promot-
ers in animal nutrition. The probiotics can
reduce or replace antibiotics used for
growth promotion in broiler chickens.

The present study demonstrated that
Lacto-Sacc® enhanced growth of benefi-
cial microorganisms and regulated the mi-
crobial environment in the cecum of broil-
ers. This probiotic significantly increased
the number of lactobacilli and enterococci
and depressed the number of coliforms
and Salmonella spp. by the low pH of ce-

Table 3
Effect of Lacto-Sacc®, Pharmastim and Avilamycin on broiler performance*

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Lacto-Sacc®</th>
<th>Pharmastim</th>
<th>Avilamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.08</td>
<td>37.73</td>
<td>37.88</td>
<td>38.08</td>
</tr>
<tr>
<td>7</td>
<td>103.00±2.00a</td>
<td>110.00±1.90a</td>
<td>100.00±2.05a</td>
<td>99.00±1.72a</td>
</tr>
<tr>
<td>28</td>
<td>818.00±8.60a</td>
<td>897.00±11.78b</td>
<td>836.00±16.30a</td>
<td>811.00±8.20a</td>
</tr>
<tr>
<td>42</td>
<td>1494.00±13.88a</td>
<td>1560.00±15.47b</td>
<td>1479.00±22.49a</td>
<td>1435.00±12.29c</td>
</tr>
<tr>
<td>49</td>
<td>1875.00±20.56a</td>
<td>2016.00±21.48b</td>
<td>1810.00±28.76a</td>
<td>1807.00±15.19a</td>
</tr>
</tbody>
</table>

Feed conversion ratio, g feed/g gain

| 1-28 | 1.970 (100)** | 1.769 (89.8) | 1.894 (96.1) | 1.949 (98.9) |
| 28-49 | 2.501 (100) | 2.315 (92.6) | 2.543 (101.7) | 2.454 (98.1) |
| 1-49 | 2.277 (100) | 2.077 (91.2) | 2.251 (98.9) | 2.233 (98.1) |

* - Georgieva et al. (2000); ** - (%) ; a – d: Different letters indicate significant differences (P < 0.001)
cal content. There was no significant difference in the number of lactobacilli, enterococci, and coliforms present in the cecum of the untreated control, Pharmastim, and Avilamycin-fed groups. Lacto-Sacc® significantly increased the LBW of broiler chicks by 7.5; 3.4 and 3.6 % respectively - compared to the untreated control and the groups fed Pharmastim and Avilamycin. The Lacto-Sacc®, Pharmastim and Avilamycin supplements improved feed conversion by 8.8; 1.1 and 1.9% (compared to the untreated control), respectively. These results suggested that feed additives based on microorganisms provide an alternative to antimicrobial substances in broiler nutrition. The probiotic Lacto-Sacc® can reduce or replace antibiotics used for growth promotion in broiler chickens.

**Conclusions**

The addition of probiotic Lacto-Sacc® to the basal diet significantly increased the count of lactobacilli and enterococci in the cecum of the broiler chickens and depressed the count of coliforms by the low pH of cecal content. There was no significant difference in the count of lactobacilli, enterococci, and coliforms present in the cecum of the untreated control, Pharmastim and Avilamycin-fed groups. Lacto-Sacc® enhanced growth of beneficial microorganisms, regulated the microbial environment in the cecum.

Lacto-Sacc® significantly increased the body weight of broiler chickens (P<0.001), compared to the untreated control and the groups fed Pharmastim and Avilamycin. The Lacto-Sacc®, Pharmastim and Avilamycin supplements improved feed conversion ratio by 8.8, 1.1 and 1.9% (P<0.001), compared to the untreated control, respectively.

These results suggested that feed additives based on microorganisms provide an alternative to antimicrobial substances in broiler nutrition. Probiotics, and especially Lacto-Sacc® can reduce or replace antibiotics used for growth promotion in broiler chickens.

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