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REGULAR ARTICLES

Dietary effects of commercial probiotics on growth performance, digestibility, and intestinal morphometry of broiler chickens

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Abstract

This study compared five commercially available probiotics *vis-à-vis* antibiotic growth promotant (AGP) supplementation and absence of feed additive based on efficiency, intestinal morphometry, and energy digestibility in improving broiler chicken production. A total of 630 straight run (Cobb) day-old broiler chicks were distributed to seven treatments following a completely randomized design, with ten replicates per treatment and nine birds per replicate per cage. Dietary treatments consisted of basal diet in combination with the following: without probiotics and AGP supplementation (treatment 1); 75 ppm each of chlorotetracycline (CTC) and Zn bacitracin (treatment 2); probiotic A, *Bacillus subtilis* (treatment 3); probiotic B, *Bacillus subtilis* (treatment 4); probiotic C, *Enterococcus faecium* (treatment 5); and probiotic D, *Bacillus subtilis* (treatment 6); probiotic E, *Enterococcus faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp. (treatment 7). At day 42, energy digestibility was determined by fasting three randomly selected birds from each treatment for 12 h and then subjecting them to their corresponding dietary treatments. Excreta were collected and pooled after 24 h of feeding. Pooled excreta were weighed, oven-dried, and subjected to energy analyses after 3-day collection. Apparent total tract metabolizable energy was then computed. At day 47, three birds were randomly selected per treatment for intestinal morphometry (villi height and crypt depth) of the duodenum, jejunum, and ileum. Dietary supplementation using probiotics showed no significant effect on overall body weight, weight gain, feed consumption, feed efficiency, dressing percentage, mortality, harvest recovery, carcass quality parameters (e.g., meat to bone ratio and abdominal fat content), intestinal morphometry, and energy digestibility. Birds under treatment 7 (basal feed + probiotic E) generated the highest income over feed and chick cost.

Keywords Production performance · Carcass quality · Probiotics · Antibiotic growth promotant · Apparent Metabolizable Energy · Intestinal morphometry

Introduction

Antibiotics are one of the most commonly known feed additives in poultry feeding. They slow down the growth of disease-causing microorganisms and increase feed efficiency, promote growth rate, and prevent intestinal infections (Bird, 1968 as cited in Balotoc 1992). Antibiotic growth promotants (AGP) are used to enhance growth, efficiency, and livability of poultry. However, AGP use was abused in many animal farms, resulting in drug residuals in meat and the development of antibiotic-resistant bacteria in the animal's gastrointestinal tract. Exposure to these pathogens could cause problems in human health (Acar and Mouglin 2006), which prompted various countries to ban the inclusion of certain antibiotics in feed rations.

Probiotics were then utilized as a substitute to antibiotics as growth promoters to competitively exclude the growth and colonization of pathogens in the intestines. Probiotic-containing feeds were found to reduce serum cholesterol levels in broilers, resulting in weight gain and feed efficiency (Mohan et al. 1996); increase feed intake and antibody production of 21-day-old broilers after a 3-week exposure

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(Zulkifli, 2000 as cited in Lope 2003); and improve antibody production in broiler chicks when combined with the injection of sheep red blood cells (Panda et al. 2000).

Probiotics were also known to reduce mortality and enhance microflora composition in the gut (Mohnl 2006). Moreover, broilers fed with probiotics have increased blood antibody titration against bronchitis (Roughani et al. 2007). Singh et al. (2009) found that supplementing poultry diet with different strains of probiotics at different levels increased feed efficiency, body weight, protein efficiency, and performance index. Tanaka and Santoso (2000) and Jin et al. (2000) observed that Lactobacillus cultures in poultry have significant effects on feed efficiency from starting to finishing period. Santoso et al. (1995) found that *Bacillus subtilis* supplementation in poultry diet can improve the utilization of nitrogen (N) by reducing the NH_3 emission in poultry houses. Probiotics were also found to improve animal health and feed conversion, increase market weight, and increase growth performance while lowering the cost of production and treatment costs for livestock (Didley, 1988 as cited in Lope 2003).

Olnood et al. (2015) reported that supplementation of different strains of *Lactobacillus* species in broiler diets did not significantly improve weight gain, feed consumption, feed conversion ratio, villous height, and crypt depth but had significant effect on villous height to crypt depth ratio obtained in the ileum of birds raised in a clean environment, which could have masked the growth-promoting effects of probiotics. In addition, the concentration of probiotics used (10^6 cfu/g of feed) was lower than the recommended inclusion rate (10^8/g of product) of probiotics available in the market. Awad et al. (2009) reported that inclusion of probiotics improved crypt depth to villi height ratio. However, Dizaji et al. (2013) reported that supplementation of *B. subtilis* has no effect on the villous height, crypt depth, and villous height to crypt depth ratio in the duodenum.

Inconsistencies in the effect of probiotics could be due to many factors, including the strains of the probiotic used, method of preparation of probiotics, dosage and mode of administration, composition of diet, age of the bird, and sanitary conditions (Mountzouris et al. 2007). Otutumi et al. (2012) also noted that variations in the effect of supplementing probiotics are attributed to species used, inclusion rate, survivability of the probiotic in the gastrointestinal tract, health and nutritional status of the animal, environment where animals are raised, and breed of chicken. They reported that supplementation of probiotics in the feed improved the performance of broilers fed with diets deficient in nutrients required by the animal. Snel et al. (2002) reported that the age of broilers could lead to differences in the effect of probiotics in digestibility due the different activities of the digestive enzymes, endogenous amino acid secretion, and bacterial metabolism.

Although studies have already been conducted on the effects of probiotics in broilers, evaluation is yet to be done using the same strain, feed, and management under Philippine conditions. This study therefore aimed at determining the effects of probiotics on production performance and carcass quality of broilers considering local conditions.

Materials and methods

Six hundred thirty (630) vaccinated straight run (Cobb) day-old broiler chicks (DOC) purchased from a reputable hatchery were weighed in groups of nine birds and randomly distributed to 70 cages. Seven (7) dietary treatments were randomly distributed to the 70 cages following the completely randomized design (CRD), replicated ten times with nine birds for each replicate. Dietary treatments are indicated below:

Treatment 1 Basal diet (without probiotics and AGP)
Treatment 2 Basal diet + (75 ppm each of CTC and Zn bacitracin)
Treatment 3 Basal diet + Probiotic A (*B. subtilis*)
Treatment 4 Basal diet + Probiotic B (*B. subtilis*)
Treatment 5 Basal diet + Probiotic C (*Enterococcus faecium*)
Treatment 6 Basal diet + Probiotic D (*B. subtilis*)
Treatment 7 Basal diet + Probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

All probiotics used were provided by Evonik Inc. Broiler rations were formulated following the nutrient recommendations of Cobb 500 broilers (Table 1). Nutrient composition and chemical analysis of basal diets are shown in Tables 2 and 3. In preparing treatments 3 to 7, the recommended inclusion rates of the probiotic manufacturer were followed.

All chicks were fed ad libitum using tube feeders, provided clean drinking water at all times, supplied with light and heat during the first 14 days of brooding through artificial light, and vaccinated against Newcastle disease (B1B1 strain) through intraocular method on the seventh day. Throughout the 5-week study period, each bird per treatment per replicate was weighed on a weekly basis.

Data gathered

Colony forming unit determination of probiotics All probiotics were determined for viability using Potato Dextrose Agar (PDA) as a source of nutrient of the bacteria. Seven dilutions in duplicate were prepared and incubated at 35 °C for 15 h. Average colony counts were expressed as colony forming units (CFU) per gram of the probiotic preparation.

Table 1 Practical levels of nutrients in feed for Cobb 00 (Coob-vantress.com, 2012)

	Starter	Grower	Finisher 1	Finisher 2
Feeding amount/bird	250 g	1000 g	–	
Feeding period (days)	0–10	11–12	23–42	43+
Crude protein	21–22	19–20	18–19	17–18
Metabolizable energy (KJ/kg)	12.7	13.0	13.3	13.4
Lysine (%)	1.32	1.19	1.05	1.0
Digestible lysine (%)	1.18	1.05	0.95	0.9
Methionine (%)	0.5	0.48	0.43	0.41
Digestible methionine (%)	0.45	0.42	0.39	0.37
Met + cys (%)	0.98	0.89	0.82	0.78
Digestible met + cys (%)	0.88	0.8	0.74	0.70
Tryptophan (%)	0.2	0.19	0.19	0.18
Digestible tryptophan (%)	0.18	0.17	0.17	0.16
Threonine (%)	0.86	0.78	0.71	0.68
Digestible threonine (%)	0.77	0.69	0.65	0.61
Arginine (%)	1.38	1.25	1.13	1.08
Digestible arginine (%)	1.24	1.10	1.03	0.97
Valine (%)	1.0	0.91	0.81	0.77
Digestible valine (%)	0.89	0.81	0.73	0.69
Calcium (%)	0.90	0.84	0.76	0.76
Available phosphorus (%)	0.45	0.42	0.38	0.38
Sodium (%)	0.16–0.23	0.16–0.23	0.15–0.23	0.15–0.23
Chloride (%)	0.17–0.35	0.16–0.35	0.15–0.35	0.15–0.35
Potassium (%)	0.60–.95	0.60–95	0.60–0.85	0.60–0.85
Linoleic acid (%)	1.0	1.0	1.0	1.0

Initial and weekly body weight Body weight of all birds per replicate per treatment were taken and recorded every week starting upon arrival until harvest time.

Body weight gain Average weekly gain in weight of all birds per replicate per treatment was obtained by subtracting the average initial body weight from the average weight of the birds at the end of each weighing period.

Feed consumption Weekly feed consumption was determined by subtracting the weight of feed left from the total amount of feed given for the week. Average cumulative feed consumption per weighing period was determined by dividing the cumulative feed consumption by the number of birds per replicate.

Feed efficiency Weekly feed efficiency was obtained by dividing the amount of feed consumed by the weight gained at the end of each feeding period.

Mortality The number of dead birds during the experimental period per treatment was recorded to determine the mortality rate per treatment. Percent mortality was computed and cause of death was identified.

Harvest recovery Percent harvest recovery was obtained by dividing the number of marketable birds (good quality broilers) after the feeding period by the initial number of birds per treatment.

Dressing percentage Two finished broilers per replicate per treatment were randomly selected and dressed to determine dressing recovery. This was computed by dividing the dressed weight by the live weight of the birds then multiplied by 100.

Abdominal fat content Abdominal fat between the keel and the pubic bone was scraped off and weighed. Abdominal fat content was computed by dividing the weight of the abdominal fat by the live weight of the bird then multiplied by 100.

Breast yield One of the two dressed broilers from each replicate was randomly selected to determine breast yield. Breast meat percentage was determined by separating the breast bone (sternum) from the meat and was computed by dividing the weight of the breast meat with the live weight of the chicken then multiplied by 100.

Meat to bone ratio All separable edible tissues (including lean, fat, and skin) from the bones were weighed. Major cuts

Table 2 Nutrient composition of feed rations used in the feeding trial

Ingredients	Chick booster	Broiler starter	Broiler finisher
Yellow corn	565.14	580.03	603.23
US soya	319.93	306.03	280.46
Hypromel	40.0000	35.00	35.00
Palm oil	38.03	45.23	48.49
MDCP	9.782	10.730	9.851
Limestone	8.176	8.737	9.226
DL-methionine	4.290	3.50	2.50
Iodized salt	3.50	3.50	2.88
L-Lysine	3.45	1.50	1.50
L-Threonine	1.69	1.50	1.40
Bro-vitamin	1.50	1.20	1.20
Co-bind	1.50	1.15	1.00
Choline	1.20	1.00	0.98
Bro-minerals	1.00	0.61	0.50
Toxicheck	0.50	0.50	0.496
Capsozyme	0.10	0.10	0.10
Ethoxyquin	0.10	0.10	0.10
L-Tryptophan	0.10	0.10	0.10
Total weight	1000.00	1000.00	1000.00
Calculated nutrient content (as fed)			
Crude protein (%)	21.00	20.50	19.50
M.E. (Kcal/kg)	3050	3100	31,400
Crude fiber (%)	2.81	2.78	2.73
Crude fat (%)	6.47	7.17	7.54
Calcium (%)	0.90	0.90	0.90
Total phosphorus (%)	0.77	0.77	0.74
Available phosphorus	0.45	0.45	0.43
Lysine (%)	1.32	1.09	1.01
Methionine (%)	0.56	0.43	0.42
Meth + cystine (%)	0.96	0.81	0.77
Threonine (%)	0.96	0.71	0.78

(breast, thigh, and drumstick) were deboned and weighed. The separated meat and bone were divided by the weight of its major cut. Percentage meat was then compared to the percentage bone of the three major cuts.

Energy digestibility determination by total collection At day 42, three broilers from each treatment were fasted for 12 h. Broilers were fed with the treatment diets for the determination of nutrient apparent total tract digestibility. Excreta were collected and pooled after 24 h of feeding. This was done for 3 consecutive days. The pooled excreta were weighed and oven-dried and were subjected to gross energy analysis following the standard methods (AOAC, 2011). Apparent metabolizable energy (AME) was computed by subtracting the total gross energy excreted in the fecal samples ($GE_{excreta}$ in kcal/kg) from the total ingested gross energy from the feed (GE_{feed} in kcal/kg):

$$\text{AME (kcal/kg)} = GE_{\text{feed intake}} - GE_{\text{excreta}}$$

Table 3 Chemical analysis of feed rations used in the feeding trial

	Chick booster	Broiler starter	Broiler finisher
Moisture (%)	9.4	9.74	10.86
Ash (%)	5.62	5.81	5.04
Crude protein (%)	21.2	20.7	20.33
Crude fiber (%)	3.45	3.14	2.7
Crude fat (%)	6.61	7.55	6.87
Calcium (%)	1.19	1.14	1.11
Phosphorus (%)	0.36	0.65	0.53

Intestinal morphometry At day 47, three birds from each treatment were randomly selected to measure the effect of probiotics on the villi height, crypt depth, and villous height to crypt depth ratio of the small intestine of broiler chickens. After dressing, tissue samples from the three points of the small intestines (at the apex of the duodenum, 10 cm distal to the point of entry of the distal bile ducts, and 5 cm proximal to the ileo-cecal junction) were taken and placed in 10% formalin. Histological sections of the tissue samples were prepared and stained using Mayer's Hematoxin and Eosin (H and E) technique. Five adjacent villi on three sections of each sample were examined and measured from the tip of the villi to the villus crypt junction using Dino Capture 2.0 v.1.5.4 digital microscope. The average villous height and crypt depth were recorded.

Profitability analysis

Income over feed and chick cost was estimated to determine the cost of producing a kilogram of broiler per treatment. The actual cost of chicks and the volume of feed consumed multiplied by the prevailing market price of feed constitute the cost

Table 4 Colony forming units (cfu) of different probiotics grown in nutrient agar incubated for 15 h

Probiotic	Cfu/g	
	Probiotic	Feed
Probiotic A *B. subtilis*	7.45×10^9	7.45×10^6
Probiotic B *B. subtilis*	6.55×10^{10}	6.55×10^7
Probiotic C *E. faecium*	1.20×10^7	1.20×10^4
Probiotic D *B. subtilis*	1.38×10^{12}	1.38×10^9
Probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)	1.55×10^7	1.55×10^4

Table 5 Average initial and weekly body weights of birds fed with different kinds of probiotics

	Live body weight (g)								
Week	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7	Ave.	C.V
Initial	47.26	47.31	46.58	47.09	46.80	47.16	46.83	47.00	2.83
1	156.92	155.11	147.28	153.06	153.56	148.50	154.19	152.66	9.27
2	410.78	404.89	411.78	385.33	393.44	400.44	405.78	401.78	6.91
3*	822.22[ab]	830.89[a]	828.56[a]	790.00[b]	789.63[b]	809.49[ab]	831.22[a]	814.57	4.45
4*	1364.89[a]	1371.89[a]	1334.56[ab]	1312.11[b]	1328.82[ab]	1336.32[ab]	1342.67[ab]	1341.61	3.32
5	1899.78	1931.83	1874.29	1884.24	1877.11	1889.47	1909.11	1895.12	3.32

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

*Means with different superscripts differ significantly ($P < 0.05$)

items. Income was estimated by multiplying the body weight per treatment with the prevailing per kilogram live weight price at the time of the study. Income over feed and chick cost (IOFCC) analysis was estimated to determine which treatment will give higher profit.

$$\mathrm{IOFCC} = \left[\text{ave.wt.of broilers, kg}\right) (\text{price/kg})\Big] - \Big[(\text{price of DOC}) + \Big(\text{total feed consumed} \times \text{price of feeds}\Big]$$

Statistical analyses

Data on growth parameters and carcass quality parameters were analyzed using general linear model procedure of SAS (V9.1.3). A statistical model for evaluating weight gain, feed consumption, feed efficiency, and livability parameters was used. Data gathered were subjected to analysis of variance (ANOVA) following CRD. Treatment mean comparison was done using Tukey's studentized range (HSD) test. Apparent total tract metabolizable energy and intestinal morphometry were analyzed using ProcMixed procedure of SAS (V9.1.3). Significance between treatment means was tested using Tukey's test. Level of significance was set at $P \leq 0.05$ for all test statistics. $P > 0.05$ is not significant for all test statistics.

Linear model

$$Yij = \mu + \alpha_i + \varepsilon_{ij} \qquad i, j = 1, 2$$

where Y_{ij} = response variable;

- Body weight (initial and average weekly body weight)
- Body weight gain (average weekly body weight gain)
- Feed consumption (average weekly feed consumption)
- Feed efficiency (average weekly feed efficiency)
- Dressing percentage
- Abdominal fat content

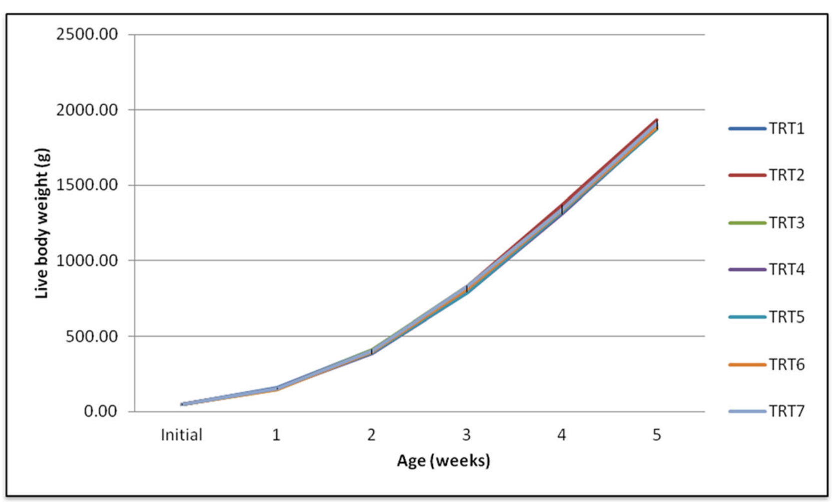

Fig. 1 Average initial and weekly body weights of birds fed with different kinds of probiotics

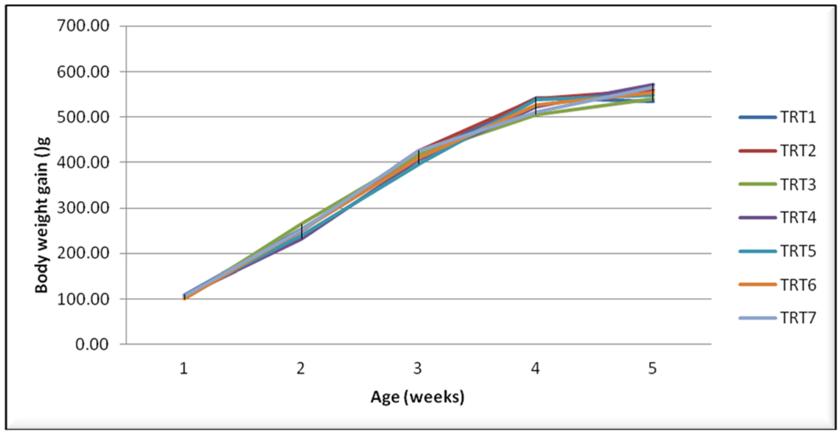

Fig. 2 Average weekly body weight gain of birds fed with different kinds of probiotics

μ = overall mean observation

α_i = effect of the *i*th probiotics

ε_{ij} = random error associated with experimental unit j given the *i*th probiotics

Results and discussion

Colony forming unit determination of probiotics

All bacteria used in the feeding trial were viable at the time of use as indicated by their ability to form colonies. Results of colony forming unit determination indicated different cfu per gram of the product and per kilogram of feed considering the differences in inclusion rates (Table 4). Variability in cfu was also observed among different probiotic preparations within the same strain as in the case of probiotic A *B. subtilis*, probiotic B *B. subtilis*, and probiotic D *B. subtilis.*

Differences in the amount of cfu per kilograms of feed could lead to different effects in broiler chickens. Although treatment 3, treatment 4, and treatment 6 used *B. subtilis*, their effectiveness could be different. These differences in their efficacy could be attributed to the cfu per kilograms of feed used. Even if there is still no universal optimal intake for probiotic efficacy reported yet, Ewing and Cole reported in their book entitled "The Living Gut: An Introduction to Micro-Organisms in Nutrition" that most probiotics are effective at dietary intake of 10^8 to 10^{11} daily (as cited in Mountzouris et al. 2007). Inclusion of *Lactobacillus* spp. at 10^6 cfu/g of feed did not have significant results in the growth performance and intestinal tract morphology of broilers (Olnood et al. 2015). However, Zhang and Kim (2014) reported that supplementing 1×10^5/kg and 2×10^5/kg of multistrain probiotics (*Lactobacillus acidophilus*, *B. subtilis*, and *Clostridium butyricum*) improved growth performance and ileal digestibility of essential amino acids which could be due to the microbial activity of the ileum that results in an improved bacterial degradation of amino acids. Mountzouris et al. (2007) reported that supplementation of *Lactobacillus reuteri*, *E. faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici*, and *Lactobacillus salivarius* at 2.5×10^8 bacteria per chick daily improves growth performance.

Table 6 Average weekly body weight gain of birds fed with different kinds of probiotics

	Weekly body weight gain (g)								
Week	TRT1[1]	TRT2[2]	TRT3[3]	TRT4[4]	TRT5[5]	TRT6[6]	TRT7[7]	Ave.	C.V.
1	109.69	107.80	100.70	105.97	106.76	101.34	107.36	105.66	13.14
2	253.83	249.78	264.50	232.28	239.89	251.94	251.59	249.12	15.13
3	411.44	426.00	416.78	404.67	396.18	409.04	425.44	412.79	7.01
4	542.67	541.00	506.00	522.11	539.19	526.83	511.44	527.03	7.27
5	534.89	559.94	539.74	572.13	548.29	553.15	566.44	553.51	8.86
Total	1852.52	1884.52	1827.72	1837.16	1830.31	1842.30	1862.27	1848.11	3.41

[1] *TRT1*, basal diet (without probiotics and AGP); [2] *TRT2*, basal diet + (CTC and Zn bacitracin); [3] *TRT3*, basal diet + probiotic A (*B. subtilis*); [4] *TRT4*, basal diet + probiotic B (*B. subtilis*); [5] *TRT5*, basal diet + probiotic C (*E. faecium*); [6] *TRT6*, basal diet + probiotic D (*B. subtilis*); [7] *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

Table 7 Average weekly feed consumption of birds fed with different kinds of probiotics

Week	Average feed consumed (g)								
	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7	Ave.	C.V.
1**	135.06[a]	125.11[abc]	114.67[bcd]	129.33[ab]	129.89[ab]	103.28[d]	107.94[cd]	120.75	16.74
2	305.18	333.56	318.94	358.32	365.33	326.94	330.89	334.17	21.61
3*	615.49[ab]	623.83[a]	590.28[b]	595.68[b]	592.01[b]	603.53[ab]	601.76[ab]	603.23	4.37
4	822.67	845.56	829.89	826.56	817.21	829.42	830.78	828.87	3.73
5	994.22	1004.24	993.40	1022.44	991.04	979.83	966.22	993.06	6.92
Total FC	2872.62	2932.30	2847.18	2932.33	2895.48	2843.00	2837.59	2880.07	4.32

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

*Means with different superscripts differ significantly ($P < 0.05$)

Body weight and gain

As shown in Table 5, the average initial body weight of day-old chicks assigned to different treatments ranged from 46.58 to 47.31 g. Average initial body weights among treatments do not significantly differ from each other, implying that initial body weights are homogeneous.

No significant differences were observed on the average body weights of birds across all treatments during the first and second weeks. On the third week, birds under treatments 7, 2, and 3 were significantly heavier than birds in treatments 4 and 5 but not in treatment 6. On the fourth week, body weights from treatment 4 were significantly lower than birds in treatments 1 and 2. Growth performance for treatment 2 was observed to be significantly higher, and together with higher third and fourth week body weights obtained, suggests that antibiotic supplementation as growth promotant is effective.

Final weights obtained on the 35th day, however, showed no significant differences among treatments, despite providing proper care and health and feeding management to the birds. In contrast, Ramlah and Tan (1995) found that broilers under probiotic treatment had significantly higher body weights on the third, fourth, and fifth weeks compared to birds fed without probiotics. Mills et al. (2011) claimed that before probiotic bacteria could perform its role in the physiology of the intestine, they must be supported by sufficient tension to ensure reaching the target for more visible effects. Figure 1 shows that growth patterns across all treatment birds have linearly increased.

The measured body weights of birds in all treatments were within the breed performance standard guide (Cobb-vantress.com, 2012). Meanwhile, no significant differences were observed on the average weekly body weight gain among treatments from the first week of age until harvest (Fig. 2 and Table 6). This conforms with the study conducted by Noh et al. (1994, as cited in Piao et al. 1999) that showed no significant effect on body weight gain of birds fed on 0.10% yeast culture supplementation. Similar results on probiotics supplementation were reported by Ramlah and Tan (1995), Piao et al. (1999), and Lope (2003). These show that under

Table 8 Average weekly feed efficiency of birds fed with different kinds of probiotics

Week	Average feed efficiency								
	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7	Ave.	C.V.
1**	1.22[a]	1.16[ab]	1.14[abc]	1.22[a]	1.21[a]	1.04[bc]	1.01[c]	1.14	12.09
2	1.24	1.40	1.24	1.63	1.63	1.35	1.37	1.41	36.41
3	1.5	1.47	1.43	1.47	1.5	1.48	1.42	1.47	6.53
4*	1.52[b]	1.56[ab]	1.65[a]	1.59[ab]	1.52[b]	1.57[ab]	1.64[a]	1.58	6.21
5	1.87	1.80	1.85	1.80	1.82	1.78	1.72	1.81	10.59
Overall (0–5)	1.55	1.56	1.56	1.60	1.58	1.54	1.52	1.56	4.82

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

*Means with different superscripts differ significantly ($P < 0.05$)

Table 9 Mortality rate and harvest recovery of birds fed with different kinds of probiotics

	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7
Mortality rate (%)[a]	0	0	0	0	1.11	1.11	0
Harvest Recovery (%)[a]	100.00	98.89	98.89	97.78	97.78	97.78	100.00
Initial no. of birds	90	90	90	90	90	90	90
No. of dead birds	0	0	0	0	1	1	0
No. of birds culled	0	1	1	2	1	1	0
No. of birds harvested	90	89	89	88	88	88	90

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

[a] Not significant ($P > 0.05$)

ideal conditions where the birds are housed properly and provided optimum nutrition, the effects of growth-enhancing additives are not required (Otutumi et al. 2012). No significant differences noted in the weekly average weights of the broilers suggest that no microbial or nutritional challenge exists in the whole flock, resulting in good growth performance.

All birds follow the same growth rate, although birds from different treatments grow slower compared to other birds at various growth stages (Table 6). An increasing trend in average body weight gain was observed during the first up to fourth week of age. Average weight gain of broilers from other treatments declined on the fifth week; thus, the inflection point or peak period of weight gain was on the fourth week of age, consistent with the harvesting schedule (25th–28th day of age) in some commercial farms. Slow growth of the broiler chickens in the last 2 weeks of the feeding trial could be the effect of summer temperature (28−39 °C at the time the birds were raised). In broilers, standard environmental temperature for brooders is 34 °C and decreases as the animal matures. For finishers, the optimum environmental temperature ranges from 24 to 26 °C. The chickens might have experienced stress resulting in decreased feed intake and poor growth rate.

Feed consumption

Significant differences on feed consumption were found only on the first and third weeks of the feeding period (Table 7). On the first week of age, birds under treatment 1 exhibited significantly higher ($P < 0.01$) feed consumption among other treatments while treatment 6 showed the lowest. Differences found in the average weekly feed consumption of birds on the first

Table 10 Average dressing percentage, percent breast yield and abdominal fat content of broilers fed with different kinds of probiotics

Treatment	Dressing percentage[a]	Breast yield (%)[a]	Abdominal fat content (%)[a]
TRT1	74.44	33.70	1.88
TRT2	72.51	36.21	2.13
TRT3	72.87	35.91	1.92
TRT4	72.96	33.76	1.64
TRT5	72.61	34.43	2.36
TRT6	75.47	34.06	1.70
TRT7	76.11	35.64	2.44
Average	73.89	34.90	2.05
C.V.	7.15	8.67	40.58

TRT1, basal diet (without probiotics and AGP)); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

[a] Not significant ($P > 0.05$)

Table 11 Meat to bone ratio of carcasses of broilers fed with different kinds of probiotics

Treatment	Meat:bone ratio		
	Breast[a]	Thigh[a]	Drumstick[a]
TRT1	6.64	5.71	2.65
TRT2	6.23	5.73	2.71
TRT3	6.46	5.47	2.46
TRT4	7.02	6.07	3.32
TRT5	5.79	5.47	2.61
TRT6	6.53	5.65	2.83
TRT7	6.34	5.75	2.72
Average	6.38	5.67	2.73
C.V.	20.06	15.19	28.84

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

[a] Not significant ($P > 0.05$)

Table 12 Average crude protein digestibility (%) and apparent metabolizable energy (AME) of broiler diets with basal diet, AGPs, and five different probiotics

Digestibility*	Treatment								
	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7	SEM	*P* value
AME kcal/kg	3670[ab]	3677[ab]	3684[ab]	3973[a]	3830[a]	3453[b]	3938[a]	72.42	0.002

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

*Nutrient digestibility measured during the feeding trial

[ab] Means in the same row with different superscript differ significantly based on Tukey's test at 5% level of confidence

week can be attributed to the unstable condition of intestinal microbiota (Fuller 2001).

On the third week, birds in treatment 2 consumed more feed compared to birds in treatments 3, 4, and 5, confirming study results by Zulkifli et al. 2000 indicating that feed intake improved significantly in ducks supplemented with lactic acid. Samli et al. (2007) and Jung et al. (2008), however, observed no significant difference in broiler feed intake after probiotic supplementation. Nunes et al. (2012) reported that the inclusion of *L. acidophilus*, *E. faecium*, and *Bifidobacterim bifidum* led to lower feed intake but has no effect on the growth performance of Cobb 500 strain of broiler both in clean and challenged environments. Differences in the results obtained in this trial and in previous studies could be due to the variation in the strains of the bacteria used (Otutumi et al. 2012). In addition, the strains of the probiotic used, method of preparation of the probiotics, dosage and mode of administration, composition of the diet, age of the bird, and sanitary conditions could also influence the efficacy of probiotic microorganism (Mountzouris et al. 2007).

Feed efficiency

Overall feed efficiency was found to not differ significantly. Significant differences were found only on the first and fourth weeks of feeding period (Table 8).

During the first week, birds in treatments 1, 4, and 5 exhibited significantly higher ($P < 0.01$) feed efficiency values compared to birds in treatment 7, indicating that the latter converts feed to meat more efficiently than other treatments. Dietary inclusion of probiotics in broiler diet has no significant effects on feed conversion ratio (FCR), consistent with the findings of Jung et al. (2008) and Salianeh et al. (2011). In contrast, Talebi et al. (2008) reported that probiotic supplementation in broiler diet improved FCR significantly.

Mortality and harvest recovery

As shown in Table 9, no significant differences were found among treatments since only two birds had died (one each from treatments 5 and 6). This agrees with the findings of Flores (2003) and Cavazzoni et al. (1998) that probiotics have no negative effects on the livability of broilers. Some studies have reported that several inoculations of probiotic in chicken feed mixture improved survival (Piao et al. 1999; Pascual, 1999 as cited in Flores 2003). Treatments 1 and 7 showed 100% harvest recovery.

Dressing percentage, percent breast yield, and abdominal fat content

Probiotics exhibited no significant influence on enhancing the dressing and percent breast yield of the treatment birds (Table 10), affirming the findings of Aceret (1988) and Mohan et al. (1996 as cited in Lope, 2003). Moreover, Weis et al. (2011) found no significant differences on carcass yield,

Table 13 Average villous height to crypt depth ratio of broilers fed with basal diet, AGPs, and five different probiotics

Section of the small intestine	Treatment							SEM	*P* value
	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7		
Duodenum	5.03	5.38	3.98	3.89	4.20	5.33	4.88	0.69	0.502
Jejunum	3.97	5.39	4.01	4.04	4.91	4.40	4.71	0.78	0.762
Ileum	4.33	4.90	4.06	4.12	4.14	4.31	4.88	0.81	0.974

TRT1, basal diet (without probiotics and AGP); *TRT2*, basal diet + (CTC and Zn bacitracin); *TRT3*, basal diet + probiotic A (*B. subtilis*); *TRT4*, basal diet + probiotic B (*B. subtilis*); *TRT5*, basal diet + probiotic C (*E. faecium*); *TRT6*, basal diet + probiotic D (*B. subtilis*); *TRT7*, basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)

Table 14 Income over feed and chick cost of birds fed with different kind of probiotics

Treatment	Ave. live weight (kg)	Feed costs	Chick cost	Probiotic costs	Total cost	Sales cost	IOFCC
T1 basal diet (control)	1.9	76.01	18.00	0.00	94.01	161.50	67.49
T2 basal diet + (CTC and Zn bacitracin)	1.93	77.60	18.00	0.04	95.65	164.05	68.40
T3 basal diet + probiotic A (*B. subtilis*)	1.87	75.34	18.00	0.57	93.91	158.95	65.04
T4 basal diet + probiotic B (*B. subtilis*)	1.88	77.60	18.00	0.59	96.19	159.80	63.61
T5 basal diet + probiotic C (*E. faecium*)	1.88	76.63	18.00	1.62	96.26	159.80	63.54
T6 basal diet + probiotic D (*B. subtilis*)	1.9	75.23	18.00	0.10	93.33	160.65	67.32
T7 basal diet + probiotic E (*E. faecium*, *Bifidobacterium* spp., *Pediococcus* spp., and *Lactobacillus* spp.)	1.91	75.09	18.00	0.71	93.80	162.35	68.55

although in another study, Weis et al. (2011) reported significantly less abdominal fat in Ross 308 broiler chickens supplemented with *S. faecium*. Pelicia et al. (2004) explained that these results could be due to absence of unbalanced microflora in the intestine. Moreover, the different biological promoters and chemicals which control the intestinal microflora could be similar.

Meat to bone ratio

No significant differences were observed among treatments for meat to bone ratio (Table 11), consistent with study results of Weis et al. (2011). Differences in valuable parts (thigh and breast) and carcass yield of Ross 308 and Hybro were not significantly different after probiotics supplementation.

Energy digestibility by total collection

Apparent metabolizable energy in treatments 4, 5, and 7 was found to be significantly higher ($P < 0.05$) than in treatment 6. Treatments 1, 2, and 3 have intermediate AMEs (Table 12). This is in contrast with the finding of Mountzouris et al. (2007), who reported that probiotic supplementation improves digestibility. Differences among values obtained in treatments 3, 4, and 6 might be due to the differences in the strain of *B. subtilis* used, as suggested in the study of Otutumi et al. (2012). Inclusion of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* in the diet results in an increased nutrient digestibility; this may improve growth performance during the starter phase, while no significant differences in growth performance were observed on day 22–42 (Bai et al. 2013).

Intestinal morphometry

No differences were found among the mean crypt depth, mean villi heights, and mean villous height to crypt depth ratio of the duodenum, jejunum, and ileum of broilers fed with basal diet and diets supplemented with AGPs and five different probiotics (Table 13). These results verified the findings of Olnood et al. (2015) who reported that supplementation of different strains of *Lactobacillus* species in broiler diets did not significantly improve villous height and crypt depth.

Profitability analysis

Table 14 shows the income over feed and chick cost of birds fed with different probiotics. Birds under treatment 2 have the highest average live body weight (1.93 kg), resulting in high sales. Meanwhile, broilers from treatment 7 had an average body weight of 1.9 kg but incurred the lowest feed and chick cost, producing the highest income.

Conclusions and recommendations

Given good biosecurity, proper management, and proper nutrition, antibiotics and/or probiotic supplementation on broiler diets has no effect on enhancing broiler production performance, carcass quality, energy digestibility, and intestinal morphometry. Supplementation of either antibiotic or probiotics shall only entail additional production cost. Further studies could explore the effects on different kinds of probiotics on broilers exposed to stressful conditions, where challenges in the gut microflora could be present.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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