Performance of layer or broiler breeder hens varies in response to different probiotic preparations

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Abstract

The effect of three probiotic dietary supplements on the laying and reproductive performance of layer hens and broiler breeder hens was investigated. A total of four hundred and eighty, 22-week-old layer hens were given diets containing one of three probiotics (1 g/kg diet for each probiotic) from weeks 22 to 49. Seven hundred and twenty female and sixty-four male breeder breeders were also subjected to the same additive feeding procedure between 21 and 50 weeks of age. The layer hen and breeder trials were initiated simultaneously and performed in pens located in the same area. Either egg production rate or egg weight was affected by the microbial cultures administered. Body weight and mortality were not affected. Egg production rate, egg weight and egg mass benefited from some of the probiotics, while, overall, the probiotics led to significant improvements in the feed conversion rate of layer hens. All of the probiotic preparations decreased the cracked-to-broken egg ratio, compared to the untreated control group. One of the three probiotic supplements significantly improved the egg production rate and increased the settable egg and chick yield per hen in broiler breeders, whereas, overall, probiotics tended to decrease settable egg weight. Probiotics enhanced hatchability, but no beneficial effects were observed on fertility. Based on these findings, it can be said that the microbial cultures in the probiotic preparations exhibited host-specific and strain-specific differences in their activities as performance-enhancer feed additives, with improvements in some selected performance parameters.

Introduction

Probiotics are defined as live cultures of microbes – often lactic acid bacteria, but also some other species – that are fed to animals to improve health and growth by altering intestinal microbial balance (Fuller, 1989; Jin et al., 1997, Patterson and Burkholder, 2003; Jernigan et al., 1985). The importance of maintaining beneficial bacteria, like Lactobacillus, in the intestinal tract of warm-blooded animals, including species of poultry, has been well documented in the past five decades. In their natural environment (the gastrointestinal tract) beneficial microorganisms produced by the host animal compete with undesirable organisms for space and nutrients and stimulate the growth of other types of bacteria that produce necessary nutrients (Jernigan et al., 1985; Miles et al., 1981; Vanbella et al., 1990).

Recently, interest in the use of probiotics to improve the production performance and general health status of all poultry species – but broiler chickens and layer hens, in particular – has been rekindled by legislation to curtail the use of sub-therapeutic doses of antibiotics in animal diets (Cook, 2000; Langhout, 2000; Mellor, 2000; Gill, 2001; Pail, 2006). Experimental studies with new probiotic cultures, either alone or in combination, under different conditions, have demonstrated that probiotics may have beneficial effects on layer hens in many, but not all, cases (Krueger et al., 1977; Hargis and Creger, 1978; Nahashon et al., 1994a,b,c; Abdulrahim et al., 1996; Gill, 1998; Yörük et al., 2004). However, with the exception of two studies (Goodling et al., 1987; Nahashon et al., 1996b), no study has yet shown that probiotic feeding has any detrimental effects on health status and productivity of layer hens.

The beneficial effects of probiotics have been related to different modes of action. The improvement in zootecchnical performances of all poultry species fed with probiotics was mostly related to the improvements that probiotics promoted in metabolic processes of digestion and utilization of nutrients (Nahashon et al., 1994c, 1996b; Jin et al., 1997; Yeo and Kim, 1997). Experimental studies have shown that probiotic dietary supplementation might influence these mechanisms by exerting enzymatic activities (Jin et al., 2000), increasing the passage rate of digestion (Dellipiani et al., 1968) and deconjugating bile salts and acids (Brown, 1977; Sung et al., 1990). It is believed that the improvements in metabolic processes that was observed after probiotics administration result from improved development of the gut and increased microvilli height, which results in the enlargement of microvilli’s absorptive surfaces and enables the optimal use of nutrients (Nahashon et al., 1994c). Additional antibacterial (Jin et al., 1997, 1998) and immunostimulatory (Inooka and Kimura, 1983; Takahashi et al., 1997) effects have been reported under different sanitary conditions.

The results of some experimental trials with probiotics have been inconsistent, due to variations in the bacterial cultures used, the age and breed of layer hens treated and other factors related to the management of production, such as the ingredients and nutritional composition of diet; husbandry practices, including environmental stress factors; and housing hygiene (Cerniglia et al., 1983; Goodling et al., 1987; Nahashon et al., 1994b, 1996b; Yörük et al., 2004).

While numerous studies have focused on probiotic microorganisms, particularly such microorganisms as the Lactobacillus species, microorganisms that originated from Aspergillus oryzae have been scarcely tested as a pre-probiotic feed additive for layer hens. Very limited scientific information is available on the effects of probiotic supplementation on the overall performance of broiler breeders, even in recent years. However, there is hardly any comparative study examining strain response to probiotic supplements, with regards to laying traits.

Therefore, the objective of this study was to investigate the effectiveness of three different probiotic preparations, intended to act as performance enhancer feed additives, on the zootecchnical performance of layer and broiler breeder hens.
Materials and methods

Laying hens

A total of 480 Nick-Brown layer hens, 22 weeks of age and with uniform body weight, were placed into cages (40x42x48 cm) in a two-tiered facility. They were then randomly assigned to be fed 1 of 4 isocaloric and isonitrogenous experimental diets: a basal diet containing no probiotics or one of three diets, each of which contained a different probiotic preparation: Prob-1, Prob-2, and Prob-3. Thus, there were 4 dietary treatments, each consisting of four replicates. The treatments were completely randomized. Each treatment was replicated in 40 cages with 3 hens. The hens in 10 adjacent cages (totally 30) were considered an experimental replicate. The trial was conducted in an open-sided, layer house under natural environmental conditions between September and April during winter and spring. The temperature in the layer house ranged from 12°C to 24°C. The trial lasted 28 weeks.

The ingredients and chemical composition of the basal layer and broiler breeder diets, as well as the microbial composition of the microbial cultures, are presented in Table 1 and Table 2, respectively. The experimental diets were formulated to meet or exceed the requirements of laying hens. Egg mass was calculated by the ratio of grams of feed consumed to grams of egg weight produced. Egg mass was calculated by

Table 1. Ingredients and chemical composition of the basal laying hen and broiler breeder diet (as fed).

| Ingredients                        | Laying hen diet, g/kg | Broiler breeder diet, g/kg |
|-----------------------------------|-----------------------|----------------------------|
| Maize                             | 539.9                 | 558.6                      |
| Wheat                             | 59.0                  | 109.0                      |
| Soybean meal, 46%                 | 188.4                 | 132.0                      |
| Sunflower meal, 29%               | 76.2                  | 88.9                       |
| Fish meal, 29%                    | 30.0                  | 11.9                       |
| Vegetable oil                     | 12.8                  | 12.5                       |
| Wheat bran                        | 2.0                   | 2.0                        |
| Salt                              | 2.5                   | 2.5                        |
| Ground limestone                  | 82.4                  | 70.9                       |
| Dicalcium phosphate               | 11.3                  | 17.3                       |
| Vitamin premix§                   | 2.5                   | 2.5                        |
| Mineral premixδ                   | 1.0                   | 1.0                        |
| DL-methionine                     | 1.0                   | 1.0                        |
| Total                             | 1000                  | 1000                       |

Composition

| Composition                       | Analytical/calculated concentration, g/kg |
|-----------------------------------|-------------------------------------------|
| Dry matter                        | 899.2                                     |
| Crude protein                     | 179.0                                     |
| Ether extract                     | 37.0                                      |
| Crude fibre                       | 38.6                                      |
| Crude ash                         | 104.4                                     |
| Starch                            | 399.3                                     |
| Sugar                             | 28.8                                      |
| Calcium                           | 36.3                                      |
| Total phosphorus                  | 6.5                                       |
| Metabolizable energy, kcal/kg³    | 2833                                      |
| Lysine†                           | 8.8                                       |
| Methionine+Cystine§               | 6.9                                       |

Table 2. Microbial composition of probiotic preparations.

| Microorganism                    | Prob-1, 60x10⁶ cfu/kg | Prob-2, 30x10⁸ cfu/kg | Prob-3, mg/kg |
|----------------------------------|-----------------------|-----------------------|---------------|
| Lactobacillus acidophilus        | +                     | 3.0x10¹⁴               |
| Lactobacillus plantarum          | +                     | 1.8x10¹⁴               |
| Lactobacillus del. subsp. bulgaricus | 3.0x10¹⁴               |
| Lactobacillus rhamnosus          | +                     | 3.0x10¹⁴               |
| Lactobacillus casei              | +                     |                       |
| Bacillus licheniformis           | +                     |                       |
| Bacillus subtilis                | +                     |                       |
| Bifidobacterium bifidum          | +                     | 3.0x10¹⁴               |
| Streptococcus sal. subsp. thermophilis | 6.1x10¹⁴               |
| Enterococcus faecium             | +                     | 8.8x10¹⁴               |
| Aspergillus oryza                | +                     | 7.9x10⁶                 |
| Aspergillus oryze                | +                     | 625 000                |
| Candida pittolopessi             | 7.9x10⁶                |

Prob-1, DI-A-ZYME 256 diet; Prob-2, Protein diet; Prob-3, Fermacto diet.
multiplying average egg weight by egg production on replicate basis. The magnitude of production variables, such as feed intake and egg production, was adjusted for hen mortality. Deaths were recorded daily as they occurred.

Additional samples of 16 eggs (4 eggs per replicate) were randomly collected from each experimental group, every 28 days, to assess eggshell quality parameters. Characteristics of egg shell quality were eggshell weight, egg shell strength and egg shell thickness. Eggshell weight is described as the ratio of eggshell weight to total egg weight, expressed as a percentage. Egg shell strength was measured using egg shell tester equipment and was measured as a unit of compression force was exposed to a unit of eggshell surface area (kg/cm²). After removing shell membranes manually, egg shell thickness (without inner and outer shell membranes) was measured at three different points (top, middle and bottom) using a micrometer. An average of three different thickness measurements was observed.

Standard techniques for proximate analysis were used to determine the nutrient concentrations in the layer and breeder diets (Naumann and Bassler, 1993). The experimental diets were also analysed for starch, sugar, calcium and phosphorus content, according to the VDLUFA (Association of German Agricultural Analysis and Research Institutes) method (Naumann and Bassler, 1993). The metabolizable energy content of the diets was calculated based on chemical composition (Anonymous, 1991).

Broiler breeders

Male and female Cobb-500 breeder pullets reared according to standard management practices in an open house with natural ventilation were moved to a breeder house at 21 weeks of age. The amount of feed they received was adjusted weekly during the growing period to maintain BW gain as recommended by Cobb Breeding Co. Ltd. (Chelmsford, UK). The experimental study was started when the birds were 22 weeks of age. Totally, 45 hens and 4 males were randomly assigned to each of 16 breeder pens (4 treatments, with 4 replicates per treatment) in a curtain-sided breeder house. Each pen was equipped with one automatic waterer, six hanging feeders and one 12-hole nest box. The floor of the breeder pens was covered with pine shavings as litter material. With the exception of feed distribution system, the housing was comparable to commercial standards. Breeder hens were exposed to natural environmental conditions in a subtropical climatic zone from September to April, throughout the laying period.

Thus, the experiment at the broiler breeder facility lasted 28 weeks (from 22 to 50 weeks of age) and was initiated simultaneously with the layer trial in the confounded area of the same experimental station. Upon movement to the breeder house at 22 weeks of age, day length was increased to 13, 14 and 15 h, with one hour increments over weekly, respectively, and 16 h at the start of laying (26 weeks), using a combination of natural and incandescent light. Feed rations were adjusted weekly to maintain recommended body weights during the pre-lay period (22 to 25 weeks). Weekly incremental increases in feed began before laying, so that feed rations were adjusted from 125 g/bird per day at housing to 165 g/bird per day at initiation of laying. After egg laying commenced (at 26 weeks), daily feed allotment remained constant at 165 g/hen/day, with energy intake 466 kcal/hen/day between weeks 22 and 50 of the experiment. Males were fed separately from hens, using separate-sex feeding equipment. Daily feed allowance per male was adjusted to 134 g during the test period. Both males and females received the same breeder diet. Diets were isonitrogenous and isocaloric; hence, breeder hens who received different treatments had similar nutritional intakes. Dietary treatments initiated at week 22, as well as all diets, were formulated to meet or exceed National Research Council (1994) specifications. The experimental protocol for probiotic dietary supplementation was similar to the layer procedure.

All of the birds were weighed individually at weeks 21 and 50. Egg production and the percentage of settable eggs per hen were recorded daily. Egg production was calculated as a percentage of the hen-day egg production of the total number of eggs laid per replicate. The percentage of settable eggs per hen was defined as the ratio of total settable eggs to total laid eggs per treatment (%). Mortality was recorded daily and feed allowance adjusted accordingly to this parameter. Eggs from each pen were collected 4 times a day and recorded as nest- or floor-laid, cracked, broken or soft-shelled. Only nest eggs that were not dirty, misshapen, broken, cracked, excessively small or double-yolked were classified as settable and stored in a cooler set at 16°C and 70% humidity. For 2 consecutive days, every week, random samples of 64 eggs (8 settable eggs/replicate/day) from each treatment were weighed individually to determine average egg weight. Additionally, 60 randomly selected newly hatched chicks (21.5-day incubation) per treatment were weighed individually at the hatchery on a weekly basis. Samples of 56 settable eggs per replicate pen were set for incubation weekly between 27 and 50 weeks of age. Eggs were incubated in Petersime model S20 incubators. Within the incubators, dry bulbs were set at 37.6°C and wet bulbs were set at 28.6°C. Eggs were candled on day 10 of incubation to determine which were infertile. All infertile eggs were opened and examined macroscopically for evidence of embryonic mortality. Embryonic mortalities were not accounted for when determining infertility. So, only the number of fertile eggs was clearly established, and fertility was expressed as the ratio of fertile eggs to total eggs set. On day 19, eggs were transferred to the hatching cabinets of the same incubators for hatching. Within the hatching incubators, dry bulbs were set at 37.5°C and wet bulbs were set at 29.2°C. The number of eggs that hatched was recorded at 21.5 days of incubation. Hatchability of fertile eggs (HFE) was expressed as the ratio of hatching chicks to fertile eggs; and percentage cumulative hatchability (HTE) was expressed as the percentage of chicks that hatched (of the total eggs set). The total number of settable eggs (TSE) and chicks (TCN) obtained per pen throughout the 24-week laying period was determined on the basis of pen, according to hen-housed egg production.

Data were analyzed as a one-way ANOVA using the General Linear Models procedures of the SAS (SAS, 1991). Significant differences between treatment means were separated using Duncan’s multiple range test, with a 5% probability (P<0.05). Each experimental unit was a replicate consisting of ten adjacent cages in laying hen trial. This experimental unit was a group of 30 layer hens for all traits studied. In broiler breeder trial, the experimental unit was a group of 45 breeder hens and 4 males reared in a floor pen. This was the replicate group for all breeder traits studied including productive and reproductive performances. There are 4 replicates per treatment in both laying hen and broiler breeder trials; thus, statistical analysis was run on the basis of 4 replicates.

Results and discussion

Laying hens

The final body weight of layer hens was variably affected (P<0.05) by the administration of different microbial cultures throughout the 28-week feeding period (Table 3). While Prob-2 supplementation significantly increased hen body weight, compared to all other treatments, no significant differences were observed between the control and other probiotic feed-
ing regimens. Layer hens given Prob-2-added diets, containing mainly *Lactobacillus* microorganisms, gained more body weight and did not experience decreased egg production, feed efficiency or egg shell quality, in spite of feed intake that was lower than that observed in the no-added program. The growth-promoting effects of dietary probiotic (*Lactobacillus*) supplementation were attributed to probiotics’ proven effects on nitrogen, fat, calcium and phosphorus retention (Nahashon et al., 1994b,c). The results of several investigations have also confirmed that probiotic supplements can promote laying hen performance without diminishing body weight and livability (Miles et al., 1981; Nahashon et al., 1994b,c; Balevi et al., 2001). In agreement with the results of the present trial, Nahashon et al., (1994b) have reported that *Lactobacillus*-added diets increased body weight by 48 g in laying hens. On the other hand, no significant effect on hen body weight was reported in response to dietary probiotic supplementation, compared to no-added control programs (Miles et al., 1981; Nahashon et al., 1996b). Thus, earlier research, as well as this study, has shown that feeding layer hens with probiotic-supplemented diets did not have detrimental effects on hen body weight; it even benefited laying performance.

The mortality of layer hens was not affected (P>0.05) by probiotic supplementation regimens (Table 3). Several authors (Balevi et al., 2001 and Yörük et al., 2004) have also reported good livability and improved performance goals when brown layer hens are fed on diets with probiotic preparations (ProteXin).

The results presented in Table 4 show the dietary effects of various microbial cultures on the performance of layer hens. The egg production rate of hens fed the Prob-1-added diet was higher than the egg production rate of hens fed the control, Prob-2 or Prob-3 diet; the latter were 1.68%, 1.24% and 1.12%, respectively, with statistical significance (P<0.01). The egg production rate for hens given Prob-2 and Prob-3 diets was similar to that of hens fed the control diet (P>0.05). Confirming these findings, most studies conducted with laying hens have pointed out the positive relationship between probiotic microbial combinations and egg production rate (Krueger et al., 1977; Hargis and Creger, 1978; Miles et al., 1981a; Tortuero and Fernandez, 1995; Yörük et al., 2004). It is of note that the egg production rate of broiler breeder hens benefited from dietary supplementation with Prob-2, as was the case with Prob-1 for layer hens (Table 4).

All of the microbial cultures led to notable improvements in table egg weight for layer hens (Table 4), whilst a contrasting pattern was observed for settable eggs in broiler breeders. Prob-2 and Prob-3 supplementation increased egg weight, in comparison to the no-added control treatment (P<0.05), while only numerical enhancement was observed in the case of Prob-1 treatment. The egg weights of hens fed Prob-2 were highest (60.70 g), whereas the egg weights of hens given the control diet were lowest (59.58 g), indicating a 1.12 g advantage in favor of Prob-2-fed hens (P<0.01). In agreement with our findings, Balevi et al. (2001) and Yörük et al. (2004) have reported 1.22 g and 0.70 g increments in egg weight, respectively, when the same commercial probiotic preparation (ProteXin) used in this study was used to supplement brown layer hens’ diets. Also consistent with the findings of this and the earlier works noted above, several preliminary studies (Nahashon et al., 1994b,c; Tortuero et al., 1995) have reported the positive effects of probiotic microbial cultures, particularly *Lactobacillus*, on egg weight in laying hens.

In association with the increased egg weight that resulted from including probiotics in hens’ diets, hens fed on diets with Prob-1, Prob-2 and Prob-3 yielded eggs with 1.21 g, 1.24 g and 0.76 g higher mass, respectively, than hens given the no-added control diet (Table 4). These results clearly show that all of the probiotic preparations used in the present study contributed to the output of eggs with more mass than did the unsupplemented control program. The beneficial effects of including direct-fed microbials in hens’ diets has also been noted elsewhere (Nahashon et al., 1994b, 1996b; Tortuero et al., 1995).

The increases in both egg weight and egg mass that resulted from supplementation with probiotic microbial preparations might be associated with improved retention of nutrients, including nitrogen, fat, calcium and phosphorus. The significant positive correlation that has been observed between probiotics (*Lactobacillus*) and egg weight, egg production rate and egg mass, as stressed by Nahashon (1996b), further support the determinations presented here.

The influence of probiotic dietary supplementation on the feed intake of layer hens is presented in Table 4 (P<0.05). Hens receiving diets with the control and Prob-1 treatments consumed more feed than hens given Prob-2, and Prob-3-added diets. No significant differences in feed consumption between Prob-2 and Prob-3 treatments, and also between Prob-1 and control treatments, were determined (P>0.05). In full agreement with our findings, Balevi et al. (2001) have indicated that feed consumption for brown layers fed a probiotic (ProteXin)-added diet was 2.69 g lower than that of hens given a control diet, while others have reported that probiotic feeding has no significant effects (Cerniglia et al., 1983; Goodling et al., 1987; Nahashon et al., 1994c, 1996a). Based on the results of the present and previous studies, the influences of dietary probiotics on feed intake could be said to be variable, but no deleterious effect on performance was observed in this case.

Compared to the unsupplemented control procedure, the different microbial cultures administered in this study led to significant

### Table 3. Initial and final body weight, and liveability of laying hens given probiotic added diets.

| Control | Prob-1 | Prob-2 | Prob-3 | SE | P   |
|---------|--------|--------|--------|----|-----|
| Initial body weight, g, 20 week | 1572 | 1571 | 1579 | 1577 | 9.86 | 0.8894 |
| Final body weight, g, 47 week | 1920  | 1923  | 1973  | 1917b | 15.94 | 0.0465 |
| Liveability, % | 97.50 | 99.19 | 97.50 | 99.19 | 0.84 | 0.6631 |

### Table 4. Productive performance of layer hens fed on diets with probiotics.

| Control | Prob-1 | Prob-2 | Prob-3 | SE | P   |
|---------|--------|--------|--------|----|-----|
| Egg production rate %, hen-day | 87.22b | 89.80b | 87.66b | 87.78b | 0.38 | 0.0001 |
| Egg weight, g (n 7168) | 59.58c | 59.81c | 60.70a | 60.06b | 0.11 | 0.0001 |
| Egg mass, g/hen/d | 51.96c | 53.17c | 53.29c | 52.72c | 0.36 | 0.0435 |
| Feed consumption, g/hen/d | 110.67c | 110.46c | 108.95b | 108.39b | 0.66 | 0.0492 |
| Feed conversion ratio, g feed/g egg | 2.13c | 2.07b | 2.04b | 2.05b | 0.03 | 0.0489 |

Prob-1, Di-A-Zyme 256 diet; Prob-2, ProteXin diet; Prob-3, Fermacto diet; SE, standard error; “c” line means with common superscripts differ significantly at P<0.05.
levels of improvements in the feed conversion ratio (Table 4). Layer hens receiving Prob-1-, Prob-2- and Prob-3-added diets exhibited increases in feed efficiency of 2.89 %, 4.41 % and 3.75 %, respectively, compared with hens fed the control diet (P<0.05). These findings indicate that all of the probiotic preparations used in the present study led to more efficient conversion of feed to egg mass. It is obvious that improvements in efficiency in layer hens fed with the probiotic supplements derive from higher egg mass yield, along with lower feed consumption, compared to the control treatment. In agreement with our findings, a great deal of studies conducted with laying hens has shown that the benefits of probiotic microbials on the feed conversion ratio were substantially different in experimental protocols (Krueger et al., 1977; Hargis and Creger, 1987; Nahashon et al., 1994b; Tortuero and Fernandez, 1995, Abdulrahim et al., 1996; Grimes et al., 1997). Interestingly, similar levels of improvement in the feed conversion ratio were obtained when the same probiotic preparation used here (Protexin) was added to layer hens’ diets (Balevi et al., 2001; Yörük et al., 2004).

It appears that the aforementioned positive correlation between probiotics and nutrient retention in layer hens might play an important role in feed conversion efficiency (Nahashon et al., 1994b,c; Grimes et al., 1997). The performance-enhancing mechanism of microbial cultures may operate on the metabolic process of digestion by increasing enzymatic activity (Jin et al., 2000) and the passage rate of digesta (Dellipiani et al., 1968), as well as by deconjugating bile salts and acids (Brown, 1977; Sung et al., 1990). Aspergillus oryzae mediated increase in metabolizabilities of gross energy and dry matter (Han et al., 1999), lipid and protein digestibilities (Grimes et al., 1997) in previous works confirm the improved performance of laying hens treated with Prob-3. Similarly, positive effect of Aspergillus oryzae on the performance of laying hens fed diets with different levels of methionine was also reported (Harms and Miles, 1988).

Eggshell quality parameters are presented in Table 5. The ratio of cracked to broken eggs was significantly reduced when the basal control diet was supplemented with different microbial cultures (P<0.05). However, no significant difference was observed between layers fed with Prob-1 and Prob-2 and layers fed on a diet that included Prob-3. The observation that all probiotic supplements tested in this study ameliorated the ratio of deformed eggs was found to be consistent with several reports (Abdulrahim et al., 1996; Balevi et al., 2001). Fittingly, characteristics of eggshell quality – e.g. shell weight, shell thickness and shell breaking strength – were not impaired, despite the increased egg weight of hens fed on diets with probiotics. As a rule of thumb, the older a hen’s age, the more likely that the quality of her eggs shells will deteriorate, due to difficulties with shell calcification metabolism. So, the beneficial impact of dietary probiotic supplementation on eggshell quality was expected in older hens. It is interesting to note that all the probiotic supplements used in the present study substantially decreased the cracked-broken egg ratio, compared with the control treatment, even though the layer hens were of younger ages (22 to 49 weeks) through the peak egg production period. Our findings are supported by those of Mohan et al. (1995) and Balevi et al. (2001), who have reported that probiotic supplementation in the diets of laying hens who are between 28 to 38 and 40 to 53 weeks of age, respectively, induced fewer egg deformations than an untreated control program, along with improved egg production rate.

**Broiler breeders**

The initial and final body weights and livability of male and female broiler breeders are presented in Table 6. Neither the body weights nor the mortality of male and female breeder hens was affected by the probiotic dietary supplements administered in the experiment (P>0.05). Unfortunately, findings related to body weight and livability cannot be discussed comprehensively, since there is a lack of information about the dietary effects of probiotics on broiler breeder performance. In fact, the findings presented here on those effects do not conflict with the numerous scientific reports that have indicated the benefits of dietary probiotics on broiler chicken body weight gain and livability (Jin et al., 1996, 1998; Panda et al., 2000). The effects of dietary treatments on the egg production performance of broiler breeders are presented in Table 7. Feeding on a diet supplemented with Prob-2 promoted hen-day egg production (P<0.05),

| Table 5. Eggshell quality characteristics of laying hens administered with probiotics. |
|---------------------------------|---------|---------|---------|---------|---------|---------|
| Cracked-broken egg rate, %      | Control | Prob-1  | Prob-2  | Prob-3  | SE      | P        |
| Shell weight, %                 | 10.77   | 11.15   | 10.89   | 11.04   | 0.11    | 0.2485   |
| Shell hardness, g/cm²            | 3.33    | 3.25    | 3.00    | 3.19    | 0.12    | 0.4516   |
| Shell thickness, mm              | 0.041   | 0.038   | 0.038   | 0.039   | 0.01    | 0.3423   |

| Table 6. Body weight and livability of male and female broiler breeders fed with probiotic added diets. |
|---------------------------------|---------|---------|---------|---------|---------|---------|
| Female body weight              | Control | Prob-1  | Prob-2  | Prob-3  | SE      | P        |
| 21 wk                           | 2323    | 2309    | 2363    | 2359    | 0.446   | 0.0001   |
| 50 wk                           | 3702    | 3700    | 3674    | 3618    | 0.538   | 0.5683   |
| Male body weight                 | Control | Prob-1  | Prob-2  | Prob-3  | SE      | P        |
| 21 wk                           | 3021    | 3002    | 3013    | 3015    | 0.8574  | 0.8574   |
| 50 wk                           | 4915    | 4788    | 4891    | 4929    | 0.2864  | 0.2864   |
| Livability, 21 to 50 week        | Control | Prob-1  | Prob-2  | Prob-3  | SE      | P        |
| Female                          | 96.87   | 96.35   | 96.87   | 95.31   | 0.86    | 0.8434   |
| Male                            | 95.00   | 90.00   | 90.00   | 95.00   | 2.25    | 0.2432   |

| Table 7. Egg production performance of broiler breeder hens fed with probiotic supplemented diets. |
|---------------------------------|---------|---------|---------|---------|---------|---------|
| Egg production rate %, hen-day  | Control | Prob-1  | Prob-2  | Prob-3  | SE      | P        |
| Egg weight, g (n = 6144)        | 89.73   | 90.85   | 89.46   | 89.90   | 0.538   | 0.3309   |
| Total number of settable eggs per hen | 87.09 | 88.50 | 89.36 | 89.03 | 1.26 | 0.0488 |
whereas no significant benefit was observed with Prob-1 and Prob-3 treatments, compared to the untreated control program. Egg production rate increased by 3.91% in Prob-2-fed hens, compared to hens given the control diet. Laying rate did not differ amongst hens that received the control, Prob-1 and Prob-3 treatments. These findings are consistent with the results of preliminary laying hen trials that have reported that probiotic dietary supplements have either some (Krueger et al., 1977; Miles et al., 1981; Nahashon et al., 1994b; Mohan et al., 1995; Yörük et al., 2004) or no beneficial effects (Cerniglia et al., 1983; Goodling et al., 1987; Nahashon et al., 1994c; 1996a,b).

Settable egg production followed the same trend as that observed in hen-day egg production rates (Table 7). Broiler breeder hens who received Prob-1 and Prob-3-added diets each laid 1.41 and 1.94 more settable eggs than hens given the control diet (P<0.05), whereas breeder breeder hens who received the Prob-2 treatment laid an average of 6.27 more settable eggs (P<0.05). Settable egg rate was not influenced by dietary treatment (P>0.05) (Table 6). The improved performance observed in breeder breeder hens treated with Prob-2 can also be explained by the beneficial effects of probiotics on the metabolic processes of digestion – i.e. by increased efficiency in the utilization and retention of nutrients (Nahashon et al., 1994c, 1996a; Jin et al., 1997; Yeo and Kim, 1997).

In contrast to the slight or notable benefits in egg production rate that are related to being fed with different microbial cultures, a depressive pattern was seen in settable egg weight (Table 7). Settable egg weight was adversely affected by all of the probiotic supplementation regimens. Settable egg weight in hens that received the Prob-3 treatment was lower than the same for hens who received the control and Prob-1 treatments (P<0.05). Prob-1 and Prob-2 dietary supplementation induced numerical reductions in settable egg weight of 0.32 g and 0.45 g, respectively, compared to the same in the control treatment. These findings demonstrate that breeder breeder hens could not maintain improvements in both egg production and egg weight with probiotic supplementation, despite differences in microbial composition. However, some research on laying hens has shown that probiotic dietary supplementation improves both egg production and egg weight, without diminishing feed efficiency (Nahashon et al., 1992a; 1994b; Tortuero and Fernandez, 1995; Yörük et al., 2004). Layer hens’ and breeder breeder hens’ conflicting responses to probiotic feeding regimens might result from differences in breeds.

The reproductive performance of breeder breeders treated with different microbial cultures is presented in Table 8. Significant improvement on the hatch rate of fertile eggs and the hatch rate of setting eggs were observed in breeder breeders fed probiotic supplemented diets compared to those fed control diet (P<0.05). However, no additional benefit to fertility was obtained with any of the different probiotic feeding regimens (P>0.05). In fact, overall hatchery performance was good – at the levels recommended for the breeder strain studied (Cobb 500). Although none of the probiotic feeding programs improved fertility, the hatch rates of fertile eggs for breeder hens fed Prob-1- and Prob-2-added diets (92.54 % and 93.31 %, respectively), were significantly higher than the same for breeders given the control diet (90.27%). The hatching pattern for all eggs set was similar to the hatching pattern of fertile eggs (Table 8). Hence, the hatch rates of all total eggs set for Prob-1 (86.99%) and Prob-2 (87.49%) treatments were significantly higher than those for control treatment (84.6%). Data on the total number of chicks produced per hen (Table 8) were similar to data on the total numbers of settable eggs per hen (Table 7). Hens fed the Prob-2-added diet yielded significantly more chickens than hens given the control, Prob-1 and Prob-3 diets, (7.94, 4.70, and 5.25 chicks, respectively), while no differences were observed amongst other treatments (P>0.05). Prob-1 dietary supplementation induced significant reductions (P<0.01) in the weights of day-old broiler chicks (1 g), compared with the control treatment, while the other two probiotic treatments did not influence chick weight.

It is evident that the total number of settable eggs and total chick yields of breeder breeders either slightly or notably benefited from the different probiotic supplements used. These results have been confirmed by a field study conducted on hens that were between 25 and 56 weeks of age (Khajerern and Ratanaesethakul, 1998). This study reported that introducing probiotic (Bacillus toyoi) dietary supplements to the diets of breeder hens improved fertility, hatchability, total number of chicks per hen and percentage of settable eggs.

As a matter of fact, restricted feeding methods induce serious stress in breeder breeders by inducing severe competition for feed. Leeson and Major (1990) have reported that direct-fed microbials have measurable benefits under the most stressful conditions when coliforms in the intestinal tract increase in number. Besides the probable beneficial effects on nutrient retention in the gastrointestinal tract (Nahashon et al., 1994c; 1996a) and microbial balance in the intestinal lumen (Jernigan et al., 1985; Fuller 1989; Jin et al., 1997), probiotic microbial dietary supplementation could stimulate the immune systems (Khajerern and Ratanaesethakul, 1998; Balevi et al., 2001) of breeder breeders in our study.

What is more, the probiotic supplements used in this study might have contributed to the physiologies, histologies and skeletal structures of developing chicken embryos, thereby ensuring more favorable conditions for embryo growth during the 21-day incubation period, compared with fertile eggs obtained from hens that have been given no microbial culture. Although reports pertaining to probiotic microbial supplementation and its effects on fertility and hatchability in breeders are very limited, similar improvements in hatchability have been observed in breeders fed a yeast culture supplement (McDaniel and Sefton, 1991, Shashidhara and Devegowda, 2003). In this study, two layer hen strains responded differently to probiotic preparations that were composed of different microbial cultures. First, the variable effects of different probiotic microorganisms may have been confounded by variations in the gut micro flora of the two breeds, since different feeding regimens (ad libitum vs restricted) were applied. Second, the beneficial microbial response observed might have been directed to different production variables.

Table 8. Reproductive performance of breeder breeders treated with probiotics.

|                      | Control | Prob-1 | Prob-2 | Prob-3 | SE   | P    |
|----------------------|---------|--------|--------|--------|------|------|
| Fertility, %         | 93.80   | 94.01  | 93.77  | 93.34  | 0.62 | 0.8977|
| Hatchability of fertile eggs, % | 90.27b | 92.54c | 93.31a | 91.984a | 0.69 | 0.0246|
| Hatchability of total eggs set, % | 84.67b | 86.99c | 87.49b | 85.85a | 0.87 | 0.00391|
| Chick weight, g      | 44.86c | 43.86b | 44.56b | 44.34b | 0.20 | 0.0041|
| Total number of hatchlings (per hen) | 73.74c | 76.98a | 81.68a | 76.43b | 1.08 | 0.0423|

Prob-1, DI-A-ZYME 256 diet; Prob-2, Protexin diet; Prob-3, Fermacto diet; SE, standard error; b,c,line means with common superscripts differ significantly at P<0.05.
Conclusions

When administered to layer and breeder hens, the same microbial cultures induced different responses, with regards to such performance traits as egg production rate and egg weight. Several explanations for the inconsistencies observed could be proposed. With regard to differences in laying rate and egg weight, it is obvious that some of the microbial cultures used in this study could potentially serve as performance enhancer feed additives either in layer or in breeder hens. In conclusion, the probiotic preparations used in this experiment had distinct host-specific and strain-specific effects. The considerable improvements observed in the reproductive performance of breeder hens fed on diets with microbial cultures are significant and are an area fertile for further research.

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