Effects of probiotic (Bacillus subtilis DSM 17299) supplementation on the caecal microflora and performance in broiler chickens

G. DENIZ1*, A. ORMAN2, F. CETINKAYA3, H. GENCÖGLU1, Y. MERAL1, I.I. TURKMEN1

1Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Uludag, 16059 Gornekle, Bursa, TURKEY.
2Department of Zootechnics, Faculty of Veterinary Medicine, University of Uludag, 16059 Gornekle, Bursa, TURKEY.
3Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Uludag, 16059 Gornekle, Bursa, TURKEY.

*Corresponding author: denizg@uludag.edu.tr

SUMMARY

The aim of the study was to determine the effects of dietary probiotic supplementation with B. subtilis spores (strain DSM 17299) on the growth performance and caecal traits in broiler chickens and to analyse the influence on the caecal microflora. A total of 364 one day-old male broiler chicks were randomly divided into 2 equal groups (not supplemented controls and birds receiving dietary addition of B. subtilis spores (8x10^5 cfu/kg of food) for 6 weeks). Body weights, body weight gains, food intake and food efficiency were weekly evaluated and the caecal parameters (hot caecal weight and yield) and the caecal microflora composition were determined at the end of the experiment. The probiotic supplementation has significantly increased the final weight gain (P < 0.05) and the hot caecal weight (P < 0.01), reduced the food intake calculated for 6 weeks (P < 0.001) and improved the food conversion ratios since the 4th week with supplementation (P < 0.001). In parallel, in the treated birds, the caecal population of bacilli was markedly enhanced (P < 0.01) whereas those of enterococci (P < 0.001) and coliforms (P < 0.05) were significantly lowered. The Enterobacteriaceae counts were also weakly depressed but not significantly and the numeration of lactobacilli has also tended to slightly increase. These results show that the dietary inclusion of B. subtilis spores improve the weight growth and the food efficiency in broilers probably through the selection of beneficial bacteria to the detriment of pathogen germs in the caecal microflora.

Keywords: Bacillus subtilis, broiler chickens, growth, carcass, performance, caecal microflora.

Introduction

Antibiotic feed supplements have been used in commercial poultry farming for over 50 years due to their growth-promoting and prophylactic properties [10, 14, 18]. However, the extensive utility of antimicrobial agents has resulted in the occurrence of an antibiotic residue problem in poultry meat and an increase of antimicrobial resistance among pathogenic bacteria which is a great problem of public health. Thus, their use in the European Union (EU) was prohibited in 2006 as part of an initiative aimed at promoting the prudent use of antibiotics [2, 45]. As a result, natural alternatives for substituting the prohibited growth promoter antibiotics with probiotics have received much attention in the recent past. Probiotic has been defined as “a live microbial feed supplement which benefits the host animal by improving its intestinal balance” [20]. A variety of microbial species have been used as probiotics, including species of Bacillus, Bifidobacterium, Enterococcus, E. coli, Lactobacillus, Lactococcus, Streptococcus, a variety of yeast species, and undefined mixed cultures [54]. Bacillus, Enterococcus, and Saccharomyces yeast have been the most common organisms used in livestock [64]. A number of probiotics used for poultry contain or consist of bacterial spores, principally of the genus Bacillus [11, 26].

RéSUMÉ

Effets du probiotique (Bacillus subtilis DSM 17299) sur la microflore caecale et les performances des poulets

Les objectifs de cette étude ont été de déterminer les effets d’une supplémentation de l’aliment avec un probiotique (souches B. subtilis, DSM 17299) sur la croissance pondérale des poulets et sur les caractéristiques des carcasses et d’analyser la composition de la microflore caecale. Au total, 364 poussins mâles de 1 jour ont été aléatoirement répartis en 2 groupes égaux (un groupe contrôle, non supplémenté, et un groupe expérimental dans lequel les oiseaux ont reçu les aliments standards enrichis en spores de B. subtilis (8x10^5 cfu/kg d’aliment) pendant 6 semaines). Les poids vifs, les gains de poids, l’ingestion alimentaire et l’efficacité de la ration ont été déterminés chaque semaine tandis que les paramètres des carcasses (poids à chaud de la carcasse et rendement) et la microflore caecale ont été analysés à la fin de la période expérimentale. La supplémentation avec le probiotique a conduit à une augmentation significative du gain de poids calculé sur 6 semaines (P < 0.05) ainsi que du poids à chaud de la carcasse (P < 0.01), à une réduction de l’ingestion alimentaire sur l’ensemble de la période (P < 0.001) et à une amélioration significative de l’efficacité alimentaire dès la 4ème semaine de supplémentation. En parallèle, chez les oiseaux traités, la population caecale des bacilles a nettement augmenté (P < 0.01) alors que celles des entérococus (P < 0.001) et celle des coliformes (P < 0.05) ont significativement diminué. La numération en Enterobactériaceae a aussi légèrement baissé mais pas significativement et celle des lactobacilles est apparue faiblement accrue. Ces résultats montrent que l’incorporation dans la ration de spores de B. subtilis améliore la croissance pondérale et l’efficacité alimentaire chez les poulets, en favorisant probablement l’émergence dans le caecum de populations bactériennes bénéfiques au détriment des populations pathogènes.

Mots clés : Bacillus subtilis, poulets, croissance, carcasse, performance, microflore caecale.
Spore-based probiotics are particularly well suited for use as live microbial products as they are metabolically dormant upon administration, they may germinate in the gastrointestinal tract of chicks and function through mechanisms which require them to be metabolically active (e.g. secretion of antimicrobial compounds and/or competition for essential nutrients) [12, 13].

Many studies demonstrated that probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces have some beneficial effects on broiler performance [6, 8, 21, 32, 36, 48, 50, 67-69]. Mechanisms by which probiotics improve host animal performance include: (i) to maintain the normal intestinal microflora by competitive exclusion and antagonism [19, 20, 30, 33, 37, 43, 59, 62]; (ii) to enhance the non-pathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide [25, 33, 43, 48, 53, 70]; (iii) to suppress the intestinal pathogens and to enhance the digestion and utilization of nutrients [3, 71]. It is reported that the major outcomes from using probiotics in livestock include improvement in growth and food efficiency [71] and reduction in mortality [38].

Consequently, the aims of the present study were to determine the effects of dietary probiotic (B. subtilis DSM 17299) supplementation on performance parameters (body weight gain, food intake, feed conversion ratio, hot carcass weight and yield as well as mortality) and on the caecal microflora, and establish connections between performance and intestinal microflora in broiler chickens.

Materials and Methods

BIRDS, MANAGEMENT AND PROTOCOL DESIGN

A total of 364 one day-old Ross 308 male broiler chicks were obtained from a commercial hatchery. The chicks were individually weighed and randomly divided into 2 groups (control and treated group), each of them being constituted by 7 replicate subgroups of 26 birds. Water and feed were provided ad libitum throughout the experiment. Birds were exposed to 23 hours of light and 1 hour of darkness per day. Wood shavings were used as litter. Experiment lasted for 42 days during spring 2010. Ventilation and heat were provided and adjusted as necessary to maintain bird comfort. Birds were vaccinated against Newcastle disease on the 7th and 21st days and against the Gumboro disease on the 14th day of the experiment. The vaccinations were administrated via drinking water. Mortality was recorded as it occurred. All birds were managed and cared according to the University of Uludag Ethical Committee recommendations.

Broiler starter, grower and finisher diets were produced by a commercial food company. The ingredients and nutrient composition of the broiler starter (0 to 21 days-crumbles), grower (21 to 35 days-pellets) and finisher diets (35 to 42 days-pellets) are shown in Table I. Whereas the control group received the basal (starter, grower and finisher) diets, chickens in the assay group were fed with basal diets supplemented with Karbiyotik-G® (Kartal Kimya San.-Tic. A.S., Gebze Ko-caeli, TURKEY) containing B. subtilis spores (strain DSM 17299) at the dose of 1g/kg of food (or 8 x 10⁵ cfu/kg of food).

MEASUREMENTS AND ANALYSES

Diets were chemically analyzed for dry matter, crude protein, crude ash, ether extract, starch and sucrose according to the Association of Official Analytical Chemists [5]. Metabolisable energy (ME) of experimental diets was calculated using the equation of HARTEL [24] as follow: ME (kcal/kg) = [(Ether extract % x 0.3431) + (Crude Protein % x 0.1551) + (Sucrose % x 0.1301) + (Starch % x 0.1669)] x 239.

All chicks were individually weighed at the beginning of the experiment (1-day-old) then weekly for the whole experimental period. Food intake was determined on a pen basis and the average bird weight gains and food conversion ratios adjusted for mortality, were determined weekly. On day 42, all birds were slaughtered in a commercial slaughterhouse and hot carcasses (without neck, giblets, and feet) were weighed in order to determine hot carcass weight and yield.

On day 42, twenty-one broilers from each main group (7 pens of 3 chicks per treatment) were euthanized, and their intestinal tracts were immediately removed. For isolation of Bacillus spp., one gram of caecal contents from each broiler were inoculated into 9 mL Tryptic Soy Broth (TSB, Oxoid CM129) supplemented with 0.6% yeast extract (YE, Oxoid CM019) and then heated at 80°C for 20 minutes. After heat treatment, the samples from each test tube were serially diluted and streaked onto Tryptic Soy Agar (TSA, Oxoid CM131) with yeast extract. Colonies were counted after incubation in an aerobic atmosphere at 37°C [66]. For the isolation and enumeration of other intestinal microflora, one gram of caecal content from each broiler was aseptically transferred into a sterile stomacher bag and homogenized with 9 mL of 0.1% peptone water in a Seward Stomacher 80 Lab System for 2 min. Serial 10-fold dilutions were made in sterile peptone water and plated in duplicate onto relevant selective media. Lactobacilli were grown on de Man Rogosa and Sharpe (MRS, Oxoid CM361) agar and enumerated after 3 days of incubation at 35°C under 5% CO². Enterococci were cultured on Sianetz Bartley agar (SB, Oxoid CM377) and enumerated after 24-48 hours of incubation at 37°C. Enterobacteriaceae and coliforms were grown on Violet Red Bile Glucose agar (VRBG, Oxoid CM485) and Violet Red Bile agar (VRB, Oxoid CM107) respectively, using the pour plate technique and enumerated after 24-48 hours of incubation at 37°C. The microbial counts were expressed as log10 cfu per gram of caecal contents.

STATISTICAL ANALYSES

Differences in bacterial counts (log10 cfu per g of caecal content) between groups (control and probiotic) were analyzed for each species by t-test as independent samples as well as carcass performance (weight and yield), body weight gain, food intake and food conversion ratio differences between treatment groups. Mann-Whitney U test was used for analysis of body weights according to weeks. Differences were considered significant at P < 0.05. All analyses were performed by
SPSS 13 (SPSS 13. 2004) statistical program. Mortality rates were analyzed by Chi-square test.

Results

The results with regard to the performance (body weight, body weight gain, food intake, food conversion ratio and mortality) and carcass performance in broilers are summarized in Tables II and III, respectively. The probiotic supplementation had no significant effect on body weight and mortality, but, compared to the controls, the total body weight gain calculated for the whole experimental period (6 weeks) was significantly increased in broilers supplemented with the B. subtilis spores \((P < 0.05)\) whereas the total food intake was significantly reduced \((P < 0.001)\). Birds receiving probiotic significantly consumed less food and gained higher body weight. Consequently, the food conversion ratio was significantly improved \((P < 0.001)\) since the 4th week until the end of experiment in the supplemented birds compared to the controls. Similarly, the hot carcass weight was found significantly higher \((P < 0.01)\) in birds receiving probiotic in comparison to the control group, but the hot carcass yield was not affected by the probiotic supplementation (Table III).

The main bacteria species found in the caecal content in 42 days old broilers supplemented or not with B. subtilis spores (DSM 17299) are presented in Table IV. It was observed that the dietary probiotic supplementation for 42 days has markedly increased the bacillus population in the caecum \((P < 0.01)\)
EFFECTS OF *BACILLUS SUBTILIS* ON BROILER PERFORMANCES

### TABLE II: Effects of the probiotic *B. subtilis* spores (strain DSM 17299), 8x10^5 cfu/kg of food] supplementation for 42 days on growth performance and mortality in broiler chickens.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>+ Probiotic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality (%)</td>
<td>6.6</td>
<td>3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>40.50 ± 0.20</td>
<td>40.36 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Week 1</td>
<td>194.56 ± 1.11</td>
<td>195.42 ± 0.98</td>
<td>NS</td>
</tr>
<tr>
<td>Week 2</td>
<td>462.00 ± 1.99</td>
<td>461.88 ± 2.30</td>
<td>NS</td>
</tr>
<tr>
<td>Week 3</td>
<td>954.53 ± 5.54</td>
<td>957.39 ± 4.87</td>
<td>NS</td>
</tr>
<tr>
<td>Week 4</td>
<td>1673.68 ± 6.04</td>
<td>1690.32 ± 6.64</td>
<td>NS</td>
</tr>
<tr>
<td>Week 5</td>
<td>2328.16 ± 9.06</td>
<td>2344.83 ± 8.74</td>
<td>NS</td>
</tr>
<tr>
<td>Week 6</td>
<td>3135.53 ± 10.28</td>
<td>3160.63 ± 12.42</td>
<td>NS</td>
</tr>
<tr>
<td>Body Weight Gain (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWG0-1</td>
<td>154.06 ± 2.36</td>
<td>155.06 ± 1.06</td>
<td>NS</td>
</tr>
<tr>
<td>BWG0-2</td>
<td>421.60 ± 2.28</td>
<td>421.51 ± 2.63</td>
<td>NS</td>
</tr>
<tr>
<td>BWG0-3</td>
<td>913.05 ± 10.63</td>
<td>917.85 ± 7.02</td>
<td>NS</td>
</tr>
<tr>
<td>BWG0-4</td>
<td>1633.05 ± 4.87</td>
<td>1650.35 ± 3.83</td>
<td>NS</td>
</tr>
<tr>
<td>BWG0-5</td>
<td>2287.15 ± 7.94</td>
<td>2306.06 ± 6.37</td>
<td>NS</td>
</tr>
<tr>
<td>BWG0-6</td>
<td>3095.03 ± 6.35</td>
<td>3120.07 ± 7.12</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Food Intake (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FI0-1</td>
<td>181.00 ± 2.69</td>
<td>183.47 ± 1.61</td>
<td>NS</td>
</tr>
<tr>
<td>FI0-2</td>
<td>565.09 ± 3.66</td>
<td>557.98 ± 2.65</td>
<td>NS</td>
</tr>
<tr>
<td>FI0-3</td>
<td>1350.16 ± 10.82</td>
<td>1342.23 ± 17.65</td>
<td>NS</td>
</tr>
<tr>
<td>FI0-4</td>
<td>2516.49 ± 8.95</td>
<td>2489.26 ± 7.73</td>
<td>NS</td>
</tr>
<tr>
<td>FI0-5</td>
<td>3775.42 ± 14.56</td>
<td>3741.11 ± 14.75</td>
<td>NS</td>
</tr>
<tr>
<td>FI0-6</td>
<td>5465.90 ± 21.36</td>
<td>5319.19 ± 12.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Food Efficiency (g/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCR0-1</td>
<td>1.18 ± 0.01</td>
<td>1.18 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>FCR0-2</td>
<td>1.34 ± 0.01</td>
<td>1.32 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>FCR0-3</td>
<td>1.48 ± 0.01</td>
<td>1.46 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>FCR0-4</td>
<td>1.54 ± 0.01</td>
<td>1.51 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FCR0-5</td>
<td>1.65 ± 0.01</td>
<td>1.62 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FCR0-6</td>
<td>1.77 ± 0.01</td>
<td>1.71 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**BWG0-i:** Body weight gain calculated for a period of *i* weeks; **FI0-i:** Food intake measured for a period of *i* weeks; **FCR0-i:** Food conversion ration (Food intake / Body weight gain) calculated for a period of *i* weeks; **NS:** not significant.

Results are expressed as mean ± SEM of 7 pens of 26 chicks per treatment.

### TABLE III: Effects of the probiotic *B. subtilis* spores (strain DSM 17299), 8x10^5 cfu/kg of food] supplementation for 42 days on carcass performance in broiler chickens.

<table>
<thead>
<tr>
<th>Parameters</th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-slaughter body weight (g)</td>
<td>3135.53 ± 10.28</td>
<td>3160.63 ± 12.42</td>
<td>NS</td>
</tr>
<tr>
<td>Hot carcass weight (g)</td>
<td>2219.25 ± 7.59</td>
<td>2258.18 ± 9.98</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hot carcass yield (%)</td>
<td>70.78 ± 0.62</td>
<td>71.41 ± 0.50</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NS:** not significant.

Results are expressed as mean ± SEM of 7 pens of 26 chicks per treatment.

whereas the Enterococci and Coliform populations were significantly lowered compared to the not supplemented controls (*P* < 0.001 and *P* < 0.05, respectively). Although not significantly, Enterobacteriaceae were also weakly diminished compared to the control group. The population of lactobacilli in the caecum tended to slightly increase in the probiotic-treated

Revue Méd. Vét., 2011, 162, 11, 538-545
Birds, but the difference with the controls was not statistically significant.

**Discussion**

**CAECAL MICROFLORA COMPOSITION**

In the current work, Enterococci populations have significantly decreased \((P < 0.001)\) in the caecum of chickens fed with *B. subtilis* DSM 17299. Contrary to our results, SIRIKEN et al. [65] did not assign detectable differences in the Enterococci counts among the study groups. In another study [70], it was suggested that these bacterial populations were slightly but not significantly diminished in faeces of kefir supplemented geese.

In the present study, there was no significant difference between the supplemented birds and the controls in changes in Enterobacteriaceae counts as reported by SIRIKEN et al. [65]. It is known that in broiler nutrition, Bacillus such as other probiotic species have a beneficial effect on maintaining normal intestinal microflora by competitive exclusion and antagonism [34]. Nevertheless, the probiotic strain *B. subtilis* 3 was previously reported to have antagonistic properties against species of the family Enterobacteriaceae [57].

The broilers fed with diet containing *B. subtilis* DSM 17299 had only slightly higher counts of lactobacilli in their caecum 42 days after supplementation but the difference with the not supplemented controls was not significant. The addition of *B. subtilis* to broiler diets has several pathways in which it may improve production parameters. *B. subtilis* consume oxygen in the gut tract and additionally it produces certain enzymes, knowing subtilisin and catalase, which results in a positive environment for beneficial bacteria such as lactobacilli [7, 61]. Our result agrees with JIN et al. [29] who found that in the caecum, lactobacilli populations were more abundant in broilers fed with diets containing lactobacillus cultures than in the controls 30 days after supplementation \((P < 0.05)\), but not after 40 days. Similarly, LIN et al. [42] showed that broilers fed with diet containing probiotics had higher amount of intestinal Lactobacillus. In contrast, SIRIKEN et al. [65] reported that there is no detectable difference in caecal lactobacilli populations among Japanese quail groups receiving diets with or without probiotics. Again, JENNY et al. [28] observed no significant effect of *B. subtilis* concentrate on populations of faecal lactobacilli in calves.

In this investigation, the dietary addition of *B. subtilis* DSM 17299 has significantly decreased the coliform population \((P < 0.05)\) in the caecum at 42 days after feeding. It is anticipated that the competitive exclusion of pathogens by *Bacillus* probiotics will result from one or more modes of action, including immune exclusion, competition for adhesion sites, and production of antimicrobial agents, such as bacteriocins [9, 49, 54]. There are reports showing that a laboratory strain of *B. subtilis* was able to suppress intestinal colonization by different avian pathogens in poultry [40, 41]. A contradictory result was reported by SIRIKEN et al. [65] that have observed no significant effect of probiotics on the caecal coliform populations in Japanese quails. JIN et al. [29] also indicated that the addition of Lactobacillus cultures in the diet of chickens did not significantly decrease \((P > 0.05)\) the numbers of coliforms in the faeces of goslings fed with kefir as a probiotic. Studies conducted by JENNY et al. [28] suggested that faecal counts of coliforms were higher in calves fed with *B. subtilis* concentrate compared to the control calves.

The different opinions related to the effects of probiotics on poultry intestinal microflora in several studies might be attributed to the strain and the form of bacteria used and their concentration in dietary supplements.

**PERFORMANCE DATA**

The probiotic effect on broiler performance is a matter of controversy. It has been stated that supplementation of probiotics has no effect on the performance of broiler chicks [17, 39, 55, 72]. However, the present results indicated that probiotic supplementation can have positive effects on broiler growth performance. Indeed, the food intake calculated for 6 weeks was significantly reduced in the birds receiving probiotic compared to the controls \((P < 0.001)\) whereas the body weight gain cumulated for 6 weeks was significantly higher \((P < 0.05)\), leading to a significant improvement in the food efficiency evidenced since the 4th week of supplementation. These results are in agreement with many investigators [6, 8,

---

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Control</th>
<th>Groups + Probiotic</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus spp.</em></td>
<td>5.98 ± 0.13</td>
<td>6.57 ± 0.16</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>6.41 ± 0.17</td>
<td>6.26 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Enterococci</td>
<td>5.75 ± 0.14</td>
<td>4.88 ± 0.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>8.58 ± 0.11</td>
<td>8.86 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Coliforms</td>
<td>6.55 ± 0.14</td>
<td>5.94 ± 0.22</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

\(NS: \) not significant.

Results are expressed as mean ± SEM of 7 pens of 3 chicks per treatment. Each value is based on means of twenty-one experiments in duplicate analysis.

**TABLE IV:** Effects of the probiotic \([B. subtilis] \) spores (strain DSM 17299), \(8 \times 10^5 \text{ cfu/kg of food}\) supplementation for 42 days on caecal bacterial counts expressed as \(\log_{10} \text{ cfu/g of caecal content}\) in broiler chickens.
EFFECTS OF BACILLUS SUBTILIS ON BROILER PERFORMANCES

21, 30, 35, 36, 48, 50, 67-69, 73] who also demonstrated increases in body weight gains in probiotic fed birds. Nevertheless, ANJUM et al. [4], RAMLAH and TAN [58] and JOYUBARI et al. [31] suggested that probiotics had no effect on food intake in broiler chickens, whereas GIL DE LOS SANTOS et al. [21] reported that 0.5% fermented product from B. subtilis inclusion significantly reduced the food intake ($P < 0.05$) compared to the controls, as it was observed in this study. Similarly, the food conversion ratio obtained when probiotic was added into the broiler diet was significantly improved ($P < 0.001$) in the current experiment, confirming in this way previous studies conducted in broilers [1, 15, 23, 27, 63]. In contrast, some other investigators [17, 22, 51, 52, 60] failed to observe any effects of probiotics on the food efficiency. The improvement in the food conversion ratio in the treated birds may be related in the present study to the presence of the B. subtilis DSM 17299. The caecal bacilli population was greater ($P < 0.01$) in the birds treated with B. subtilis DSM 17299 than in the controls. Additionally, the population of lactobacilli in the caecum has also tended to slightly but not significantly increase. It is reported [16] that the inclusion of desirable microorganisms (probiotics) in the diet allows rapid development of beneficial bacteria in the digestive tract of the host. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes [56], which may explain the improvement in the food efficiency observed in the present study.

In this study, the hot carcass weight was significantly improved ($P < 0.01$) in birds receiving probiotic in comparison to the control group, but the hot carcass yield was not affected by the probiotic supplementation. MAHAJAN et al. [44] recorded that the hot carcass weight and yield were significantly higher in probiotic fed broilers. MIDILLI and TUNCER [46] and KABIR et al. [32] noticed significant effect on the hot carcass yield in broilers supplemented with probiotic. In contrast, ERGUN et al. [17], MOHAN et al. [47] and ANJUM et al. [4] did not determine any effect of probiotics on the hot carcass yield, as it was observed in this study.

Moreover, the group of B. subtilis treated birds exhibited a lower mortality ratio (3.3%) than the control group (6.6%). Although the difference was not significant, it is probably due to the fact that continuous feeding with probiotic may have suppressed the undesirable microorganisms such as Enterococci and coliforms, leading to the improvement of the health status.

As a conclusion, it was demonstrated in the present study that dietary supplementation of broiler diets with B. subtilis DSM 17299 (8x10⁵ cfu/kg of food) for 42 days significantly decreased the caecal populations of coliforms and enterococci, slightly increased counts of lactobacilli, and did not influence Enterobacteriaceae. Body weight gain, food efficiency, food intake and hot carcass weight were significantly improved. The caecal microflora data indicates that there are an improvement in the intestinal environment, and an increase in the nutrient digestion and absorption processes, which may explain the positive effects on the broiler growth performance in the present study. It is possible that the use of B. subtilis spores in broiler diets may improve performance by modulation of intestinal microflora and may increase economical profits in this way.

Acknowledgement

Appreciation is extended to Charoen Pokphand Group (Bangkok, Thailand) for supporting this project.

References

5. - AOAC: Official Methods of Analytical Chemist, 17th edition, revision 2. AOAC international, Gaithersburg, Maryland, USA, 2003, Chapter 4, pp.: 1-56.


KUMPRECHT I., ZOBAC P.: The effect of probiotic preparations containing Saccharomyces cerevisiae and Enterococcus faecium in diets with different levels of B-vitamins on chicken broiler performance. Zvoznicova Vyroba, 1998, 43, 63-70.


SANDERS M.E., MORELLI L., TOMPKINS T.A.: Spore formers


