Investigating the Effects of Commercial Probiotics on Broiler Chick Quality and Production Efficiency

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ABSTRACT A study was undertaken to test the effect of 2 commercially available probiotics on the production efficiency of broiler chickens hatched from the same breeder flock at 3 different ages (28, 43, and 57 wk). At each of the 3 breeder flock ages, 1,600 broiler chickens were hatched and randomly allocated to 1 of 4 treatments: 1) no probiotics (control), 2) probiotic 1 administered in the drinking water, 3) probiotic 1 administered as a spray, and 4) probiotic 2 administered in the feed. A coccidiostat was included in the feed, but no other antimicrobial agents were given. Broilers were then reared on straw litter in identical floor pens for a period of 6 wk. There were no significant differences in broiler BW, feed conversion, or mortality between the probiotic treatments and the control group in any of the trials. The 43-wk-old breeder flock had the highest fertility and hatchability and the lowest percentage of chicks culled at hatching. Throughout the broiler production period, the broilers from the 43- and 57-wk-old breeder flocks had higher BW and weight gains than the broilers produced at 28 wk of breeder flock age. Broiler feed conversion over the 6-wk production period decreased as the breeder flock aged. Probiotics had no effect on chick quality or production efficiency in broilers produced by the breeder flock ages examined.

Key words: probiotic, broiler production, gastrointestinal tract, chick quality, breeder flock age

INTRODUCTION

Over the past several decades, the physiological stresses that have been placed on broilers in commercial production have increased. This increased stress is the result of practices used in modern broiler production, such as processing at the hatchery and high stocking densities (Pinchasov and Noy, 1993). Genetic selection for faster, more efficient growth may also place increased physiological stress on broilers. This is evidenced by reduced immune function in modern broilers vs. older genetic stock that has been less selected for production traits (Qureshi and Havenstein, 1994)

Chick viability and broiler growth are influenced by breeder flock age, with younger breeder flocks typically producing smaller, poorer-quality broiler chicks (McNaughton et al., 1978; Sinclair et al., 1990) with lower market BW (Morris et al., 1968; Sklan et al., 2003). The combination of environment, parental age, and genetic factors can negatively affect early chick viability. These increased stressors may weaken immune function and thus predispose broilers to colonization of the gastrointestinal tract (**GIT**) by bacterial pathogens or other unfavorable microorganisms, posing a threat to food safety and bird health (Barnes, 1979; Hume et al., 2003).

Probiotics, also called direct-fed viable microbial products, often consist of live microbial cultures that are isolated from the GIT of a healthy adult animal of the same species to which the probiotic product will be administered. Commercially produced probiotic products are usually species-specific, with products intended for use in chickens comprised of bacterial species that would have been isolated from the GIT of chickens. The use of probiotics may provide an alternative to the administration of subtherapeutic levels of antibiotics in preventing the colonization of the GIT by unfavorable microorganisms.

Microbial populations within the GIT colonize very quickly after hatching (Guan et al., 2004). Contact with microorganisms on the eggshell (Coates and Fuller, 1977) or in feed (Jones and Richardson, 2004) contribute to microbial colonization of the GIT. It is during this early period, when a stable gut microflora has not yet been established, that the chick is most vulnerable to colonization by pathogens, and establishment of a healthy GIT microflora in newly hatched broiler chicks provides vital

protection against these undesirable organisms. It is thought that probiotics act to promote the development of a healthy GIT microflora (Blankenship et al., 1993; Chambers and Lu, 2002).

If a GIT microflora composed of bacterial species that are beneficial to the bird can be established, the colonization of pathogenic bacteria in the GIT can be avoided, even when the bird is eventually exposed to these microorganisms in the environment (Blankenship et al., 1993; Palmu and Camelin, 1997; Kubena et al., 2001; Chambers and Lu, 2002). This is accomplished through competitive exclusion (Nurmi and Rantala, 1973; Nisbet et al., 1993) and a lowering of the pH in the GIT caused by lactic acid production (Fuller, 1977; Chateau et al., 1993).

Past research has shown that administering probiotics can provide the same protection as a naturally developed commensal GIT microflora (Nurmi and Rantala, 1973; Pascual et al., 1999; Kubena et al., 2001; LaRagione et al., 2001). Improvements in broiler weight gain (Nurmi and Rantala, 1973), feed conversion ratio (**FCR**; Jin et al., 1998), and food safety, through a reduction in the numbers of pathogenic bacteria colonizing the GIT (Chambers and Lu, 2002), have been demonstrated. Previous research indicates that effective probiotic products may provide a viable alternative to antibiotic use in broiler production.

The objectives of this experiment were to examine the efficacy (in broiler chickens) of the only 2 commercially available probiotics approved for use in poultry in Canada and to determine if the effectiveness of these products in broilers varied with breeder flock age. It was hypothesized that each of the probiotic treatments would result in improved chick viability, increased weight gains, and improved feed conversion compared with that of the broilers not administered the probiotics. Because broiler chicks from younger breeder flocks can often be of poorer quality, it was also anticipated that the probiotic treatments would have a greater effect on the performance of broilers from a young breeder flock as opposed to broiler chicks produced by an old breeder flock.

MATERIALS AND METHODS

Incubation and Hatching

The experimental protocol was approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta, in accordance with the guidelines set forth by the Canadian Council on Animal Care (1993). Hubbard Hi-Yield hatching eggs (2,500) were obtained from the same commercial broiler breeder flock at each of 3 flock ages: 28, 43, and 57 wk of age. Any cracked eggs or eggs weighing less than 52 g were not used in this experiment, as per commercial hatchery standards in Canada. All settable eggs were individually weighed, numbered, and randomly divided into groups of 18 eggs. Each group of 18 eggs was randomly placed within a 5,000-egg capacity Jamesway single stage setter (Jamesway Incubator Co. Inc., Cambridge, Ontario, Canada) and incubated for 18

d at a dry bulb temperature of 37.5°C and a wet bulb temperature of 29.4°C.

At 7 d of incubation, all eggs were removed from the setter and candled. Any eggs thought to contain nonviable embryos were broken open to assess fertility, and, if fertile, the approximate day of embryonic death was recorded. At 18 d of incubation, the eggs were removed from the setter, individually weighed, and each group of 18 eggs was transferred to a 5,000-egg capacity Jamesway hatcher in which they were incubated for an additional 3.5 d at a dry bulb temperature of 35.2°C and a wet bulb temperature of 29.4°C.

Broiler Production Period

After 21.5 d of incubation, all hatched chicks were counted, and chick quality was visually assessed according to commercial hatchery standards. All unhatched eggs were broken open to determine the approximate day of embryonic death. Embryonic mortality was grouped into 3 developmental stages: early (1 to 7 d), mid (8 to 14 d), and late (15 to 21 d). All chicks deemed to be saleable were individually weighed, neck-tagged (Heartland Animal Health Inc., Fair Play, MO), and randomly allocated to 1 of 4 treatment groups: 1) control (no probiotics administered), 2) probiotic 1 water (**P1W**; probiotic 1 administered in distilled drinking water as directed by the manufacturer at 1, 2, 19, and 20 d of age), 3) probiotic 1 spray (**P1S**; probiotic 1 administered as a spray at hatch as directed by the manufacturer), and 4) probiotic 2 feed (**P2F**; probiotic 2 administered as a feed additive; 0.5 g of probiotic 2/kg of feed) throughout the production period.

The bacterial species included in probiotic 1, as listed by the manufacturer, were *Lactobacillus acidophilus*, *Lactobacillus bifidus*, and *Streptococcus faecalis* (now reclassified as *Enterococcus faecalis*). The manufacturer listed *L. acidophilus*, *E. faecalis*, and bifidobacteria (no specific species identified) as the bacteria included in probiotic 2.

Chicks allocated to each treatment were randomly placed at a stocking density of $0.07 \, \text{m}^2$/bird into 2 isolated environmental chambers, with each chamber divided into 2 pens. The number of chicks placed in each pen varied among trials (28 wk of breeder flock age: 110 chicks/pen; 43 wk of breeder flock age: 124 chicks/pen; 57 wk of breeder flock age: 104 chicks/pen) due to differences in the number of saleable chicks hatched, but stocking density was held constant across the 3 trials by adjusting the floor space available. Each environmental chamber was equipped with an isolated ventilation system vented to the exterior of the building, individual access doors, and disinfectant boot dips (filled with Virkon; Antec International Ltd., Chilton Industrial Estate, Sudbury, Suffolk, UK). Separate coveralls and boots were allocated to each chamber to prevent the transmission of microorganisms among chambers and, thus, among treatments. Between trials, the chambers were fumigated using formaldehyde after the removal of old litter and washing but before the placement of fresh straw in the pens. Once the birds were

Table 1. Ingredient and nutrient composition of the broiler starter and grower diets fed in the present experiment

Ingredients (g/kg)	Starter diet (0 to 21 d)	Grower diet (22 to 42 d)
Wheat	621	535.3
Corn	18.7	148.5
Soybean meal	245.1	218.7
Canola meal	16	0
Canola oil	55	56
Dicalcium P	14.8	11.1
Limestone	13	14
Choline chloride premix[1] (60%)	5	5
Broiler vitamin premix[2]	5	5
Salt	4	3.5
DL-Met	1.6	0.8
L-Lys	0.4	1.1
Amprol[3]	0.5	0.5
Avizyme 1302[4]	0.5	0.5
Nutrient (%) and energy content		
Protein	21.35	19.6
ME (kcal/kg)	3,000	3,098
Ca	0.93	0.86
Available P	0.42	0.34
Cl	0.29	0.25
Na	0.19	0.17
Ly	1.02	0.97
Met	0.46	0.37
Met + Cys	0.84	0.71

[1]Choline chloride premix provided 100 mg/kg of diet.

[2]Broiler premix provided the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E, 35 IU; menadione, 2 mg; D-pantothenic acid, 14 mg; riboflavin, 5 mg; folic acid, 0.8 mg; niacin, 65 mg; thiamine, 2 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.015 mg; biotin, 0.18 mg; I, 0.5 mg; Fe, 100 mg; Zn, 80 mg; Mn, 70 mg; Cu, 8.5 mg; Se, 0.1 mg.

[3]Merial Canada Inc., Baie d'Urfe, Quebec, Canada.

[4]Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

placed in their pens, the probiotic treatments were administered according to the manufacturer's recommendations. All treatment groups received chlorinated, city-supplied drinking water, except for the P1W birds, which were provided with distilled drinking water only during the administration of the treatment.

The broilers were reared on straw litter for 6 wk. Birds were fed a broiler starter diet for the first 3 wk, and a broiler grower diet for the remaining 3 wk of the production period (Table 1). A coccidiostat (Amprol; Merial Canada Inc., Baie d'Urfe, Quebec, Canada) was included in the feed, but no other antimicrobial agents were administered.

The viability of the probiotic cultures and their persistence in the water for the P1W treatment and in the feed for the P2F treatment were confirmed to remain at or above levels specified by the manufacturer throughout the 6-wk broiler production period. This was accomplished by plating serial dilutions of the feed or water sample on de Man, Rogosa, and Sharpe agar to culture lactic acid bacteria. The number of colony-forming units was then calculated and compared with the manufacturer's guaranteed minimum number of colony-forming units.

A sample of 30 broilers, nearest the average chick weight from each pen (120 broilers/treatment group), were individually weighed at 7, 14, 21, 28, and 35 d of

Table 2. Average egg weights at setting and transfer and percentage of weight loss of eggs produced by a breeder flock at 3 ages

Breeder flock age	Set weight[1] (g)	Transfer weight (g)	Weight loss[2] (%)
28 wk	55.8[c]	49.4[c]	11.4[c]
n[3]	2,250	1,998	1,998
43 wk	61.9[b]	53.8[b]	13.0[b]
n	2,592	2,374	2,374
57 wk	66.4[a]	57.5[a]	13.3[a]
n	2,664	2,076	2,076
Pooled SEM	0.1	0.1	0.1

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Eggs weighing <52.0 g were not set.

[2]Weight loss (%) = [(egg set weight − egg weight at transfer)/egg set weight] × 100.

[3]n = number of experimental units; each experimental unit = 1 egg.

age. Feed consumption in each pen was measured on a weekly basis. At 42 d of age, all broilers were individually weighed before shipping. Mortality in each pen was recorded on a daily basis, and all birds that died during the production period were necropsied after the trials.

Statistical Analysis

All data were analyzed using the GLM procedure of SAS (SAS Institute, 1999). All percentage data were transformed using arc sine transformation before analysis. Due to differences in chick weight at placement among treatments, BW and weight gains for subsequent weeks were analyzed as a covariate analysis. Significance was assessed at $P < 0.05$. Where the model indicated significance, the means were separated using the P-DIFF procedure of SAS. A significant interaction among main effects occurred only for BW at d 0; therefore, only main effects will be presented for all variables other than BW.

RESULTS AND DISCUSSION

Egg Weights and Weight Loss

Average egg weights at setting increased as the breeder flock aged (Table 2). This is in agreement with previous research (McNaughton et al., 1978; Wyatt et al., 1985). Average egg weights at transfer and percentage of egg weight loss at the time of transfer followed the same trend as egg weight. Percentage of weight loss increased as the flock aged. This was expected, because as egg size increases, shell thickness decreases, resulting in increased eggshell conductance (Ar et al., 1974) and, thus, greater moisture loss.

Fertility, Hatchability, Embryonic Mortality, and Culled Chicks

Because probiotic treatments were imposed after hatching, hatch characteristics and egg weights will only be discussed concerning breeder flock age. Fertility was dif-

Table 3. Percentage of fertility; hatchability; hatch of fertile; early, mid, and late embryonic mortality; and culled chicks from eggs produced by a breeder flock at 3 ages

Breeder flock age (wk)	n[1]	Fertility[2] (%)	Hatchability[3] (%)	Hatch of fertile[4] (%)	Early dead[5] (%)	Mid dead[6] (%)	Late dead[7] (%)	Culls[8] (%)
28	125	93.1[b]	78.8[b]	84.6[b]	5.9[a]	0.9[b]	4.9[b]	3.3[a]
43	144	96.4[a]	83.7[a]	86.8[a]	4.2[b]	1.6[b]	4.9[b]	2.1[b]
57	148	84.0[c]	63.6[c]	75.6[c]	7.0[a]	2.4[a]	10.7[a]	3.3[a]
Pooled SEM		0.6	1.0	0.9	0.5	0.3	0.6	0.4

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]n = number of experimental units; each experimental unit = 18 eggs.

[2]Fertility (%) = (number of fertile eggs/number of eggs set) × 100.

[3]Hatchability (%) = (total number of chicks hatched/number of eggs set) × 100.

[4]Hatch of fertile (%) = (total number of chicks hatched/number of fertile eggs set) × 100.

[5]Early dead (%) = (number of embryos that died from 1 to 7 d of incubation/number of eggs set) × 100.

[6]Mid dead (%) = (number of embryos that died from 8 to 14 d of incubation/number of eggs set) × 100.

[7]Late dead (%) = (number of embryos that died from 15 to 21 d of incubation/number of eggs set) × 100.

[8]Culls (%) = (number of chicks culled at hatching/number of eggs set) × 100.

ferent among all 3 flock ages, with the highest fertility at 43 wk and the lowest at 57 wk (Table 3). Hatchability of all eggs set and of fertile eggs followed the same pattern as fertility, with eggs from the oldest flock having the poorest hatchability. There were also differences in early, mid, and late embryonic mortality due to flock age. Early embryonic mortality was lowest in the 43-wk-old flock compared with the 28 and 53 wk old flocks, which did not differ from one another. Both mid and late embryonic mortality was highest in the 57-wk-old flock compared with the 28- and 43-wk-old flocks, which did not differ from each other. There were also higher percentages of culled chicks in both the 28- and 57-wk-old flocks compared with the 43-wk-old flock (Table 3). This indicates that both the youngest and oldest flocks had poorer overall chick quality than did the chicks from the breeder flock nearest peak production.

These results are not consistent with previous research showing that the percentage of saleable chicks hatched is higher in eggs from younger breeder flocks (McNaughton et al., 1978). However, the results in the present study do agree with previous findings of poor hatchability in extremely small and large eggs from extremely young and old breeder flocks, respectively (Lerner and Gunns, 1952; Morris et al., 1968).

BW and Weight Gains

Effect of Breeder Flock Age. Chick weight increased as the breeder flock aged (Table 4). This was expected, because past research has determined that smaller eggs from younger breeder flocks produce smaller chicks (McNaughton et al., 1978; Wyatt et al., 1985). Chick weight is traditionally used as an evaluation of chick quality; lighter broiler chicks are usually of poorer quality, having lower weight gains and final market weights (Sklan et al., 2003).

At both 7 and 14 d of age, the broilers from the 28-wk-old breeder flock were lighter than those from either the 43- or 57-wk-old breeder flocks. At 21 d, there were differences among the broilers from each breeder flock age,

with broiler weights increasing as breeder flock age increased. From 28 d of age until the end of the production period, the broilers from the 28-wk-old breeder flock were lighter than the broilers from either the 43- or 57-wk-old breeder flocks, which did not differ from one another.

There were also differences in weight gains among the chicks from different breeder flock ages (Table 5). Up to 35 d of age, broilers from the 28-wk-old flock consistently had lower weight gains than broilers produced when the flock was 43 and 57 wk of age. During wk 2 and 3, the BW gains were different among all breeder flock ages, with weight gain increasing with breeder flock age. The overall weight gain was lowest in broilers from the 28-wk-old flock compared with the other flock ages, which did not differ from one another.

These results were expected, because it is well documented that as breeder flock age increases, so does egg size (McNaughton et al., 1978). Larger eggs from older breeder flocks subsequently produce larger chicks and broilers with heavier final BW (Merritt and Gowe, 1965; Morris et al., 1968; Sklan et al., 2003).

Effect of Probiotic Treatments. There were differences in chick weight at d 0 among treatments (Table 4). However, average weight differences were <0.25 g. Consequently, covariate analysis was used for the BW and BW gains for subsequent weeks. From d 7 on, there were no differences in broiler BW among the probiotic treatments (Table 4).

There were no differences in weekly BW gains among probiotic treatments from 0 to 35 d (Table 5). However, the BW gain from 36 to 42 d was higher in the P1W, P1S, and P2F treatments compared with the control treatment. This indicates that administering the probiotics may improve BW gain in the final week of production. However, this did not result in significant differences in final market BW. The overall BW gains over the 6-wk growout period did not differ among probiotic treatments (Table 5).

The available body of literature offers a variety of conflicting results concerning the efficacy of probiotics for increasing BW and BW gains in broilers. Whereas undefined or complex, defined probiotic cultures (that include

Table 4. Effect of breeder flock age, probiotic treatment, and the interaction on average broiler BW (mean ± SEM)

Item	n[1]	Day (g)						
		0	7	14	21	28	35	42
Breeder flock age (wk)								
28	16	38.9 ± 0.1[c]	93.4 ± 1.5[b]	214.2 ± 6.0[b]	408.6 ± 7.7[c]	722.8 ± 12.4[b]	1,160.8 ± 16.9[b]	1,725.47 ± 20.6[b]
43	15	41.5 ± 0.1[b]	116.6 ± 1.2[a]	281.5 ± 3.2[a]	571.1 ± 6.1[b]	1,008.6 ± 9.8[a]	1,534.7 ± 13.4[a]	2,112.29 ± 16.4[a]
57	14	43.4 ± 0.1[a]	119.7 ± 1.6[a]	291.7 ± 4.2[a]	612.9 ± 8.1[a]	1,036.2 ± 12.9[a]	1,540.5 ± 17.7[a]	2,099.91 ± 21.7[a]
Probiotic treatment[2]								
Control	11	41.1 ± 0.1[b]	109.0 ± 1.4	260.0 ± 3.7	530.2 ± 7.1	929.1 ± 11.4	1,419.2 ± 15.6	1,961.9 ± 19.0
P1W	12	41.3 ± 0.1[ab]	110.3 ± 1.3	263.8 ± 3.5	530.1 ± 6.8	916.2 ± 10.9	1,406.4 ± 15.0	1,974.0 ± 18.4
P1S	11	41.4 ± 0.1[a]	111.1 ± 1.4	265.8 ± 3.7	534.7 ± 7.2	929.2 ± 11.4	1,422.1 ± 15.7	1,999.3 ± 19.3
P2F	11	41.2 ± 0.1[b]	109.2 ± 1.4	260.3 ± 3.7	528.5 ± 7.1	915.5 ± 11.3	4,100.1 ± 15.6	1,981.7 ± 19.0
Breeder flock age (wk) × probiotic treatment								
28 × control	4	38.3 ± 0.1[e]	92.3 ± 2.6	208.0 ± 6.9	402.5 ± 13.3[a]	732.8 ± 21.3	1,171.2 ± 29.1	1,723.9 ± 35.5
28 × P1W	4	39.0 ± 0.1[c]	93.0 ± 2.5	215.5 ± 6.6	412.4 ± 12.7[a]	704.9 ± 20.6	1,147.3 ± 28.1	1,704.9 ± 34.6
28 × P1S	4	39.4 ± 0.1[d]	97.4 ± 2.4	224.9 ± 6.3	420.3 ± 12.3[a]	739.5 ± 19.7	1,198.8 ± 26.8	1,774.8 ± 32.7
28 × P2F	4	38.8 ± 0.1[cd]	91.0 ± 2.5	208.6 ± 6.6	399.3 ± 12.9[a]	713.9 ± 20.5	1,126.0 ± 28.1	1,698.2 ± 34.2
43 × control	3	41.5 ± 0.2[b]	115.0 ± 2.6	278.8 ± 7.0	564.3 ± 13.5[a]	998.8 ± 21.4	1,518.5 ± 29.2	2,084.6 ± 35.9
43 × P1W	4	41.5 ± 0.1[b]	116.9 ± 2.3	275.8 ± 6.1	558.6 ± 11.7[a]	987.4 ± 18.8	1,514.3 ± 25.8	2,106.1 ± 31.4
43 × P1S	4	41.5 ± 0.1[b]	116.2 ± 2.3	281.0 ± 6.0	569.7 ± 11.8[a]	1,010.0 ± 18.9	1,533.8 ± 26.1	2,104.9 ± 32.0
43 × P2F	4	41.4 ± 0.1[b]	118.1 ± 2.3	290.6 ± 6.0	591.8 ± 11.7[a]	1,038.1 ± 18.5	1,572.0 ± 25.7	2,153.7 ± 31.9
57 × CON	4	43.5 ± 0.1[a]	119.8 ± 2.5	293.3 ± 6.6	623.9 ± 12.9[a]	1,055.7 ± 20.5	1,567.9 ± 28.3	2,077.1 ± 34.5
57 × P1W	4	43.5 ± 0.1[a]	120.8 ± 2.5	300.1 ± 6.6	619.1 ± 12.8[a]	1,056.3 ± 20.3	1,557.8 ± 27.9	2,111.1 ± 34.3
57 × P1S	3	43.4 ± 0.2[a]	119.5 ± 2.8	291.4 ± 7.5	614.2 ± 14.5	1,038.1 ± 22.9	1,533.7 ± 31.5	2,118.4 ± 38.9
57 × P2F	3	43.4 ± 0.2[a]	118.6 ± 2.8	281.9 ± 7.4	594.2 ± 14.4	994.7 ± 22.8	1,502.4 ± 31.5	2,093.1 ± 38.4

[a-e]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]n = number of experimental units; each experimental unit = 1 broiler pen.

[2]P1W = probiotic 1 administered in distilled drinking water as directed by the manufacturer at 1, 2, 19, and 20 d of age; P1S = probiotic 1 administered as a spray at hatch as directed by the manufacturer; P2F = probiotic 2 administered as a feed additive (0.5 g of probiotic 2/kg of feed) throughout the production period.

Table 5. Effect of breeder flock age and probiotic treatment on average weekly broiler BW gains (mean ± SEM)

Item	n[1]	0 to 7	8 to 14	15 to 21	22 to 28	29 to 35	36 to 42	0 to 42
					Day (g)			
Breeder flock age (wk)								
28	16	53 ± 1.1[b]	119 ± 2.4[c]	198 ± 4.1[c]	306 ± 7.7[b]	434 ± 7.4[b]	557 ± 7.6[b]	1,672 ± 17.0[b]
43	15	75 ± 1.2[a]	165 ± 2.5[b]	289 ± 4.3[b]	439 ± 7.9[a]	527 ± 7.7[a]	579 ± 7.9[a]	2,069 ± 17.0[a]
57	14	78 ± 1.2[a]	174 ± 2.6[a]	320 ± 4.4[a]	432 ± 8.2[a]	516 ± 8.0[a]	572 ± 8.2[ab]	2,089 ± 17.0[a]
Probiotic treatment[2]								
Control	11	68 ± 1.4	151 ± 2.9	270 ± 5.0	398 ± 9.2	489 ± 8.9	542 ± 9.1[b]	1,926 ± 18.6
P1W	12	69 ± 1.3	154 ± 2.9	269 ± 4.7	387 ± 8.9	499 ± 8.9	575 ± 8.8[a]	1,941 ± 18.6
P1S	11	70 ± 1.4	155 ± 2.9	269 ± 5.0	396 ± 9.3	495 ± 9.0	579 ± 9.2[a]	1,963 ± 18.6
P2F	11	68 ± 1.4	151 ± 2.8	268 ± 5.0	386 ± 9.2	487 ± 8.6	582 ± 9.1[a]	1,943 ± 18.6

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]n = number of experimental units; each experimental unit = 1 broiler pen.

[2]P1W = probiotic 1 administered in distilled drinking water as directed by the manufacturer at 1, 2, 19, and 20 d of age; P1S = probiotic 1 administered as a spray at hatch as directed by the manufacturer; P2F = probiotic 2 administered as a feed additive (0.5 g of probiotic 2/kg of feed) throughout the production period.

numerous bacterial species) have generally improved weight gains and market BW (Nurmi and Rantala, 1973; Tortuero, 1973), simple, defined probiotics (that include only a few bacterial species) have been used with mixed results (Watkins and Kratzer, 1983, 1984; Yeo and Kim, 1997; Jin et al., 1998; Estrada et al., 2001; Hofacre et al., 2003). It is, thus, possible that, in the present study, the low number of bacterial strains in the probiotic products may have limited their efficacy in improving BW and BW gains.

Effect of Breeder Flock Age × Probiotic Treatment. Chick weight at placement was different among probiotic treatments (Table 4). For this reason, subsequent BW were analyzed as a covariate analysis to account for these initial differences in chick weight. There was no effect of the interaction on BW (Table 4) or BW gains (data not shown) for the entire production period. This was contrary to the hypothesis, because it was anticipated that the probiotics would benefit the broilers from the younger breeder flock to a greater extent than the broilers from the peak and older breeder flocks

Feed Conversion

Effect of Breeder Flock Age. During 0 to 7 d, the broilers from the 28-wk-old breeder flock had a higher FCR than did the broilers from either of the other 2 older breeder flock ages (Table 6). This changed from d 8 to 14, with the broilers from the 43-wk-old breeder flock having a higher FCR than the broilers from the 28- and 57-wk-old breeder flocks. During d 15 to 42, there were no differences in FCR due to breeder flock age. From d 22 to 28, the broilers from the 43-wk-old breeder flock had a lower FCR compared with the broilers from the 28-wk-old flock, with the broilers from neither of these flocks being significantly different from the broilers from the 57-wk-old flock. Over the entire production period, the broilers from the 28-wk-old flock had the best FCR compared with the broilers from the 57-wk-old flock. The FCR of the broilers from the 43-wk-old flock did not differ from either of the other 2 flock ages. This agrees with

previous findings that smaller chicks exhibit a better FCR than larger chicks (O'Neil, 1955; Morris et al., 1968; Proudfoot and Hulan, 1981; Wyatt et al., 1985; Hearn, 1986). It is unclear whether this is an effect of breeder flock age or simply of chick size.

Effect of Probiotic Treatments. There was no effect of any of the probiotic treatments on FCR at any point during the production period or on the overall FCR over the entire production period (Table 6). Past studies using simple, defined probiotics have found improvements in broiler FCR (Jin et al., 1998; Zulkifli et al., 2000). However, others have not found differences in FCR between probiotic-treated birds and untreated control birds (Watkins and Kratzer, 1983, 1984; Estrada et al., 2001; Huang et al., 2004).

These varying results may be due to differences in the bacterial strains used in the above-mentioned studies and the origins of these strains. Because in most studies no information is provided as to whether the strain used was isolated from poultry, it is not possible to assess whether it is host-specific and would be able to attach to the GIT epithelial cells (Jin et al., 1998; Cox et al., 2001). In the present study, no information as to the origin of the bacterial strains used was provided by the manufacturer, so it was impossible to assess whether this may have played a role.

Broiler Mortality

Effect of Breeder Flock Age. Breeder flock age had an effect on d-7 broiler mortality and d-14 cumulative mortality. Broilers produced by the 57-wk-old breeder flock had higher mortality than broilers produced from the 28- and the 43-wk-old breeder flocks, which did not differ from one another (Table 7). From 21 d of age until the end of the production period, there were no differences in cumulative broiler mortality due to breeder flock age. Although the younger flock produced smaller chicks with lower weight gains, there was no difference in total mortality between the broilers from the young flock and broilers produced by the 2 older flocks. This is in contrast

Table 6. Effect of breeder flock age and probiotic treatment on average weekly broiler feed conversion ratios (mean ± SEM)

Item	n[1]	Day (g of feed/g of gain)						
		0 to 7	8 to 14	15 to 21	22 to 28	29 to 35	36 to 42	0 to 42
Breeder flock age (wk)								
28	16	1.59 ± 0.03[a]	1.55 ± 0.02[b]	1.55 ± 0.03[a]	1.74 ± 0.03[a]	1.83 ± 0.04	1.89 ± 0.27	1.73 ± 0.01[b]
43	15	1.31 ± 0.03[b]	1.73 ± 0.03[a]	1.54 ± 0.03[a]	1.63 ± 0.03[b]	1.82 ± 0.04	2.00 ± 0.28	1.76 ± 0.01[ab]
57	14	1.38 ± 0.03[b]	1.50 ± 0.03[b]	1.48 ± 0.03[a]	1.72 ± 0.03[ab]	1.83 ± 0.04	2.55 ± 0.29	1.78 ± 0.01[a]
Probiotic treatment[2]								
Control	11	1.44 ± 0.03	1.59 ± 0.03	1.52 ± 0.03	1.67 ± 0.04	1.84 ± 0.05	2.10 ± 0.32	1.77 ± 0.01
P1W	12	1.40 ± 0.03	1.61 ± 0.03	1.52 ± 0.03	1.71 ± 0.04	1.76 ± 0.05	2.59 ± 0.32	1.74 ± 0.01
P1S	11	1.39 ± 0.03	1.58 ± 0.03	1.53 ± 0.03	1.69 ± 0.04	1.85 ± 0.05	1.95 ± 0.32	1.75 ± 0.01
P2F	11	1.47 ± 0.03	1.60 ± 0.03	1.53 ± 0.03	1.72 ± 0.04	1.87 ± 0.04	1.94 ± 0.31	1.76 ± 0.01

[a,b]Means in a column with different superscripts differ significantly ($P < 0.05$).

[1]n = number of experimental units; each experimental unit = 1 broiler pen.

[2]P1W = probiotic 1 administered in distilled drinking water as directed by the manufacturer at 1, 2, 19, and 20 d of age; P1S = probiotic 1 administered as a spray at hatch as directed by the manufacturer; P2F = probiotic 2 administered as a feed additive (0.5 g of probiotic 2/kg of feed) throughout the production period.

to previous research showing that smaller chicks experience a higher mortality rate (McClung and Smith, 1949; O'Neil, 1950; Hays and Spear, 1952; McNaughton et al., 1978; Wyatt et al., 1985; Hearn, 1986). This may be due to the fact that whereas the broilers were all reared in straw floor pens, the rearing conditions were very sanitary, so chicks from the younger breeder flock may not have faced as many pathogen challenges as they would in a commercial situation.

Effect of Probiotic Treatments. There was no effect of probiotic treatment on cumulative mortality from 0 to 35 d of age (Table 7). However, the probiotic treatments did have an effect on 42-d cumulative broiler mortality. Both the P1W and the P1S treatments had higher mortality than the P2F treatment. However, none of the probiotic treatments (P1W, P1S, or P2F) were significantly different from the control. This result was not unexpected, because there appears to be no evidence that probiotics, either complex or simple, are capable of reducing broiler mortality (Bilgili and Moran, 1990; Palmu and Camelin, 1997; Jin et al., 1998; Estrada et al., 2001), despite their efficacy in reducing the number of pathogens colonizing the GIT (Blankenship et al., 1993; Palmu and Camelin, 1997; Chambers and Lu, 2002).

Incidence of Necrotic Enteritis

In some of the broiler pens at each of the breeder flock ages, there were cases of necrotic enteritis (**NE**), as confirmed by a veterinarian. There were subclinical cases of NE (resulting in possible growth depression but low or no mortality) in several pens during each of the 3 trials. There was a clinical outbreak (resulting in a rapid rise in the rates of morbidity and mortality) in 1 control pen in the trial in which broilers were hatched from the 43-wk-old breeder flock. The outbreak occurred during wk 3 of this trial. Clinical outbreaks also occurred in 1 P2F and 1 P1S pen during wk 4 of the trial in which broilers were hatched from the 57-wk-old breeder flock. The clinical outbreaks necessitated the treatment of the affected pens with therapeutic levels of antibiotics administered in the drinking water. Subsequently, the decision was made to completely remove all data collected from these pens from all data analysis. No data from these pens are pre-

Table 7. Effect of breeder flock age and probiotic treatment on weekly cumulative mortality of broilers (mean ± SEM)

Item	n[1]	Day (%)					
		0 to 7	0 to 14	0 to 21	0 to 28	0 to 35	0 to 42
Breeder flock age (wk)							
28	16	0.4 ± 0.2[b]	1.1 ± 0.3[b]	1.9 ± 0.4	2.7 ± 0.5	3.4 ± 0.6	3.6 ± 0.6
43	15	0.3 ± 0.2[b]	1.0 ± 0.3[b]	1.8 ± 0.4	2.5 ± 0.6	3.7 ± 0.6	4.6 ± 0.7
57	14	1.3 ± 0.2[a]	2.1 ± 0.3[a]	2.7 ± 0.4	3.7 ± 0.6	4.4 ± 0.7	5.1 ± 0.7
Probiotic treatment[2]							
Control	11	0.5 ± 0.2	1.4 ± 0.4	2.3 ± 0.5	3.0 ± 0.6	3.6 ± 0.7	4.1 ± 0.8[ab]
P1W	12	0.7 ± 0.2	1.4 ± 0.4	2.3 ± 0.5	3.9 ± 0.6	4.8 ± 0.7	5.3 ± 0.8[a]
P1S	11	0.9 ± 0.2	1.7 ± 0.4	2.6 ± 0.5	3.3 ± 0.6	4.6 ± 0.7	5.4 ± 0.8[a]
P2F	11	0.6 ± 0.2	1.1 ± 0.4	1.4 ± 0.5	1.7 ± 0.6	2.4 ± 0.7	2.9 ± 0.7[b]

[a,b]Means in a column with different superscripts differ significantly ($P < 0.05$).

[1]n = number of experimental units; each experimental unit = 1 broiler pen.

[2]P1W = probiotic 1 administered in distilled drinking water as directed by the manufacturer at 1, 2, 19, and 20 d of age; P1S = probiotic 1 administered as a spray at hatch as directed by the manufacturer; P2F = probiotic 2 administered as a feed additive (0.5 g of probiotic 2/kg of feed) throughout the production period.

sented in this paper or the associated data tables. In the remaining pens, there were no significant differences in the incidence of NE between probiotic treatments or flock ages, as confirmed by postmortem examination of all birds.

Because NE infects the bird by colonizing the small intestine (Culter, 2002), the incidence of NE in all treatment groups provides further evidence indicating that the probiotic products investigated in this research did not protect the GIT from colonization by pathogenic microorganisms such as *Clostridium perfringens*, the causative agent of NE. This is contrary to past research, which has shown that either a simple probiotic culture or an undefined culture consisting of adult cecal material is able to prevent colonization of the GIT by *C. perfringens* (Fukata et al., 1991; Hofacre et al., 2003), reduce the toxicity of *C. perfringens* (Fukata et al., 1991), and reduce NE-associated mortality (Hofacre et al., 2003). In general, it appears that most probiotics, both simple and complex, are more effective in competitively excluding *Salmonella* than other potentially harmful bacteria (Blankenship et al., 1993; Pascual et al., 1999; Kubena et al., 2001; Chambers and Lu, 2002). No microscopic or microbiological analysis was performed on broiler GIT samples in the present study, so the ability of the probiotics to prevent the colonization of the broiler GIT by pathogens such as *Salmonella* could not be confirmed.

The diet used in the present study was wheat-based. This may also have increased the likelihood of NE infection, because diets high in wheat have been linked to a higher incidence of NE than corn-based diets (Riddell and Kong, 1992; Annett et al., 2002).

It was hypothesized that the broilers in the P1W, P1S, and P2F probiotic treatments would all have higher BW gains and BW, lower mortality, and better FCR than the broilers in the control group. Data from the current study does not support the hypothesis. It was also determined that there was no effect of the probiotic treatments on broiler performance in chicks produced by a young breeder flock, even though the young (28 wk) and old (57 wk) breeder flocks produced poorer quality chicks than the chicks produced from the flock near peak (43 wk). During the broiler growout period, the 43- and 57-wk-old breeder flocks produced broilers with higher overall BW gains, although the broilers from the younger flock had a lower FCR. There was no effect of the interaction between treatment and breeder flock age on any production parameter.

Previous research has shown that probiotics can be capable of improving broiler BW gains and market BW (Nurmi and Rantala, 1973; Mohan et al., 1996; Jin et al., 1998). However, the probiotics tested in the current trial did not result in improvements in broiler production efficiency over the control group.

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