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Effects of Lactobacilli and an Acidophilic Fungus on the Production Performance and Immune Responses in Broiler Chickens

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ABSTRACT Accumulated lines of evidence indicate that inactivated probiotics could have beneficial effects similar to those of live probiotics. Two strains of disrupted, cobalt-enriched, lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus casei*) and a disrupted fungal mycelium (*Scytalidium acidophilum*) were spray-mixed onto a mash basal feed, in 2 concentrations, prior to pelleting. The effects of these probiotics on production performance and immune response in broiler chickens were investigated. The production parameters, including BW, feed intake (FI), BW gain (BWG), and feed conversion ratio (FCR), were monitored weekly during a 6-wk trial. The immune response was evaluated by immunizing the birds with the antigen keyhole limpet hemocyanin (KLH) followed by a serological assay to measure blood IgA and IgG titers. Some of the production parameters were significantly improved by low *L. casei* (LCL; for BW and BWG), high *L. acidophilus* (LAH; for BW and BWG), and high fungal (FH; for BW, BWG, and FI) in comparison with the nonadditive control (NC–). However, these 3 treatments (LCL, LAH, and FH) did not enhance the measured immune responses. Instead, the titers of serum KLH-specific IgA in high *L. casei* (LCH) and low *L. acidophilus* (LAL) were significantly higher than those of NC–, 10 d after immunization. None of the probiotic treatments increased the titer of KLH-specific IgG in blood. Our results indicate that disrupted and cobalt-enriched *L. acidophilus* or *L. casei* was able to enhance production performance of broiler chickens. The fungal mycelium, *S. acidophilum*, when used at a high concentration, also demonstrated its potential for the first time to be used as a probiotic. In addition, the optimal concentration for administering probiotics is strain dependent. A higher dose does not always result in a better performance.

(*Key words*: *Lactobacillus*, fungus, performance, immune response, broiler chicken)

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INTRODUCTION

The intestinal microflora of an animal is the first barrier in protecting the host from diseases caused by colonization of pathogens in the gastrointestinal tract. Probiotics, defined as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" by Fuller (1989), have been administered to farm animals to enhance production performance and immune responses. In the poultry industry, probiotic supplementation has been shown to improve BW gain, feed conversion ratio, and mortality rate in broiler chickens (Kalbande et al., 1992; Jin et al., 1996; Mohan et al., 1996). Moreover, it has been shown that probiotics could protect broilers against pathogens by colonization in the gastrointestinal tract (Nisbet et al., 1993; Hejlicek et al., 1995; Pascual et al., 1999) and stimulation of systemic immune responses (Muir et al., 1998; Quéré and Girard, 1999). Nevertheless, contradictory results have been reported by other researchers (Watkins and Kratzer, 1984; Maiolino et al., 1992; Senanl et al., 1997; Panda et al., 1999). The strain of selected microorganisms, the dosage, method of preparation, and condition of animals could be partially responsible for such discrepancies.

The number of viable microorganisms in probiotics has been considered a critical factor affecting the efficacy of probiotics. Theoretically, probiotic microorganisms have to be viable to accomplish their putative beneficial effects,

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Abbreviation Key: BWG = body weight gain; FCR = feed conversion rate; FH = high (fungus) *Scytalidium acidophilum*; FI = feed intake; FL = low (fungus) *S. acidophilum*; KLH = keyhole limpet hemocyanin; LAH = high *Lactobacillus acidophilus*; LAL = low *L. acidophilus*; LCH = high *Lactobacillus casei*; LCL = low *L. casei*; NC– = negative control, no additives; NC+ = negative control, whey permeate medium only; PCL = low positive control; PCH = high positive control; PBS-T = PBS containing 0.05% Tween 20; WPM = whey permeate medium.

TABLE 1. The compositions and concentrations of cobalt-enriched probiotics

Treatment[1]	Additive name	Concentration of additive in feed (as mg/kg of dry matter)		Added cobalt concentration in feed[2] (μg/kg = ppb)	
		Starter	Finisher	Starter	Finisher
Negative control (NC–)	None	0	0	0	0
Whey control (NC+)	Whey permeate media	115	76	0.23	0.15
Positive control-low (PCL)	Nutragen-PCW[3]	21	15	1.68	1.20
Positive control-high (PCH)	Nutragen-PCW	122	103	9.76	8.24
L. acidophilus low (LAL)	*L. acidophilus* disrupted cells	72	56	214.1	151.0
L. acidophilus high (LAH)	*L. acidophilus* disrupted cells	425	332	1,263.8	895.2
L. casei low (LCL)	*L. casei* disrupted cells	77	62	189.8	135.4
L. casei high (LCH)	*L. casei* disrupted cells	457	368	1,126.5	803.7
Fungus low (FL)	*S. acidophilum* disrupted cells	68	54	4.7	2.9
Fungus high (FH)	*S. acidophilum* disrupted cells	412	326	28.5	17.5

[1]We used 2 negative controls, one was nonadditive, the other was whey permeate medium. The probiotic-treated groups include 2 levels of disrupted *Lactobacillus acidophilus* with cobalt, disrupted *Lactobacillus casei* with cobalt, and disrupted fungus *Scytalidium acidophilum*.

[2]The original level of cobalt in feed was 200 and 150 ppb for the starter and the finisher diets, respectively. The concentration of cobalt was calculated on a dry matter basis.

[3]Nutragen-PCW, NutriBios Inc., Oshawa, ON, Canada.

such as producing antimicrobial substances and competing for colonization sites and nutrients. However, accumulated evidence indicates that inactivated, or nonviable, probiotics have beneficial effects similar to those of viable probiotics. For example, the ability to inhibit the adhesion of the pathogens *Eschericia coli* and *Salmonella typhimurium* to human Caco-2 cells is not affected by the viability of *Lactobacillus acidophilus* (Coconnier et al., 1993; Ouwehand and Salminen, 1998). In addition, inactivated probiotics have been shown to enhance immune responses and increase the resistance to pathogens (Shkarupeta et al., 1988; Aattouri and Lemonnier, 1997; Wagner et al., 1997). Nonviable probiotics have prolonged shelf life, reduced cost of transportation, and broadened usage compared with viable probiotics. However, the performance of nonviable probiotics can be affected by different methods of inactivation, including heat, γ-irradiation, or ultraviolet light, and this influence is species dependent. For example, heat increased the adhesion of *Propionibacterium freudenreichii* but inhibited the adhesion of *Lactobacillus* species (Ouwehand et al., 2000). Therefore, selecting the optimal strains and methods of inactivation are important in preparing inactivated probiotics without compromising their beneficial effects.

Lactobacillus species have been widely studied as probiotics, whereas the information on using fungal strains is scarce. *Aspergillus oryzae* is probably the only strain that has been described. Supplementation of this viable fungal probiotic has been demonstrated to improve the digestibility of plant cell wall in sheep (Jouany et al., 1998) but has no effects on ruminal volatile fatty acids and bacterial composition in sheep and dairy heifers (Mathieu et al., 1996; Chiquette and Benchaar, 1997). It remains unclear whether viability is essential to the performance of fungal probiotics. Because resistance to acid is one factor for selection of potential probiotic microorganisms, *Scytalidium acidopilum*, an extremely acid-tolerant fungus, was included in this trial.

A unique function of microflora, or probiotics, is synthesis of vitamin B_{12} from dietary cobalt to meet the requirements of the host. Cobalt is essential for the growth and metabolic processes of microorganisms as well (Swift, 1980). However, the effect of cobalt supplementation on the performance of probiotics has never been studied. In the present study, 2 strains of cobalt-enriched lactobacilli (*Lactobacillus casei* CL96 and *L. acidophilus* ATCC[2] 43121) and 1 strain of acidophilic fungus (*S. acidophilum*) were disrupted by a homogenizer and supplemented to the diet at 2 levels (high and low). Their effects on the production performance and the immune response in broiler chicks were evaluated.

MATERIALS AND METHODS

Probiotics

L. casei and L. acidophilus. *L. casei* CL96 was previously isolated from a high-quality Cheddar cheese and was tentatively identified by API 50 CHL system.[3] *L. acidophilus* ATCC 43121 was purchased from ATCC.[2] The culture stocks were kept in whey permeate medium (WPM)/glycerol (50:50, vol/vol) at –70°C. Working cultures were prepared by 2 successive transfers of stock culture in WPM broth for 24 h at 30°C (*L. casei*) or 37°C (*L. acidophilus*). The bacteria were grown anaerobically in WPM, supplemented with cobalt (200 ppm) in the form of ammonium lactate complex, for 30 h in flasks. Thereafter, cells were collected by centrifugation (15 min, 12,000 × *g*). A small portion of the cell pellet was used to verify viability, and the rest of cells were disrupted by high-pressure homogenization. Briefly, the pellet was washed twice with 50 m*M* sodium phosphate buffer (pH 7.0),

[2]American Type Culture Collection, Rockville, MD.
[3]BioMerieux, Montreal, QC, Canada.

TABLE 2. Compositions and calculated contents of starter and finisher feeds

Calculated content	Starter (d 1–21)[1]	Finisher (d 22–42)[1]
Crude protein (%)	21.0	17.5
Crude fat (%)	2.0	4.0
Crude fiber (%)	4.0	5.5
Sodium (%)	0.15	0.13
Calcium (%)	1.0	0.9
Phosphorus (%)	0.8	0.7
Stabilized vitamin A (IU/kg)	9,000	7,500
Stabilized vitamin D_3 (IU/kg)	2,100	1,750
Stabilized vitamin E (IU/kg)	40	33
Selenium (mg/kg)	0.3	0.3
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.40
Cysteine (%)	0.35	0.35
Threonine (%)	0.75	0.65
Tryptophan (%)	0.25	0.20
Fe (ppm)	280	225
Cu (ppm)	35	30
Zn (ppm)	110	85
Iodine (ppm)	1.35	1.05
Cobalt (ppm)	0.2	0.15

[1]These feed products were provided by a local feed manufacturer (Nutribec, Quebec, QC, Canada) and contained corn, soybean meal (48%), wheat, wheat middlings, micronized soybean, canola meal, dicalcium phosphate, limestone, corn gluten meal, and salt.

resuspended in the washing buffer, and disintegrated by using a C-5 high-pressure homogenizer[4] through 6 passes with the pressure of 12,000 psi (pounds per square inch). The cell slurry was then diluted with cold buffer and adjusted to the desired concentrations in dry cell material. The viability was confirmed by plating a portion of the homogenized cell mixture on agar plates.

S. acidophilum. The fungus, *S. acidophilum*,[3] was maintained in acidified WPM at 30°C. The culture medium was basal WPM with 1% glucose, and the pH was adjusted to 2.5 with H_2SO_4. High-strength inocula were obtained after 3 d of growth at 30°C in flasks, with 250 rpm agitation. These inocula were then seeded into fresh medium (1:9, vol/vol) to obtain sufficient mycelium for subsequent high-pressure disintegration, which was accomplished by 5 passes of a 20% mycelium suspension in 0.5 *M* lactate buffer (pH 4) through an Avestin C-5 homogenizer.

The Treatments

The acid-tolerant fungus *S. acidophilum* and cobalt-enriched *L. casei* and *L. acidophilus* were disrupted as described. Each of these strains, as well as the positive control, Nutragen-PCW,[5] was administrated at 2 levels, low and high [L. casei, low (LCL) and high (LCH); L. acidophilus, low (LAL) and high (LHL); fungus S. acidophilum, low (FL) and high (FH); positive control, low (PCL) and high (PCH)] in this trial (Table 1). Furthermore, 2 negative controls (NC–, no additives; NC+, whey permeate medium only) were included in this trial. All additives were spray-mixed with the mash basal diet before pelleting and then crumbled.

Experimental Design

Nine hundred twenty (920) 1-d-old, vaccinated (against Marek's disease and infectious bronchitis), male broiler chickens (Cobb) were obtained from a local hatchery.[6] The birds were allocated to 40 floor pens (23 birds per pen) followed by randomly assigning the pens to the 10 treatments (4 pens per treatment). All the chickens were maintained under uniform temperature and lighting control system during the entire period of study. During the first week of the trial, heat lamps were used to maintain an optimal environment for the chicks. Thereafter, the lamps were removed and the room temperature was gradually adjusted from 30 to 22°C by lowering 1°C every 3 d. There were 4 stages for the lighting system: 1) d 1 to 3; 23L:1D; 2) d 4 to 13; 6L:8.5D:1L:8.5D; 3) d 14 to 20; 10L:14D, 4) d 21 to 42; 14L:10D. Birds in each group were fed with the ration, including the starter and the finisher, supplemented with its assigned probiotic treatment (Table 2) without antibiotics. The feeding trial was conducted in the poultry house of MacDonald Campus, McGill University for 6 wk. The experimental protocol was approved by the Animal Care Committee of the university. BW, BWG, feed intake (FI), feed conversion ratio (FCR), and mortality rate were recorded weekly and analyzed.

Evaluation of Immune Responses

At the age of 21-d, 2 chickens in each pen were randomly selected and immunized intravenously with keyhole limpet hemocyanin (KLH)[7] at 200 μg per chick. Blood samples were collected from the chickens' wing veins (cutaneous vein of the elbow) at 10 d postimmunization. The titers of KLH-specific IgG and IgA in serum were measured by ELISA as described (Quéré and Girard, 1999). Briefly, 96-well microplates were coated with 10 μg/mL soluble KLH in carbonate/bicarbonate buffer (pH 9.6), 100 μL/well, at 4°C overnight. After the coating, the wells were washed twice with PBS containing 0.05% Tween 20[7] (PBS-T) and then saturated with 200 μL/well of 1% BSA[7] for 1 h at 37°C. Thereafter, 100 μL of diluted sera (1/200 for IgA and 1/30 for IgG in PBS-T) was added to each well and allowed to react for 1 h at 37°C. After 3 washes, 100 μL of PBS-T containing either horseradish peroxidase-conjugated goat anti-chicken IgA α-chain[8] (1:4,000) or alkaline phosphatase-conjugated goat anti-chicken IgG Fc fragment[8] (1:1,000) was added to each well, and the plates were incubated at 37°C for another hour. At the end of incubation, the plates were washed 3 times, and 100 μL of the substrate solution, either 0.3 mg/mL 2-2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) diammonium[7] in citrate buffer (pH4) plus 30% H_2O_2 (for IgA) or 1 mg/mL *p*-nitrophenylphosphate[7] in diethanolamine (pH 9.8) (for IgG), was added for color develop-

[4]Avestin Inc., Ottawa, ON, Canada.
[5]NutriBios Inc., Oshawa, ON, Canada.
[6]Couvoir Ramsay, St-Felix-de-Valois, QC, Canada.
[7]Sigma Chemical Co., St. Louis, MO.
[8]Bethyl Laboratories, Montgomery, TX.

ment. Finally, the absorbance values of IgA and IgG titers were measured at 405 nm in a Multiskan MCC 340 plate reader.[9]

Statistical Analyses

The parameters including BW, FI, BW gain (BWG), FCR, and serum Ig titers (IgA and IgG) were monitored. All data were analyzed by ANOVA with the repeated model mixed procedure of SAS software (2000) and compared by least squares means. Mortality was analyzed by the GENMOD procedure of SAS software (2000) with the significance tested by chi square. Means were considered statistically different at $P < 0.05$. The statistical models were described as follows.

For BW, FI, BWG and FCR:

$$Y_{ij} = \mu + TRT_i + blk_j + e_{ij} \text{— weekly}$$

$$Y_{ijkl} = \mu + TRT_i + blk_j + pen_{1(ij)} + WK_k + e_{ijkl} \text{ — whole period (excluded BW)}$$

For IgA and IgG titers:

$$Y_{ij} = \mu + TRT_i + pen(TRT)_{ij} + e_{ij}$$

where,

Y_{ij}, Y_{ijkl}, Y_{ij} = dependent observation;
μ = overall mean;
TRT_i = fixed effect of treatment, i = 1,2,3,...., 9,10;
blk_j = random effect of block, j = 1,2,3,4;
WK_k = fixed effect of week, k = 1,2,3,4,5,6;
$pen_{l(ij)}$ = random effect of pen nested in treatment and block, l = 1,2,3,....,39,40;
$pen(TRT)_{ij}$ = random effect of pen nested in treatment, i = 1,2,3,4; j = 1,2,...,7,8; and
e_{ij}, e_{ijkl}, e_{ij} = residual error.

All other interactions were tested for significance ($P < 0.05$) and were eliminated from the model because they were not significant.

RESULTS

The BW, FI, BWG, and FCR were monitored weekly throughout the trial (6 wk). The least squares means of these parameters are presented in Tables 3 to 6. The weekly average for BWG among treatments was statistically significant. On the other hand, weekly averages for FI, FCR, or the total BW were not significantly different among the treatments. However, when the data were analyzed on a weekly basis, some of the treatments showed significant differences during certain growth periods. For example, the differences of BW among treatments from wk 2, 3, 4, 5, and 6 were significant. In the case of BWG, the differences among treatments were significant in the second and the fifth weeks. For FI and FCR, significant differences among treatments were observed in the second and third weeks, respectively.

At the end of the feeding trial, BW for 4 of the treatments, including FH, LAH, LCL, and PCH, were significantly higher (approximately 5 to 6%) than that of NC– or NC+. The data of BWG and FI exhibited similar tendencies. For BWG, all 4 treatments were significantly different from the NC-. In the case of FI, the 4 treatments were all higher than the controls. However, FH was the only treatment significantly different from NC–. The FCR was not affected by any of these treatments.

An immunization protocol was carried out to evaluate the effect of probiotics on the immune system of broiler chickens. Ten days after immunization, the concentrations of KLH-specific IgA in the serum, as represented in optical density values, were significantly higher in birds selected from PCL, LCH, and LAL in comparison with those from NC (Table 7). On the other hand, none of the probiotic treatments increased production of KLH-specific IgG in serum. The mortality rates among treatments were not statistically different ($P > 0.05$). The mortalities of all groups were between 2.17 and 7.61% (data not shown).

DISCUSSION

The effects of 3 potential homogenizer-disrupted probiotic strains, *L. casei, L. acidophilus* and *S. acidophilum*, on the production performance of broiler chickens were evaluated in this study. A preliminary, small-scale trial was carried out to investigate the effect of viability and cobalt enrichment (*Lactobacillus* strains) of these probiotics on the production performance of broiler chickens. Our results indicated that the production performance was not affected by viability; however, cobalt-enriched lactobacilli were better than those without cobalt supplementation (data not shown). The purpose of the present study was to further determine the potential of these 3 disrupted probiotics. In addition, the effect of probiotics on immune responses was evaluated by measuring KLH-specific antibody titers (IgA and IgG) in serum 10 d postimmunization. This work is the first to investigate the effect of disrupted probiotics enriched with cobalt on the performance and immune response of broiler chickens. Furthermore, this study is the first to evaluate an acid-tolerant fungus *S. acidophilum*, as a probiotic in farm animals. From the current study, 3 probiotic treatments, FH, LAH, and LCL, demonstrated the potential to be used as probiotics for broiler chickens. However, these 3 treatments (FH, LAH, and LCL) did not affect the immune response, characterized by production of antigen-specific antibodies.

Nonviable probiotics have more economical advantages, such as prolonged shelf life, reduced cost of transportation, and broadened usage. It has been demonstrated that nonviable probiotics were able to shorten the duration of diarrhea (Kaila et al., 1995) and increase

[9]Titertek, Huntsville, AL.

TABLE 3. The effect of probiotics on BW (g)

Treatment[1]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
NC–	135.9^{ab}	342.7^{cd}	709.3^{de}	1,210.2^{cd}	1,845.3^{c}	2,536.3^{c}
NC+	140.3^{ab}	349.9^{cd}	720.5^{cde}	1,214.5^{cd}	1,852.6^{c}	2,527.1^{c}
PCL	141.2^{ab}	353.2^{bc}	726.4^{bcde}	1,245.1^{abcd}	1,915.8^{abc}	2,572.8^{bc}
PCH	141.2^{ab}	354.8^{abc}	740.2^{abcd}	1,265.7^{abc}	1,923.7^{abc}	2,656.0^{ab}
LCL	143.9^{a}	370.4^{ab}	768.3^{a}	1,304.6^{a}	1,973.8^{ab}	2,662.2^{ab}
LCH	137.9^{ab}	359.2^{abc}	745.3^{abcd}	1,266.7^{abc}	1,917.8^{abc}	2,608.2^{abc}
LAL	140.3^{ab}	357.8^{abc}	735.6^{abcd}	1,242.0^{bcd}	1,892.4^{bc}	2,565.4^{bc}
LAH	143.3^{ab}	371.9^{a}	756.2^{abc}	1,290.3^{ab}	1,957.5^{ab}	2,665.4^{ab}
FL	135.2^{b}	333.7^{d}	697.1^{e}	1,203.9^{d}	1,864.9^{c}	2,534.3^{c}
FH	143.7^{a}	368.7^{ab}	760.0^{ab}	1,291.3^{ab}	1,995.1^{a}	2,688.0^{a}
SE	±3.2	±7.3	±14.7	±22.8	±30.7	±39.3

$^{a–e}$Least squares means with different letters within the same column differ significantly ($P < 0.05$). All numbers shown are least squares means.

[1]NC– = negative control, nonadditive. NC+ = negative control, whey permeate medium; PCL = commercial probiotic, Nutragen-PCW (NutriBios Inc., Oshawa, ON, Canada), low level. PCH = commercial probiotic, Nutragen-PCW, high level. LAL = *Lactobacillus acidophilus*, disrupted cells with cobalt, low level. LAH = *L. acidophilus*, disrupted cells with cobalt, high level. LCL = *Lactobacillus casei*, disrupted cells with cobalt, low level. LCH = *L. casei*, disrupted cells with cobalt, high level. FL = fungus *Scytalidium acidophilum*, disrupted cells, low level. FH = fungus *S. acidophilum*, disrupted cells, high level.

TABLE 4. The effect of probiotics on BW gain (g)

Treatment[1]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weekly average
NC–	95.7	206.8^{de}	366.6^{c}	500.9^{bc}	635.2^{b}	668.5^{ab}	412.6^{c}
NC+	99.7	209.5^{cde}	370.7^{bc}	494.0^{c}	638.1^{b}	674.5^{ab}	415.0^{c}
PCL	100.4	212.0^{bcde}	373.2^{bc}	518.7^{abc}	670.6^{ab}	657.1^{b}	421.3^{bc}
PCH	99.5	213.6^{abcd}	385.4^{abc}	525.5^{abc}	658.1^{b}	732.3^{a}	436.9^{ab}
LCL	102.4	226.5^{ab}	397.9^{a}	536.3^{a}	669.2^{ab}	688.3^{ab}	436.2^{ab}
LCH	96.5	221.3^{abcd}	386.1^{abc}	521.5^{abc}	651.1^{b}	690.5^{ab}	427.8^{abc}
LAL	99.0	217.5^{abcd}	377.8^{abc}	506.4^{abc}	650.3^{b}	673.0^{ab}	420.6^{bc}
LAH	102.2	228.6^{a}	384.3^{abc}	534.1^{a}	667.2^{ab}	707.9^{ab}	437.5^{ab}
FL	94.4	198.5^{e}	363.5^{c}	506.8^{abc}	661.0^{b}	669.4^{ab}	415.6^{c}
FH	102.0	225.0^{abc}	391.3^{ab}	531.3^{ab}	703.9^{a}	692.9^{ab}	440.3^{a}
SE	±3.1	±6.2	±8.9	±11.1	±12.6	±23.6	±6.1

$^{a–e}$Least squares means with different letters within the same column differ significantly ($P < 0.05$). All numbers shown are least squares means.

[1]NC– = negative control, nonadditive. NC+ = negative control, whey permeate medium; PCL = commercial probiotic, Nutragen-PCW (NutriBios Inc., Oshawa, ON, Canada), low level. PCH = commercial probiotic, Nutragen-PCW, high level. LAL = *Lactobacillus acidophilus*, disrupted cells with cobalt, low level. LAH = *L. acidophilus*, disrupted cells with cobalt, high level. LCL = *Lactobacillus casei*, disrupted cells with cobalt, low level. LCH = *L. casei*, disrupted cells with cobalt, high level. FL = fungus *Scytalidium acidophilum*, disrupted cells, low level. FH = fungus *S. acidophilum*, disrupted cells, high level.

TABLE 5. The effect of probiotics on feed intake (g)

Treatment[1]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weekly average
NC–	119.7^{c}	287.7^{c}	582.3^{ab}	946.6^{abc}	1,188.8^{c}	1,445.0	762.9^{a}
NC+	124.3^{abc}	303.0^{cde}	590.1^{ab}	917.2^{abc}	1,207.0^{bc}	1,445.5	765.6^{a}
PCL	123.5^{abc}	306.0^{bcde}	601.3^{ab}	958.9^{abc}	1,251.3^{abc}	1,440.7	775.3^{ab}
PCH	124.7^{abc}	329.3^{ab}	595.5^{ab}	944.8^{abc}	1,238.9^{abc}	1,529.7	799.2^{ab}
LCL	128.9^{a}	314.8^{abcd}	610.0^{a}	966.4^{ab}	1,258.4^{abc}	1,478.5	790.8^{ab}
LCH	125.8^{abc}	304.5^{bcde}	596.5^{ab}	928.3^{abc}	1,249.7^{abc}	1,441.1	771.8^{ab}
LAL	124.3^{abc}	302.8^{cde}	568.7^{b}	914.0^{bc}	1,205.6^{bc}	1,446.4	763.0^{a}
LAH	129.6^{a}	333.7^{a}	610.2^{a}	975.5^{a}	1,270.8^{ab}	1,484.6	797.3^{ab}
FL	122.3^{bc}	292.9^{de}	583.6^{ab}	900.6^{c}	1,204.5^{bc}	1,458.6	764.7^{a}
FH	127.0^{ab}	322.0^{abc}	605.3^{ab}	976.8^{a}	1,308.1^{a}	1,515.8	807.6^{b}
SE	±2.3	±10.2	±13.5	±21.3	±25.6	±36.2	±14.6

$^{a–e}$Least squares means with different letters within the same column differ significantly ($P < 0.05$). All numbers shown are least squares means.

[1]NC– = negative control, nonadditive. NC+ = negative control, whey permeate medium; PCL = commercial probiotic, Nutragen-PCW (NutriBios Inc., Oshawa, ON, Canada), low level. PCH = commercial probiotic, Nutragen-PCW, high level. LAL = *Lactobacillus acidophilus*, disrupted cells with cobalt, low level. LAH = *L. acidophilus*, disrupted cells with cobalt, high level. LCL = *Lactobacillus casei*, disrupted cells with cobalt, low level. LCH = *L. casei*, disrupted cells with cobalt, high level. FL = fungus *Scytalidium acidophilum*, disrupted cells, low level. FH = fungus *S. acidophilum*, disrupted cells, high level.

TABLE 6. The effect of probiotics on feed conversion ratio (feed intake/BW gain)

Treatment[1]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weekly average
NC–	1.25	1.40^{b}	1.59^{ab}	1.89^{a}	1.87	2.18	1.70
NC+	1.25	1.45^{ab}	1.59^{ab}	1.86^{ab}	1.90	2.14	1.70
PCL	1.23	1.45^{ab}	1.61^{a}	1.85^{abc}	1.87	2.20	1.70
PCH	1.28	1.54^{a}	1.55^{abc}	1.80^{bc}	1.89	2.09	1.69
LCL	1.26	1.39^{b}	1.53^{bc}	1.80^{bc}	1.88	2.15	1.67
LCH	1.30	1.38^{b}	1.55^{abc}	1.78^{c}	1.93	2.09	1.67
LAL	1.26	1.39^{b}	1.51^{c}	1.80^{bc}	1.85	2.16	1.66
LAH	1.27	1.46^{ab}	1.59^{ab}	1.83^{abc}	1.91	2.10	1.69
FL	1.30	1.47^{ab}	1.61^{a}	1.78^{c}	1.82	2.18	1.69
FH	1.25	1.43^{ab}	1.55^{abc}	1.84^{abc}	1.86	2.19	1.69
SE	±0.03	±0.04	±0.02	±0.03	±0.04	±0.05	±0.02

[a–c]Least squares means with different letters within the same column differ significantly ($P < 0.05$). All numbers shown are least squares means.

[1]NC– = negative control, nonadditive. NC+ = negative control, whey permeate medium; PCL = commercial probiotic, Nutragen-PCW (NutriBios Inc., Oshawa, ON, Canada), low level. PCH = commercial probiotic, Nutragen-PCW, high level. LAL = *Lactobacillus acidophilus*, disrupted cells with cobalt, low level. LAH = *L. acidophilus*, disrupted cells with cobalt, high level. LCL = *Lactobacillus casei*, disrupted cells with cobalt, low level. LCH = *L. casei*, disrupted cells with cobalt, high level. FL = fungus *Scytalidium acidophilum*, disrupted cells, low level. FH = fungus *S. acidophilum*, disrupted cells, high level.

resistance to *Candida* infections (Shalev et al., 1996) in humans. The effect of nonviable probiotics on broiler chickens has never been evaluated. Whether viability is required for probiotics to exert their benefits on host animals is still unclear. It is noteworthy that different methods of inactivation, including heat, irradiation, and ultraviolet light, have been shown to affect the efficacy of probiotics (Ouwehand and Salminen, 1998). Results from the present trial demonstrated that inactivated probiotics, disrupted by a high-pressure homogenizer, have positive effects on the production performance of broiler chickens when used at certain concentrations.

Cobalt is an important component for the synthesis of vitamin B12, an essential requirement in poultry feed. However, the direct addition of cobalt into chicken feed depresses performance and causes a high mortality rate (Southern and Baker, 1981; Diaz et al., 1994). It is unclear why cobalt-enriched lactobacilli are able to enhance the beneficial effects of probiotics. The growth curves of *L. acidophilus* and *L. casei* were not affected by cobalt supplementation (data not shown). Because cobalt is an essential element for some bacteria, it is possible that the supplementation maintains better metabolism of probiotics, which make them more active in providing vitamin B_{12} required for the host. In addition, it has been demonstrated that cobalt chloride induced expression of surface adhesive proteins of human endothelial cells (Sultana et al., 1999). Therefore, cobalt absorption by probiotics may alter the enzymes involved in membrane metabolism and upregulate their binding ability to the intestinal wall. Further investigation is required to verify these hypotheses.

TABLE 7. The effect of probiotic on production of serum IgA and IgG in response to the antigen KLH[1]

Treatment	IgA[2] (OD)[3]	IgG[2] (OD)
NC–	0.400 ± 0.080^{a}	0.834 ± 0.080^{ab}
NC+	0.410 ± 0.080^{ac}	0.901 ± 0.080^{ab}
PCL	0.768 ± 0.080^{b}	0.987 ± 0.080^{a}
PCH	0.572 ± 0.080^{abcd}	0.990 ± 0.080^{a}
LCL	0.619 ± 0.080^{abcd}	0.746 ± 0.080^{b}
LCH	0.640 ± 0.080^{bc}	0.963 ± 0.080^{ab}
LAL	0.646 ± 0.080^{bd}	0.759 ± 0.080^{ab}
LAH	0.443 ± 0.085^{acd}	0.932 ± 0.085^{ab}
FL	0.543 ± 0.080^{abcd}	0.951 ± 0.080^{ab}
FH	0.523 ± 0.080^{acd}	0.926 ± 0.080^{ab}

[a–d]Least squares means with different letters within the same column differ significantly ($P < 0.05$). All data were calculated as least squares means.

[1]KLH = keyhole limpet hemocyanin.

[2]Blood samples were collected from broilers 10 d after the immunization with KLH (at the age of 21 d).

[3] OD = optical density.

In consideration of future commercial application, all probiotics were mixed into the basal mash feed before pelleting. At the end of the feeding trial, FH, LAH, and LCL significantly improved BW and BWG in comparison with NC–. Although the FCR of these 3 treatments were also lower, the differences were not significant. It is noteworthy that LAH had the best performance among the treatments during the second week, followed by LCL (third and fourth weeks). The lead was then taken over by FH during the last 2 wk. The change in diet could be responsible for this phenomenon. The starter feed was replaced by the finisher feed at the beginning of the fourth week (21 d). *Lactobacillus* strains seemed to work better with the starter feed. In contrast, *S. acidophilum* performed better when provided with the finisher feed. These results imply that the composition of the base diet might affect the efficacy of probiotics. The finisher feed contains more crude fat and fiber but less crude protein. The amount of single-cell protein produced is not sufficient to explain the growth-promoting effect of the fungus. The underlining mechanisms could be complex. Nevertheless, the very first application of this fungus showed promising results. Chickens fed with FH had the best BW, BWG, and FI among all the treatments.

Unlike *L. acidophilus*, the lower dose of *L. casei* showed a better performance in broiler chickens. This finding

implies that the optimal dose for probiotics varies from one strain to another, and a higher dose does not always lead to a better performance. This observation is in agreement with previous studies. A probiotic supplementation of 100 mg/kg in the diet improved the daily egg production and antibody response during the declining phase of layers in comparison with a higher dose (150 mg/kg diet) or the untreated groups (Panda et al., 2000a). In contrast, Senanl et al. (1997) found that higher levels of *L. casei* (5.1×10^7 and 7.0×10^7 cfu) performed better than the lower levels (1.7×10^7 and 3.5×10^7 cfu) in terms of increasing BW.

The mortality of groups treated with lactobacilli was not different from that of the NC−, indicating that cobalt administration is not toxic to broilers. Although the 3 treatments (PCL, LCH, and LAL) showed a higher production of antigen-specific IgA in the serum, they did not improve the performance of the chickens. The effect of probiotic administration on the immune response is controversial. For example, Panda et al. (2000a,b) indicated that antibody production, in response to the SRBC antigen, in broilers and layers, was significantly enhanced by a commercial probiotic (Probiolac), which disagreed with one of their earlier studies (Panda et al., 1999). In our study, it is unclear how these disrupted probiotics could enhance immune responses. It is possible that disrupted probiotics contain a certain amount of bacterial antigens that are able to stimulate the gastrointestinal immune system. The mechanisms involved in the mucosal immune system are very complex. Thus, a better-designed approach is required to evaluate the effect of probiotics on the immune system of broiler chickens.

In conclusion, cobalt-enriched and disrupted *Lactobacillus* strains, *L. casei* (low dose) and *L. acidophilus* (high dose), were able to promote the growth of broiler chickens. Moreover, disrupted *S. acidophilum* (high dose), a strain of fungus, was introduced to this field, and its growth-promoting effects on broiler chickens were demonstrated. Application of these nonviable probiotics may improve the performance of broiler chickens.

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