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Research Article
The Supplementation Effects of Multienzymes and Synbiotics on Production Performance, Nutrient Utilization, Economic Value and *Salmonella* spp. Content of Broilers

Catootjie L. Nalle, Helda, D. Kusumaningrum, J. Pobas, A.A. Lay Riwu and Max A.J. Supit

Department of Animal Husbandry, Polytechnic of Agriculture Kupang, East Nusa Tenggara, Indonesia

Abstract

Background and Objectives: The use of enzymes either individually or in combination with other feed additives in poultry production has been studied thoroughly but the results are still contradictory. Thus, the objective of the present study was to evaluate the production performance, nutrient utilization, economic value and *Salmonella* spp. content of broilers fed diets containing multienzymes and synbiotics. **Materials and Methods:** The experiment was designed using a completely randomized design consisting of three treatments and six replicates. A total of 180 one-day-old unsexed Cobb chicks were randomly distributed into 18 pens (10 birds/pen) and fed a control diet (R0), diets supplemented with multienzymes (R1) and diets supplemented with multienzymes and synbiotics through drinking water (R2). The assay diets were offered *ad libitum* during the experiment. **Results:** No differences ($p>0.05$) were observed in the growth performance, apparent metabolizable energy values (AME/n), dry matter (DM), crude protein (CP) and phosphor (P) digestibilities, carcass traits, or economic value of broilers in all treatments. Significant differences ($p<0.05$ to $p<0.01$) were observed in the digestibility of neutral detergent fiber (NDF) and phytate as well as the *Salmonella* spp. content. Birds given R1 and R2 treatment diets had higher ($p<0.05$) NDF and phytic acid digestibilities than did those receiving the R0 treatment. The *Salmonella* spp. content of birds given the R1 and R2 treatments was lower ($p<0.05$) than that of birds fed the control diet. The *Salmonella* spp. content was similar ($p>0.05$) between the R1 and R2 treatments. **Conclusion:** The supplementation of multienzymes, alone or in combination with synbiotics, resulted in higher NDF and phytate digestibilities and a lower *Salmonella* spp. content. The AME/n and P digestibility were slightly improved by the treatments. The growth performance, dry matter and CP digestibilities, carcass traits and economic value of broilers were not influenced by the treatments.

Key words: Carcass traits, corn based diet, enzymes, nutrient utilization, poultry diet, production performance, synbiotics

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Corresponding Author: Catootjie L. Nalle, Department of Animal Husbandry, Polytechnic of Agriculture Kupang, East Nusa Tenggara, Indonesia
Tel: +62-82266275937 Fax: +62-380-881600

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobial growth promoters (AGPs) have been routinely used for a long time in poultry diets to improve the production performance of birds. According to Kiess[1], the US Food and Drug Administration has given permission to animal agriculture to use AGPs without the supervision of veterinarians since 1952. However, since it was found that AGPs caused bacterial resistance in birds and humans, the use of AGPs has been banned in some parts of the world, including Indonesia. The Indonesian government has launched a regulation regarding the ban on using AGPs in animal feed and drinking water since 2017 but the implementation of this regulation started in January 2018. In contrast, in European countries, Kocher[2] reported that the banned use of AGPs was practically implemented in January 1, 2006 (EU Regulation 1831/2003).

Based on this reality, it is clear that public awareness and concern about food safety and quality assurance has grown. Thus, it is crucial to use natural growth promoters (NGPs) in poultry diets. Enzymes, prebiotics, probiotics and synbiotics are examples of natural growth promoters that can be used safely in poultry diets. Chemically, the majority of enzymes are proteins and collectively are able to accelerate thousands of chemical reactions. The aims of using enzymes in poultry diets were to reduce the negative effects of antinutritional factors and improve nutrient digestion and bird performance.

A study conducted by Dersjant-Li *et al.*[3] showed that the growth performance of birds given diets containing phytase, xylanase, amylase and protease did not vary with that of birds given a control diet containing phytase, xylanase, amylase and protease plus probiotics. Ravindran[4] explained from his review that the inclusion of xylanase and phytase in a wheat basal diet improved nutrient utilization, growth performance and litter quality.

Probiotics, on the other hand, are produced from selected beneficial microbes such as Lactobacilli, Streptococci and Bacillus species and are used to improve intestinal health and animal performance. Prebiotics are fiber substrates that are used to stimulate the growth and/or activity of beneficial microorganisms (Lactobacilli and Bifidobacteria) to increase their beneficial effects[5]. A synbiotic product is a combination of prebiotics and probiotics. Synbiotics improve the growth and metabolism activity of probiotic bacteria and thus will improve their survival in the gastro-intestinal tract[5].

The efficacy of using probiotics and enzymes in poultry diets to improve digestion and performance, as well as to reduce pathogenic bacteria, has been studied extensively[3,4,6-9]; however, the published results are still inconsistent. In addition, the combination use of probiotics and enzymes as AGPs is still limited. Mountzouris *et al.*[10] reported from their study that the performance of broilers at 42 days of age given multistrain probiotics through diet and drinking water was similar to that of broilers given a control diet without probiotics. Caldwell *et al.*[11] suggested that birds fed a diet containing multistrain probiotics had better growth performance than those fed a control diet without probiotics. Vicente *et al.*[12] reported from their studies that probiotic administration reduced the incidence of *Salmonella* in commercial turkey flocks.

Recently, Polytechnic of Agriculture Kupang in West Timor Indonesia commercially produced a new brand of probiotics called Synbiotics Probio FM^{plus}. This new product, containing prebiotics and probiotics together, was produced through a research collaboration between the Faculty of Veterinary Science, Jambi University and Polytechnic of Agriculture Kupang, Indonesia. The investigation of using this product in combination with multienzymes has not yet been conducted. Thus, this research was designed to investigate the effects of supplementation of multienzymes and Synbiotics Probio FM^{plus} on the production performance, carcass percentage and quality, nutrient digestibility, economic value and health status of broilers fed corn-based diets containing rice bran and *putak* meal (sago).

MATERIALS AND METHODS

Chicks: A total of 180 one-day-old broiler chicks (mixed female and male, Cobb) obtained from local commercial hatchery were randomly distributed into 18 pens (10 birds/pen). The birds were kept for 21 days in the floor pens. Then, on the 22nd day, the birds were moved to 36 metabolic cages (five birds each). The separation of birds was conducted for digestibility and AME assays.

Feedstuffs

Sago (*Putak* meal): Sago was obtained from the pith stem of *gebang* tree (*Corypha utan* Lamk). The thick bark of the stem was removed to obtain the pith. The pith rods were then cut into 8-10 parts. Each part was chopped into small pieces and ground using a hammer mill (3 mm screen size), sun-dried, sieved (2 mm screen size) and mixed (Fig. 1). Avizyme 1502 and Phyzyme XP 5000 G are the commercial products from Danisco Animal Nutrition. Phyzyme XP 5000 G contains phytase from an *Escherichia coli* strain, while Avizyme 1502 contains amylase, protease and xylanase.

Fig. 1: Sago (*putak* meal)

Synbiotics Probio FM^{plus} contains lactic acid bacteria (*Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Pediococcus pentosaceus*) in an amount ranging from 36.1×10^{11} to 210×10^{11} CFU mL^{-1}, with the pH between 3.00 and 3.40. This product was created through a research collaboration between the Polytechnic of Agriculture Kupang and the University of Jambi, Indonesia. The lactic acid bacteria were taken from the Faculty of Veterinary Science, Jambi University-Indonesia. Sago (*putak* meal) from the pith of the gebang tree trunk (*Corypha utan* Lamk) and liquid palm sugar from the *lontar* tree (*Borassus flabellifer)* as substrates (prebiotics) for probiotics are local products that are found abundantly in West Timor, East Nusa Tenggara Province, Indonesia. The procedure of making Synbiotics Probio FM^{plus} was as follows: sago (*putak* meal) was mixed with water and liquid palm sugar, boiled for ten minutes and then cooled it down. Lactic acid bacteria were then added into the solution and incubated for 48 h at 38°C. After incubation, the product was ready to use at a dose of 20 mL L^{-1} drinking water.

Experimental design: A completed randomized design with three treatments and six replications was used during the 35-day experiment. The treatments were R0 (control diet), R1 (supplemented with Avizyme 1502 and Phyzyme XP G 5000) and R2 (supplemented with Avizyme 1502 and Phyzyme XP G 5000, plus Synbiotics Probio FM^{plus} 20 mL L^{-1} in drinking water). The treatment diets based on corn-rice bran-sago (isonitrogenous-isoenergetic) (Table 1) were offered *ad libitum* in crumble form to six replicate pens of broilers (10 birds/pen) during the 35-day experiment. Synbiotics Probio FM^{plus} was given through drinking water (20 mL L^{-1}) for 8 h a day.

Bird management: Birds were housed in floor pens in a semi-open house during the starter period (0-1 days) and then, they were moved to metabolic cages until day 35. The birds were fed the treatment diets *ad libitum* and given free access to drinking water. The birds in the R2 treatment group were given drinking water supplemented with Synbiotics Probio FM^{plus} for eight hours; then, they were given drinking water without Synbiotics Probio FM^{plus}. The respective average minimum and maximum temperatures of housing during the

Table 1: Treatment diets

	R0 (g kg^{-1})	R1 (g kg^{-1})	R2 (g kg^{-1})
Yellow corn (8.5% CP)	403.000	403.000	403.000
Rice bran	50.000	50.000	50.000
sago (*putak* meal)	100.000	100.000	100.000
Soybean meal (44.0% CP)	308.500	304.900	304.900
Meat and bone meal	700.000	70.000	70.000
Fish meal (local)	29.300	29.300	29.300
Vegetable oil	20.000	20.000	20.000
L-Lysine	2.500	2.500	2.500
DL-Methionine	2.500	2.500	2.500
Limestone feed grade	1.000	1.000	1.000
Dicalcium phosphate	10.000	10.000	10.000
Salt	2.500	2.500	2.500
Sodium bicarbonate	0.700	0.700	0.700
Vitamin-Mineral Premix[1]	3.000	3.000	3.000
Avizyme 1502[2]	-	0.500	0.500
Phyzyme XP 5000 G[2]	-	0.100	0.100
Synbiotics Probio FM^{plus} (20 mL L^{-1} drinking water)[3]	-	-	+
Total	100.000	100.000	100.000
Calculated analysis			
AME (kcal kg^{-1} DM)	2,787.000	2.779	2.779
Crude protein (g kg^{-1})	229.000	228.000	228.000
Crude fiber (g kg^{-1})	19.560	19.560	19.560
Lysine (g kg^{-1})	14.900	14.800	14.800
Met+Cys (g kg^{-1})	11.300	11.300	11.300
Ca (g kg^{-1})	12.900	12.900	12.900
Av P (g kg^{-1})	5.500	5.500	5.500
Laboratory analysis			
AME (kcal kg^{-1} DM)	2.941	2.890	2.910
Crude protein (g kg^{-1} DM)	217.300	214.700	213.300
NDF (g kg^{-1} DM)	516.400	688.100	745.600
P (g kg^{-1} DM)	5.510	5.210	3.140
Phytic acid (g kg^{-1} DM)	34.900	34.400	36.900

[1]Sanmix, PT Sanbe Farma, per kg provided; Vit A: 1250000 IU, Vit D3: 250000 IU, Vit E: 750 IU, Vit K: 200 mg, Vit B1: 150 mg, Vit B2: 500 mg, Vit B6: 500 mg, Vit B12: 1012 mcg, Vit C: 3000 mg, Ca-d-pantothenate: 500 mg, Niacin: 3500 mg, Methionine: 3500 mg, lysine: 3500 mg, Manganese: 10000 mg, Iron: 2500 mg, Iodine: 20 mg, Zinc: 10000 mg, Cobalt: 20 mg, Copper: 300 mg and Antioxidant: 1000 mg, [2]Bran products of Danisco Animal Nutrition, [3]Bran product of the Polytechnic of Agriculture Kupang, Indonesia

experimental period were 31°C and 33°C (days 1-7), 29.4°C and 32°C (days 8-14), 29.7°C and 36.3°C (days 15-21), 26.4°C and 37.5°C (days 22-28) and 27.3°C and 37.5°C (days 29-35). The respective indoor relative humidity (minimum and maximum) was 43 and 55% (days 1-7), 36 and 54% (days 8-14), 37 and 54% (days 15-21), 38 and 52% (days 22-28) and 48 and 60% (days 29-35).

AME and digestibility assay: The AME assay was conducted on day 28 until day 35 using the classical total excreta collection method. Feed intake and excreta were collected from day 32-day 35. The excreta were then mixed, sub sampled and oven-dried (60°C) for two days. The excreta and treatment diets were ground (0.5 mm sieve) and stored in a sealed plastic bag for the determination of the dry matter, gross energy, nitrogen, phosphor and phytic acid contents.

Identification and quantification of *Salmonella* spp: (1) Ten birds from each treatment (2) birds/cage) were physically euthanized by cervical dislocation; then, the birds were dissected and the ileum part of the small intestine was removed.

Pre-enrichment: One gram of ileal digesta was then collected and diluted into lactose broth (LB) solution, homogenized and incubated for 24 h (37°C).

Enrichment: One milliliter of the solution from step one was taken and diluted into tetrathionate broth solution and incubated for 24 h (37°C).

Inoculation of bacteria into selective media: One single colony from step 3 was taken and inoculated in bismuth sulfite agar (BSA) media and incubated for 24 h (37°C).

Dilution: One single colony of bacteria from BSA media was taken and diluted three times into tryptone broth (TB) solution and then homogenized (10^{-3}).

***Salmonella* spp. identification:** Triple sugar iron agar (TSIA) and peroxide tests were conducted to prove the presence of *Salmonella* spp. The TSIA test was conducted on bacterial isolates inoculated onager medium containing glucose, lactose, sucrose and maltose. Then, the medium containing bacteria was incubated for 2×24 h at 37°C. If the color of the medium turns yellow, it means that the conditions are acidic. If the color turns red, it means that the conditions are basic. A black color shows that H_2S was formed. A peroxide test was conducted by dropping 2-3 drops of H_2O_2 into tubes containing bacteria. If the reaction shows gas bubbles, it means that the sample positively contains *Salmonella* spp.

Quantification of *Salmonella* spp. using total plate count (TPC): One milliliter of TB solution containing *Salmonella* spp. (Step 4) was pipetted and poured into plate count agar media (PCA) and incubated for 24 h (37°C). Then, the *Salmonella* spp. colonies were quantified using a colony counter (Funke Gerber, ART No 8500-Deutsch).

Carcass yield and carcass quality measurement: On day 35, three birds with weight nearest to the average weight of the pen were randomly selected, identified and then slaughtered (killed, bled, plucked and eviscerated) and dried before being reweighed without a head, neck, feet or gut to obtain the carcass weight. Before weighing, the carcass was dried using a clean and dry cloth. The quality of broiler meat was then examined. Breast and chest parts of broilers (2×2 cm) were evaluated to measure the quality of broiler meat using a texture analyzer (Brookfield).

Data collection: The body weight of birds was measured at days 1, 21 and 35 and then, body weight gain was calculated. Feed intake was recorded weekly. Mortality was recorded daily. Feed intake and mortality data were used to calculate FCR. The carcass percentage, economic value, apparent metabolizable energy (AME/n) and nutrient digestibility were calculated using the following formulas:

$$\text{Carcass percentage}^{13} = \frac{\text{Carcass weight}}{\text{Live weight}} \times 100\%$$

$$\text{Broiler feed efficiency (BFE)}^{14} = \frac{\text{BWG (g bird}^{-1})}{\text{FI (g bird}^{-1})}$$

$$\text{Income over feed cost (IOFC)}^{15} = \text{Income-production cost}$$

$$\text{Feed cost per gain (FCG)}^{16} = \frac{\text{Feed cost (Rp kg}^{-1})}{\text{Weight gain (g bird}^{-1})}$$

$$\text{PI}^{17} = \frac{(100\text{-D}) \times \text{BW} \times 100}{\text{FCR} \times \text{DF}}$$

PI : Performance index
D : Depletion (% mortality)
BW : Body weight (g bird^{-1})
FCR : Feed conversion ratio
DF : Duration fattening (days)

$$\text{AME}_{\text{diet}}\ (\text{MJ kg}^{-1})^{18} = \frac{(\text{Feed intake} \times \text{GEdiet}) - (\text{excreta output} \times \text{GE}_{\text{excreta}})}{\text{Total feed intake}}$$

A factor of 36.54 kJ g^{-1} N retained in the body was used to calculate zero nitrogen retention[19].

$$\text{Digestible coefficient nutrient diet}^{18} = \frac{(\text{Feed intake} \times \text{nutrient}_{\text{diet}}) \times (\text{total excreta} \times \text{nutrient}_{\text{excreta}})}{\text{Feed intake} \times \text{nutrient}_{\text{diet}}}$$

Chemical analysis: The dry matter content was determined using AOAC method No. 930.15[20]. The crude protein content was analyzed using AOAC 2001.11[20] and AOAC 942.5[20] (van Soest method) was used to determine the NDF content. Phytic acid was analyzed using a spectrophotometry method. A PARR 1341 Plain Oxygen Bomb Calorimeter was used to measure the gross energy level.

Statistical analysis: Experimental data were analyzed by the GLM procedure of SAS version 9.1 (SAS Institute University Edition)[21]. Differences between treatments were calculated to be significant at $P < 0.05$. Significant differences among the treatments were calculated using Fisher's least significant difference test.

RESULTS

Production performance: As shown in Table 2, no significant differences ($p>0.05$) were found in any of the parameters observed. However, the group of birds fed R1 and R2 treatment diets showed a numerically improvement in feed efficiency. The lowest FCR was observed in the R2 treatment (2.177), followed by the R1 treatment (2.215) and R0 treatment (2.284).

Nutrient digestibility coefficient: The results showed that significant differences ($p<0.05$ to $p<0.01$) were observed in

Table 2: The Effect of Treatments on Production Performance of Broilers (35 days)[1]

	Treatments				
Variables	R0	R1	R2	SEM	p-value
0-21 days					
Initial BW (g bird^{-1})	51.200	51.670	51.610	0.126	0.275
BW 21 day (g bird^{-1})	585.100	577.600	582.100	18.160	0.987
BWG (g bird^{-1})	533.900	525.900	530.500	33.300	0.986
FI (g bird^{-1})	1173.000	1090.000	1173.000	42.900	0.419
FCR (g g^{-1})	2.284	2.215	2.177	0.170	0.905
Mortality (%)	0.030	0.000	0.000	0.007	0.116
22-35 days					
BWG (g bird^{-1})	753.190	738.860	692.000	24.740	0.229
FI (g bird^{-1})	1557.000	1430.000	1477.000	73.230	0.592
FCR (g g^{-1})	2.079	2.079	2.079	0.104	0.405
Mortality (%)	0.000	0.000	0.000	0.000	0.000
0-35 days					
BW 35 day (g bird^{-1})	1338.000	1316.000	1274.000	24.840	0.591
BWG (g bird^{-1})	1287.000	1264.000	1222.000	42.430	0.589
FI (g bird^{-1})	2704.000	2443.000	2525.000	91.960	0.171
FCR (g g^{-1})	2.153	1.937	2.111	0.190	0.404
Mortality (%)	0.030	0.000	0.000	0.007	0.116

Not significant difference, p>0.05, [1]Each value was the average of 6 replicates (10 birds each)

Table 3: The Effect of treatments on the apparent metabolizable energy and nutrient digestibility coefficient of broiler chickens (35 days)

	Treatments				
Variables	R0	R1	R2	SEM	p-value
Crude protein consumed (g bird^{-1})	565.000	524.000	502.000		
Crude protein output (g bird^{-1})	202.000	206.000	208.000		
Crude protein digestibility coefficient	0.639^{a}	0.607^{a}	0.595^{a}	0.012	0.090
Dry matter digestibility coefficient	0.652^{a}	0.625^{a}	0.619^{a}	0.323	0.085
Phosphor consumed (g bird^{-1})	14.320	12.970	12.210		
Phosphor consumed (g bird^{-1})	7.050	5.900	5.440		
Phosphor dig coeff	0.506^{a}	0.545^{a}	0.553^{a}	0.021	0.269
NDF consumed (g bird^{-1})	1242.000	1754.000	1680.000		
NDF output (g bird^{-1})	373.000	384.000	363.000		
NDF digestibility coefficient	0.699^{b}	0.781^{a}	0.785^{a}	0.010	0.0004
Phytic acid (g bird^{-1})	83.920	77.570	79.940		
Phytic acid output (g bird^{-1})	23.240	15.010	15.920		
Phytic acid dig coeff	0.723^{b}	0.806^{a}	0.802^{a}	0.018	0.021
Apparent metabolizable energy (AME, kcal kg^{-1} DM)	2888.000^{a}	2900.000^{a}	2936.000^{a}	15.690	0.065
Nitrogen-corrected apparent metabolizable energy (AMEn, kcal kg^{-1} DM)	2,714.000^{a}	2,718.000^{a}	2,743.000^{a}	14.040	0.078

^{a,b}Mean values in the same row with different superscript indicate significant differences, (p <0.05), each value was the average of 6 replicates (5 birds each)

the digestibility coefficient of NDF and phytic acid (Table 3). Birds given the R1 and R2 treatments had higher (p<0.05) NDF and phytic acid digestibility coefficients than did those receiving the R0 treatment. The P and crude protein digestibility coefficients were not improved (p>0.05) by the treatments.

Health status of broilers: Both sugar and catalase tests proved that all treatments positively contained *Salmonella* spp. bacteria (Table 4). Significant differences (p<0.001) were found in the *Salmonella* spp. content. The group of broilers fed a control diet (R0) had higher (p<0.05) *Salmonella* spp. content than did those receiving the R1 and R2 treatments. The Salmonella spp content of broilers fed diets containing multienzymes (R1) was similar (p>0.05) to that in broilers given a control diet supplemented with multienzymes along with Synbiotics Probio FM*plus* in the drinking water (R2).

Economic value: It can be seen from Table 5 that the income over feed cost (IOFC), broiler feed efficiency (BFE), feed cost per gain (FCG) and performance index (PI) were not affected (p>0.05) by all treatments.

Table 4: Qualitative and quantitative tests of *Salmonella* spp. On broilers given three different treatments (35 days)[1]

	Treatments				
Variables	R0	R1	R2	SEM	p-value
TSIA[2] test	+	+	+		
Peroxide test	+	+	+		
Salmonella spp content (CFU mL^{-1}, $\times 10^3$)	534[a]	281[b]	304[b]	23.75	0.0007

[a,b]Mean values in the same row with different superscript indicate significant differences, ($p < 0.05$), [1]Each value was the average of 4 replicates (2 birds each)
[2]TSIA: Triple sugar iron test

Table 5: The effect of treatments on economic value of broilers (35 days)[1]

	Treatments				
Variables	R0	R1	R2	SEM	p-value
IOFC (Rp kg^{-1})	16.445	17.761	15.558	1152.000	0.4371
BFE (%)	47.630	52.070	48.570	1.511	0.1339
FCG (Rp kg^{-1})	19.999	18.471	19.755	567.810	0.1609
PI	183.180	202.350	166.260	14.190	0.2590

Not significant difference, $p > 0.05$, [1]Each value was the average of 6 replicates (10 birds each)

Table 6: The effect of treatments on carcass quality of broilers (35 days)

	Treatments				
Variables	R0	R1	R2	SEM	p-value
Carcass percentage (%)	66.69[ab]	65.22[b]	68.32[a]	0.850	0.023
Hardness (g)	4.01[a]	4.13[a]	4.27[a]	0.186	0.663
Adhesiveness (g sec^{-1})	24.45[a]	24.10[a]	3.20[a]	3.260	0.963
Fracturability	4.01[a]	4.13[a]	4.27[a]	0.186	0.623
Cohesiveness	-304.50[a]	-245.30[a]	-187.90[a]	67.130	0.484
Gumminess	1097.00[a]	1013.00[a]	1039.00[a]	235.600	0.968

[a,b]Mean values in the same row with different superscript indicate significant difference, ($p < 0.05$), [1]Each value was the average of 6 replicates (3 birds each)

Carcass percentage and quality: Values of carcass percentage and quality are presented in Table 6. The treatment diets significantly affected ($p<0.05$) the carcass percentage of broilers but did not affect ($p>0.05$) the hardness, adhesiveness, fracturability, cohesiveness and gumminess of broiler meat in all treatments. The carcass percentage of broilers given the combination of Synbiotics Probio FM*plus* and multienzymes (R2) was higher ($p<0.05$) than that in broilers receiving the diet containing only multienzymes (R1). No significant differences were observed between the R2 and R0 treatments or between the R0 and R1 treatments.

DISCUSSION

It appears that the treatments did not affect the growth performance, carcass percentage, or quality and economic value of broiler chickens during the 35-day experiment. However, the feed per gain of broilers in the R1 and R2 treatments was numerically lower than that in broilers fed the R0treatment. The lowest FCR was observed in the R2 treatment (2.177), followed by the R1 treatment (2.215) and R0 treatment (2.284). Except for weight gain and feed intake, Nalle and Yowi[22] similarly observed that combination use of Avizyme and Phyzyme did not improve the feed per gain and mortality rate in 21-day-old birds. The result of the present study is in contrast to the results of Attia *et al.*[23], who reported that the supplementation of multienzymes containing Avizyme and Phyzyme improved the feed intake, body weight gain and feed efficiency of broilers at day 20 of the experiment. Hartini *et al.*[24]also found that supplementation of Phyzyme in a basal diet improved the growth performance of broilers at day 21 of the experiment. Such contradictory evidence from studies was probably due to the different methodologies used. In this experiment, the measurement of growth performance was conducted on day 35, while early works conducted by Attia *et al.*[23] and Nalle and Yowi[16] measured growth performance on days 20 and 21, respectively.

The results obtained between the R1 and R2 treatments in terms of weight gain were in agreement with those of Dersjant-Li *et al.*[3], who found that the weight gain of birds fed control diets containing phytase, xylanase, amylase and protease was similar to that of birds fed a control diet containing phytase, xylanase, amylase and protease plus

directly fed microbials. Thus, it is indicated from the present results that the supplementation of Synbiotics Probio^{Fmplus} through drinking water did not provide a beneficial effect to the body weight gain of birds.

Regarding the digestibility assay, broilers given diets containing Avizyme and Phyzyme (R1) or a combination of Avizyme and Phyzyme and Synbiotics Probio FM^{plus} (R2) had no effect on the dry matter and protein digestibility coefficients but the treatment increased the phytic acid and NDF digestibility coefficients. Attia *et al.*[23] similarly found that supplementation with Avizyme and Phyzyme did not have a beneficial impact on dry matter and crude protein digestibility. In contrast, a study conducted by Cowieson and Ravindran[7] demonstrated that the supplementation of phytase, xylanase, amylase and protease improved the digestibility of protein and carbohydrate. Ravindran[4] explained from his review that simultaneous inclusions of xylanase and phytase in wheat-based broiler diets resulted in an improvement in protein and energy utilization, growth performance and litter quality.

Regarding phytic acid digestibility, the results agreed with those of Selle *et al.*[25,26] who explained that phytases have the capacity to hydrolyze one phytate molecule (myo-inositol hexaphosphate; IP_6) completely to inositol and to release six P moieties. Thus, these enzymes will reduce the excretion of phytate phosphorus[4]. The use of exogenous phytase has been proven to improve P digestibility and utilization, hence decreasing P excretion into the environment[6].

The improvement of phytic acid digestibility observed in the present study was between 10.93 and 11.48%. These values were lower than that noted by Slominsky[8], who reported approximately 20% in his review. According to the author, the inclusion rate of exogenous phytase was not the main cause of the low liberation of P from phytate by exogenous phytase but was more likely due to the inaccessibility of phytate molecules for hydrolysis. This condition was triggered by the formation of insoluble phytate-Ca complexes that are resistant to enzymatic hydrolysis by phytase[7,25,26]. Thus, according to Selle *et al.*[26], calcium is the limiting factor for phytate hydrolysis in the gastrointestinal tract. Furthermore, the authors also explained that Ca-phytate complex formation is affected by constituent molar ratios and the gut pH and the reduced solubility means that this phytate is less readily degraded by phytase.

The improvement of the NDF digestibility coefficient in the present study was approximately 11.71-12.30%. No differences were found in the NDF digestibility coefficients between the R1 and R2 treatments. This result indicated that the supplementation of Synbiotics Probio FM^{plus} through drinking water did not have a beneficial effect on NDF and phytic acid digestibility.

It is interesting to note that broilers fed R1 and R2 treatment diets had a lower Salmonella spp content than that of broilers fed control diets. However, the Salmonella content found in the R1 and R2 treatments was not significantly different. This result indicated that the decreased intestinal Salmonella spp content in both treatments was solely due to the supplementation of multienzymes, not because of Synbiotics Probio FM^{plus}. This was an unexpected result because the supplementation of Synbiotics Probio FM^{plus} was expected to decrease the *Salmonella* spp. content more in the broiler intestine. Therefore, the lack of increased Salmonella content in the R2 treatment might be due to the change in pH of the drinking water leading to a reduction in lactic acid bacteria activity.

The mechanism of enzymes reducing the intestinal *Salmonella* spp. content in this research could be explained as follows: the fiber-degrading enzyme (xylanase) improved fiber (NDF) digestion. The improvement in fiber digestion would in turn reduce the gut viscosity. It is well known that gut viscosity can cause anaerobic conditions. These conditions provide a good medium for pathogenic bacteria to proliferate. As the gut viscosity decreases, the proliferation of pathogenic bacteria in the poultry gut decreases.

The present study indicated that the improvement in NDF and phytic acid digestibilities as well as the decrease in Salmonella spp content did not produce any improvement in growth performance, apparent metabolizable energy (AME/n), or protein digestibility of broilers during the experiment. The lack of improvement in growth performance, (AME/n) and protein digestibility was probably due to two main factors. The first factor was probably due to the low improvement of NDF and phytic acid digestibilities (11.71-12.30% for NDF and 10.93-11.48% for phytic acid). The second factor was the low percentage decrease in the abundance of *Salmonella* spp. In addition, no improvement in protein digestibility was probably another factor that was responsible for the lack of improvement in the growth performance of growing birds.

The income over feed cost, broiler feed efficiency, feed cost per gain and performance index of broilers were not affected by the supplementation of multienzymes or the combination of multienzymes and Synbiotics Probio FM^{plus}. Regarding the performance index, the present result did not agree with that of Attia *et al.*[23], who found that supplementation of enzymes resulted in a greater production

index. This difference was probably due to the methodology used and the efficacy of the enzymes used. The results of this study imply that multienzymes can be used to improve the digestibility of nutrients and digestive tract health, as well as reduce the negative effects of fiber and phytic acid in broiler diets that contain putak and rice bran. However, in this study, it was observed that the combination of multienzymes and synbiotics did not elicit an increase in production performance, nutrient digestibility and gut health, so it is necessary to conduct a further study of what factors caused the multienzymes and synbiotics to not work synergistically. In addition, it is also necessary to compare the effects of using multienzymes and synbiotics separately and at higher doses than those recommended.

CONCLUSION

The supplementation of enzymes or combined use of enzymes and probiotics resulted in higher NDF and phytate digestibilities, a lowered *Salmonella* spp. content and a slight influence on AME/n and phosphor digestibility but had no effects on the growth performance, dry matter and crude protein digestibilities, carcass traits, or economic value of broilers.

SIGNIFICANCE STATEMENT

This study discovers the response of broiler chickens fed corn-sago-soybean meal diets containing commercial multienzymes and Synbiotics Probio FM^{plus}. This study is essential to help researchers reveal the role of multienzymes and synbiotics in replacing antibiotic growth promoters. The Synbiotics Probio FM^{plus} used in the present study is a product produced through a collaboration between the Polytechnic of Agriculture Kupang and the University of Jambi. Thus, a new theory regarding the use of multienzymes and synbiotics may be developed to maximize the genetic potential of broiler chickens through improvements in nutrient digestibility and gut health.

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REFERENCES

1. Kiess, A.S., 2018. *In ovo* delivery of probiotics: A novel strategy to the antibiotic dilemma. Proceedings of the 20th Massey University Poultry Industry Conference, May 15, 2018, Monogastric Research Center, Massey University, Palmerston North, New Zealand, pp: 23.
2. Kocher, A., 2006. Threats and challenges affecting the viability of poultry production in Europe. Proceeding of New Zealand Poultry Industry Conference in Collaboration with Massey University, (NZPI'06), Palmerston North, New Zealand, pp: 31.
3. Dersjant-Li, Y., A. Awati, C. Kromm and C. Evans, 2014. A direct fed microbial containing a combination of three-strain Bacillus sp. can be used as an alternative to feed antibiotic growth promoters in broiler production. J. Applied Anim. Nutr., Vol. 2. 10.1017/jan.2014.4
4. Ravindran, V., 2013. Feed enzymes: The science, practice and metabolic realities. J. Applied Poult. Res., 22: 628-636.
5. Ravindran, V., 2013. Feed enzymes: The science, practice and metabolic realities. J. Applied Poult. Res., 22: 628-636.
6. Sohail, S.S., M.M., Bryant, D.A., Roland, H.A. Apajalahti and E.M. Pierson, 2003. Influence of Avizyme 1500 on performance of commercial leghorns. J. Applied Poult. Res., 12: 284-290.
7. Cowieson, A.J., T. Acamovic and M.R. Bedford, 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci., 45: 101-108.
8. Slominski, B.A., 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci., 90: 2013-2023.
9. Hassan, H.M.A., A. Samy, A.W. Youssef and M.A. Mohamed, 2018. Using different feed additives as alternative to antibiotic growth promoter to improve growth performance and carcass traits of broilers. Int. J. Poult. Sci., 17: 255-261.
10. Mountzouris, K.C., P. Tsirtsikos, E. Kalamara, S. Nitsch, G. Schatzmayr and K. Fegeros, 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult. Sci., 86: 309-317.
11. Caldwell, D.J., N.H. Eckert, J.T. Lee, S.M. Stevens, R. Beltran, M. Mohnl and G. Schatzmayr, 2007. Evaluation of drinking water or post-pelleting probiotic administration alone or in combination with a phytogenic product on broiler performance. Proceedings of the International Poultry Scientific Forum, January 22-23, 2007, Atlanta, Georgia, USA., pp: 25.
12. Vicente, J.L., G.M. Nava, R.L. Jarquin, A. Torres-Rodriguez and C.D. Sartor *et al*., 2004. Evaluation of probiotic cultures on Salmonella prevalence in commercial Turkey houses. Proceedings of the International Poultry Scientific Forum, January 26-27, 2004, Atlanta, Georgia, pp: 10.

13. Nalle, C.L., V. Ravindran and G. Ravindran, 2010. Evaluation of faba beans, white lupins and peas as protein sources in broiler diets. Int. J. Poult. Sci., 9: 567-573.
14. Lumpkins, B.S., A.B. Batal and N.M. Dale, 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poult. Sci., 83: 1891-1896.
15. Santoso, S.I., S. Susanti and A. Setiadi, 2017. Economic analysis of male broiler chickens fed diets supplemented with *Salvinia molesta*. Int. J. Poult. Sci., 16: 233-237.
16. Mateo, C.D. and N.F. Carandang, 2006. Feeding and economic evaluation of corn, wheat and sorghum based-diets in broilers. Philip. J. Sci., 135: 49-58.
17. Mitrović, S., V. Dermanović, M. Radivojević, Z. Rajić, D. Zivković, D. Ostojić and N. Filipović, 2010. The influence of population density and duration of breeding on broiler chickens productivity and profitability. Afr. J. Biotechnol., 9: 4486-4490.
18. Nalle, C.L., G. Ravindran and V. Ravindran, 2010. Nutritional value of faba beans (*Vicia faba* L.) for broilers: Apparent metabolisable energy, ileal amino acid digestibility and production performance. Anim. Feed Sci. Technol., 156: 104-111.
19. Hill, F.W. and D.L. Anderson, 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr., 64: 587-603.
20. AOAC., 2005. Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 978-0935584752.
21. SAS., 2013. SAS user's guide statistics. Software Version 9.4. SAS Inst Institute, Inc., Cary, NC, USA.
22. Nalle, C.L. and M.R.K. Yowi, 2018. The efficacy of multy-enzymes supplementation on low energy corn-soybean meal basal diet containing rice bran on growth performance of broilers. Int. J. Agric. Syst., 6: 138-145.
23. Attia, Y.A., W.S. El-Tahawy, A.E.E. Abd El-Hamid, S.S. Hassan, A. Nizza and M.I. El-Kelaway, 2012. Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci., 11: 303-308.
24. Hartini, S., D.D. Rahardjo and P. Purwaningsih, 2019. The effects of rice hull inclusion and enzyme supplementation on the growth performance, digestive traits, dry matter and phosphorus content of intestinal digesta and feces of broiler chickens. Int. J. Poult. Sci., 18: 21-27.
25. Selle, P.H., A.J. Cowieson and V. Ravindran, 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci., 124: 126-141.
26. Selle, P.H., V. Ravindran, A.J. Cowieson and M.R. Bedford, 2010. Phytate and phytase. In: Enzymes in farm animal nutrition. 2nd Edition. Bedford, M.R. and G.G. Partridge, CAB International, Wallingford, Oxforshire, UK.