2023 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 13, Issue 2: 105-110; March 30, 2023

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2023.16

PHYSICO-CHEMICAL PROPERTIES AND DIGESTIBILITY OF AMMONIATED BAMBARA GROUNDNUT (*Vigna subterranea*) SHELL FOR RUMINANTS

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Supporting Information

RESEARCH ARTICLE

PII: S222877012300016-13

Received: November 13, 2022

Revised: March 17, 2023

Accepted: March 20, 2023

ABSTRACT: This experiment was conducted to evaluate the physical properties, chemical quality, and digestibility of the ammoniated Bambara groundnut (*Vigna subterranea*) shell as ruminant feed. Bambara groundnut shell (BGS) were collected, ground with a grinder machine, afterward added 0, 3 and 5 (% DM) urea levels into 500 g of sample. Samples were mixed until homogeneous, then put into plastic bottles, after that stored for 7 and 14 days. Opened, dried in the oven at 65^{0}C for 48 hours and ground. A completely randomized design (CRD) was used with 5 treatments of BGS ammoniation (T0= control, T1= BGS + 3% urea and 7 days storage, T2= BGS +5% urea and 7 days storage, T3= BGS + 3% urea and 14 days storage, and T4= BGS + 5% urea and 14 days storage time), 4 replications each. The result of this study showed that the increase of urea level and days storage time, can decrease crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose contents of all samples (P<0.05) and increase the value of bulk density, tapped density, in vitro dry matter digestibility and in vitro organic matter digestibility in comparison to untreated samples (P<0.05). It was concluded that the T4 was the best treatment. The BGS ammoniated with 5% urea for a period 14 days of storage causes the lowest value of crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose, and also causes the highest value of bulk density, tapped density, crude protein, in vitro dry matter digestibility and in vitro organic matter.

Keywords: Ammoniation, Fibrous feed, Bambara groundnut shell, Ruminant, Urea.

INTRODUCTION

Bambara groundnut (*Vigna Subterranea*) is more easily adaptable in the Bogor area and the eastern part of West Java, so it is better known as Bambara groundnut (Adhi and Wahyudi, 2018). Bambara groundnut shell is an agricultural by-product, that has high fiber and low protein content, so it can be used as a source of fiber feed but low quality so processing techniques are needed (Dewi et al., 2018). Treatment is needed to break down fiber so it can increase the quality of agricultural by-products (Sarnklong et al., 2010). Therefore, Bambara groundnut shells can be used as an alternative feed for ruminants. The treatment that reduces the fiber content and increases the quality of agricultural by-products is ammoniation (Lukman et al., 2020). The ammoniation process is an effort using urea which will be converted to ammonia in gastrointestinal tract livestock (Belanche et al., 2021; Datsomor et al., 2022). The ammonia process server increases crude protein levels (Tampoebolon et al., 2019), through the addition of non-protein nitrogen (NPN) components from ammonia in urea $(NH_2)_2CO$ (Amin et al., 2016).

The function of urea (ammonia) as an alkali can also stretch fiber bonds, and break lignin, and cellulose bonds as well as lignin and hemicellulose bonds (PPrastyawan et al., 2012). Using urea levels of 5% in empty palm oil marks using Fiber Cracking Technology (FCT) to improve the nutrient quality of empty palm marks and reduce the content of fiber fractions (Dewi et al., 2018). The urea can be converted to ammonia and induce cleavage in lignocellulose structures (Shi et al., 2015; Hildebrant et al., 2017). According to Lukman et al. (2020) two sources of ammonia that can be used for the ammoniation process, namely ammonium hydroxide (NH_4OH) in the form of a solution, ammonia (NH_3) in gas form and urea $(NH_2)_2CO$ in solid form. The advantages of urea from the use of the other two forms are ease of handling (because it is solid) and ease to obtain.

Urea contains 42-45% nitrogen (Cantarella et al., 2018; Lukman et al., 2020). According to Van Soest (2006) the level of urea that is safe to use is 1-6% of dry matter. Ammoniation has been used in several agricultural by-products such as rice straw (Bata et al., 2014), corn straw (Elkholy et al., 2009), and coconut leaves (Filho et al., 2013), and has been reported to increase crude protein content as much as 9.63%, 11.45%, 17.35%. Wanapat et al. (2013) stated of 2-3% urea on rice straw was able to increase dry matter intake, nutrient digestibility and rumen ecology. Dewi et al., (2018) reported the addition of 5% urea in corn plant waste (corn straw, corn husks and corn cobs) and sugarcane waste (sugarcane shoots and bagasse) had increased dry matter digestibility and organic matter digestibility in ruminants. The treatment with 14 days incubation time was the best method that could be used in ammoniated of banana stems so

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that, increasing crude protein and decreasing crude fiber in the ammoniated feed from banana stems (Irianto et al., 2022). Feeding with amniotic fiber has also been reported to increase livestock body weight gain by 147.1 g/head/day in sheep (Walandari et al., 2014) and by 120.0 g/head/day in cattle (Bata et al., 2014).

Evaluation of the degradation of feed components in the rumen can be done by several methods; one of them is the in vitro technique. In vitro technology is an indirect method of estimating digestibility in the laboratory by imitating the digestive process in the digestive tract of ruminants (Oluwo and Yaman, 2019; Mayulu et al., 2022). Compared to in vivo methods, in vitro techniques have the advantage of a short time and low cost (Getachew et al., 2004). Another advantage of in vitro is that feed samples are single-use and can measure the digestibility of one type of feed ingredient (Dijkstra et al., 2005).

This experiment was conducted to evaluate the physical properties, chemical quality, and digestibility of the ammoniated Bambara groundnut shell (*Vigna subterranea)* as ruminant feed, due to in vitro method.

MATERIALS AND METHODS

Location and time

This research was conducted in May–July 2022 at the Faculty of Animal Husbandry, IPB University, Indonesia. Proximate and Van Soest analyses were carried out at the Feed Science and Technology Laboratory. Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) analyses were carried out at the Dairy Animal Nutrition Laboratory.

Experimental design

This study used a completely randomized design (CRD), 5 treatments with 4 replications. The treatment consists of T0= Bambara groundnut shell + 0% urea (control), T1= T0 + urea 3% + 7 day storage, T2= T0 + urea 5% + 7 day storage, T3= T0 + urea 3% +14 day storage, T4= T0 +urea 5% + 14 day storage.

Research procedure

A 10 kg sample of Bambara groundnut shell have been used (randomly selected from Bogor City) and divided into 20 research samples (500 g per sample). To reduce the size of the Bambara groundnut shells, they were milled with a milling machine without a sieve. The ammoniation process is carried out by adding as much urea as 0, 3 and 5 from (%DM into 500 g of sample. Mixing is carried out until the homogenous first dissolving urea into distilled water in comparison (1:5). The treatment samples were put into 500 mL plastic bottles, then stored for 7 and 14 days. At the end of each time, the research sample storage is opened. Dried in the oven at 65^{0}C for 48 hours then ground. Milling result were used to the analysis of physical properties, proximate, Van Soest and in vitro digestibility.

Sample analysis procedure

Analysis of the characteristics of physical properties of bulk density (BS) and tapped density (TD) was carried out according to the method of Amidon et al. (2017). Dry matter analysis (DM), crude protein (CP), and crude fiber (CF) using method of AOAC (1990). Content Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by the method of Van Soest (2006). In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were analyzed by the method of Tilley and Terry (1963).

Statistical analysis

The data from this was an analysis of variance in Completely Randomized Design (CRD) using SPSS version 25 software. Data were analyzed of variance (ANOVA) if a difference is found ($P<0.05$) and further tested Duncan Multiple Range Test (DMRT) (Duncan, 1955). The significance value was determined based on a 5% significance level. Mathematical model of experimental design follows the mathematical model of Steel et al. (1997). The linear model used was: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

Where Y_{ij} is the observation values of each of the response variables arising as a result of μ= the overall mean; τ_i= observed effect of the ith dietary treatment; ε_{ij}= random or residual error due to the experimentation.

RESULTS

Composition physical properties and chemical structure

In present experiment, all samples are given the treatment value of physical properties bulk density (BS) and tapped density (TD; $P<0.05$) increasing along with an increase in urea level and storage time (Table 1). The addition of urea (ammonia) and storage time ($P<0.05$) increased the crude protein content of Bambara groundnut shell (Table 2). Untreated samples have higher values of crude fiber, NDF, ADF, and Hemicellulose than the samples with the addition of 3 and 5% urea (Table 3). All samples with the addition of 5% urea and storage time of 14 days have the lowest content of crude fiber, NDF, ADF, and hemicellulose.

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In vitro characteristics

All samples with the addition of urea and storage time can increase the digestibility of livestock. The addition of urea 0, 3 and 5% levels with storage time of 7 and 14 days on the samples increased IVDMD and IVOMD of Bambara groundnut shell (P<0.05; Table 4).

Table 1 - Physical properties of ammoniated Bambara groundnut shell bulk density (BS) and tapped density (TD)

Treatment	Bulk density (g L^{-1})	Tapped density (g L^{1})
T0	230±0.82^{e}	383±1.63^{e}
T1	237±0.58^{d}	395±0.82^{d}
T2	257±1.15^{b}	428±2.3b^{b}
T3	246±0.50^{c}	402±1.29^{c}
T4	266±0.96^{a}	444±1.41^{a}

T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). P>0.05; ns, not significant; CP: crude protein; DM: Dry Matter.

Table 2 - The crude protein content of ammoniated Bambara groundnut shell

Treatment	DM (%)	CP (%)
T0	92.30±0.97^{b}	7.9±0.66^{c}
T1	87.28±1.54^{a}	10.85±0.64^{b}
T2	87.57±0.62^{a}	10.92±0.65^{b}
T3	87.63±1.29^{a}	12.15±0.68^{a}
T4	87.18±0.51^{a}	12.39±0.89^{a}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% +7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). P>0.05; ns, not significant; CP: crude protein; DM: Dry Matter.

Table 3 - Content of ammoniation Bambara groundnut shell fiber components

Treatment	CF	NDF	ADF	Hemicellulose
T0	29.83±0.40^{a}	55.74±4.21^{a}	34.94±0.76^{a}	20.77±3.56^{a}
T1	26.51±0.45^{b}	49.25±2.11^{b}	33.12±0.52^{b}	16.13±1.64^{b}
T2	26.06±0.80^{bc}	47.99±2.06^{bc}	33.07±0.45^{bc}	14.91±2.11^{bc}
T3	25.34±1.19^{c}	44.81±02.03^{c}	32.01±0.68^{c}	12.80±1.57^{c}
T4	25.02±0.53^{c}	44.56±2.05^{c}	31.99±0.82^{c}	12.57±1.63^{ac}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). *, P>0.05; ns, not significant; CF: crude fiber (CF); NDF: Neutral detergent fiber; ADF: Acid detergent fiber; HS: Hemicellulose.

Table 4 - *In vitro* digestibility of ammoniated Bambara groundnut shell

Treatment	IVDMD	IVOMD
T0	59.75±0.57^{c}	59.21±0.55^{c}
T1	61.44±1.33^{b}	60.85±1.52^{b}
T2	62.14±0.69^{b}	61.57±0.56^{b}
T3	62.35±0.93^{b}	61.88±1.06^{b}
T4	64.25±0.71^{a}	63.78±0.57^{a}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% +14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with different superscripts differ significantly (P<0.05). *, P<0.05; ns, non-significant; IVDMD: in vitro dry matter digestibility; IVOMD: in vitro organic matter digestibility.

DISCUSSION

Bulk density (BS) is a method used to assess the physical quality of a material. The value of density is determined with calculating the volume of the material to the weight that has been determined in a measuring cup (Amidon et al., 2017). Particle size affects space-filling, coarse particles occupy more space while fine particles tend to be denser (Toit et al., 2019). Stack density affects the space between particle size storage times (Jaelani et al., 2016). The tapped density obtained was measured by first inserting a known sample mass into a measuring cup and carefully leveling it and then shaking it for 10 minutes (Amidon et al., 2017).

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The results of the study showed that increased levels of urea and increased time of storage can higher the content of BD and TD. This is caused by the content of values BD and TD are affected by moisture content and particle size. Ridla et al. (2023) reported that physical properties are highly correlated with nutrient values, they reported that crude protein positive correlation with the values of bulk density and tapped density. BS and TD values are increasing along with the increase in urea level and length of storage time. Compared to the control the value of physical BS and TD was found to be highest at the level of 5% urea addition with a storage time of 14 days of 266 – 444 g L^{1} the next is the treatments of urea doses of 3% 14 days of storage 246 – 402 g L^{1} the 5% urea treatment with a storage time 7 days and smallest treatment doses of 3% urea addition with storage time of 7 days of 237 -395 g L^{1}. This increase in BS and TD value is suspected because there has been a change in the nutrient content on Bambara groundnut shell treatment due to the addition of urea.

The addition of urea up to 5% and stored for 14 days can increase the crude protein content that is 12.39%, 7 days storage 10.92%, 0 days storage control 7.90%. This means that the higher the N content in a material the higher the value of crude protein. Bambara groundnut shells with 3 and 5% urea based on DM material, no changes were observed compared to the control treatment. The content of CP at 14 days of storage was found to be higher, presumably because the nitrogen fixation time ammonia formed in the ammoniated Bambara groundnut shell was higher than the fixation time of 7 days. The increase in crude protein content of feed ingredients (rice straw) due to ammoniating with urea is the result of the hydrolysis of urea which is fixed into the fiber network and the fixed nitrogen will be measured as crude protein (Amin et al., 2016). The increase in crude protein levels in ammoniated bagasse at 21 days of storage from 4.0% to 12.7% was reported by Lunsin et al. (2018).

The addition of urea level decreased the fiber component because of the changes in the structure of the cell wall due to ammonia treatment, the hydrolysis process of urea is able to break down lignocellulosic and lignohemicellulose bonds. Urea is alkali component, because it contains ammonia (NH_3) so in addition to having the ability to increase CP levels, it can also degrade fiber components (CF, NDF, ADF, and Hs) (Elihasridas et al., 2015). Urea ($(NH_2)_2CO$) causes fiber expansion so that it remodels complex carbohydrate components into simple carbohydrates (Lukman et al., 2020). In addition, high pressure can encourage the release of acetyl groups from the fiber structure which leads to an increase in substrate acidity and an increase in the solubility of the fiber (Thomsen et al., 2014; Jayanegara et al., 2018). Urea can be converted into ammonia and induce cleavage in lignocellulose structures (Shi et al., 2015; Hildebrandt et al., 2017). The combination of 1% urea and autoclave in rice straw increased digestibility but did not change the fiber content (Jayanegara et al., 2017). In another study, Jayanegara et al. (2018) reported that the combination treatment of 5% urea under pressure using an FCT (Fiber Cracking Technology) machine on empty oil palm fruit bunches could reduce fiber fraction and increase digestibility. According to Tampoebolon et al. (2018) different storage times (7,14,21 and 28 days) affect the crude protein, ash and crude fiber, 14 days of storage is the best treatment, because increasing the crude protein and decreases the crude fiber content.

In the present study, the storage time also does not affect the fiber component (Table 3); it is suspected that the added urea in the treatment sample has not worked optimally in hydrolyzing fiber bonds Bambara groundnut shell. Decrease in NDF content and weight it is also suspected that the increase in cell contents is supported by the increase in amniotic crude protein ammoniated Bambara groundnut shell.

Acid detergent fiber is a part of NDF, which is insoluble in acidic solutions composed of cellulose, lignin, and silica (Jannnah, et al., 2019). A higher concentration of ammonia can reduce the hemicellulose content (Yuan et al., 2015). Nuswantara et al. (2020) it was reported that ammonia reduced the content of NDF, ADF, Cellulose, Hemicellulose and lignin in the coconut belt. Decreased ADF levels are suspected because microorganism activity produces the enzyme cellulase in the ammonia process (Nuswantara et al. 2020). The hemicellulose content has decreased along with the increasing concentration of ammonia in the treatment (Table 3). Therefore, adding treatment 3 and 5% urea increased the fibrous feed quality of Bambara groundnut shell by lowering the fiber content substantially (Table 3). The decrease in the value of CF, NDF, ADF and Hemicellulose in Bambara groundnut shell after the addition of 5% urea in a row is 25.02%, 44.56%, 31.99% and 12.57% were observed.

The Increasing level of urea affects the higher value of IVDMD and IVOMD Bambara groundnut shells (Table 4), this is validated by the results of the NDF and ADF, which are decreasing simultaneously with increased urea levels (Table 3). Higher levels of urea addition of 5% with 14 days storage increased IVDMD and IVOMD of ammoniated Bambara groundnut shell by 64.25% and 63.78% (Table 4).

This is reinforced by the results of NDF and ADF a decrease with an increase in urea level with storage in Table 3. The highest increase in ammoniated Bambara groundnut shell IVDMD and IVOMD at the level 5% with a storage time of 14 days was due to the degradation of lignocellulose during the ammoniated process being able to break the bond between lignin and cellulose resulting in a simpler form of carbohydrates. According to Zulkiple et al., (2015), 5% urea treatment can loosen fiber components and open the lignocellulose structure so that cellulose becomes more accessible to fiber digestion microbes which break down polysaccharide polymers into sugar monomers. This makes microbial developments in the process of degradation of dry matter components and organic matter. According to Amin et al. (2016), IVDMD value increases as the storage of ammoniated increases. In addition, Dewi et al., (2018) reported that the 5% urea level causes a decrease in fiber in feed ingredients, significantly increasing the digestibility of dry matter and organic matter.

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CONCLUSION

Best treatment in present study was the treatment at 5% urea content and 14 days of storage. Bambara groundnut shell ammoniated with the additional level of urea 5% for 14 days of storage can reduce the lowest component of fiber such as crude fiber, Neutral Detergent Fiber, Acid Detergent Fiber, and hemicellulose, increase bulk density, tapped density, in vitro dry matter digestibility and in vitro organic matter digestibility highest. Therefore, the processed Bambara groundnut shell is promising for ruminants.

DECLARATIONS

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Authors' contribution

Perform the experiment, data analysis by SPSS software and writing the original draft was done by R. Putri; conceptualization, investigation methodology, review and editing of the paper was done by S. P. Dewi; supervise the experiment and revise the paper was done by M. Ridla and Y. Retnani; supervise the experiment and review the paper was done by F. A. Kurniawan.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Citation: Putri R, Dewi SP, Kurniawan FA, Ridla M and Retnani Y (2023). Physico-chemical properties and digestibility of ammoniated Bambara groundnut (*Vigna subterranea*) shell for ruminants. *Online J. Anim. Feed Res.*, 13(2): 105-110. DOI: https://dx.doi.org/10.51227/ojafr.2023.16