Effects of feeding a probiotic blend on live performance of broiler chickens from 0 to 49 days of age

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Primary Audience: Live Production Personnel, Nutritionists, Veterinarians

SUMMARY

Probiotic use has increased as the use of antibiotics in broiler feeds has decreased. This has largely occurred due to a desire to maintain improvements in growth and feed conversion which had typically been observed from subtherapeutic use of antibiotics. As such, two (2) commercially applicable trials were conducted to examine the response from a proprietary blend of five (5) encapsulated Lactobacillus organisms (1-GP; Life Products, Inc.; Norfolk, NE), fed at different dosages across feed phases. In Trial 1, diets supplemented with 1-GP at 0.1%, providing 1,000,000 CFU/g of feed, gave the highest BW at d 14 and 42. In Trial 2, diets supplemented with 1-GP at or above 0.075% gave the best BW and FCRa at d 28. When 1-GP was removed from the diet between d 29 and 49, performance was the lowest, suggesting that 1-GP needs to be fed till time of market. When data were combined from the 2 trials and Quadratic Polynomial (**QP**) and Quadratic Broken Line (**QBL**) models were fit, diets supplemented with 1-GP at or above 0.075% gave the best BW and FCRa at d 28 as noted in Trial 2. Model analysis indicated, on average, that the optimal 1-GP inclusion was 0.115% and 0.100% for BW and FCRa, respectively for the 0 to 28 d growing period. Inclusion which maximized or minimized response was shown to be 0.130 and 0.155%, on average, for BW and FCRa, respectively for the 28 to 42 d growing period. This suggests that 1-GP inclusion should not be decreased in the latter phases of production.

Key words: probiotic, broiler, probiotic blend, titration model

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DESCRIPTION OF PROBLEM

Over the last decade, the broiler industry has responded to changing consumer preferences and increased regulation of medically important antibiotics, by reducing or eliminating the use of subtherapeutic antibiotics as growth promotants (Singer et al. 2020). Although the mechanisms by which in-feed antibiotics exert growth-promotant effects are not fully understood, it is clear that antibiotics directly impact the composition of the gastrointestinal microflora (Dibner and Richards, 2005; Gaskins et al., 2002). In addition to reducing reliance on in-feed antibiotics, many broiler producers have also ceased administering prophylactic antibiotics during vaccination for Marek's disease at the hatchery. This is particularly problematic because in ovo injection of the vaccine provides a potential entry point for pathogenic

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bacteria to access the embryo, leading to reduced hatchability and increased chick mortality in the first week of life (Smith, 2019). After hatch, populations of commensal bacteria in the gastrointestinal tract of chicks require several weeks to stabilize (Lan et al., 2005) – a duration that might exceed the length of a broiler grow-out, depending on the targeted weight at harvest. In the absence of in-feed antibiotics, the broiler industry has increasingly relied upon other feed additives aimed at reducing the colonization of pathogenic bacteria and which support gastrointestinal health.

Probiotics are direct-fed live microorganisms that are specifically selected for their beneficial effects on the gastrointestinal microbiome of the host. These beneficial effects are largely attributable to competition with pathogens for access to colonization sites and nutrients, production of antimicrobial metabolites that are inhibitory to pathogenic bacteria, or direct stimulation of the immune system (Dalloul et al., 2003; Servin and Coconnier, 2003; Yang et al., 2009). In particular, many strains of *Lactobacillus* have been cultured for probiotic applications as they are ubiquitous in the gastrointestinal tract of healthy broilers and can competitively exclude pathogens such as *Clostridium perfringens, Salmonella* spp., and *Campylobacter jejuni* (Hofacre et al. 2003; Servin and Coconnier, 2003; Stanley et al. 2014). However, performance responses to probiotic feed additives have been notably variable (Applegate et al., 2010; Stanley et al, 2014; Yang et al., 2009). This is likely due to the complex interaction between the host microbiome, diet composition, environment, and other unknown factors that may affect the ability of the probiotic microbe to successfully colonize the host. Multistrain probiotics can capitalize on their unique mode of action, gastrointestinal niche, and target key pathogens with each of their component bacteria, reducing the potential impact of host variation.

The objective of the current study was to evaluate the effects of a multistrain probiotic blend (**1-GP**), with a patented liquid encapsulation, on the live performance of broilers under conditions applicable to commercial production. The bacteria used in 1-GP were selected using in vitro pathogen inhibition zone testing (unpublished data). These studies were conducted to evaluate the efficacy of individual probiotic strains: *Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus brevis*, and *Lactobacillus plantarum* and a cocktail of these 5 strains against pathogenic strains of *Salmonella, Campylobacter, Clostridium, E. Coli. Listeria, and Staphylococcus.* These studies showed consistent inhibition zones at pH 3.78 and 7 against these pathogens. 1-GP was initially tested by a large commercial layer company, in battery cages, against several competing products. Life Products encapsulation technology has been shown to maintain bacterial viability for up to 1 yr, and can protect those bacteria from environmental conditions (moisture and antimicrobial components in feed). This helps to ensure that the bacteria used in 1-GP are still alive in the feed when they are consumed by the bird. In order to evaluate the efficacy of 1-GP, 2 trials were performed and reported individually. A combined data set from both of the trials was used for further modeling analysis to determine the most effective dose of the product.

MATERIALS AND METHODS

Bird Husbandry

An experiment consisting of 2 trials was conducted by Poultry Research Partners, LLC (BC Farm; Hoschton, GA) under the supervision of Dr. Bradley Turner, DVM, MAM. This research met the guidelines approved by the institutional animal care and use committee (IACUC, Trial 1:PRP-2020-03, Trial 2:PRP-2020-06). For each trial, a total of 2,600-day-old Ross 708 by-product male broiler chickens were selected for health and uniformity before each group of 40 chicks was weighed and randomly allocated to 65 (4' X 8') floor pens, providing 0.80 square feet (0.074 square meters) per bird. Birds were raised on used litter with adequate ventilation and specific breed lighting recommendations. Feed and water were provided ad libitum. At time of bird placement, a uniform average bird weight was maintained amongst experimental pens. Each pen was assigned to one of 5 dietary treatments (**TRT**),

Table 1. Description of the dietary treatments.

	Trial 1[1] 0 to 42d		Trial 2[2] 0 to 28 d		Trial 2[2] 29 to 49 d	
Treatment	1-GP Inclusion (%)	Targeted CFU/g feed	1-GP Inclusion (%)	Targeted CFU/g feed	1-GP Inclusion (%)	Targeted CFU/g feed
1	0	0	0	0	0	0
2	0.025	250,000	0.025	250,000	0.1	1,000,000
3	0.05	500,000	0.075	750,000	0.134	1,340,000
4	0.1	1,000,000	0.1	1,000,000	0.1	1,000,000
5	0.2	2,000,000	0.1	1,000,000	0	0

[1]Trial 1 was conducted using mash feed with 1-GP added to the mixer in dry form (liquid encapsulated bacteria sprayed onto a dry carrier; 1.0×10^9 CFU/g concentration).

[2]Trial 2 was conducted using pelleted feed with 1-GP added post-pelleting in liquid encapsulated form (2×10^{10} CFU/g concentration) converted to standard inclusion rate of the dry form at the same targeted CFU/g of feed.

with 13 replicate pens per TRT. Feed was supplemented with an encapsulated, multistrain probiotic blend (1-GP; Life Products, Inc.; Norfolk, NE) containing *Lactobacillus acidophilus, Enterococcus faecium, Pediococcus pentosaceus, Lactobacillus brevis*, and *Lactobacillus plantarum*.

Trial 1. This trial objective was to verify the optimum dosage of 1-GP while avoiding any potential errors that could be caused by feed manufacturing. Thus, mash feed was manufactured and 1-GP in a dry form, with 1.0×10^9 CFU/g concentration was used. Pens were assigned to one of the following dietary treatments (Table 1): TRT1: corn-soybean meal mash diet as a negative control (**NC**); TRT2: NC+1-GP at 0.025% (targeted 250,000 CFU/g of feed); TRT3: NC+1-GP at 0.05% (targeted 500,000 CFU/g of feed); TRT4: NC+1-GP at 0.1% (targeted 1,000,000 CFU/g of feed); and **TRT5**: NC + 1-GP at 0.2% (targeted 2,000,000 CFU/g of feed). Bird weights and feed intake (**FI**) were recorded on d 0, 14, 28, and 42. Mortality adjusted feed conversion (**FCRa**) was calculated by accounting for mortality weight.

Trial 2. To more closely replicate commercial broiler production, Trial 2 was conducted using pelleted feed. Because the lactic-acid producing bacteria in 1-GP cannot survive the high temperature conditions of the feed pelleting process, it was necessary to apply the treatment in liquid form (liquid encapsulated bacteria, 2×10^{10} CFU/g concentration) post-pelleting. For comparison purposes, the liquid inclusion rate was converted to the standard inclusion rate of the dry form, targeting the same CFU/g of feed. After pelleting and cooling, feed for each treatment was weighed and placed into a horizontal ribbon mixer. While the mixer was running, the appropriate amount of 1-GP for each treatment was hand sprayed onto the feed. Once it was established that the appropriate amount of product had been applied, the mixer was allowed to run for an additional 5 min. For untreated diets, the same spraying procedure was followed using soybean oil instead of 1-GP. Untreated diets were mixed prior to treated diets to avoid cross contamination of any product into the NC treatment. Pens were assigned to one of the following dietary treatments (Table 1): **TRT1**: corn-soybean meal diet as a negative control (NC); **TRT2**: NC+ 1-GP at 0.025% (targeted 250,000 CFU/g of feed) from 0 to 28 d and 0.1% (targeted 1,000,000 CFU/g of feed) from 29 to 49 d; **TRT3**: NC+ 1-GP at 0.075% (targeted 750,000 CFU/g of feed) from 0 to 28 d and 0.134% (targeted 1,340,000 CFU/g of feed) from 29 to 49 d; **TRT4:** NC+ 1-GP at 0.1% (targeted 1,000,000 CFU/g of feed) from 0 to 49 d; and **TRT5**: NC+ 1-GP at 0.1% (targeted 1,000,000 CFU/g of feed) from 0 to 28 d and no supplementation from 29 to 49 d. Bird weights and feed consumption were recorded on d 0, 14, 28, 42, and 49 d of age. Mortality weight was used to calculate FCRa.

Statistical Analysis

The experimental design for both trials was a randomized complete block design with pen as

Table 2. Effects of different levels of a probiotic blend (1-GP) on live performance of broilers from 0 to 42 d (Trial 1).

		0 to 14 d				0 to 28 d				0 to 42 d			
Treatment	1-GP[1] Inclusion (%)	BW (kg)	FCRa[2] (kg:kg)	Feed Intake (kg)	Mortality (%)	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)
1	0	0.415[a,b]	1.014	0.420	1.54	1.090	1.662	1.809	3.65	1.891[b]	1.791[ab]	3.387[b]	4.62
2	0.025	0.419[a,b]	1.005	0.419	1.81	1.105	1.645	1.813	3.89	1.923[b]	1.800[a]	3.461[ab]	4.87
3	0.05	0.398[b]	1.039	0.414	1.68	1.080	1.645	1.777	2.87	1.916[b]	1.764[b,c]	3.377[b]	4.23
4	0.1	0.431[a]	0.987	0.426	1.90	1.110	1.633	1.811	2.27	2.023[a]	1.745[c]	3.529[a]	3.40
5	0.2	0.409[b]	1.030	0.419	1.73	1.096	1.650	1.806	2.31	1.934[a,b]	1.762[b,c]	3.407[a,b]	2.89
	SEM[3]	0.002	0.008	0.003	0.140	0.004	0.005	0.005	0.223	0.010	0.004	0.016	0.279
	P-value	0.001	0.284	0.779	0.947	0.151	0.487	0.136	0.086	0.003	0.0003	0.022	0.152

[a,b,c]Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Tukey HSD.

[1]Diets were supplemented with a proprietary blend of lactic-acid producing bacteria (1-GP; 1.0×10^9 CFU/g; Life Products, Inc., Norfolk, NE).

[2]FCR adjusted to account for mortality weight.

[3]Pooled standard error of mean.

the experimental unit. In each trial, the 5 dietary TRT were each fed to 13 replicate pens per TRT, for a total of 65 pens with 40 birds per pen at placement. Data from each trial was analyzed separately using a one-way ANOVA in JMP® Version 15 (SAS Institute, 2019). The combined data from both trials was also analyzed using one-way ANOVA where trial was treated as a random factor. The mean values among treatments were compared using Tukey HSD with statistical significance considered at $P \leq 0.05$ unless otherwise indicated. For the combined data set, 2 models were used to fit the biological responses: 1) Quadratic broken-line (**QBL**) model: $[Y = a + b(R - X)^2,\ if\ X \leq R;$ or $Y = a,\ if\ X > R]$, where Y = responses, X = 1-GP levels, a = maximum or minimum response, b = rate constant, and R = requirement or breakpoint; and 2) Quadratic polynomial (**QP**) model: $Y = a + bX + cX^2$, where Y = responses; X = 1-GP levels; and a, b, and c are constants. The requirement or breakpoint based on the QP model was calculated as: $\left(\frac{-b}{2c}\right)$.

RESULTS AND DISCUSSION

Trial 1

Live performance results for supplementation of mash feed with 1-GP from 0 to 42 d are contained in Table 2. At 14 d of age, birds receiving diets supplemented with 0.1% inclusion rate or targeted 1,000,000 CFU/g feed (TRT 4) had the highest BW (0.431, $P = 0.001$). Supplementation with either 0.05% or targeted 500,000 CFU/g feed or 0.2% or targeted 2,000,000 CFU/g feed resulted in lower BW (0.398 or 0.409 kg). No differences in FI, mortality, or FCRa were observed at 14 d. At 28 d, no differences in live performance were detected ($P > 0.05$). However, at 42 d, supplementation with 0.1% inclusion rate or targeted 1,000,000 CFU/g feed (TRT4) resulted in increased BW (2.203 kg; $P = 0.003$) in comparison with all other TRT, except with 0.2%. Similarly, TRT 4 also had increased cumulative FI (3.529 kg; $P = 0.022$) at 42 d. Supplementation at 0.1% inclusion rate or targeted 1,000,000 CFU/g feed resulted in lower ($P = 0.0003$) FCRa when compared to 0.025%

Table 3. Effects of different levels of a probiotic blend (1-GP) on live performance of broilers from 0 to 49 d (Trial 2).

	1-GP[1] Inclusion (%)		0 to 14 d				0 to 28 d			
Treatment	0 to 28 d	29 to 49 d	BW (kg)	FCRa[2] (kg:kg)	Feed Intake (kg)	Mortality (%)	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)
1	0	0	0.423	1.074	0.454	0.39	1.322[c]	1.387[a]	1.833	2.12
2	0.025	0.1	0.424	1.076	0.457	0.77	1.341[bc]	1.369[a,b]	1.837	2.50
3	0.075	0.134	0.424	1.061	0.450	0.77	1.359[a,b]	1.341[bc]	1.823	1.73
4	0.1	0.1	0.422	1.074	0.453	0.39	1.358[a,b]	1.348[bc]	1.830	1.35
5	0.1	0	0.425	1.068	0.454	0.77	1.374[a]	1.332[c]	1.830	1.73
		SEM[3]	0.001	0.004	0.001	0.137	0.003	0.003	0.005	0.21
		P-value	0.859	0.717	0.712	0.755	<0.0001	<0.0001	0.912	0.503
	1-GP[1] Inclusion (%)		0 to 42 d				0 to 49 d			
Treatment	0 to 28 d	29 to 49 d	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)
1	0	0	2.680[b]	1.688[a]	4.521	3.65[a]	2.934[c]	1.819[a]	5.339	5.00[a]
2	0.025	0.1	2.699[b]	1.665[a]	4.494	3.46[a]	3.003[b]	1.775[b]	5.329	4.62[a,b]
3	0.075	0.134	2.756[a]	1.617[b]	4.456	2.69[a,b]	3.070[a]	1.740[b,c]	5.341	2.89[c]
4	0.1	0.1	2.762[a]	1.616[b]	4.463	1.92[b]	3.085[a]	1.718[c]	5.297	3.27[b,c]
5	0.1	0	2.765[a]	1.631[b]	4.508	3.08[a]	3.029[a,b]	1.770[b]	5.361	3.65[a,b,c]
		SEM	0.006	0.004	0.010	0.178	0.006	0.004	0.014	0.185
		P-value	<0.0001	<0.0001	0.164	0.026	<0.0001	<0.0001	0.696	0.002

[a,b,c]Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Tukey HSD.

[1]Diets were supplemented with a proprietary blend of lactic-acid producing bacteria (1-GP; 2×10^{10} CFU/g; Life Products, Inc., Norfolk, NE).

[2]FCR adjusted to account for mortality weight.

[3]Pooled standard error of mean.

Table 4. Effects of different levels of a probiotic blend (1-GP) on live performance of broilers from 0 to 28 d (Trials 1 and 2 combined).

	0 to 14 d				0 to 28 d			
1-GP[1] Inclusion (%)	BW (kg)	FCRa[2] (kg:kg)	Feed Intake (kg)	Mortality (%)	BW (kg)	FCRa (kg:kg)	Feed Intake (kg)	Mortality (%)
0	0.419	1.044	0.437	0.96	1.206[b]	1.524[a]	1.821	2.88
0.025	0.421	1.041	0.438	1.25	1.220[a,b]	1.508[a,b]	1.822	3.17
0.050	0.399	1.065	0.429	1.17	1.205[a,b]	1.507[a,b]	1.792	2.36
0.075	0.426	1.028	0.434	1.33	1.238[a,b]	1.482[a,b]	1.812	2.25
0.1	0.426	1.034	0.439	1.21	1.241[a]	1.483[b]	1.821	1.97
0.2	0.407	1.064	0.435	1.17	1.217[a,b]	1.509[a,b]	1.816	1.78
SEM[3]	0.002	0.005	0.002	0.100	0.004	0.004	0.004	0.157
P-value	0.999	0.581	0.704	0.938	0.014	0.008	0.411	0.068

[a,b,c]Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Tukey HSD.

[1]Diets were supplemented with a proprietary blend of lactic-acid producing bacteria (1-GP; 1.0×10^9 (Trial 1) or 2×10^{10} CFU/g (Trial 2); Life Products, Inc., Norfolk, NE).

[2]FCR adjusted to account for mortality weight.

[3]Pooled standard error of mean.

inclusion rate or no supplementation. These results indicate that providing an in-feed probiotic blend in the Starter phase (0−14 d) at 0.1% inclusion rate may improve early BW, but these gains may not persist into the Grower phase (15−28 d). This may be attributable to the rapid development of the intestinal microflora during this time period, particularly the cycling of coccidia. Mild dysbiosis due to cocci cycling may temporarily disrupt the efficacy of the probiotic bacteria. This trend was not observed in trial 2, so the lack of differences during the grower phase may have been due to inhibited growth caused by the mash diet limiting intake. Regardless, the improved performance observed in TRT 4, with 0.1% inclusion rate, at 42 d suggests that continuous supplementation with a high concentration of the probiotic bacteria may be sufficient to overcome any setbacks due to typical fluctuations of the intestinal microbiome.

Trial 2

Live performance results for birds receiving pelleted feed with post-pelleting supplementation of 1-GP from 0 to 49 d are contained in Table 3. No differences in performance ($P >$

Table 5. Effects of different levels of a probiotic blend (1-GP) on live performance of broilers from 0 to 42 d (Trials 1 and 2 combined).

1-GP[1] Inclusion (%)		0 to 42 d			
0 to 28 d	29 to 42 d	BW (kg)	FCRa[2] (kg:kg)	Feed Intake (kg)	Mortality (%)
0	0	2.285[c]	1.739[a]	3.954	4.13[a]
0.025	0.025	2.304[c]	1.742[a]	3.976	4.30[a]
0.025	0.1	2.318[c]	1.723[a,b]	3.978	3.97[a,b]
0.05	0.05	2.297[c]	1.706[b,c]	3.895	3.72[ab]
0.075	0.134	2.374[a,b]	1.675[d,e]	3.940	3.20[a,b]
0.1	0	2.383[a]	1.689[c,d,e]	3.991	3.59[a,b]
0.1	0.10	2.393[a]	1.681[e]	3.996	2.69[b]
0.2	0.2	2.316[b,c]	1.704[b,c,d]	3.923	2.37[b]
	SEM[3]	0.006	0.003	0.010	0.169
	P-value	<0.0001	<0.0001	0.233	0.042

[a,b,c]Means within a column with no common superscripts are significantly different ($P \leq 0.05$) using Tukey HSD.

[1]Diets were supplemented with a proprietary blend of lactic-acid producing bacteria (1-GP; 1.0×10^9 (Trial 1) or 2×10^{10} CFU/g (Trial 2); Life Products, Inc., Norfolk, NE).

[2]FCR adjusted to account for mortality weight.

[3]Pooled standard error of mean.

Table 6. Comparison of Quadratic Broken Line (QBL) and Quadratic Polynomial (QP) models fitted to difference BW and FCR responses of broilers receiving a probiotic blend (1-GP[1]) from 0 to 42 d (Trials 1 and 2 combined).

		Quadratic broken line[2]			Quadratic polynomial[3]			
Age	Response	Probiotic supplementation breakpoint (% Inclusion)	Sum of square error	R-square	Probiotic supplementation breakpoint (CFU/g feed)	Sum of square error	R-square	Average of both models Probiotic supplementation breakpoint (% inclusion)
0 to 28 d	BW	0.11	0.0007	37.97	0.12	0.0005	55.96	0.11
	FCRa[4]	0.09	0.0005	59.47	0.11	0.0001	89.17	0.10
28 to 42 d	BW	0.14	0.0015	42.86	0.12	0.0010	61.34	0.13
	FCRa	0.16	0.0038	61.85	0.15	0.0036	63.24	0.16

[1]Diets were supplemented with a proprietary blend of lactic acid-producing bacteria (1-GP; 1.0×10^9 (Trial 1) or 2×10^{10} CFU/g (Trial 2); Life Products, Inc., Norfolk, NE).
[2]Quadratic Broken-Line model: $Y = a + b*(R-X)^2$, if $X \leq R$ or $Y = a$, if $X > R$ where Y = responses, X = 1-GP levels, a = maximum or minimum response, b = rate constant, R = requirement or breakpoint.
[3]Quadratic polynomial model: $Y = a + bX + cX^2$ where Y = responses, X = 1-GP levels, and a, b, c are constants. The requirement based on QP model can be calculated using -b/2c.
[4]FCR adjusted to account for mortality weight.

0.05) were observed from 0 to 14 d. At 28 d, supplementation at or above 0.075% inclusion rate or targeted 750,000 CFU/g feed improved BW ($P < 0.0001$) and FCRa ($P < 0.0001$). These improvements in BW ($P < 0.0001$) and FCRa ($P < 0.0001$) persisted for TRT3, TRT4, and TRT5 at 42 d, despite the removal of the probiotic blend from TRT5 between 29 and 42 d. Additionally, supplementation at 0.1% inclusion rate or targeted 1,000,000 CFU/g feed from 0 to 42 d resulted in reduced mortality (TRT4; 1.923%; $P = 0.026$). However, at 49 d, TRT3 (0.075% or targeted 750,000 CFU/ g feed from 0 to 28 d; 0.134% or targeted 1,340,000 CFU/g feed from 29 to 49 d) and TRT4 (0.1% or targeted 1,000,000 CFU/g feed from 0 to 49 d) had the highest cumulative BW's (3.070 and 3.085 kg, respectively; $P < 0.0001$) and lowest FCRa (1.740 and 1.718, respectively; $P < 0.001$). These treatments also had the lowest cumulative mortality ($P = 0.002$). It is possible that removal of the probiotic blend from TRT5 from 29 to 49 d resulted in poorer performance.

Combined Data

Live performance results for the combined data set from Trial 1 and Trial 2 are displayed in Table 4 (0−28 d) and Table 5 (29−42 d). In contrast to Trial 1, no differences ($P > 0.05$) in performance were observed from 0 to 14 d for the combined data set. At 28 d, however, supplementation at 0.1% inclusion rate or 1,000,000 CFU/g feed resulted in improved BW ($P = 0.014$) and FCRa ($P = 0.008$) when compared to the NC (TRT1). Probiotic supplementation at any other level from 0 to 28 d resulted in intermediate BW and FCRa. At 42 d, birds receiving more than 750,000 CFU/g, but not in excess of 1,000,000 CFU/g, from 0 to 28 d had improved BW ($P < 0.0001$) regardless of the level of supplementation after 28 d. Birds receiving 1,000,000 CFU/g feed throughout d 0 to 42 had the lowest FCRa (1.681; $P < 0.0001$). Birds receiving any combination of supplementation levels at or above 500,000 CFUs/g feed had improved FCRa ($P < 0.0001$) in comparison with the negative control (TRT1). These improvements in FCRa may be partially attributable to a reduction in mortality among TRT

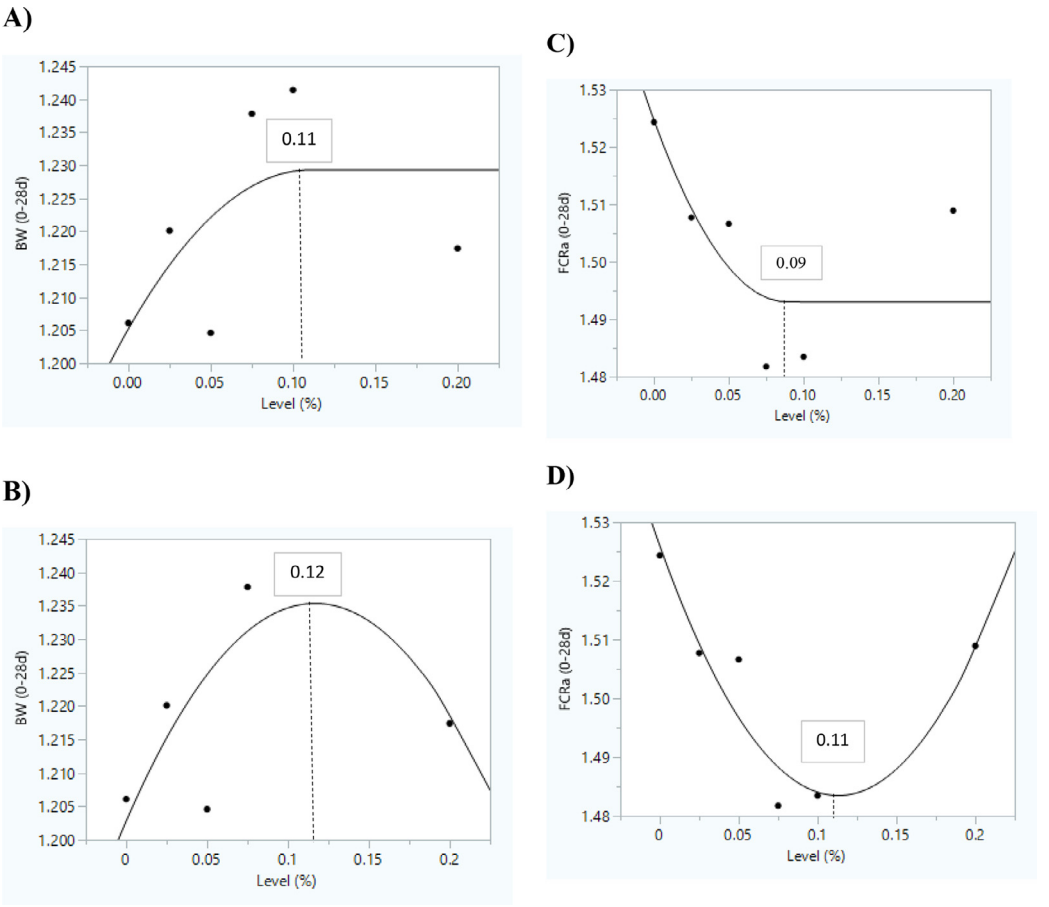

Figure 1. (A-D) Quadratic broken-line (QBL) and quadratic polynomial (QP) models fit for BW and mortality-adjusted FCR (FCRa) responses of broilers to increasing levels of Lactobacillus probiotic supplementation (1-GP) from 0 to 28 d of age using combined data from 2 trials. (A) Effect of 1-GP on BW using QBL model; (B) Effect of 1-GP on BW using QP model; (C) Effect of 1-GP on FCRa using QBL model; (D) Effect of 1-GP on FCRa using QP model.

receiving probiotic supplementation, with those birds receiving 1,000,000 to 2,000,000 CFU/g feed from d 0 to 42 having significantly lower mortality ($P = 0.042$).

Results from QBL and QP models fit to the combined data set from Trial 1 and Trial 2 are displayed in Table 6. Due to an observed high variation between 2 trials, least square means which were adjusted for each trial variation were used to determine the optimum supplementation level which maximized or minimized the response from each model. The maximum/minimum response was observed for QP when the ascending/descending portion of the curve increased/decreased at a declining rate until the maximum/minimum is reached. The maximum/minimum response was observed for QBL when the ascending/descending portion of the curve reached a plateau. Based on these trials, the probiotic supplementation level which reached the maximum BW and minimum FCRa were slightly higher using the QP vs. the QBL model from 0 to 28 d (BW: 0.12 vs. 0.11%, Figures 1A and 1B) and FCRa: 0.11 vs. 0.09%, Figures 1C and 1D). In contrast, from 28 to 42 d, the probiotic supplementation level which reached the maximum BW and minimum FCRa were slightly lower using QP vs. the QBL model (BW: 0.12 vs. 0.14%, Figures 2A and 2B) and FCRa: 0.15 vs. 0.16%, Figures 2C and 2D). Using both models, the optimal probiotic supplementation level ranged from 0.09 to 0.12% during 0 to 28 d while the optimal probiotic supplementation levels were higher during

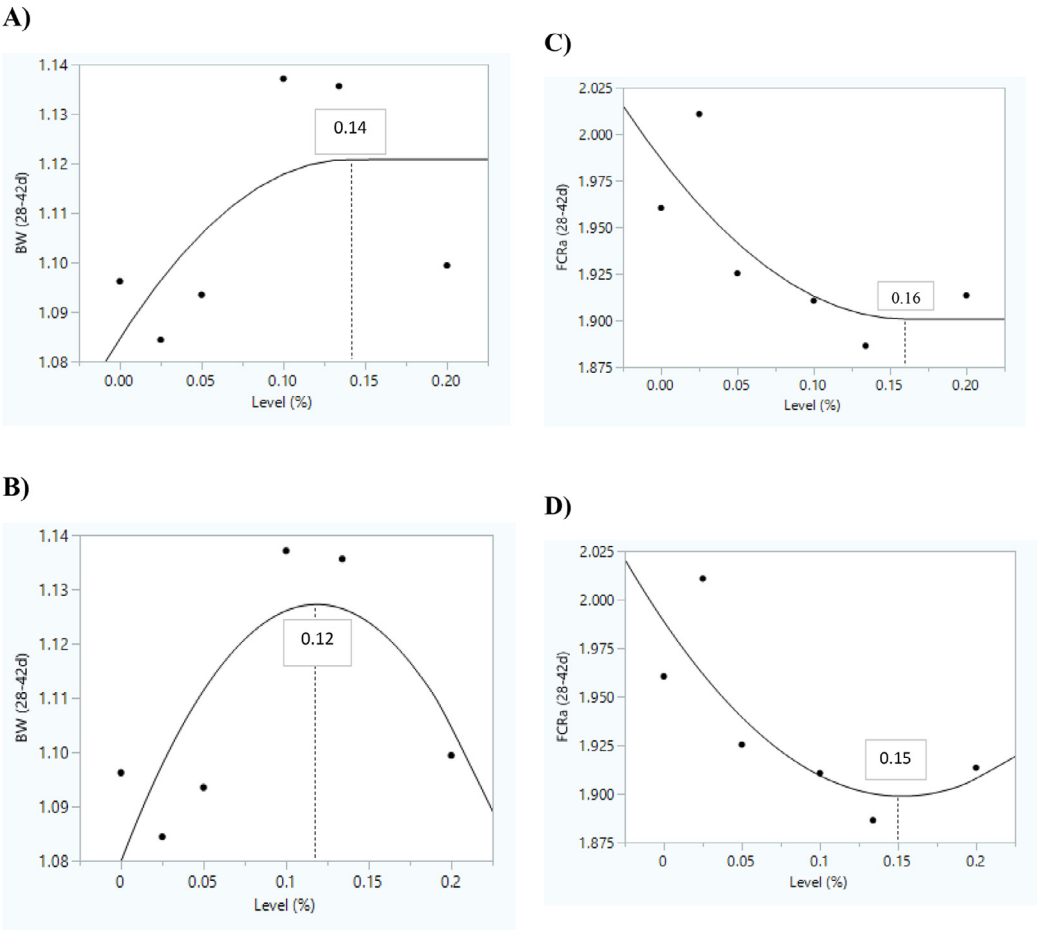

Figure 2. (A-D) Quadratic broken-line (QBL) and quadratic polynomial (QP) models fitted for BW and mortality-adjusted FCR (FCRa) responses of broilers to increasing levels of Lactobacillus probiotic supplementation (1-GP) from 28 to 42 d of age using combined data from 2 trials. (A) Effect of 1-GP on BW using QBL model; (B) Effect of 1-GP on BW using QP model; (C) Effect of 1-GP on FCRa using QBL model; (D) Effect of 1-GP on FCRa using QP model.

28 to 42 d and ranged from 0.12 to 0.16%. Either the QP or the QBL model gave similar estimates as to the optimal level of 1-GP. These results suggest that the effective 1-GP inclusion should not be reduced during the latter phases of production.

CONCLUSIONS AND APPLICATIONS

1. Supplementation with a multistrain probiotic (1-GP) improved BW and FCR at 42 d when fed continuously at a minimum of 1,000,000 CFU/g feed for mash feed.
2. Removal of the multistrain product (1-GP) at 29 d resulted in decreased BW, increased FCR, and increased mortality, suggesting that probiotic products should be fed continuously for the duration of a broiler grow-out, to achieve optimal results.
3. Based on combined trial results between 0 and 28 d, the average from the QBL and QP models predicted the optimal 1-GP dosage to be 0.12% for BW and 0.10% for FCRa.
4. Based on combined trial results between 28 and 42 d, the average from the QBL and QP models predicted the optimal 1-GP dosage to be 0.13% for BW and 0.16% for FCRa.
5. The combined results noted above between the 2 phases, suggests that a higher dosage

of 1-GP might be required in the latter phase vs. the dosage used earlier.
6. Additional research is necessary to characterize the effect of a multistrain probiotic (1-GP) in commercial or challenged environments.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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