Research Article

Lack of Effect of Xylanase and Cellulase Supplementation on Nutrient Utilization in KUB Chickens Fed a Low-NSP Diet

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Abstract | The Indonesian local KUB chicken, a selected strain developed by the Agricultural Research and Development Agency, has gained increasing popularity due to its dual-purpose performance and resemblance to native chicken meat quality. However, the sustainability of KUB chicken production remains challenged by high feed costs, which account for 60–70% of total expenses. This study investigated the effects of xylanase and cellulase supplementation on nutrient digestibility and digestive organ development in KUB chickens fed a low-nonspecific phosphate (NSP) diet. A total of 27 male day-old chicks were allocated in a 3 × 3 factorial design with three xylanase levels (0, 200, and 600 mg/kg diet) and three cellulase levels (0, 300, and 700 mg/kg diet), yielding nine dietary treatments. Birds were fed a corn–soybean meal–rice bran–based diet for 10 weeks, after which nutrient digestibility and organ biometry were assessed. Enzyme supplementation, either individually or in combination, did not significantly affect feed intake and fecal output ($p > 0.05$). In addition, no significant effects were observed on organic matter digestibility, ash digestibility, or the relative weights of digestive organs ($p > 0.05$). Organic matter digestibility remained high across all treatments, likely due to the good quality and low NSP content of the basal diet. Although numerical variations were observed, no consistent biological responses were detected. These findings suggest that the lack of enzyme response is attributable to the low-NSP formulation, reinforcing that the efficacy of xylanase and cellulase in KUB chickens is diet-dependent and reliant on substrate availability. Future studies using diets with higher fiber content and larger sample sizes are warranted to further clarify enzyme benefits and support sustainable local poultry production.

Keywords | Cellulase, Digestive organ development, Nutrient utilization, KUB chicken, Nutrient digestibility, Xylanase

Received | October 27, 2025; **Accepted** | November 12, 2025; **Published** | December 13, 2025
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Citation | Al-Arif MA, Lamid M, Rimayanti R, Rosyada ZNA, Ayuti SR, Khairullah AR, Gangil R (2025). Lack of effect of xylanase and cellulase supplementation on nutrient utilization in KUB chickens fed a low-NSP diet. Adv. Anim. Vet. Sci., 13(12):2693-2701.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.12.2693.2701
ISSN (Online) | 2307-8316

INTRODUCTION

The Indonesian local KUB chicken is a selected strain developed by the Agricultural Research and Development Agency (Balitbangtan) as an improvement of kampung chickens, and it has been introduced to farmers across almost all provinces in Indonesia (Komarudin *et al*., 2021). This breed is increasingly in demand in the market, primarily because its meat quality closely resembles that of native chickens while offering higher productivity than ordinary local chickens (Saragih *et al*., 2019). In addition to meat, KUB chickens also exhibit good egg-laying performance, with hen-day production reaching up to 66% (Setiasih *et al*., 2021). These advantages have driven the continuous development of KUB chicken farming; however, its sustainability is constrained by the high cost of feed, which contributes 60–70% of the total production expenses (Kirana *et al*., 2024). Therefore, innovations to improve feed efficiency and reduce production costs are essential to strengthen the competitiveness of KUB chickens in meeting the growing demand for both meat and eggs.

Local chicken breeds, including KUB, differ from commercial broilers in physiological growth patterns, digestive system development, and metabolic efficiency (Wahyuningtyas *et al*., 2025). Indigenous chickens generally exhibit slower growth rates, higher gastrointestinal tract proportions, and potentially lower endogenous enzyme production than fast-growing strains (Al-Arif *et al*., 2024). These breed-specific characteristics suggest that the digestive capacity and nutrient utilization mechanisms of KUB chickens may respond differently to exogenous enzymes (Wongnaa *et al*., 2023). Therefore, evaluating enzyme supplementation in KUB chickens is important to determine whether strategies proven effective in commercial broilers can be directly extrapolated to this local genotype or require tailored nutritional approaches to achieve optimal performance.

Feed utilization and efficiency are crucial determinants of productivity and profitability in poultry farming, including local breeds such as KUB chickens (Masey-O'Neill *et al*., 2014; Warsito *et al*. 2024). One of the main challenges is the relatively high fiber content of many feed ingredients, which limits nutrient availability and reduces feed efficiency in monogastric animals (Liu and Kim, 2017; Prawasa *et al*., 2023). This inefficiency hampers optimal growth and production and increases production costs owing to lower nutrient digestibility (Liu and Kim, 2017). To address this issue, various dietary strategies have been developed, among which enzyme supplementation has received increasing attention as an effective approach to enhance nutrient value and reduce feed costs (Francesch *et al*., 2012; Hidayat *et al*., 2021). Non-starch polysaccharides (NSP), a major component of plant-based feedstuffs, are particularly difficult for poultry to digest because their digestive system lacks the enzymes needed to break down these complex fiber structures (Craig *et al*., 2019). Supplementation with exogenous enzymes, such as xylanase and cellulase, has been reported to improve nutrient release by breaking down cell wall components and lowering feed viscosity, thereby enhancing nutrient digestibility and overall performance in poultry (Gonzalez-Ortiz *et al*., 2017; Lee *et al*., 2019).

Although numerous studies have demonstrated the clear benefits of xylanase and cellulase supplementation, these responses are strongly context-dependent and influenced by the NSP content and composition of the diet. Enzyme supplementation tends to be more effective in high-NSP viscous grain-based diets, such as wheat or barley (Zhang *et al*., 2014). In contrast, corn-soybean meal diets generally contain lower NSP levels and may show limited responsiveness unless additional fiber sources are included (Gonzalez-Ortiz *et al*., 2017). Despite this, corn-based rations supplemented with rice bran are widely used in Indonesian smallholder poultry production, including in KUB chickens. Therefore, this study employed a low-NSP diet to reflect typical feeding practices and provide baseline information on whether xylanase and cellulase supplementation can offer measurable benefits under practical production conditions. Future studies using higher fiber formulations may better reveal the potential of enzyme inclusion in KUB chicken diets.

MATERIALS AND METHODS

Animals and housing

A total of 27 day-old male KUB chickens were obtained from the Central Poultry Breeding Center, Malang, East Java, and transported to the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. During the first week, the chicks were housed in floor pens (200 × 100 cm) with rice hull litter and provided with supplemental heating using light bulbs. The birds were fed a commercial starter diet (CP-511) and water ad libitum. From the second week onward, the birds were individually housed in battery cages (one bird per cage) with free access to feed and water throughout the 10-week experimental period. Temperature and lighting were maintained according to the standard management practices for KUB chickens, a recognized Indonesian local breed.

Experimental design

The experiment followed a completely randomized design in a 3 × 3 factorial arrangement, consisting of three xylanase levels (0, 200, and 600 mg/kg diet) and three cellulase levels (0, 300, and 700 mg/kg diet). This resulted in nine dietary treatments, each replicated thrice (n= 3 birds per treatment). The treatments were coded P1–P9 as follows:

- P1 : 0 mg/kg xylanase + 0 mg/kg cellulase
- P2 :200 mg/kg xylanase + 0 mg/kg cellulase
- P3 :600 mg/kg xylanase + 0 mg/kg cellulase
- P4 : 0 mg/kg xylanase + 300 mg/kg cellulase
- P5 : 0 mg/kg xylanase + 700 mg/kg cellulase
- P6 : 200 mg/kg xylanase + 300 mg/kg cellulase
- P7 : 200 mg/kg xylanase + 700 mg/kg cellulase

- P8 : 600 mg/kg xylanase + 300 mg/kg cellulase
- P9 : 600 mg/kg xylanase + 700 mg/kg cellulase

This experiment served as a preliminary physiological and digestibility assessment to establish the baseline responses of KUB chickens to xylanase and cellulase supplementation. Owing to individual housing constraints and ethical considerations regarding animal use, three birds per treatment were employed as biological replicates. While individual-bird units increased data precision, we acknowledge that the sample size may limit statistical power and increase the risk of Type II error. Future studies with larger sample sizes are planned to strengthen and validate these findings.

Diets and enzyme products

A basal grower/finisher diet (weeks 2–10) was formulated using locally available ingredients (as-fed basis): maize (42.5%), wheat pollard (25.0%), rice bran (11.0%), soybean meal (7.0%), corn gluten meal (6.0%), meat and bone meal (6.0%), and mineral premix (2.5%).

Enzyme treatments were prepared by adding feed-grade xylanase (HaBio, Mianyang HaBio Bioengineering Co., Ltd., Sichuan, China; ≥20,000 U/g) and cellulase (Chatson Jaya Lab, Bogor, Indonesia). The cellulase product was derived from Trichoderma reesei fermentation and had a declared activity of ≥10,000 U/g. Both enzyme products were supplied as dry powders intended for use in poultry feed applications. The calculated enzyme dosage (mg/kg feed) was first premixed with a small portion of the basal feed and then blended into the bulk feed to ensure a homogeneous distribution.

Wheat pollard was included as a representative ingredient frequently used in Indonesian smallholder poultry systems and as a moderate source of arabinoxylans. Although wheat pollard contains fermentable NSP, the diet was formulated to reflect a practical low-to-moderate NSP feed scenario that is typical for local poultry production. Direct laboratory analysis of dietary NSP was not conducted due to facility constraints. Instead, the NSP content was estimated based on standard feed composition tables (Nguyen *et al.*, 2022), yielding an estimated total NSP content of 13–15% and a crude fiber content of 4.2%. Although these values indicate a low to-moderate NSP diet relative to wheat or barley-based poultry feeds, we acknowledge the limitation of not performing direct NSP quantification in this preliminary study.

The cellulase product used in this study was declared by the manufacturer to contain ≥10,000 U/g of activity, which falls within the typical activity range of commercial cellulase preparations used in poultry research. The inclusion levels of 300 and 700 mg/kg feed were selected based on prior enzyme feasibility studies in poultry and monogastrics receiving fiber-containing diets and were formulated to represent practical field-applicable dosages for smallholder systems. These dosages corresponded to approximately ≥3,000 and ≥7,000 U/kg of cellulase activity, respectively. To ensure functional stability, the product was sourced fresh from the supplier and stored under the recommended conditions (cool, dry environment), and feed was prepared weekly to avoid enzyme degradation. Although direct post-mixing enzyme activity assays were not conducted in this preliminary study, the declared activity, storage management, and feed preparation procedures support the assumption that the enzymes remained active and biologically functional. The observed high basal digestibility and low dietary NSP levels may have contributed to the limited response to the inclusion of exogenous enzymes.

Digestibility trial

At week 10, an apparent nutrient digestibility trial was performed. The birds were adapted to metabolism cages, and excreta were collected for three consecutive days. The daily feed intake, refusals, and excreta were recorded. Representative samples of feed and excreta were analyzed for dry matter (DM) and ash content, according to the AOAC (2016). Organic matter (OM) was calculated as 100- ash (%DM). The descriptive measurements included feed intake (g DM/bird/day), fecal output (g DM/bird/day), and the OM content of the feed (%) and OM content of feces (%).

The apparent digestibility (%) was calculated as follows:

$$\text{Digestibility (\%)} = \frac{Intake - Excretion}{Intake} \times 100$$

The following parameters were evaluated:

Organic matter digestibility (OMD):

$$\text{OMD(\%)} = \frac{OM\ intake - OM\ Excretion}{OM\ Intake} \times 100$$

Ash digestibility (AD):

$$\text{AD (\%)} = \frac{Ash\ intake - Ash\ Excretion}{Ash\ Intake} \times 100$$

Digestive organ measurements

At the end of the experiment (week 10), all birds were fasted for 12 h with free access to water, weighed individually, and then slaughtered humanely by the triple vein-cutting method (severing the jugular veins, carotid arteries, and esophagus). After complete bleeding, the abdominal cavity was opened, and the digestive organs were excised, blotted dry, and weighed. The following organs were recorded: proventriculus, ventriculus (gizzard), liver, pancreas, spleen, and small intestine. Organ weights were expressed as absolute weight (g) and relative weight (% of live body weight).

Table 1: Descriptive statistics of feed intake, fecal output, and organic matter of feed and feces in KUB chickens fed diets supplemented with xylanase and cellulase enzymes.

Treatment	Xylanase (mg/kg)	Cellulase (mg/kg)	Feed intake (g/bird/day)	Fecal output (g/bird/day)	OM feed (%)	OM feces (%)
P1 control	0	0	88.69 ± 5.15	18.50 ± 2.77	82.14 ± 1.21	56.91 ± 2.50
P2	200	0	63.28 ± 20.46	13.53 ± 1.79	82.25 ± 1.05	57.23 ± 1.98
P3	600	0	97.22 ± 9.62	20.23 ± 4.02	82.11 ± 1.09	56.45 ± 0.70
P4	0	300	81.56 ± 7.30	13.37 ± 0.85	82.37 ± 0.94	60.70 ± 2.00
P5	0	700	61.85 ± 8.57	14.77 ± 1.72	81.92 ± 1.47	56.23 ± 2.49
P6	200	300	81.39 ± 12.07	16.03 ± 3.18	82.08 ± 0.87	55.85 ± 2.22
P7	200	700	76.98 ± 13.44	20.50 ± 4.25	82.31 ± 1.32	55.07 ± 1.55
P8	600	300	73.31 ± 21.92	19.17 ± 2.32	82.46 ± 1.42	54.12 ± 1.60
P9	600	700	72.51 ± 18.17	17.60 ± 4.44	81.89 ± 1.03	56.25 ± 2.21

Values are presented as mean ± standard deviation (SD); OM Feed: Organic matter feed; OM Feces: Organic matter feces.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) for a 3 × 3 factorial arrangement (xylanase × cellulase). The statistical model included the main effects of xylanase and cellulase and their interactions. When significant differences were detected ($P < 0.05$), means were separated using Tukey's test. Results are expressed as mean ± standard deviation (SD) for descriptive parameters (Table 1) and as mean ± standard error of the mean (SEM) for ANOVA-based parameters (Tables 2 and 3). Statistical analyses were performed using the SPSS software (IBM Corp., 2019).

Table 2: Effect of dietary xylanase and cellulase on organic matter digestibility (OMD) and ash digestibility (AD) in KUB Chicken

Treatments	Xylanase (mg/kg)	Cellulase (mg/kg)	OMD (%)	AD (%)
P1 Control	0	0	89.55 ± 2.64	85.98 ± 2.16
P2	200	0	88.60 ± 3.42	83.71 ± 6.40
P3	600	0	89.83 ± 0.91	84.52 ± 2.61
P4	0	300	90.81 ± 1.10	88.46 ± 1.92
P5	0	700	88.30 ± 2.01	83.06 ± 5.05
P6	200	300	90.51 ± 2.02	87.30 ± 0.99
P7	200	700	86.93 ± 5.47	80.82 ± 8.36
P8	600	300	87.64 ± 1.88	79.93 ± 3.76
P9	600	700	88.13 ± 2.40	84.56 ± 2.73

P= treatment code; xylanase; cellulase (mg enzyme/kg diet). Values are expressed as mean ± SEM (n = 3). Two-way ANOVA revealed no significant effects of xylanase, cellulase, or their interaction on OMD or AD ($p > 0.05$).

Prior to the experiment, an a priori statistical power estimation was performed based on the effect sizes reported in previous enzyme supplementation studies in poultry. Assuming a medium effect size (Cohen's f= 0.40), α = 0.05, and power (1-β)= 0.80, a minimum of 5–6 birds per treatment group was suggested to detect significant differences. However, owing to ethical considerations, individual housing requirements for physiological measurements, and the exploratory nature of this study, only three birds per treatment group were used. This preliminary design allows for biological insights into enzyme responses in KUB chickens under low-NSP diets while acknowledging the increased risk of Type II error. Therefore, the non-significant findings should be interpreted with careful consideration of sample size limitations, and future studies with larger groups are planned to validate these results.

RESULTS AND DISCUSSION

Feed intake, fecal output, and organic matter composition

The results showed that xylanase and cellulase enzyme supplementation, either singly or in combination, had no significant effect on feed consumption, fecal production, or fecal organic matter (OM) content in KUB chickens (Table 1). Average feed consumption ranged from 61.85 g/head/day in the treatment with 700 mg/kg of cellulase (P5) to 97.22 g/head/day in the treatment with 600 mg/kg of xylanase (P3). This variation fluctuated and did not show a consistent pattern of increase or decrease, thus concluding that consumption levels were more influenced by individual variation than by the enzyme treatment. A similar pattern has also been reported in several studies on local chickens, indicating that exogenous enzyme supplementation does not always increase feed consumption. The response that emerged was strongly influenced by the ration composition, particularly the non-starch fiber (NSP) content, growth phase, and genetic differences between chicken types (Sinurat *et al*., 2022). Several studies have also explained that the effectiveness of enzymes is highly dependent on the availability of substrates in the feed and digestive conditions; therefore, insignificant results do not mean they are not important, but rather indicate that the benefits of new enzymes are clearly visible in rations with

Table 3: Effect of dietary xylanase and cellulase supplementation on digestive organ weights of KUB chickens.

Treatment	Proventriculus (g)	Gizzard (g)	Liver (g)	Pancreas (g)	Spleen (g)
P1 Control	2.43 ± 0.96	18.60 ± 1.11	14.56 ± 2.50	1.30 ± 0.51	1.80 ± 1.25
P2	1.86 ± 0.49	12.53 ± 3.29	4.46 ± 0.87	0.40 ± 0.30	0.63 ± 0.57
P3	2.63 ± 0.80	15.83 ± 2.77	10.63 ± 0.95	0.93 ± 0.15	0.53 ± 0.32
P4	2.83 ± 0.94	15.40 ± 0.65	7.23 ± 1.65	0.73 ± 0.37	0.53 ± 0.40
P5	2.46 ± 0.49	11.66 ± 1.17	6.23 ± 1.90	0.73 ± 0.28	1.00 ± 0.75
P6	1.73 ± 0.57	11.26 ± 0.66	5.76 ± 1.19	0.46 ± 0.15	0.40 ± 0.10
P7	1.76 ± 0.32	11.93 ± 1.42	7.86 ± 2.05	0.73 ± 0.32	0.30 ± 0.17
P8	3.80 ± 0.78	19.00 ± 3.47	9.33 ± 2.77	0.90 ± 0.45	0.86 ± 0.50
P9	2.53 ± 1.72	15.93 ± 3.25	7.33 ± 0.96	1.00 ± 0.40	0.63 ± 0.49

P = treatment code; xylanase; cellulase (mg enzyme/kg diet). Values are expressed as mean ± SEM (n = 3). Two-way ANOVA revealed no significant effects of xylanase, cellulase, or their interaction on OMD or AD ($p > 0.05$).

high NSP content (Seyedalmoosavi *et al*., 2022; Bedford and Apajalahti, 2022).

Fecal production also showed a similar pattern, with values ranging from 13.37 g/head/day in the 300 mg/kg cellulase treatment (P4) to 20.50 g/head/day in the 200 mg/kg xylanase and 700 mg/kg cellulase combination treatment (P7). The lack of significant differences indicates that enzyme supplementation does not directly alter the amount of excreta produced but has the potential to influence the quality and composition of excreta. In the context of digestive physiology, excreta produced not only reflect undigested feed residues but also involve endogenous secretions; therefore, the effect of enzymes on excreta quantity is often inconsistent (Perim *et al*., 2024).

The fecal organic matter content ranged from 54.12% in the combination treatment of 600 mg/kg xylanase with 300 mg/kg cellulase (P8) to 60.70% in the single cellulase treatment of 300 mg/kg (P4). Although this difference was not significant, the tendency for lower values in the enzyme combination suggests a possible increase in the utilization of the organic fraction of the feed when both enzymes are administered simultaneously. Conversely, the higher values observed with single cellulase treatment can be interpreted as an increase in the proportion of undigested organic matter that is ultimately excreted. This supports the findings of Zou *et al*. (2013), who reported that the response to cellulase is substrate-specific, and although the digestibility of some cell wall components increases, some organic components are still excreted in the feces due to incomplete degradation.

Overall, although the results of this study did not show a statistically significant effect, the presence of numerical variation between treatments is important to note. The unclear response pattern can be explained by several factors, including the limited sample size, genetic heterogeneity of KUB chickens, and composition of the corn- and rice bran-based basal diet, which is relatively low in non-starch polysaccharides (NSP). The low levels of NSP in the basal diet can result in suboptimal xylanase and cellulase activity. This is consistent with the report by Zhang *et al*. (2014), who found that the effectiveness of exogenous enzymes is more pronounced in wheat-based diets with high NSP content than in corn-based diets.

In this study, the absence of significant differences in fecal output aligns with the lack of improvement in nutrient digestibility. Although an improvement in digestibility is theoretically expected to reduce undigested residue and thus fecal mass, fecal output in poultry also reflects endogenous contributions, such as mucin, sloughed epithelial cells, and digestive secretions (Abd El-Wahab *et al*., 2022). These components can vary independently of dietary digestibility, particularly in short-term digestibility trials. Therefore, the unchanged fecal production in the present study should not be interpreted as evidence that enzymes cannot influence excreta volume in general, but rather that under the current low-NSP dietary conditions, no measurable enhancement of nutrient utilization occurred, which translated into reduced excreta output (Walker *et al*., 2024). This outcome is consistent with the digestibility results and reinforces that enzyme supplementation did not elicit meaningful improvements in nutrient breakdown under the tested conditions.

Thus, this study confirmed that xylanase and cellulase supplementation in KUB chickens did not been able to significantly affect feed consumption, fecal production, or fecal organic matter content. However, the observed numerical variation trend indicates the potential benefits of enzymes that require further verification through studies with larger sample sizes, longer rearing periods, and measurements of nutrient-specific digestibility coefficients. Further research is also recommended to evaluate the interaction between NSP levels in the basal feed and enzyme supplementation, so that the true role of xylanase and cellulase in improving feed efficiency in local Indonesian chickens can be more comprehensively understood.

Organic matter and ash digestibility

The results of the factorial analysis of variance (3 × 3; two-way ANOVA) showed that supplementation with xylanase and cellulase, either singly or in combination, did not have a significant effect (P > 0.05) on organic matter digestibility (OMD) or ash digestibility (AD) in KUB chickens (Table 2). OMD values in all treatments were relatively high, ranging from 86.93 to 90.81%, while AD ranged from 79.93% to 88.46%. Although there were numerical variations between the treatments, these differences were not consistent enough to form a pattern that could be directly linked to the enzyme dosage. The relatively high OMD values across treatments indicate that the rations used in this study were of good quality, with a relatively low non-starch fiber (NSP) content. This likely reduces the effects of exogenous enzymes, as reported in several studies in which poultry responded more effectively to enzyme supplementation in diets with high NSP content (Zou *et al*., 2013; Nguyen *et al*., 2022). In diets with low NSP content, enzymes lack sufficient substrate to break down, and thus, the differences in digestibility between treatments tend to be minimal.

Meanwhile, in terms of ash digestibility (AD), although not significantly different, there was a tendency for higher values in treatments with medium doses of cellulase (P4) and enzyme combinations (P6). This is consistent with the report by Perim *et al.* (2024), who showed that cellulase can increase nitrogen utilization and mineral release through plant cell wall degradation, as well as with the results of Irmak *et al.* (2024), who reported that multi-enzyme supplementation in cereal-based diets can increase ash digestibility through the release of minerals previously bound to the fiber fraction. However, the large standard error in some high-dose enzyme combination treatments (e.g., P7 and P8) indicates considerable individual variability among chickens, resulting in less consistent biological effects of the enzymes. This variability may stem from differences in the physiological digestive abilities of individuals, which are commonly reported in local poultry with relatively high genetic diversity.

These non-significant findings support previous research on local and broiler chickens, which found that enzyme supplementation does not always improve nutrient digestibility, especially when the ration quality is already relatively good (Sinurat *et al*., 2022; Dewi *et al*., 2025). Enzyme effectiveness is highly dependent on substrate composition, physiological conditions of the bird, and interactions between enzymes in the digestive tract. Therefore, despite the numerical variation in OMD and AD values, these data confirm that the use of xylanase and cellulase in rations with low NSP content does not provide significant digestibility benefits for KUB chickens.

Digestive organ weights

The results of this study indicate that xylanase and cellulase supplementation, either singly or in combination, had no significant effect (p > 0.05) on the weights of the KUB chicken digestive organs, including the proventriculus, gizzard, liver, pancreas, and spleen (Table 3). In general, the average organ weight values were relatively uniform between treatments, although there were numerical variations in some groups, for example P8 (600 mg/kg xylanase + 300 mg/kg cellulase) which showed a tendency for the weight of the proventriculus and gizzard to be higher than the control. In general, the average organ weight values were relatively uniform between treatments, although there were numerical variations in some groups, for example P8 (600 mg/kg xylanase + 300 mg/kg cellulase) which showed a tendency for the weight of the proventriculus and gizzard to be higher than the control.

The lack of difference in results is likely influenced by the quality of the basal ration used. The feed formulation used in this study was dominated by corn (42.5%) and soybean meal (7.0%), with the addition of wheat pollard (25.0%) and rice bran (11.0%). This composition is relatively low in non-starch fiber (NSP) and has high organic matter digestibility, thus limiting the availability of substrate for exogenous enzymes. Under these conditions, the additional activity of xylanase or cellulase was insufficient to induce significant physiological changes in the digestive organs. This is in line with reports by Souza *et al.* (2023) and Perim *et al.* (2024), who emphasized that enzyme responses are strongly influenced by diet quality and NSP content and that non-significant results are often found in diets with low NSP.

Nevertheless, the numerical variations that emerged in some organs, such as the gizzard and proventriculus at P8, are interesting to observe. Several previous studies, such as Macambira *et al.* (2025), have shown that certain xylanases can increase the mechanical activity of the gizzard when the diet contains specific fiber fractions. Similarly, Attia *et al.* (2022) reported that multi-enzyme combinations can modify digestive organ size under certain conditions, although the effects were inconsistent across studies. These results strengthen the evidence that the effectiveness of exogenous enzymes in local poultry, such as KUB chickens, is highly contextual, depending on feed composition, NSP levels, and the physiological condition of the animal. Non-significant findings do not imply a lack of enzyme potential, but rather indicate that in corn-based diets with low NSP content, the response to xylanase and cellulase supplementation tends to be minimal. This also provides an important basis for further studies using formulations with various fiber sources or higher NSP.

It is also important to note that the primary objective of this study was to evaluate the direct effects of xylanase and cellulase supplementation on digestibility-related physiological responses rather than growth performance. Therefore, key production parameters such as body weight gain (BWG) and feed conversion ratio (FCR) were not included. Although parameters such as digestibility and organ morphology are valuable early indicators of nutrient utilization efficiency, they may not detect subtle improvements in nutrient absorption that could manifest more clearly through performance metrics over a longer rearing period (Zhang *et al*., 2023). As this trial was designed as a short-duration digestibility experiment with a limited sample size, growth performance was beyond its scope. Future studies are planned to incorporate BWG, FCR, and carcass composition measurements in conjunction with digestibility assessments to provide a more comprehensive evaluation of enzyme efficacy in KUB chickens under different dietary NSP conditions.

In addition to the small sample size, one notable numerical variation observed in this study was the markedly lower liver weight in treatment P2 compared to the control (P1). Because each treatment group only consisted of three birds, such differences may reflect biological variation rather than a true treatment effect. Prior to statistical analysis, the data were screened for obvious recording errors and abnormal biological values; however, no data point met the exclusion criteria, and thus, all values were retained. Given the limited sample size, a single unusually low or high value has the potential to influence group means, which may explain the large numerical gap observed for certain parameters, such as liver weight. Therefore, these variations should be interpreted with caution and not as conclusive evidence of the treatment effects. Future studies with larger sample sizes will allow more robust outlier detection procedures and help confirm whether such observations reflect true physiological responses or random variations.

Despite providing physiological insights into the enzyme responses in KUB chickens, it is important to acknowledge that the small sample size in this preliminary study represents a major limitation. With only three birds per treatment, individual biological variation may mask the subtle effects of enzyme supplementation, thereby increasing the risk of Type II error. Therefore, the absence of statistically significant differences should not be interpreted as definitive evidence of inefficacy, but rather as an indication that the enzyme response under low-NSP diet conditions could not be fully detected with the current experimental power used. Future studies with larger sample sizes and expanded treatment groups are required to validate these findings and to more accurately characterize the interaction between NSP levels and exogenous enzyme efficacy in local chicken strains.

CONCLUSION

This study found no statistically significant effects of xylanase or cellulase supplementation, either individually or in combination, on feed intake, nutrient digestibility, or digestive organ development in KUB chickens fed a corn rice bran soybean meal based diet with a relatively low non-starch polysaccharide (NSP) content. These findings indicate that under low-NSP dietary conditions, enzyme efficacy may be limited due to insufficient substrate availability. However, this study did not definitively demonstrate the absence of enzyme benefits. Rather, it reflects that under the current experimental conditions, including a small sample size and a basal diet that already supports high digestibility, no measurable improvements were detected. The numerical variation observed in the digestibility parameters suggests the potential for subtle responses that require greater statistical power and diets with higher NSP levels to be accurately quantified. Future research with larger sample sizes, clearly defined enzyme activity specifications, longer rearing periods, and higher-NSP diet formulations is recommended to evaluate the role of xylanase and cellulase in enhancing feed efficiency and supporting sustainable indigenous chicken production systems.

ACKNOWLEDGMENTS

This research was supported by the Airlangga Research Fund (ARF) Program, Penelitian Dasar Unggulan, Universitas Airlangga, in the fiscal year 2025, with the grant number 1804/UN3.LPPM/PT.01.03/2025.

AUTHOR'S CONTRIBUTION

MAAA and ML conceived the idea and drafted the manuscript. ARK and SRA: Acquisition, analysis, and interpretation of the data. ZNAR, RR, and RG critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

NOVELTY STATEMENT

This study provides the first evidence demonstrating that supplementation of xylanase and cellulase does not improve nutrient utilization in KUB chickens when fed a low-NSP (non-starch polysaccharide) diet. Unlike previous research conducted on commercial broilers or high-

NSP diets, this work specifically evaluates the unique digestive characteristics of KUB chickens, showing that enzyme supplementation may offer no additional benefit under low-NSP nutritional conditions. These findings highlight the importance of diet–enzyme compatibility and contribute new insight into precision feeding strategies for indigenous poultry breeds.

Ethical approval

This research was permitted by the procedures in this experiment regarding the use of live animals according to the Animal Ethics Commission, Faculty of Veterinary Medicine, Universitas Airlangga, with the approval of the Animal Care and Use Committee (ACUC) No: 2.KEH.81.06.2025.

Generative AI and AI-assisted technology statement

No generative AI or AI-assisted technologies were used in the design of the study, data collection, data analysis, or interpretation of the results. Only standard statistical software and conventional writing tools were used during manuscript preparation. All text, tables, and conclusions were produced by the authors without assistance from generative AI systems.

Conflict of interest

The authors have declared no conflict of interest.

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