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Research Article
Effect of Cellulase Supplementation on *in vitro* Digestibility and Energy, Crude Fiber and Cellulose Content of Sago Palm (*Metroxylon* sp.) Waste as Broiler Chicken Feed

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Abstract
Objective: The purpose of this study is to determine the amount of cellulase enzyme needed to optimize sago palm waste feed digestibility *in vitro*. This information can provide the basis for use of sago palm waste in broiler chicken feed. **Methodology:** Cellulase was added to the sago palm waste feed material at 5 different concentrations and five trials were conducted for each feed mixture. The parameters measured in this study were: *In vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD) and *in vitro* crude protein digestibility (IVCPD). The metabolic energy, crude fiber and cellulose contents in sago palm waste were also determined. The resulting data were analyzed statistically with a directional completely randomized design. **Results:** Sago palm waste with cellulase added to 0.75 g cellulase kg^{-1} waste had the best IVDMD (35.94 vs 19.32% for 0 g cellulase) and IVOMD (35.25 vs 19.32% for 0 g cellulase) values. Optimal values for the crude fiber and cellulose content were also seen for 0.75 g cellulase kg^{-1} waste with a maximum reduction to 12.79% crude fiber content (decreased by 23.69% compared to untreated waste) and 7.91% cellulose content (decreased by 29.56% relative to untreated waste). The crude protein digestibility and sago palm waste metabolic energy content at various cellulase concentrations were similar but 0.75 g cellulose kg^{-1} sago palm waste produced optimal crude protein digestibility and metabolism content. **Conclusion:** Addition of cellulase enzyme to sago palm waste at 0.75 g cellulose kg^{-1} sago palm waste improves IVDMD and IVOMD. This amount of cellulase also produced the best crude fiber and cellulose contents. Together these results suggest that cellulase can be used to improve the qualities of sago palm waste as a feed for broiler chickens.

Key words: Sago palm waste, cellulase enzyme, *in vitro* digestibility, crude fiber, cellulose

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

INTRODUCTION

Materials used for poultry feed must consider nutritional characteristics that are suitable to maintain health and productivity but should also avoid competition between livestock needs and human needs, so that price spikes are minimized[1]. Currently, use of agricultural waste feed materials, especially waste from sago palm (*Metroxylon* sp.) processing, has not been optimized. In Indonesia, sago palms occupy 1,128 million hectares that are spread across several provinces, including the Sulawesi Province in the southeastern part of the country[2]. Sago palm plants in Southeast Sulawesi, especially those that grow in the Kendari peninsula are agricultural plants that provide a local source of animal feed[3]. Sago palm vegetation covers 4,899 ha in Southeast Sulawesi and 41.33% of the plants are located in Konawe Regency[4].

During sago palm processing, between 75 and 83% of the material generated is waste[5]. Unused waste can create foul odors and increase soil acidity[6,7]. As a source of livestock feed material, sago palm waste has 11.65% crude fiber, 63.32% nitrogen free extract and 2.08% crude protein[8]. Furthermore, the sago palm waste nutrient content is 3.84% crude protein, 1.48% ether extract, 14.51% crude fiber and 0.32% calcium by weight with a metabolic energy[9] of 1,352 kcal kg^{-1}.

Sago palm waste contains paddy (58%), cellulose (23%), hemicellulose (9.2%), pectin (5.8%) and lignin (3.9%)[10]. The crude fiber fraction contains cellulose and hemicellulose[11], wherein the cellulose component is 20% and the remainder is lignin and ash extracts[12]. High levels of crude fiber in broiler chicken feed can be detrimental to growth, because these birds lack enzymes to digest the feed fiber component, which compromises the activity of endogenous enzymes and inhibits nutrient uptake[13-15]. The addition of enzymes as feed additives can be used to enhance uptake of sago palm waste nutrients (e.g., carbohydrates, lipids and proteins) that cannot be digested by native digestive enzymes[15]. In addition to improved nutrient uptake, enzyme additives can increase the metabolic energy value of the feed to enhance fowl productivity and reduce environmental impact if the waste went otherwise unused[16-18].

Since cellulose is a dominant component of sago palm waste, cellulase could be used to increase digestibility by catalyzing the conversion of cellulose to glucose[19]. The temperature and pH needed for optimal enzyme activity are dependent on the enzyme type[20]. In this study, we examined the optimal cellulase concentration needed to enhance *in vitro* digestibility and metabolic energy content, while optimizing crude fiber and cellulose contents in sago palm waste used as broiler chicken feed.

MATERIALS AND METHODS

This study was conducted over the course of 3 months at the Nutrition Biochemistry Laboratory and Feed Animal Science Faculty and Nutrition Unit at the Intra-University Center Laboratory, Gadjah Mada University in Indonesia. The materials used were sago palm waste, HCl, pepsin, $NaHCO_3$, NaCl, sodium succinate and concentrated sulfate acid. Sago palm waste generated from sago palm processing was collected from Konawe Regency, Southeast Sulawesi province and treated mechanically. The sago palm waste was dried for use as primary feed material. The cellulase enzyme was added to the sago palm waste feed material at 5 different concentrations, which were tested in 5 trials. The treatment groups were: R1 (1 kg sago palm waste+0.00 g cellulase enzyme), R2 (1 kg sago palm waste+0.25 g cellulase enzyme), R3 (1 kg sago palm waste+0.50 g cellulase enzyme), R4 (1 kg sago palm waste+0.75 g cellulase enzyme) and R5 (1 kg sago palm waste+1.00 g cellulase enzyme).

We evaluated *in vitro* digestibility of sago palm waste feed dry matter (IVDMD), organic matter (IVOMD) and crude protein (IVCPD) using methods described by Han and Persons[21]. The energy content of the feed was analyzed by bomb calorimetry. Crude fiber and cellulose content of sago palm waste were analyzed by an AOAC method and a procedure described by Datta *et al.*[22], respectively.

The data were analyzed using a directional, completely randomized design. Real average differences between treatments were subjected to a Duncan's New Multiple Range Test (DMRT)[23].

RESULTS AND DISCUSSION

***In vitro* digestibility of sago palm (*Metroxylon* sp.) waste with added cellulase:** The *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD) and *in vitro* crude protein digestibility (IVCPD) of sago palm waste with different concentrations of cellulase enzyme for use as broiler chicken feed was tested (Table 1).

The IVDMD of sago palm waste increased with increasing amounts of cellulase enzyme with values ranging between 30.13% for the R1 treatment (control without the cellulase enzyme) and 36.65% for the R5 treatment (1.00 g cellulase kg^{-1} sago palm waste) (Table 1). The variations in the results showed that added cellulase produced true differences in IVDMD values ($p<0.01$). Similarly, a Duncan's Multiple Range Test (DMRT) showed that the R1 treatment (control) had significant differences from the R2, R3,

Table 1: Average *in vitro* digestibility values for dry matter, organic matter and crude protein of sago palm waste supplemented with cellulase

	Cellulase enzyme level (g kg^{-1} sago palm waste)				
Parameters	R1	R2	R3	R4	R5
Dry matter (%)	30.12^a	32.28^b	35.08^c	35.94^{cd}	36.65^d
Organic matter (%)	29.46^a	31.88^b	33.87^c	35.25^d	35.49^d
Crude protein (%)	28.47	29.84	30.81	32.15	32.31

Source: Biochemistry Lab, UGM Animal Husbandry Faculty, 2015, ^{abc}Different superscripts on the same line indicate significant difference , R: Treatment R1 control (no cellulase), R2 0.25 g cellulase kg^{-1} sago palm waste, R3 0.5 g cellulase kg^{-1} sago palm waste, R4 0.75 g kg^{-1} sago palm waste and R5 1.00 g cellulase kg^{-1} sago palm waste

R4 and R5 treatments wherein the cellulase content increased from 0.25-1.00 g enzyme kg^{-1} waste. Furthermore, the IVDMD for the R2 treatment, which had the lowest cellulase content, differed from that for the R3, R4 and R5 treatments. As the cellulase content reached 0.5 g kg^{-1} waste (R3 treatment), the IVDMD values differed significantly from those for the R4 (0.75 g kg^{-1} waste) and R5 (1.00 g kg^{-1} waste) treatments, whereas the R4 treatment and R5 treatment values were similar.

The IVDMD is a general indicator of whether a feed fulfills livestock nutritional needs. Higher IVDMD values translate to greater nutrient availability to fulfill the nutritional needs of livestock[24]. Nutrient digestibility can be influenced by several factors, including the addition of enzymes to feed[25]. The low IVDMD value for the R1 treatment (control) suggested that the high level of crude fiber in sago palm waste negatively affected digestibility. Indeed, crude fiber in feed acts as an anti-nutritional factor[26], especially for young livestock or non-ruminant livestock. In addition to low digestibility, high crude fiber contents in feed decrease feed palatability and overall nutritional value[27]. The higher IVDMD levels seen for feed with cellulase suggests that the added enzyme can hydrolyze the crude fiber in sago palm waste, particularly because cellulose is the dominant crude fiber component.

The IVOMD of sago palm waste also increased with added cellulase enzyme. The R1 treatment (control) had the lowest IVOMD values (29.46%) and the highest values (35.49%) were seen for the R5 treatment (1.00 g added cellulase). The IVOMD and IVDMD values are strongly related[28] such that reduced IVDMD values are often associated with reduced IVOMD values.

Variations in analytical results revealed that adding cellulose to sago palm waste produced a significant influence ($p<0.01$) on IVOMD. Moreover, the Duncan's Multiple Range Test (DMRT) results showed that the R1 treatment (control) IVOMD differed from that for the R2, R3, R4 and R5 treatments. Similarly, the R2 treatment had an IVOMD value that was significantly different from the R3, R4 and R5 treatments. The R3 treatment IVOMD differed from that for the R4 and R5 treatments, whereas the R4 and R5 treatments were similar.

Added cellulase increased the IVOMD value of sago palm waste feed matter due to the ability of cellulase to degrade sago palm waste cellulose. A Duncan's Multiple Range Test (DMRT) showed that 0.75 g cellulase kg^{-1} palm waste reduced the cellulose content to affect the IVOMD values. Through this activity, cellulase could degrade crude fiber into useable sugars. Cellulase converts cellulose from a linear chain of disaccharide units into glucose[29] by breaking polysaccharide bonds that in turn increase the digestibility value and reduce the crude fiber content[30].

The IVCPD of sago palm waste with added cellulase enzyme ranged from 28.47-32.31% (Table 1). The IVCPD values in this study were consistent with Kusmayadi *et al.*[31] who discovered that the *in vitro* digestion of chicken feather-based monogastric feed crude protein ranged from 28.4-39.6% but was lower than those reported by Hanim[14], who showed that broiler chicken feed supplemented with 2.25 g kg^{-1} xylanase had an IVCPD between 41.70 and 48.38%. These differences in crude protein digestibility level could be caused by several factors, such as feed ingredients, nutrient composition, feed ingredient processing and crude fiber content[11].

The variant analytical results showed that added cellulase enzyme, even at 1 g enzyme/1 kg waste did not contribute to real differences in IVCPD of sago palm waste. Nonetheless, the cellulase enzyme could effectively function in palm waste mixtures according to its expected substrate specificities and activity[32]. Although not statistically significant, cellulase enzyme could increase the IVCPD to values that were higher than that of untreated waste, with the highest value seen for the R5 treatment (32.31%).

Nutrient content of sago palm waste with added cellulase: The metabolic energy content of sago palm waste supplemented with 1.00 g cellulase kg^{-1} waste ranged between 2,597.05 and 2,612.59 kcal kg^{-1} (Table 2). These values are consistent with an earlier study showing an energy content[33] of 2,900 kcal kg^{-1} but higher than another study that reported[9] 1,352 kcal kg^{-1}. Sago palm waste had a gross energy value[33,34] of 4,148 kcal kg^{-1}. For a converted form of

Table 2: Average metabolic energy, crude fiber and cellulose content in sago palm waste supplemented with cellulase

	Cellulase enzyme level (g kg^{-1} of sago palm waste)				
Parameters	R1	R2	R3	R4	R5
Metabolic energy (kcal kg^{-1})	2.609	2.601	2.597	2.611	2.613
Crude fiber (%)	16.76^{a}	15.98^{b}	14.98^{c}	12.79^{d}	12.95^{d}
Cellulose (%)	11.23^{a}	10.49^{b}	9.60^{c}	7.91^{d}	8.26^{e}

Source: UGM Intra University Center Lab, 2015, $^{a-e}$Different superscripts on the same line indicates a significant difference, R: Treatment R1 control (no cellulase), R2 0.25 g cellulase kg^{-1} sago palm waste, R3 0.5 g cellulase kg^{-1} sago palm waste, R4 0.75 g kg^{-1} sago palm waste and R5 1.00 g cellulase kg^{-1} sago palm waste

metabolic energy, value of 3,765.91 kcal kg^{-1} for sago palm waste[15]. Several analyses of sago palm waste metabolic energy contents revealed that sago palm waste could represent a readily available energy source for livestock. However, a constraining factor of this material was its low crude protein content and high crude fiber content.

Here, added cellulase even at 1.00 g kg^{-1} did not produce significant differences in the metabolic energy content of sago palm waste. This result is consistent with the finding of Melati and Sunarno[29], who showed that added commercial cellulase did not increase the metabolic energy of wheat bran. Cellulase enzyme did not increase the metabolic energy of soybean-based feeds, since the cellulase enzyme activity depended on the material used, the energy content and the feed protein[35]. The ability of cellulase to improve sago palm waste metabolic energy content is also affected by the raw fiber content and fiber type. Feed metabolic energy values are positively influenced by the fat, carbohydrate and protein contents but are negatively influenced by the ash and crude fiber content[36].

The sago palm waste crude fiber content was between 12.79 and 16.76% (Table 2). The addition of cellulase enzyme to sago palm waste significantly influenced ($p<0.01$) the crude fiber content. The Duncan's Multiple Range Test (DRMT) test results showed that the R2, R3, R4 and R5 treatments decreased the amount of crude fiber relative to the R1 treatment. The R2 treatment crude fiber differed from that for the R3, R4 and R5 treatments as did the R3 treatment from the R4 and R5 treatments. However, the R4 and R5 treatments had similar crude fiber contents.

Based on the DMRT test results, cellulase enzyme added to 0.75 g kg^{-1} sago palm reduced the crude fiber content to 12.79% (Table 2), suggesting that cellulase is an effective biocatalyst for degrading fiber in cellulose-rich feeds. This result is similar to previous study studies that examined the use of *Aspergillus* cellulase in fermentation processes showed that cellulase supplementation of yam skin-based feeds reduced the crude fiber content[37] from 21.20-14.96%. Another study showed that supplementation of cocoa pod husk-based feed with an enzyme cocktail also reduced cellulose and hemicellulose levels to 11.62 and 15.29%, respectively[38].

The dominant crude fiber component in sago palm waste is cellulose, which is composed of hundreds of glucose units that must be broken down into digestible units. Here the addition of cellulase to sago palm waste produced cellulose contents ranging between 7.91 and 11.23% (Table 2), which is lower than earlier studies of enzyme supplementation in feed materials that showed cellulose fractions[39-41] of 23, 22.4 and 9.28%.

The addition of cellulase to sago palm waste indeed significantly ($p<0.01$) affected the sago palm waste cellulose content. Duncan's Multiple Range Test (DRMT) showed that cellulase reduced the cellulose fraction in sago palm waste, in that the R2-R5 treatments all had less cellulose than the unsupplemented R1 treatment. The lowest cellulose content was seen for the R4 treatment (7.91%) and the highest was the control R1 treatment (11.23%). These results support the ability of cellulase to degrade and transform cellulose macromolecules into simpler glucose molecules[42] that can be more easily digested by poultry.

CONCLUSION

Adding cellulase enzyme at 0.75 g kg^{-1} to sago palm waste incorporated in dry feed improves IVDMD and IVOMD as well as the crude fiber and cellulose contents. This study showed that, relative to untreated waste, treatment with 0.75 g cellulase kg^{-1} waste produced 35.94% IVDMD (increased by 19.32%), 35.35% IVOMD (increased by 19.65%), 12.79% crude fiber (decreased by 23.69%) and 7.91% cellulose (decreased by 29.56%). Meanwhile, adding the cellulase enzyme to the sago palm waste at various levels did not significantly affect crude protein digestibility (IVCPD) or metabolic energy content.

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