METABOLISM AND NUTRITION

Performance of Broiler Chickens Fed Diets Supplemented with a Direct-Fed Microbial

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ABSTRACT From hatch to 18 d of age broilers were fed starter diets with (0.9 kg/ton) or without direct fed microbial (DFM). At 18 d, birds were weighed and, within DFM treatment (trt), randomly assigned to battery pens. In Exp 1, a 2 × 2 factorial arrangement of nutrient density [control (C, 19.3% protein (CP), 0.84%, Ca 0.37% nonphytin P (nPP); and 17.1% CP, 0.8% Ca, and 0.3% nPP in the grower (Gr) and finisher (Fn) diets, respectively) and moderate (M) (17% CP, 0.69% Ca, 0.30% nPP; 15% CP, 0.66% Ca, 0.25% nPP in the Gr and Fn diets, respectively)] and DFM concentration [0 or 0.9 kg/ton (++)] was used. Exp 2 was a 2 (DSM at 0 and 0.45 kg/ton) × 3 (nutrient densities) factorial. Exp 2 included a low (L) nutrient density that differed from diet M only in Ca and nPP concentrations and an added trt, diet M with 0.45 kg/ton DFM as in Exp 1. At the end of the Gr and Fn weight, feed efficiency, apparent nutrient retention were determined, and 4 birds per pen were sampled for tibia ash. In Exp 2, gains in the Gr phase were 1,122.0, 983.7, 1,121.5, 930.7, and 1,151.5 g in birds fed the C, M, M+, L, and L+ diets, respectively. Addition of DFM to the M diet overcame the negative effect of nutrient concentration on performance but not when the L diet was fed. Nutrient level and DFM affected apparent protein, Ca, and P retention at 32 or 42 d of age with retention increasing as nutrient level decreased and with DFM added to the diet. Ca and P retention at 28 d (Exp 1) was higher in birds fed M++ (45.8 and 46%, respectively) than in those fed the C diet (38.7 and 40.0%, respectively). Feeding the M and L diets resulted in lower tibia ash than that of birds fed the C diet, but the addition of DFM to low nutrient diets overcame this negative effect.

(*Key words*: direct-fed microbial, *Lactobacillus*, performance, protein, phosphorus)

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INTRODUCTION

Fuller (1989) defined direct-fed microbials (DFM) as live microbial feed supplements that improve microbial balance in the animal gastrointestinal tract and, therefore, are beneficial. The Food and Drug Administration considers DFM to be a source of live (viable) naturally occurring microorganisms (Miles and Bootwalla, 1991). Whereas evidence has been presented that *Lactobacillus*-based DFM improves performance and feed utilization in poultry (Nahashon et al., 1994; England et al., 1996; Mohan et al., 1996; Jin et al., 1998; Schneitz et al., 1998; Zulkifli et al., 2000), several other studies did not report any beneficial effects (Goodling et al., 1987; Maiolino et al., 1992; Owings, 1992).

Proposed mechanisms for the beneficial effect of DFM are (1) to help in maintaining a beneficial microflora in the gastrointestinal tract by inhibiting the growth of pathogenic microorganisms (Jin et al., 1996) and (2) to increase nutrient utilization through improved intestinal health resulting in greater intestinal enzyme activities and nutrient availability (Nahashon et al., 1994). Work done with laying hens has shown that the addition of *Lactobacillus*-based DFM to the diet improves N, Ca, and P retentions (Nahashon et al., 1994, 1996).

Given current regulatory emphasis on lowering N and P in animal manures and the existing published literature on the impact of DFM on nutrient retention, the work presented here was based on the hypothesis that inclusion of DFM in the diet increases nutrient retention, thus allowing for the feeding of low nutrient diets without impacting performance negatively. The objectives of these experiments were to determine if the addition of a commercial DFM could overcome the negative effect of feeding grower (Gr) and finisher (Fn) diets containing low concentrations of protein, lysine, methionine, Ca, and P to broiler chickens on performance and result in improved utilization of these nutrients.

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Abbreviation Key: C = control; DFM = direct fed microbial; Exp = experiment; Fn = finisher; Gr = grower; L = Low; M = moderate; nPP = nonphytin phosphorus; PP = phytin phosphorus; St = starter; trt = treatment.

TABLE 1. Ingredient (%) and nutrient content of diets, experiments 1 and 2

		Grower			Finisher		
Ingredient	Starter	Control	Moderate	Low	Control	Moderate	Low
Corn	60.3	67.4	75.3	75.4	73.4	80.9	81.0
Soybean meal (48%)	32.0	24.5	18.1	18.1	18.9	13.1	13.1
Corn gluten meal (60%)	1.80	2.0	2.0	2.0	2.0	2.0	2.0
Soybean oil	2.25	2.17	0.88	0.88	2.19	0.84	0.84
Calcium carbonate	1.08	1.19	1.11	1.03	1.31	1.25	1.18
Dicalcium phosphate	1.58	1.37	1.31	1.04	1.06	0.88	0.79
Lysine	0.11	0.18	0.22	0.22	0.18	0.22	0.22
Methionine	0.22	0.24	0.19	0.19	0.19	0.15	0.15
Diatomaceous earth[1]	0.00	0.23	0.27	0.52	0.29	0.17	0.23
Other[2]	0.65	0.62	0.62	0.62	0.48	0.48	0.48
Nutrients (formulated)							
Protein, %	22.0	19.3	17.0	17.0	17.1	15.0	15.0
Lysine, %	1.23	1.10	0.97	0.97	0.95	0.84	0.84
Methionine, %	0.57	0.55	0.48	0.48	0.48	0.41	0.41
Metabolizable energy, kcal/kg	3,070	3,150	3,150	3,150	3,200	3,200	3,200
Ca, %	1.0	0.85	0.69	0.64	0.80	0.66	0.61
Total P	0.75	0.63	0.54	0.51	0.58	0.50	0.46
Nonphytin P (nPP),[3] %	0.45	0.35	0.29	0.26	0.30	0.24	0.21
Nutrients (analyzed)							
Experiment 1							
Protein, %	22.2	19.5	17.3		17.0	15.3	
Lysine, %	1.25	1.12	0.97		0.93	0.86	
Methionine, %	0.57	0.54	0.46		0.47	0.43	
Ca, %	0.99	0.87	0.71		0.80	0.64	
Total P, %	0.73	0.60	0.52		0.54	0.46	
Phytin P, %	0.28	0.27	0.27		0.25	0.26	
nPP, %	0.45	0.33	0.25		0.29	0.20	
Experiment 2							
Protein, %	21.9	19.2	16.8	17.0	16.8	14.7	14.8
Lysine, %	1.17	1.05	0.92	0.93	0.92	0.83	0.81
Methionine, %	0.53	0.50	0.43	0.44	0.46	0.40	0.41
Ca, %	0.95	0.84	0.70	0.65	0.78	0.63	0.60
Total P, %	0.71	0.59	0.51	0.47	0.54	0.45	0.42
Phytin P, %	0.27	0.27	0.27	0.27	0.26	0.26	0.26
nPP, %	0.44	0.32	0.24	0.20	0.28	0.19	0.16

[1]The direct-fed microbial (DFM) was added to the diets at the expense of diatomaceous earth.

[2]Other included salt, choline chloride, vitamin premix, and mineral premix. The vitamin premix supplied per kilogram of diet: vitamin A, 14,991 IU from retinyl acetate; vitamin D, 5,291 ICU from cholecalciferol; vitamin E, 52.9 IU from DL-α-tocopheryl acetate; vitamin B_{12}, 0.026 mg from cyanocobalamin; riboflavin, 17.64 mg; niacin, 70.55 mg from nicotinic acid; pantothenic acid, 24.6 mg from D-pantothenic acid; vitamin K, 3.2 mg from menadion sodium bisulfite complex; folic acid, 2.12 mg; vitamin B_6, 6.17 mg from pyridoxine hydrochloride; thiamine, 4.4 mg from thiamine mononitrate; and biotin, 0.149 mg from D-biotin. The mineral premix supplied per kilogram of diet: Ca, 98 mg from $CaCO_3$; Zn, 210 mg from ZnO; Mn, 120 mg equally from MnO and $MnSO_4$, Fe, 40 mg from $FeSO_4$; Cu, 20 mg from CuO; I, 3.0 mg from $Ca(IO_3)_2$; and Co, 0.05 mg from $CoCO_3$.

[3]Nonphytin P (nPP) was determined by subtracting analyzed phytin P minus total P.

MATERIALS AND METHODS

Bird Management

All procedures used in this work were approved by the institutional Animal Care and Use Committee. In 2 experiments (Exp), Ross 308 male broiler chicks hatchlings were obtained commercially and placed in 8 floor pens in 1 room. The room was divided in half such that no movement of litter or caretakers could occur between the 2 sides in order to prevent cross-inoculation with the DFM.

In the first Exp, 400 hatchlings were placed in floor pens, 50 chicks per pen, with 4 pens fed a starter (St) with the DFM (Primalac[2] at 0.9 kg/ton), and the other 4 pens were fed the same St without DFM. At the end of the St phase (hatch to 18 d of age), birds were weighed individually within treatment (trt; with or without added DFM). Thus, birds fed the DFM in the St feed were allocated only to the DFM trt, and birds fed the St without DFM were allocated only to trt without added DFM. Heavy and light birds were discarded (40%), and birds closest to the mean within St trt weight were reallocated to 16 grower battery pens for the no-added DFM trt and to 16 pens for the DFM added trt. This resulted in 4 trt and 8 pens of 8 birds per trt. The DFM, Primalac,[3] used in these Exp is commercially available and contains primarily *Lactobacillus acidophilus* and *Lactobacillus casei* (in addition to other genera); specifics on blend concentrations are proprietary.

In Exp 2, 500 hatchlings were placed in floor pens with 200 birds fed on a St diet without DFM and 300 birds on the same St diet but with DFM (Primalac at 0.9 kg/ton).

[2]Star Labs, Inc., Clarksdale, MO.

[3]California Pellet Mill (Model 3016-4, 125 hp, with a production capability of 10 to 12 tons/h, 5/32 CPX die, steam hardness of 600 to 1,100 lb/h), California Pellet Mill Co., Crawfordsville, IN.

TABLE 2. Effect of adding a direct-fed microbial (DFM) to broiler feed on performance in the grower (18 to 28 d of age) and finisher phases (28 to 42 d of age; experiment 1)

		18 to 28 d of age[2]				28 to 42 d of age[3]		
		Body weight						
Nutrient level	DFM[1]	18 d	28 d	Weight gain	Feed to gain	42 d Weigh	Weight gain	Feed to gain
		(g/bird)				(g/bird)		
C[4]	–	628.3	1,341.0^{ab}	712.7^{a}	1.58^{bc}	2,684.2^{a}	1,293.7^{a}	1.90^{b}
C	++	673.5	1,399.3^{a}	725.8^{a}	1.55^{c}	2,704.7^{a}	1,315.7^{a}	1.88^{c}
M[5]	–	623.1	1,285.4^{b}	662.3^{c}	1.68^{a}	2,523.4^{b}	1,044.9^{b}	1.95^{a}
M	++	670.6	1,368.6^{a}	698.0^{b}	1.60^{b}	2,702.6^{a}	1,311.1^{a}	1.87^{c}
SEM		8.6	19.2	10	0.009	41	31	0.015
Overall probability		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Main effect probabilities								
Nutrient Level		NA[6]	0.008	0.001	0.001	0.002	0.006	0.026
DFM		<0.001	0.0052	0.008	0.008	0.010	<0.001	0.015
Nutrient Level × DFM		NA	0.2765	0.301	0.332	0.073	0.025	0.092
Main effect means								
Nutrient level								
C			1,370.0	719.3	1.57	2,694.5	1,304.7	1.89
M			1,326.5	680.2	1.64	2,613.0	1,178.0	1.91
DFM								
None (–)		625.5	1,313.1	687.5	1.63	2,603.8	1,169.3	1.93
Added (++)		672.3	1,383.5	711.9	1.58	2,703.7	1,313.4	1.88

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO) at inclusion levels of 0 (–) or 0.9 kg/ton (++).

[2]Means of 8 pens per treatment (8 birds/pen).

[3]Means of 8 pens per treatment (4 birds/pen).

[4]The control (C) diet contained (by analysis): grower, 19.5% protein, 0.87% Ca, and 0.33% nonphytin P (nPP); finisher, 17.0% protein, 0.80% Ca, and 0.29% nPP.

[5]The moderate (M) diet contained (by analysis): grower, 17.3% protein, 0.71% Ca, and 0.25% nPP, finisher, 15.3% protein, 0.64% Ca, and 0.20% nPP.

[6]Not applicable (NA). All broiler chickens were fed diets with the same nutrient levels in the starter phase. The diets differed only in the presence or absence of DFM.

At the end of the St phase (hatch to 18 d of age) birds were weighed individually and reallocated as described for Exp 1, except that they were allocated to 15 battery pens for the no-added DFM trt and 20 pens for the DFM added trt resulting in 5 pens of 8 birds per trt.

Both Exp had 3 phases: St, as described previously; Gr phase (18 to 28 and 18 to 32 d of age in Exp 1 and 2, respectively); and Fn phase (28 to 42 and 32 to 42 d of age, Exp 1 and 2, respectively). Feed and water were provided ad libitum, and a light program was followed. Hours of light per 24-h period were 24 (hatch to 4 d), 14 (4 to 12 d), 16 (12 to 24 d), 18 (24 to 32 d), and 22 (32 to 42 d of age).

To prevent cross-inoculation of *Lactobacillus*, dietary assignments to cages were not completely random. Instead, cage and battery arrangements were carefully selected to ensure that 1) each diet was represented in each level of a battery, 2) there was sufficient lateral separation to prevent cross-contamination of feed or water, and 3) a cage without DFM was never under a cage with a DFM diet to prevent inoculation by water spillage or spillage of fecal matter from above. In addition, bird care was scheduled such that birds without DFM were serviced first followed by those on DFM diets. Whenever possible, 2 separate animal care crews were used to minimize cross contamination.

Diets

For both Exp, the St diet was a corn-soybean meal based diet that met or exceeded all National Research Council (1994) nutrient recommendations (Table 1). In Exp 1, the dietary trt consisted of 2 levels of DFM (0 and 0.9 kg/ton (++) and 2 nutrient densities [control (C) and moderate (M)] arranged in a 2 × 2 factorial arrangement. The 2 diets (Table 1) differed in their protein, methionine, lysine, Ca, and nPP contents. The C diets were formulated to meet or exceeded National Research Council (1994) recommendations for all nutrients. The M diets were formulated to be 12% lower in protein, lysine, and methionine and 18% lower in Ca and nPP compared with the C diets.

In Exp 2, a 2 × 3 factorial arrangement with an additional trt was used. The factorial arrangement was 2 DFM concentrations [0 (–) and 0.45 kg/ton (+)] and 3 nutrient densities C, M (as in Exp 1), and low (L) (Table 1). The DFM was used in Exp 2 at an inclusion level of 0.45 kg/ton in the Gr and Fn diets. The reason for the change in DFM inclusion level versus that used in Exp 1 (0.9 kg/ton) was to reflect more closely commercial recommendations and usage in Gr and Fn diets. To test for a level of inclusion effect, an additional trt was used, diet M with 0.9 kg of DFM/ton (M++). The L diet nutrient densities were

TABLE 3. Effect of adding a direct-fed microbial (DFM) to broiler feed on performance in the grower (18 to 32 d of age) and finisher phases (32 to 42 d of age) (experiment 2)

		18 to 32 d of age[2]			32 to 42 d of age[3]		
Nutrient level	DFM[1]	32 d Weight	Gain	Feed to gain	42 d Weight	Gain	Feed to gain
		(g/bird)			(g/bird)		
C[4]	–	1,838.1[a]	1,122.0[a]	1.70[c]	2,819.2[a]	1,018.2[ab]	1.87[c]
C	+	1,822.4[a]	1,109.3[a]	1.70[c]	2,859.2[a]	1,036.8[a]	1.87[c]
M[5]	–	1,650.0[cd]	983.7[c]	1.74[b]	2,589.8[c]	939.8[bc]	1.90[b]
M	+	1,825.1[a]	1,121.5[a]	1.70[c]	2,824.1[a]	999.0[ab]	1.87[c]
M	++	1,824.8[a]	1,121.0[a]	1.69[c]	2,833.4[a]	998.8[ab]	1.87[c]
L[6]	–	1,595.2[d]	930.7[d]	1.78[a]	2,476.0[d]	880.8[c]	1.94[a]
L	+	1,755.1[bc]	1,051.5[b]	1.72[bc]	2,707.3[b]	952.2[b]	1.88[bc]
SEM		12.8	13.2	0.006	21.9	18.2	0.006
Overall probabilities		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Main effect probabilities[7]							
Nutrient level		<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
DFM		<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001
Nutrient level × DFM		0.0052	0.0043	0.0001	0.0912	0.9452	0.0006
Main effect means							
Nutrient level							
C		1,827.2	1,115.1	1.70	2,832.1	1,113.4	1.87
M		1,737.6	1,052.6	1.72	2,707.0	969.4	1.89
L		1,675.1	991.1	1.75	2,591.6	916.5	1.91
DFM							
Not added (–)		1,687.1	1,009.3	1.74	2,622.6	944.1	1.90
Added (+)		1,800.9	1,097.4	1.70	2,796.9	996.0	1.87

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO), (–) none added, (+) 0.45 kg/ton added, (++) 0.95 kg/ton added.

[2]Means of 5 pens per treatment (8 birds/pen).

[3]Means of 5 pens per treatment (4 birds/pen).

[4]The control (C) diet contained (by analysis): grower 19.2% protein, 0.84% Ca, and 0.32% nonphytin P (nPP); finisher, 16.8% protein, 0.78% Ca, and 0.28% nPP.

[5]The moderate (M) diet contained (by analysis): grower, 16.8% protein, 0.70% Ca, and 0.24% nPP; finisher, 14.7% protein, 0.63% Ca, and 0.19% nPP.

[6]The low (L) diet contained (by analysis): grower, 17.0% protein, 0.65% Ca, 0.20% nPP; finisher, 14.9% protein, 0.60% Ca, and 0.16% nPP.

[7]Data were analyzed as a factorial of 3 nutrients × 2 DFM without the moderate ++ treatment.

similar in contents of protein, methionine, and lysine to those in the M diets, but Ca and nPP content were decreased by 7% below that of the M diets or 25% below that of the C diets.

A basal batch of each diet (C, M, and L) was mixed for the Gr and Fn phases. Each basal was subdivided, the DFM, diatomaceous earth, calcium carbonate, and dicalcium phosphate were added; and the diet was mixed and pelleted. DFM was added at the expense of diatomaceous earth. Feed was pelleted between 82.2 and 87.7°C(180 and 190°F) in a California Pellet Mill[3]. The St diet was crumbled after pelleting. To assess the *Lactobacillus* status of the experimental diets, random samples of DFM product and diets were collected, coded, and tested in a blind fashion by a commercial laboratory[4] for viable counts by standard methods.

Measurements

Body weights were determined by pen at the start and end of each period. Feed consumption was measured for each period by pen. Weight gain and feed efficiency (feed to gain ratio) were determined based on BW and feed consumption data. Mortalities were recorded daily, and performance was corrected for mortality.

Excreta total collection was done during the last 2 d of the Gr and Fn phases. Feed consumption and total excreta voided were determined for this 48-h period. Excreta were dried in a forced draft oven, at 60°C for 48-h. After drying, excreta were ground through a 0.25-mm screen and stored at 4°C until analyzed. Feeds were analyzed for moisture (AOAC, 1980); N by combustion;[5] lysine and methionine (AOAC, 1990) and total P, colorimetrically (Heinonen and Lahti, 1981); Ca by atomic absorption (Perkin Elmer, 1982), and phytin P (PP) by high performance liquid chromatography according to the method of Rounds and Nielsen (1993) as modified by Newkirk and Classen (1998). Excreta were analyzed for moisture, N, Ca, and total P by the methods specified previously.

At the end of the Gr and Fn phases, 4 birds per pen were sampled. The 4 birds sampled at the end of the Gr phase were chosen at random among the 8 birds per pen, and at the end of the Fn phase all birds left were sampled. Birds were euthanized by cervical dislocation, and the left tibia was removed (defleshed and cartilage caps removed) and stored (–20°C) until analyzed. Tibial breaking strength was determined following the method of

[4]Forage Research Microbiology Laboratory, Houston, TX.

[5]Leco Corporation. St. Joseph, MI.

TABLE 4. Effect of adding a direct-fed microbial (DFM) to broiler feed on bone measurements in the grower (18 to 28 d of age) and finisher phases (28 to 42 d of age) (experiment 1)

		28 d of age[2]		42 d of age	
Nutrient level	DFM[1]	Bone breaking strength (kg)	Tibia ash (%)	Bone breaking strength (kg)	Tibia ash (%)
C[3]	–	18.5[a]	36.8[a]	22.9[a]	42.1[a]
C	++	19.1[a]	36.6[a]	23.5[a]	42.7[a]
M[4]	–	16.7[b]	34.7[b]	20.1[b]	39.8[b]
M	++	18.4[a]	36.3[a]	23.0[a]	42.3[a]
SEM		0.37	0.34	0.55	0.49
Overall probability		0.0145	0.0036	0.0005	0.0002
Main effect probabilities					
Nutrient level		0.0325	0.0323	0.0421	0.0325
DFM		0.0455	0.0412	0.0064	0.0187
Nutrient level × DFM		0.0683	0.0577	0.0347	0.0641
Main effect means					
Nutrient level					
C		18.8	36.7	23.2	42.4
M		17.5	35.5	21.6	41.1
DFM					
Not added (–)		17.6	35.8	21.5	41.0
Added (++)		18.8	36.5	23.3	42.5

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO) included at 0 (–) or 0.9 kg/ton (++).

[2]Means of 8 pens per treatment and 4 left tibias per pen.

[3]The control (C) diet contained (by analysis): grower, 19.5% protein, 0.87% Ca, and 0.33% nonphytin P (nPP); finisher, 17.0% protein, 0.80% Ca, and 0.29% nPP.

[4]The moderate (M) diet contained (by analysis): grower, 17.3% protein, 0.71% Ca, and 0.25% nPP; finisher, 15.3% protein, 0.64% Ca, and 0.20% nPP.

Crenshaw et al. (1981) and using a Sintech (I/G)[6] machine. Ash percentage was determined on a dry defatted basis on individual bones (AOAC, 1990). Tibial breaking strength and ash measurements were averaged by pen (experimental unit), and statistical analysis was conducted on pen means.

Statistical Analysis

Data from Exp 1 were analyzed as a complete factorial design of 2 nutrient levels and 2 levels of DFM using the GLM procedure of SAS software (1999). Data for Exp 2 were analyzed as a complete factorial of 3 nutrient levels and 2 levels of DFM. To determine differences of specific trt with the additional diet (M++) treatment, all trt in the Exp were analyzed as a completely randomized block design with blocks being battery and tier within a battery. Starting BW (18 d) was used as a covariate to remove starting weight effects because starting weight was different between the 2 populations (no added DFM and added DFM). Individual as well as main effect means are reported. Pair wise comparisons using Tukey's significant difference method (Kramer, 1956) were done when the model was significant. For interpretation purposes, main effect means were used when the interaction term was not significant, and individual means were used when the interaction term was significant. Means were considered significant at $P \leq 0.05$.

[6]MTS Systems Corporation, Eden Prairie, MN.

RESULTS AND DISCUSSION

The DFM product used in both Exp tested at 7.1×10^8 cfu *Lactobacillus* spp./g, and feed samples without added DFM were negative for lactobacilli. In Exp 1, diets to which DFM had been added (0.9 kg/ton equivalent) and then pelleted averaged 6.3×10^5, 5.1×10^5, 7.1×10^5, and 6.5×10^5 cfu/g for Gr C++, Gr M++, Fn C++, and Fn M++ diets, respectively. In Exp 2, diets to which DFM had been added and then pelleted averaged 3.0×10^5, 2.9×10^5, 3.2×10^5, 2.7×10^5, 3.1×10^5, and 3.0×10^5 cfu/g for the Gr C+, Gr M+, Gr L+, Fn C+, Fn M+, and Fn L+ diets, respectively. The additional diet (M++) used in Exp 2 contained 7.3×10^5 and 7.1×10^5 cfu/g in the Gr and Fn phases, respectively. These data established that DFM treatments contained large populations of viable lactobacilli after pelleting and that non-DFM diets were not naturally nor inadvertently inoculated with *Lactobacillus* species.

Nutrient analysis of the Exp diets (Table 1) showed that the analyzed and calculated levels of protein, lysine, methionine, and Ca were similar. Total P levels, when analyzed, were lower than formulated for the M diets in Exp. 1 and 2 and in the L diet in Exp 2. This difference only served to exacerbate the nPP deficiency of these diets. Based on analyzed values, the actual decreases in the M vs. the C Gr diets in Exp 1 were 18.3% for Ca and 24.3% for nPP and in the Fn diets were 20% for Ca and 31% for nPP. In Exp 2, the M and C Gr diets differed, based on analyzed levels, by 16.7% for Ca and 25.0% for nPP, and the L and C Gr diets differed by 22.6% Ca and 37.5% nPP. In the Fn phase, the C and M diets (Exp 2)

TABLE 5. Effect of adding a direct-fed microbial (DFM) to broiler feed on bone measurements in the grower (18 to 32 d of age) and finisher phases (32 to 42 d of age) (experiment 2)

		32 d of age[2]		42 d of age	
Nutrient level	DFM[1]	Bone breaking strength (kg)	Tibia ash (%)	Bone breaking strength (kg)	Tibia ash (%)
C[3]	–	18.9[a]	37.5[a]	22.9[a]	42.2[ab]
C	+	18.9[a]	37.6[a]	23.5[a]	42.7[a]
M[4]	–	17.1[b]	36.9[a]	21.7[ab]	40.8[c]
M	+	18.8[a]	37.6[a]	23.1[a]	42.5[a]
M	++	19.1[a]	37.5[a]	23.2[a]	42.5[a]
L[5]	–	16.1[c]	35.2[b]	20.5[b]	39.3[d]
L	+	17.7[ab]	37.0[a]	22.1[ab]	41.2[bc]
SEM		0.38	0.30	0.40	0.25
Overall probabilities		<0.0001	<0.0001	<0.0001	<0.0001
Main effect probabilities[6]					
Nutrient level		0.0002	0.0002	0.0004	<0.0001
DFM		0.0025	0.0018	0.0015	<0.0001
Nutrient level × DFM		0.0799	0.0366	0.3853	0.0121
Main effect means					
Nutrient level					
C		18.9	37.6	23.1	42.4
M		18.0	37.2	22.4	41.7
L		16.9	36.1	21.3	40.2
DFM					
Not added (–)		17.4	36.5	21.7	40.7
Added (+)		18.5	37.4	22.9	42.1

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO) included at (–) none added, (+) 0.45 or (++) 0.9 kg/ton added.

[2]Means of 5 pens per treatment and 4 left tibias per pen.

[3]The control (C) diet contained (by analysis): grower 19.2% protein, 0.84% Ca, and 0.32% nonphytin P (nPP); finisher, 16.8% protein, 0.78% Ca, and 0.28% nPP.

[4]The moderate (M) diet contained (by analysis): grower, 16.8% protein, 0.70% Ca, and 0.24% nPP; finisher, 14.7% protein, 0.63% Ca, and 0.19% nPP.

[5]The low (L) diet contained (by analysis): grower, 17.0% protein, 0.65% Ca, 0.20% nPP; finisher, 14.9% protein, 0.60% Ca, and 0.16% nPP.

[6]Data were analyzed as a factorial of 3 nutrients × 2 DFM without the moderate ++ treatment.

differed by 19.2% Ca and 32.1% nPP, and the C and L diets differed by 23.1% for Ca and 42.9% nPP. Actual nutrient changes, rather than formulated diet, will be referred to in this section.

Decreasing the nutrient levels moderately (diet M) in Exp 1 resulted in lower gain in the Gr and Fn phases than for birds fed diets containing the DFM, regardless of diet nutrient level (C++, M++; Table 2). In the Fn phase (28 to 42 d of age) birds fed the moderate nutrient diet with DFM (M++) gained as well as those fed the higher nutrient density (C) diet, with or without added DFM, and only for this measurement at this age was there an interaction between nutrient level and DFM. This interaction was probably due to the difference in magnitude of the DFM effect, depending on diet any nutrient concentration. In the Fn phase, birds fed the M nutrient diet with DFM (M++) were as efficient as those fed the C++ diet but were more efficient than those fed the C and M diets. Birds fed the M nutrient diet without DFM (M) had the lowest feed conversion of all birds. Performance results in Exp 2 were similar to those in Exp 1.

In Exp 2, birds fed the M+ and M++ diets had similar growth and feed efficiencies to those fed the C and C+ diets (Table 3). Based on these results there was no difference between adding 0.45 and 0.9 kg/ton of the DFM. This finding is consistent with the results of Mohan et al. (1996), who reported that the maximum effect of adding DFM on gain was at 0.75% (6.8 kg/ton) addition of a mix of viable organisms with no further improvements at higher levels.

A further decrease in Ca and nPP levels but not in protein, lysine, and methionine (L diets) resulted in lower gain and impaired feed efficiency than in birds fed the C or M nutrient level diet. Adding the DFM to the L diet at 0.45 kg/ton (L+) resulted in similar gain to that of birds fed the C and M++ diets but was lower than that of birds fed the C+ diet in the Fn phases. Thus, adding the DFM to the diet containing between 10 and 12% less protein, 8.0 to 13.4% less lysine, 9.0 to 14.9% less methionine, 16.7 to 20.0% less Ca, and 24.0 to 32.1% less nPP vs. the C diet allowed broilers to grow and convert feed to gain as well as those fed the higher nutrient density C diet. When Ca and nPP were decreased further as compared with the C diet (22.6 to 23.1% lower in Ca and 37.5 to 42.9% lower in nPP), the addition of DFM allowed the birds to perform similarly to those on the highest nutrient diet (C) except for lower gain as compared with the C+ fed birds.

These results are not without precedent. Zulkifli et al. (2000) reported improved gain and feed efficiency in broilers when a mixture of *Lactobacillus* strains were added to diets. Similarly, Abdulrahim et al. (1999) reported improvements in gain and feed efficiency of broil-

TABLE 6. Effect of adding a direct-fed microbial (DFM) to broiler feed on apparent nutrient retention (%) in the grower (18 to 28 d of age) and finisher phases (28 to 42 d of age) (experiment 1)

		Apparent nutrient retention (%)					
		26 to 28 d of age[2]			40 to 42 d of age[3]		
Nutrient level	DFM[1]	Protein	Ca	P	Protein	Ca	P
C[4]	–	68.9[c]	38.7[c]	40.0[b]	68.8[b]	39.3[c]	38.9[bc]
C	++	72.3[b]	42.9[b]	44.9[a]	71.1[ab]	43.0[ab]	40.2[ab]
M[5]	–	71.9[b]	42.3[b]	41.2[b]	70.3[b]	41.0[bc]	39.9[bc]
M	++	75.6[a]	45.8[a]	46.0[a]	73.9[a]	44.9[a]	42.7[a]
SEM		0.52	0.66	0.48	0.69	0.77	0.66
Overall probabilities		0.0008	0.0012	0.0012	<0.0001	<0.0001	<0.0001
Main effect probabilities							
Nutrient level		0.0432	0.0412	0.0362	0.0445	0.0321	0.0221
DFM		0.0013	0.0076	0.0178	0.00021	0.0006	0.0008
Nutrient level × DFM		0.4598	0.3554	0.4355	0.3021	0.7133	0.6118
Main effect means							
Nutrient level							
C		70.6	40.8	42.5	70.0	41.2	39.7
M		73.8	44.1	43.6	72.1	43.0	41.3
DFM							
Not added (–)		70.4	40.5	40.6	69.5	40.2	39.0
Added (++)		73.5	44.9	45.5	72.5	44.0	41.5

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO) at 0.9 kg/ton (++).

[2]Means of 8 pens per treatment (8 birds per pen).

[3]Means of 8 pens per treatment and (4 birds per pen).

[4]The control (C) diet contained (by analysis): grower, 19.5% protein, 0.87% Ca, and 0.33% nonphytin Ps (nPP); finisher, 17.0% protein, 0.80% Ca, and 0.29% nPP.

[5]The moderate (M) diet contained (by analysis): grower, 17.3% protein, 0.71 Ca and 0.25% nPP, finisher, 15.3% protein, 0.64% Ca, and 0.20% nPP.

ers with dietary inclusion of a culture of *Lactobacillus acidophilus*. In contrast, others have reported no effect of addition of *Lactobacillus*-based preparations on performance measures (Watkins and Kratzer, 1984; Maiolino et al., 1992).

The contradictory results that have been reported can partly be explained by strain of microorganisms and amount of live organisms added to the water or diet, the survivability of the microorganisms in the feed, dietary nutrient levels used and incidence of subclinical health problems prevalent in the testing facility. It is also important to note that in some studies in which no differences have been observed, the methods used did not include steps that minimized the potential for cross-contamination between treatments by the DFM (England et al., 1996). If birds were fed diets that did not contain DFM, but due to lack of steps to prevent cross-contamination they were colonized early with the DFM, then performance differences would be expected to disappear or be minimized. In the present study, the microorganisms in the culture used were various and the mix details were proprietary. Extreme care was taken to prevent cross-contamination with the DFM across treatments; survivability of the organisms in the DFM after pelleting was documented, and levels were close to those formulated. In this study, unfortunately, the diets were not sampled prior to pelleting, and, thus, the percentage pelleting survivability of this DFM could be calculated. Based on the results of these Exp, it would appear that if cross-contamination occurred it occurred late and did not occur at high concentrations, but this cannot be stated with complete certainty as no intestinal colonization work was done in these studies.

Neither bone ash nor bone breaking strength was different in birds fed the M+ and M++ in Exp 1 and 2 as compared with these of birds fed the C and C+ diets but were higher than those of birds fed the M diet without any added DFM (Tables 4 and 5). Feeding a DFM containing diet that met NRC (1994) recommended nutrient levels (C) did not change bone ash or bone breaking strength when compared with feeding the same diet without any added DFM. When the DFM was added to a diet containing 18% less Ca and nPP than NRC (1994) recommended levels (M+ and M++), bone ash and bone breaking strength were similar to those of broilers fed the C diets but were higher than those of broilers fed the M diet without any added DFM, possibly because these broilers were able to retain Ca and nPP more efficiently. Even when Ca and nPP were decreased by 25% (L diets, Exp 2) below NRC (1994) recommended levels, the addition of DFM allowed broilers fed these L nutrient diets to have bone mineralization that was not different than that of the birds fed the C diet (Table 5). Lowering Ca and nPP in the diet by 25% (L diet) greatly decreased both bone ash and bone breaking strength versus all other diets fed, but the addition of DFM was enough to help broilers overcome this apparent deficiency. Others have reported decreased bone ash as the levels of Ca and P decreased in the diet (Nelson et al., 1990). Requirements for male Ross 308 birds, 0.31% nPP for the Gr (18 to 32 d of age) phase (Angel et al., 2000) and 0.21% nPP for the Fn (32 to 42 d of age) phase (Dhandu and Angel,

TABLE 7. Effect of adding a direct-fed microbial (DFM) to broiler feed on apparent nutrient retention (%) at the end of the grower (30 to 32 d of age) and finisher phases (40 to 42 d of age) (experiment 2)

		Apparent nutrient retention (%)					
		30 to 32 d of age[2]			40 to 42 d of age[3]		
Nutrient level	DFM[1]	Protein	Ca	P	Protein	Ca	P
C[4]	–	71.7^{c}	39.6^{d}	40.9^{c}	69.3^{c}	38.2^{c}	38.9^{c}
C	+	73.8^{b}	43.0^{c}	43.2^{b}	71.1^{abc}	41.4^{c}	42.2^{abc}
M[5]	–	73.1^{b}	42.6^{c}	41.8^{bc}	70.6^{bc}	41.2^{bc}	40.4^{b}
M	+	75.6^{a}	46.4^{b}	45.9^{a}	72.9^{ab}	44.4^{a}	43.2^{ab}
M	++	75.8^{a}	46.6^{b}	45.6^{a}	73.1^{ab}	44.6^{a}	43.7^{a}
L[6]	–	73.8^{b}	43.2^{c}	41.3^{bc}	71.0^{abc}	42.9^{ab}	40.5^{bc}
L	+	76.0^{a}	47.4^{a}	46.1^{a}	74.0^{a}	45.7^{a}	44.0^{a}
SEM		0.59	0.61	0.59	0.70	0.68	0.64
Overall probabilities		<0.0001	<0.0001	<0.0001	0.0006	<0.0001	0.0001
Main effect probabilities[7]							
Nutrient level		0.0003	<0.0001	0.0053	0.0099	<0.0001	0.2116
DFM		<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001
Nutrient level × DFM		0.7558	0.8159	0.0849	0.7325	0.9733	0.6461
Main effect means							
Nutrient level							
C		72.2	41.3	42.1	70.2	39.8	41.1
M		74.4	44.5	43.9	71.8	42.8	41.8
L		74.9	45.3	43.7	72.5	44.3	42.2
DFM							
Not added (–)		72.5	41.8	41.3	70.3	40.8	40.3
Added (+)		75.2	45.6	45.1	72.7	43.8	43.1

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

[1]The DFM used was Primalac (Star Labs, Inc., Clarksdale, MO) at (–) none added, (+) 0.45 or (++) 0.9 kg/ton added.

[2]Means of 5 pens per treatment (8 birds/pen).

[3]Means of 5 pens per treatment (4 birds/pen).

[4]The control (C) diet contained (by analysis): grower 19.2% protein, 0.84% Ca, and 0.32% nonphytin P (nPP); finisher, 16.8% protein, 0.78% Ca, and 0.28% nPP.

[5]The moderate (M) diet contained (by analysis): grower, 16.8% protein, 0.70 Ca, and 0.24% nPP; finisher, 14.7% protein, 0.63% Ca, and 0.19% nPP.

[6]The low (L) diet contained (by analysis): grower, 17.0% protein, 0.65% Ca, and 0.20% nPP; finisher, 14.9% protein, 0.60% Ca, and 0.16% nPP.

[7]Data were analyzed as a factorial of 3 nutrients × 2 DFM without the moderate ++ treatment.

2003), indicate that the levels used in these Exp for the Gr and Fn phases for the M and L diets were deficient in nPP.

Other researchers have reported that improved performance observed when DFM are used is associated with increases in nutrient retention in broilers (Mohan et al., 1996). These researchers explained, in part, the improved performance is a consequence of improved nutrient retention. In the present study, the ability of birds fed the lower nutrient diets to perform as well as those fed the control diets could be, in part, explained by the higher nutrient retention observed for birds fed the DFM. The inclusion of the DFM in the diet improved nutrient retention as measured by apparent retention (amount consumed minus amount excreted) regardless of diet nutrient density (no interactions seen). Diet density as well as addition of DFM affected protein, Ca, and to a lesser extent P retentions. The improved Ca and P retentions associated by addition of DFM to M and L nutrient diets determined with bone ash were confirmed by the apparent Ca and P retention levels determined in the DFM added diets. The addition of DFM to all diets, regardless of period, in both Exp resulted in increased N, Ca, and P retentions as compared with diets with no added DFM (Tables 6 and 7). There was a main effect of nutrient level and inclusion of DFM on N, Ca, and P apparent retentions but no nutrient level by DFM inclusion interactions. Feeding the L nutrient diet resulted in improved ($P < 0.05$) apparent N, Ca, and P retentions in the Gr and Fn phases of Exp 1 (Table 6).

Addition of the DFM also resulted in increased nutrient retentions in both age phases studied in Exp 1. Adding DFM to the diet had a greater impact on P retention than that of feeding the lower nutrient (M) diet. The inclusion of the DFM resulted in 10.8 and 5.1% increases in P retention in the Gr and Fn phases, respectively, whereas decreasing dietary nutrients resulted in 2.5 and 3.9% increases in P retention in the Gr and Fn phases, respectively. Similar results were observed in Exp 2. Decreasing diet nutrient content to a M level improved apparent nutrient retention but further decreases (L) had no further impact. The effect of adding DFM improved apparent nutrient retention to a greater extent than decreasing nutrient level as was observed in Exp 1.

Retention of N has been reported by others to increase when DFM are added to poultry diets, but no published research was found by the authors directly exploring the effect of DFM on Ca and P retention in broilers (Mohan

et al., 1996; Schneitz et al., 1998. Mohan et al. (1996) found that the addition of a DFM to a broiler diet resulted in an improvement in protein efficiency ratio as well as N retention, even in broilers fed diets that met recommended levels. The DFM used by these researchers was a dried mixed culture of 5 viable strains of microorganisms. Schneitz et al. (1998) inoculated broiler chicks with a commercial DFM preparation of selected bacteria from the ceca of laying hens and found that N retention and metabolizable energy of the diet were higher in chicks inoculated with the DFM. The diet used by Schneitz et al. (1998) was a wheat and barley based diet and contained L energy (2,386 kcal/kg). These authors hypothesized that the higher energy content in the DFM-containing diet might have been, in part, the result of the decreased digesta viscosity observed in the DFM-fed birds. These researchers documented actual intestinal colonization by the microorganisms used to inoculate the birds, and they hypothesized that competitive exclusion might have been a contributor to the improved nutrient retention measured. In the present study, corn-soy based diets were used, and thus, viscosity should not have been a factor. In this study intestinal colonization was not tested, and thus, the improvements in apparent retention of nutrients could not be conclusively attributed to competitive exclusion mechanisms.

In contrast to the results obtained for Ca and P retention in these 2 Exp, Nahashon et al. (1994) found that the level of available P in the diet did not affect P retention in Single Comb White Leghorn layers during the laying period. They did find that adding a *Lactobacillus*-based DFM to a diet containing low available P improved P retention (32.4% retention) vs. that of the control diet (17.8% retention). In the present study, the use of a DFM had a positive effect on P retention in diets containing lower nPP levels (diets M and L), but in contrast to what Nahashon et al. (1994) reported, P retention was improved regardless of level of nPP when a DFM was added to the diet. In 2 studies reported by Nahashon et al. (1994, 1996), contrary results were obtained for Ca and P retentions, when DFM were tested in layers. They reported from their first study no effect of DFM on Ca retention but a positive effect on P retention (Nahashon et al., 1994), and in their second study (Nahashon et al., 1996) they found that addition of DFM to layer diets had no effect on P retention but had a positive effect on Ca retention. Contrasting results appear to be normal when studies on the impact of DFM are compared. As stated earlier, numerous factors can account for contrasting results. The impact of DFM would be expected to be dependant on the viability and number of the microorganisms in the diet as fed as well as management and health status of birds on test and on nutrient levels used in the diet.

The disparities in findings reported in the literature prompted this controlled battery study to determine whether a benefit could be ascribed to the feeding of *Lactobacillus* under practical feeding conditions. The data show that in the absence of any particular stress or disease pressure, as might be expected under field conditions, addition of 0.45 to 0.9 kg of DFM/ton of feed in a full-nutrient or control ration did not produce any performance advantage with the possible exception of slight but significant feed-to-gain improvement observed in Exp 1 during the Fn phase. However, the impact of DFM was highly significant when it was added to a diet containing 12% less protein and 18% less nPP than the C diets. Not only were growth (gain) and feed conversion better in DFM-supplemented birds than in those on reduced nutrients without DFM, but the improvement placed the lower nutrient DFM-supplemented birds (M++ diet) at the same performance level as the full-fed, unsupplemented (C diet) birds in both trials. These results offered clear evidence of the capacity of one *Lactobacillus*-based DFM product to sustain commercially desirable performance goals while lowering feed nutrient inputs. The implications are substantial in terms of the possible use of such a DFM to enhance nutrient retention, allowing for use of lower nutrient levels and potentially reducing diet any costs and nutrient levels in broiler excreta.

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