

Effects of enzyme and synbiotic supplementation on energy, nutrient digestibility, and health status of broilers fed high sago-rice bran diets

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ABSTRACT

Studies on enzyme and synbiotic supplementation in sago- and rice-bran-based diets are limited. This study examined whether enzyme and synbiotic supplementation could improve energy utilisation, nutrient digestibility, and health status in broilers fed diets containing 25% sago and 10% rice bran. Two hundred broilers were assigned to a completely randomised design with four treatments (control, synbiotics, enzymes, and combined supplementation). Feed intake (FI), nitrogen-corrected apparent metabolisable energy (AMEn), nutrient digestibility, and *Salmonella* counts were measured. FI differed significantly among treatments, decreasing progressively from control (1,992 g/bird) to combined treatment (1,588 g/bird). AMEn was also reduced in the combined treatment (9.6 MJ/kg) compared to other groups (11.2–11.5 MJ/kg). Neither synbiotics nor enzymes alone affected crude protein or NDF digestibility or *Salmonella* counts. Combine supplementation reduced both AMEn and phosphorus digestibility, potentially due to altered mineral solubility and microbial phosphorus incorporation. Overall, neither supplement improved AMEn or nutrient digestibility in these high-fibre diets. Although *Salmonella* counts did not differ significantly, synbiotic-supplemented birds showed numerically lower loads, suggesting a potential beneficial trend in gut microbial balance.

INTRODUCTION

Dry-shelled maize, including both white and yellow varieties, constitutes the primary energy source in the feed industry because of its high carbohydrate content, excellent palatability, and relatively low concentrations of antinutritional factors such as crude fibre and phytic acid. However, heavy reliance on maize presents economic challenges because of price volatility and competition with the human food and other industrial sectors. In local markets, maize prices fluctuate seasonally; during the rainy season, mass production may reduce prices to approximately IDR 5,000/kg, whereas in the dry season, limited supply can increase prices to between IDR 7,000 and 12,000/kg. National price variation, including in East Nusa Tenggara,

is also influenced by grain moisture content, with maize with higher moisture content generally priced lower than properly dried grain.

Rising maize prices directly increase feed production costs, thereby reducing profitability in poultry enterprises. Consequently, there is a need to explore locally available and sustainable alternative feed resources. Sago (*putak meal*) derived from the gebang palm (*Corypha utan Lamk*) (Nalle et al., 2021), and rice bran are abundant local feedstuffs in East Nusa Tenggara with potential to partially replace maize in poultry and swine diets. Nevertheless, both ingredients contain substantial levels of antinutritional compounds, including non-starch polysaccharides, phytic acid, tannins, phenolic compounds, and flavonoids, which limit their dietary inclusion (Manzoor et al.,



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2023; Irakly et al., 2021; Nalle et al., 2017; Nalle et al., 2021). Rice bran was reported to contain 16.6% crude fibre (Isah et al., 2023). These antinutritional factors adversely affect nutrient digestibility, energy utilization, and mineral availability. Non-starch polysaccharides increase intestinal viscosity, thereby hindering the activity of amylase, protease, and lipase in degrading starch, protein, and lipids within the small intestine (Raza et al., 2019; Tejeda et al., 2021). Consequently, nutrients remain unavailable and are excreted, potentially leading to suboptimal growth. Phytic acid chelates essential minerals such as phosphorus, calcium, iron, and magnesium, rendering them unavailable to poultry. Rice bran, comprising the pericarp, aleurone layer, and germ, contains 5.94–6.09% phytic acid (Manzoor et al., 2023). Poultry lack endogenous enzymes capable of degrading phytic acid and complex fibre fractions; therefore, inclusion levels of rice bran and sago are generally < 200 g/kg in broiler diets (Rizwanuddin et al., 2023; Raza et al., 2019; Nalle et al., 2017).

Strategies to enhance the nutritional value of sago and rice bran include fermentation, supplementation with exogenous enzymes or synbiotics, extrusion cooking, and roasting (Adekunle et al., 2024; Habibollahi et al., 2019; Mujahid et al., 2004; Supriyati et al., 2015). Fermentation of rice bran with *Aspergillus niger* reduced fibre content by 47.8% (Wolayan et al., 2019). Enzymes such as xylanase and phytase can hydrolyze non-starch polysaccharides and phytic acid, respectively, thereby reducing digesta viscosity and releasing bound minerals, while synbiotics, which are a combination of probiotics and prebiotics, may further support nutrient utilization by modulating gut microbiota, enhancing fermentation of residual fibre, and improving intestinal health. However, most previous studies have evaluated enzymes and synbiotics separately, and evidence on their combined use in maize–sago–rice bran-based broiler diets remains scarce. In particular, supplementation with avizyme and phyzyme, alone or combined with synbiotics, in diets containing sago (*putak meal*) did not improve live weight or carcass percentage of broilers (Helda et al., 2022), yet the underlying effects of this enzyme–synbiotic combination on apparent metabolizable energy and nutrient digestibility-

key determinants of feed efficiency—were not examined in that study and remain unclear.

Based on these considerations, the present study was conducted to evaluate the effects of dietary supplementation with complex enzymes and synbiotics, individually and in combination, on nitrogen-corrected apparent metabolisable energy (AMEn) and nutrient digestibility in broilers fed maize–sago–rice bran-based diets. The findings are expected to provide scientific evidence on the efficacy and potential complementary effects of enzyme and synbiotic supplementation in improving nutrient utilisation efficiency, thereby supporting more economical, efficient, and sustainable broiler production systems based on locally available feed resources.

MATERIALS AND METHODS

Broiler Chickens and Feed Ingredients

A total of 200 Cobb broiler chicks (22 days old) were randomly distributed among 20 cages, with five birds per cage. The main feed ingredient was sago (*putak meal*) derived from the pith of the gebang tree (*Corypha utan Lamk*), which was ground through a 3-mm screen, sun-dried to below 10% moisture, and sieved to remove fibre.

The study used a multi-enzyme complex containing seven enzymes: amylase, protease, cellulase, β -glucanase, pectinase, xylanase, and phytase. A synbiotic product, developed through collaboration between the State Polytechnic of Agriculture Kupang and the University of Jambi, contained lactic acid bacteria (*Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Pediococcus pentosaceus*) with a concentration of 36.10×10^{11} to 210×10^{11} CFU/mL and pH 3.00–3.40. The synbiotic was administered at 20 mL per liter of drinking water.

Experimental Design and Diets

The study employed a completely randomised design consisting of four dietary treatments, each with five replicates, from 22 to 35 days of age. *Salmonella* spp. counts were measured at 42 days of age. The treatments were as follows: D0 (control), D1 (synbiotics supplied through drinking water at 20 mL/L for 8 hours

per day), D2 (supplementation with a complex enzyme preparation), and D3 (a combination of complex enzyme preparation and synbiotics).

All diets were formulated to meet nutritional requirements (Table 1) and were offered in

crumble form on an ad libitum basis during the experimental period. The level of sago incorporated into the diets was higher than the level recommended by (Nalle et al., 2017).

Table 1. Experimental diets and estimated price of diets

Ingredients	D0	D1	D2	D3
	g/100 g as fed			
Maize	20.30	20.30	20.30	20.30
Sago (Putak meal)	25.00	25.00	25.00	25.00
Rice bran	10.00	10.00	10.00	10.00
Soybean meal	33.20	33.20	33.20	33.20
Meat and bone meal	5.00	5.00	5.00	5.00
Vegetable oil	2.50	2.50	2.50	2.50
L-Lysine HCl	0.25	0.25	0.25	0.25
DL-Methionine	0.05	0.05	0.05	0.05
Limestone feed grade	0.01	0.01	0.01	0.01
Dicalcium phosphate	0.60	0.60	0.60	0.60
Sea Salt	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.07	0.07	0.07	0.07
Vitamin–mineral premix ¹	0.30	0.30	0.30	0.30
Complex enzymes ²	-	-	0.02	0.02
Synbiotics	-	+	-	+
Total	100.00	100.00	100.00	100.00
Estimated diet price (Rp/kg)	7,419	7,449	7,459	7,49
Calculated analysis (g/kg as fed)				
Apparent metabolisable energy (MJ/kg DM)	11.80	11.80	11.80	11.80
Crude protein	226.00	226.00	226.00	226.00
Crude fiber	33.90	33.90	33.90	33.90
Lysine	14.80	14.80	14.80	14.80
Methionine + Cysteine	10.10	10.10	10.10	10.10
Calcium	13.60	13.50	13.50	13.50
Non-phytate phosphorus	5.30	5.30	5.30	5.30

Note: ¹Sanmix, PT Sanbe Farma, Bandung, Indonesia. Per kg provided: vitamin A (1,250,000 IU), vitamin D3 (250,000 IU), vitamin E (750 IU), vitamin K (200 mg), vitamin B1 (150 mg), vitamin B2 (500 mg), vitamin B6 (500 mg), vitamin B12 (1,012 mcg), vitamin C (3,000 mg), calcium D-pantothenate (500 mg), niacin (3,500 mg), methionine (3,500 mg), lysine (3,500 mg), manganese (10,000 mg), iron (2,500 mg), iodine (20 mg), zinc (10,000 mg), cobalt (20 mg), copper (300 mg), and antioxidant (1,000 mg). ²Avizyme.

Apparent Metabolisable Energy (AME) and Total Tract Nutrient Digestibility Assay

Apparent metabolisable energy (AME) and total tract nutrient digestibility coefficients (TTNDC) were evaluated from 28 to 35 days of age using the conventional total excreta collection technique (Nalle et al., 2021). Feed consumption and excreta production

were recorded between days 32 and 35. The collected excreta were thoroughly mixed, and representative subsamples were taken and oven-dried at 60°C until constant weight. The dried samples were then ground using a laboratory mill fitted with a 0.5 mm screen before undergoing chemical and nutrient analyses.

Identification and Quantification of *Salmonella* spp.

On day 42, two birds per cage were euthanized, and ileal digesta samples were collected for *Salmonella* spp. analysis. The procedure for *Salmonella* identification and quantification refers to (Nalle et al., 2020). The identification procedure involved pre-enrichment in lactose broth followed by enrichment in tetrathionate broth, with subsequent isolation on bismuth sulphite agar (BSA). Selected colonies were serially diluted in tryptone broth. Confirmation of *Salmonella* spp. was performed using Triple Sugar Iron Agar (TSIA) and peroxide tests. Finally, bacterial quantification was conducted using the total plate count (TPC) method on plate count agar (PCA), and colonies were enumerated with a colony counter.

Data Collection

Apparent metabolisable energy corrected for nitrogen (AMEn) and the total tract nutrient digestibility coefficients were determined according to the following equations (Nalle et al., 2021):

$$AME_{diet} (MJ/kg) = \frac{(feed\ intake \times GE_{diet}) - (excreta\ output - GE_{excreta})}{total\ feed\ intake}$$

A factor of 36.54 kJ/g N retained in the body was used to calculate zero nitrogen retention (Hill et al., 1958).

Total Tract Nutrient Digestibility Coefficient diet

$$= \frac{(feed\ intake \times nutrient\ diet) - (total\ excreta \times nutrient\ excreta)}{feed\ intake \times nutrient\ diet}$$

Chemical Analysis

Crude protein content was determined according to AOAC 2001.11 (AOAC, 2005) and NDF content was analysed according to AOAC 942.5 (van Soest method) (AOAC, 2005). Phosphorus was analysed using a spectrophotometric method.

Statistical Analysis

The experimental data were subjected to one-way Analysis of variance (ANOVA) using SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Statistical significance was declared at $P < 0.05$. Whenever a significant

treatment effect was detected, differences among means were separated using Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

Feed Intake

Table 2 presents the effect of synbiotic, enzyme (D2), and combined synbiotic + enzyme (D3) supplementation on feed intake relative to the control (D0). During the starter period (0–21 days), feed intake ranged from 938.1 to 1,070.2 g/bird and did not differ ($P > 0.05$) significantly among treatments. During the cumulative period (0–35 days), treatment had a significant effect ($P < 0.05$) on feed intake. This indicates that enzyme supplementation, whether alone or combined with synbiotics, significantly reduced ($P < 0.05$) cumulative feed intake ($P < 0.05$), whereas synbiotics alone (D1) did not differ significantly ($P > 0.05$) from the control.

The absence of a significant difference at this early stage is plausible because the digestive tract of young chicks is still developing, meaning the physiological effects of synbiotics and enzymes on feed intake regulation may not yet be fully expressed. A similar pattern was reported by Perera et al. (2026) in a study on synbiotics based on *Bacillus subtilis* and *Saccharomyces cerevisiae* in Cobb 200 broilers, where feed intake was unaffected by synbiotic supplementation from days 14–21, even though feed conversion efficiency was reduced. This supports the notion that synbiotic effects on feed intake become more apparent over a longer rearing period.

The significant reduction in feed intake in D2 (enzyme) and D3 (synbiotic + enzyme) is consistent with Nisar et al. (2021) who reported a decrease in feed intake accompanied by a marked improvement in feed conversion ratio in broilers supplemented with synbiotics at 1,200 g/tonne of feed. This pattern suggests that reduced feed intake does not necessarily indicate a disadvantage - it can instead reflect improved feed utilization efficiency, where nutrient requirements are met with a smaller quantity of feed.

For the enzyme treatment, the present findings actually diverge from the general trend reported in much of the international literature.

Table 2. Effect of treatments on feed intake (g/bird) during the starter and finisher periods.

Variable	Treatment				SEM	P-value
	D0	D1	D2	D3		
Feed intake (g/bird)						
0-21 d	1005.6	1070.2	956.9	938.1	77.6	0.061
0-35 d	1992.0 ^a	1915.0 ^a	1623.0 ^b	1588.0 ^b	104.2	0.032

Notes: ^{a,b} Means of the rows with the superscripts indicate significant differences (P<0.05). P: Probability. SEM: Standard error mean; D0 = Control; D1 = Synbiotics; D2 = Enzymes; D3 = Synbiotics + Enzymes

Several studies report that exogenous enzyme supplementation (xylanase, protease, amylase, phytase, or multi-enzyme combinations) generally increases feed intake and growth performance, particularly in corn-soybean or wheat-based diets with high non-starch polysaccharide (NSP) content. Saleh et al. (2018) noted that enzyme supplementation is a strategy to enhance broiler growth performance, including feed intake, feed conversion ratio, and weight gain, by reducing digesta viscosity and modulating gut microbiota. The discrepancy in decreased intake in the present study versus increased intake in most of the literature could be due to (1) differences in enzyme type/dose and basal diet composition; 2) Interaction with synbiotics; and 3) variation in broiler strain, climate, and management practices.

Apparent Metabolisable Energy

Evaluating apparent metabolisable energy (AME) is essential for optimising feed

efficiency and poultry performance because it directly affects growth and feed conversion. Supplementation with enzymes and synbiotics can enhance nutrient utilisation by improving substrate degradation and gut health. Understanding their effects on AME and nutrient digestibility is important for developing efficient and sustainable poultry diets.

Table 3 demonstrates that dietary treatments significantly influenced (P<0.05) both apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn). The AME values ranged from 10.2 to 12.2 MJ/kg DM and AMEn values from 9.6 to 11.5 MJ/kg DM, which were lower than those reported by (Nalle et al., 2021). These differences may be related to the higher sago inclusion level (250 g/kg) and the multi-enzyme complex used in the present study, compared with 100-150 g/kg sago in earlier studies (Nalle et al., 2020; Nalle et al., 2021). However, the studies differed in other respects as well, so this comparison is indicative only.

Table 3. Effect of treatments on apparent metabolisable energy (AME, MJ/kg DM) and nitrogen-corrected AME (AMEn, MJ/kg DM) in broiler diets

Variable	Treatment				SEM	P-value
	D0	D1	D2	D3		
AME	12.2 ^a	12.1 ^a	11.9 ^a	10.2 ^b	0.498	0.044
AMEn	11.3 ^a	11.2 ^a	11.5 ^a	9.6 ^b	0.469	0.035

Notes: ^{a,b} Means of the rows with the superscripts indicate significant differences (P<0.05). P: Probability. SEM: Standard error mean; D0 = Control; D1 = Synbiotics; D2 = Enzymes; D3 = Synbiotics + Enzymes

The Fisher’s least significant difference (LSD) test indicated that the AME and AMEn of D0, D1, and D2 did not differ significantly (P>0.05) from one another, whereas D3 exhibited significantly lower (P<0.05) AME and AMEn values compared with the other treatments. Comparable metabolisable energy (AME and AMEn) levels among D0 (control), D1 (synbiotics), and D2 (enzymes) suggest that use

of synbiotics or enzymes as sole supplements did not improve dietary energy utilization compared with the control diet, which disagrees with (Dev et al., 2022; Walker et al., 2024).

The lower AME and AMEn in D3 were observed only when enzymes and synbiotics were combined. The mechanism behind the lower AME and AMEn in D3 cannot be determined from the present data because gut

microbial composition, fermentation products, endogenous nutrient losses, and intestinal pH were not measured. One possible hypothesis is that the combination of enzymatic hydrolysis and synbiotic-driven microbial activity altered fermentation or nutrient competition in the gastrointestinal tract, thereby reducing net energy available to the host. This remains speculative because as presented in Table 5 that *Salmonella* counts did not differ among treatments, thus there is no evidence of a measurable shift in at least this microbial population. Further studies measuring microbiota composition and fermentation profiles are needed to test this hypothesis. According to Yue et al. (2025), probiotics play a role in controlling gut microbial populations, promote the proliferation of beneficial bacteria, and support nutrient absorption as well as metabolic activities. Modifications in microbial populations could affect how fermentation occurs in the gut and how efficiently dietary energy is utilized (Wen et al., 2021).

Overall, the combined inclusion of enzymes and synbiotics did not improve, and was associated with lower, AME/n values in diets containing 25% sago and 10% rice bran, whereas individual supplementation enzymes or synbiotics maintained AME/n at the control treatment. The result of the present study partly agreed with Nalle et al. (2021b), who used the same feed additives but the level of sago is lower than in the present study and reported improved AME/AMEn from complex enzyme preparations in corn-based diets. This may suggest that such enzymes work better in corn-soy diets than in diets with high sago (*putak*) and rice bran, although dietary composition was not varied within the present study. NDF digestibility (Table 4) did not differ among treatments ($P = 0.740$) and was numerically highest in D3, the group with the lowest AME/n. Thus, NDF digestibility does not explain the lower energy values in D3. It is consistent only with the absence of AME improvement in D1 and D2.

Nutrient Digestibility

Table 4 presents the total tract nutrient digestibility coefficients (TTNDC) of broiler diets supplemented with enzymes and the synbiotic Probio FMplus. Supplementation of broiler diets with enzymes, synbiotics, or their

combination did not exert a significant effect ($P > 0.05$) on the TTNDC of crude protein (CP) or neutral detergent fibre (NDF), which contrasts with previous findings (Jalal et al., 2025; Song et al., 2022). The absence of significant effects ($P > 0.05$) on CP and NDF digestibility suggests that the complexity of fibrous substrates and the interactions among components in maize-sago-rice bran-based diets may limit the efficacy of both enzymes and synbiotics. In addition, synbiotics primarily modulate gut microbiota and fermentation patterns, but these changes do not necessarily enhance host proteolytic capacity or amino acid absorption, particularly when endogenous enzyme secretion is already sufficient (Jha et al., 2021). The lack of significant effects likely may reflect substrate complexity, enzyme specificity, and limited scope for further improvement under the given dietary conditions, although these factors were not directly evaluated.

Statistical analysis revealed that dietary treatments significantly ($P < 0.05$) affected the total tract digestibility coefficient (TTDC) of phosphorus. The Fisher's least significant difference (LSD) test indicated that D0 and D2 did not differ significantly ($P > 0.05$) from each other, nor did D1 and D3 ($P > 0.05$). However, the phosphorus digestibility values in D1 and D3 were significantly lower ($P < 0.05$) than those observed in D0 and D2. These findings suggest that enzyme supplementation alone (D2) was able to maintain phosphorus digestibility at a level comparable to the control, whereas synbiotic supplementation, either alone or in combination with enzymes, resulted in reduced phosphorus digestibility.

Phosphorus digestibility in D2 (0.581) was comparable to that of D0 (0.596), indicating that the multi-enzyme complex, which includes phytase, did not improve phosphorus digestibility beyond the control level. Phytase is known to hydrolyse phytic acid and increase phosphorus availability (Adabi et al., 2025; Moita et al., 2022). A possible reason for the absence of an additional effect is that the control diet already supplied adequate (inorganic) phosphorus, but phytate-bound phosphorus was not partitioned in this study, so this cannot be confirmed.

Phosphorus digestibility was lower in both synbiotic-containing groups (D1 and D3) than

Table 4. Effect of treatments on the total tract nutrient digestibility coefficients of broiler diets

Variable	Treatment				SEM	P-value
	D0	D1	D2	D3		
Crude protein intake (g/bird)	529	460	287	309		
Crude protein output (g/bird)	202	227	134	166		
Crude protein digestibility Coefficient	0.609	0.507	0.538	0.463	0.034	0.366
Phosphorus intake (g/bird)	17.8	15.0	12.5	11.87		
Phosphorus output (g/bird)	7.09	7.88	5.28	6.30		
Phosphorus digestibility coefficient	0.596 ^a	0.475 ^b	0.581 ^a	0.469 ^b	0.034	0.030
Neutral detergent fiber intake (g/bird)	1088	1209	859	956		
Neutral detergent fiber output (g/bird)	442	460	326	320		
Neutral detergent fiber digestibility coefficient	0.591	0.619	0.622	0.663	0.031	0.740

Note: ^{a,b} Means of the row with the superscripts indicate significant differences ($P < 0.05$). P: Probability. SEM: Standard error mean; NDF: Neutral detergent fiber; D0 = Control; D1 = Synbiotics; D2 = Enzymes; D3 = Synbiotics + Enzymes

in D0 and D2, indicating an association with the synbiotic irrespective of enzyme addition. Previous studies reported improved phosphorus digestibility with synbiotics (Jalal et al., 2025; Song et al., 2022), in contrast to the present results; responses may vary with microbial strain, diet composition, and physiological conditions. One possible explanation is that increased intestinal microbial activity competes for available phosphorus or alters gut pH and mineral solubility (Atuahene et al., 2025). However, microbial biomass, fermentation products, intestinal pH, and phosphorus retention were not measured, so this explanation remains hypothetical.

Considered together, the responses of the different variables were not consistent. AME/n were reduced only in D3, phosphorus digestibility was reduced in both D1 and D3, and NDF and CP digestibility were unaffected. Because reduced phosphorus digestibility in D1 was not accompanied by lower energy values, and NDF digestibility did not follow the pattern of AME, the present data do not support

a single mechanism linking microbial activity, fibre digestibility, phosphorus utilisation, and metabolisable energy. These outcomes are therefore treated as separate responses, and the proposed mechanisms should be regarded as hypotheses requiring direct verification.

Health Status of Birds

Table 5 presents the qualitative and quantitative evaluation of *Salmonella* spp. in broilers fed the experimental diets. The TSIA assay showed positive reactions in all treatment groups, indicating *Salmonella* colonisation irrespective of dietary supplementation. Quantitatively, *Salmonella* counts ranged from 243.00×10^3 to 396.00×10^3 CFU/mL; although the numerically lowest count was observed in D3 and the highest in D2, statistical analysis showed no significant difference ($P > 0.05$) among treatments, and this result should therefore be interpreted as an absence of a measurable effect of synbiotic or enzyme supplementation on *Salmonella* load, rather than as evidence of a true reduction.

Table 5. Quantitative and qualitative tests for *Salmonella* spp. in broilers exposed to the experimental diets

Variable	Treatment				SEM	P-value
	D0	D1	D2	D3		
TSIA	+	+	+	+		
<i>Salmonella</i> spp. (CFU/mL)	379.00×10^3	341.00×10^3	396.00×10^3	243.00×10^3	44.21×10^3	0.658

Note: ^{a,b} Means of the row with the superscripts indicate significant difference ($P < 0.05$); P: Probability. SEM: Standard error mean; TSIA: Triple sugar iron agar; CFU: Colony forming unit

The lack of a significant reduction is consistent with Nalle et al. (2020) who reported that supplementation with the same synbiotic product did not significantly lower *Salmonella* spp. Counts in broilers fed sago-based diets. A plausible mechanism relates to the pH-dependent activity of the lactic acid bacteria strains in the synbiotic product: while competitive exclusion by lactic acid bacteria is a well-documented mode of pathogen suppression, its efficacy depends on the strain maintaining viability and metabolic activity across the physiological pH range of the gastrointestinal tract. If gastrointestinal pH fluctuations fell outside the optimal range for these strains, bacterial viability and thus competitive exclusion capacity may have been reduced, limiting synbiotic efficacy under the high-fibre, antinutrient-rich dietary conditions used here.

Although the numerically lowest count was observed in D3 and the highest in D2, these differences were not statistically significant ($P = 0.658$) and are therefore reported as descriptive observations only. The results could not be interpreted as evidence of a synergistic interaction between the complex enzyme preparation and the synbiotic, or of any beneficial effect on gut microbial balance. The wide variation among replicates ($SEM = 4.42 \times 10^4$ CFU/mL) suggests that the response was inconsistent. Factors such as dosage, duration of supplementation, and the level of environmental challenge may have contributed to this outcome, but these were not tested in the present study. Whether the combined supplementation influences *Salmonella* colonisation therefore remains to be confirmed in further studies with greater statistical power.

The inclusion of synbiotics, either alone or combined with complex enzyme preparation, was expected to suppress pathogenic bacteria, including *Salmonella* spp., in the small intestine. However, the results did not demonstrate a reduction in bacterial counts (Nalle et al., 2020). The synbiotic product (pH 3.00–3.50) contained four lactic acid bacteria strains: *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus pentosaceus*. Although *L. brevis* can tolerate acidic conditions (pH 2.00–3.0) for several hours, optimal growth

of *L. fermentum* occurs at pH 5.50 and 25°C, while *L. plantarum* and *L. brevis* grow best at pH 4–6. Therefore, fluctuations in gastrointestinal pH may have reduced bacterial viability and limited synbiotic efficacy.

The complex enzyme product contained cellulase, β -glucanase, xylanase, and pectinase intended to improve neutral detergent fibre (NDF) digestibility. However, NDF digestibility was not enhanced. This could be due to the presence of enzyme inhibitors or bioactive compounds at high levels as a result of high inclusion levels of sago (25%) and rice bran (10%) in the diet, or intestinal pH alterations. Previous reports indicate that sago contains tannins, phenolic compounds, and flavonoids (Nalle et al., 2019; Nalle et al., 2021) that may interfere with enzyme activity by binding nutrients or impairing intestinal integrity. Consequently, undigested soluble fibre may have increased the viscosity of the intestine, creating favourable conditions for pathogenic bacterial proliferation. This mechanism may explain why *Salmonella* populations were not reduced in broilers fed diets containing 250 g/kg sago and 10% rice bran supplemented with complex enzyme preparation.

CONCLUSIONS

In conclusion, supplementation with synbiotics and enzymes, either individually or in combination, did not improve energy utilisation or the digestibility of crude protein, phosphorus, or fibre in broilers fed diets containing 25% sago and 10% rice bran. Combined supplementation additionally reduced AMEn and phosphorus digestibility. Although *Salmonella* counts did not differ significantly among treatments, synbiotic-supplemented broilers showed a numerically lower *Salmonella* load than the control, suggesting a possible, though not statistically confirmed, trend towards improved gut microbial balance. Overall, these findings indicate that under high-fibre, antinutrient-rich dietary conditions, synbiotics and enzymes provided limited benefit for nutrient utilisation, and any potential contribution to intestinal health warrants further investigation with larger sample sizes or more sensitive microbial assays.

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