Buletin Peternakan 44 (3): 81-84, August 2020

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v44i3.53409

Nutrient Quality and in Vitro Digestibility of Bio-Ammoniated Rice Straw Bales

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Article history
Submitted: 14 January 2020
Accepted: 27 August 2020

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ABSTRACT

This study was done to determine the effects of bale sizes of bio-ammoniated rice straw on its nutrient quality and *in vitro* digestibility. Rice straw were bio-ammoniated by adding 2 g urea and 1 g probiotic/kg DM. By following a completely randomized design, rice straw was baled in 3 different weights (15, 25, and 35 kg) with six replications for each treatment, and then stored for 3 weeks. In the end of the week 3, bales were opened, aired, and then sampled for proximate analysis (dry matter, organic matter, crude protein, and crude fiber) and digestibility by *in vitro* gas production method. Using analysis of variance method, the proximate data showed that greater the bales size (15, 25, and 35 kg) increased crude protein (7.59, 7.86, and 9.95%, respectively; $P<0.05$) and decreased crude fiber contents (24.1, 22.1, and 18.8%, respectively; $P<0.05$). By increasing the size of bales also increased *a, b,* and *c* fractions (-0.79, 0.26, and 0.82 mL/100 mg DM; 20.2, 18.2, and 17.6 mL/100 mg DM; 0.012, 0.014, and 0.019 mL/h, respectively; $P<0.05$) as well as gas production (11.4, 11.5, and 13.8 mL/100 mg DM, respectively; $P<0.05$). It can be concluded that packing bio-ammoniated rice straw in 35 kg bale is the most effective way in increasing nutrient quality and digestibility of rice straw.

Keywords: Bales, Bio-ammoniated rice straw, Digestibility, In vitro, Nutrient quality

Introduction

Climatic conditions in Indonesia led to the inconsistent availability of forages for ruminants throughout the year. Forages are abundant during the rainy seasons and the opposite occurs during the dry seasons. This fluctuation in the availability of forages ultimately gives a negative impact on ruminants' productivity, since forages is one of crucial factor in the ruminants' industry. This problem can be solved by finding alternative feed that meet the requirements in terms of availability, quality, and price.

Rice straw is an agricultural by-product that can be used as an alternative feed in dealing with fluctuations in the availability of feed. Since rice is the staple food for Indonesian, rice straw as a by-product of rice production is abundant throughout the year. In 2019, Indonesia produced 54.60 million tons of dry milled unhulled rice (BPS, 2020), and with each ton of harvested unhulled rice capable of producing 1.35 tons of rice straw (Putun et al., 2004), it can be estimated that rice straw production was 73.7 tons.

Sarwono and Arianto (2003) reported that rice straw contain 92% dry matter (DM), 77.8% organic matter (OM), 5.31% crude protein (CP), 32.1% crude fiber (CF), 3.32 % ether extract (EE), 36.7% nitrogen free extract (NFE), 3,799 kcal/kg metabolizable energy (ME), 73.8% neutral detergent fiber (NDF), 51.5% acid detergent fiber (ADF), and 8.81% lignin. However, rice straw still contains polysaccharides as a potential energy source for ruminants, although its soluble nutrients content and digestibility values were low (Hartadi *et al*., 2005). The high level of lignification and silicification, the slow and limited ruminal degradation of the carbohydrates, and the low content of nitrogen are the main deficiencies of rice straw, affecting its value as feed for ruminants (Van Soest, 2006). Another problem of rice straw utilization as feed is its bulk density that causing problems in transportation and storage.

The low nutritive value of rice straw can be overcome by several methods, such as physical, chemical, or biological treatments. Treatments using probiotics is one variant of biological treatment using microorganisms producing fiber-digester enzymes (Sarnklong *et al*., 2010; Yang *et al*., 2011). This method was chosen because it is easy to be applied on field, effective in increasing nutritive value of rice straw, and does not cause environmental pollution. In application, probiotics are usually added with urea that serves as N source for microbial protein synthesis. This biological method can also be combined with physical method, such as baling (compaction) to overcome the bulky nature of rice straw, thus will save storage spaces as well as easier for handling. In addition, the size of rice straw bales might affect the activity of probiotic microorganisms due to the size of bales is correlated with rice straw surface area exposed to air, which in turn would affects the anaerobic conditions in the rice straw bales. Thus, rice straw digestibility will increase as the bales size increases. This study was done to determine the most optimal size of bio-fermented rice straw bales based on its nutrient quality and *in vitro* digestibility.

Materials and Methods

The experimental design used in the study was a one-way experimental design with weight of bales as the treatments. In the bio-ammoniation process, the probiotic used was Biofad® at a dose of 0.3% of dry matter (DM) of rice straw; with probiotic-to-urea ratio was 1: 2 (1 g probiotic + 2 g urea/kg DM). After mixed with probiotic and urea mixture, rice straw were baled in different weights of 15, 25, and 35 kg (T15, T25, and T35 treatments, respectively) with 5 replications for each treatment, and then stored in the room temperature for 3 weeks.

The chemical compositions of bio-ammoniated rice straw were analyzed by proximate analysis (AOAC, 2005). The digestibility analysis was carried out using *in vitro* gas production method (Menke and Steingass, 1988). The fermentation rates were measured by inserting gas production data during the incubation period into exponential formula of Ørskov and McDonald (1979) *cit*. Blümmel and Ørskov (1993):

$$P = a + b\,(1\text{-}e^{-ct})$$

Where: P is the gas production at time t (mL/100 mg DM), a is the gas production from soluble fraction (mL/100 mg DM), b is the gas production from insoluble fraction (mL/100 mg DM), c is the gas production rate constant (mL/h), $a + b$ is the potential gas production (mL/100 mg DM), and t is the incubation time (h). The values of a, b, and c were obtained from calculation using Excel program (Neway Excel) from Chen (1994).

The chemical composition and *in vitro* gas production data were analyzed by one-way analysis of variance (ANOVA) using SPSS ver. 22 (IBM, USA). Comparisons of means for treatments were done by contrast test with Duncan's new multiple range test (Gomez and Gomez, 1984) when the effects of treatments (P≤0.05) were detected.

Results and Discussion

Chemical composition of bio-ammoniated rice straw bales

In general, bales with greater size increased DM, crude protein (CP), and decreased crude fiber (CF) contents of bio-ammoniated rice straws (P<0.05; Table 1), but no significant effects was showed on organic matter (OM) content.

The CP content of bio-ammoniated rice straw increased (P<0.05) in line with the increasing of bales size (Table 1). The increasing levels of CP content in this case is due to the differences in the level of probiotic microorganisms' activity in rice straw bales, with the highest level of activity was assessed in the T35. Baling rice straw in 35 kg caused anaerobic conditions in the bale is easier to be achieved, thus the anaerobic microorganisms in the probiotic can grow more optimal. The increasing CP content of rice straw bales in this case might derived from microbial protein, which its population grew optimally due to anaerobic condition in bales with greater size. In addition, CP content in bio-ammoniated rice straw bales (8.47%) was greater than those in rice straw bio-ammoniated without baling (7.18%; Agus *et al*., 2006). This suggests that the activity for microbial protein synthesis in baled bio-ammoniated rice straw was better than those in non-baled bio-ammoniated rice straw, thus the improvement of nutrient availability, especially CP content, of baled rice straw was better.

By increasing the weight of bales also positively affected CF content of bio-ammoniated rice straw. The T35 showed the lowest CF content (P<0.05) than the T15 and T25 (18.8 vs. 24.1 and 22.1%, respectively). The differences in CF content due to different weight of bale were caused by the differences in the level of microbial activity in each bale. Baling rice straw causing the surface of rice straw that facing the air was reduced thus less contact with air would be happened, and then create a better anaerobic condition in rice straw bales. Storing hay in the form of bales were compacted the hay and less surface area exposed to air (Ball *et al*., 2015; Davis, 2016). Anaerobic microorganisms in the probiotic grew most optimal in the T35 since the most anaerobic condition was achieved in those bales. The increase of microbe population leads to greater production of cellulolytic and hemicellulolytic enzymes, thus increased cell wall degradation, resulted in lower CF content of the

Table 1. Chemical composition of bio-ammoniated rice straw with different bale sizes

Parameters (%)	Treatments		
	T15	T25	T35
Dry matter	76.4±2.02[a]	79.3±1.13[ab]	80.8±1.78[b]
Organic matter	72.7±0.98	73.2±1.53	72.0±1.11
Crude protein	7.59±0.05[a]	7.86±0.48[b]	9.95±0.81[c]
Crude fiber	24.1±0.60[a]	22.1±0.56[b]	18.8±0.22[c]

T15: bio-ammoniated rice straw weighing in 15 kg bales, T25: bio-ammoniated rice straw weighing in 25 kg bales, T35: bio-ammoniated rice straw weighing in 35 kg bales.

[a,b,c] Means in the same row with different superscripts differ significantly (P<0.05).

Table 2. Effect of bale sizes on gas production of bio-ammoniated rice straw

Parameters	Treatments bio-ammoniated rice straw		
	T15	T25	T35
a fraction (mL/100 mg DM)	-0.79±0.037 [a]	0.26±0.028[b]	0.82±0.075[c]
b fraction (mL/100 mg DM)	20.2±1.46[a]	18.2[b]±1.15[b]	17.6±1.33[b]
c fraction (mL/h)	0.012±0.0033[a]	0.014[b]±0.0023[b]	0.019±0.0032[c]
Gas production (mL/100 mg DM)	11.4±0.39[a]	11.5[a]±0.27[a]	13.8±0.87[b]

T15: bio-ammoniated rice straw weighing in 15 kg bales, T25: bio-ammoniated rice straw weighing in 25 kg bales, T35: bio-ammoniated rice straw weighing in 35 kg bales.
[a,b,c] Means in the same row with different superscripts differ significantly (P<0.05).

T35. The decline in CF content of rice straw bio-ammoniated with probiotic was caused by the addition of probiotics, which have ability to secrete enzymes that are able to breakdown cellulose and hemicellulose into smaller polysaccharides (Dean *et al*., 2008). In addition, baled bio-ammoniated rice straw had lower CF content compared with non-baled ones. Crude fiber content of rice straw bio-ammoniated with the same level of probiotic and urea without baling (Agus *et al*., 2007) was greater than those of the T35 (20.0 vs. 18.8%, respectively).

In vitro digestibility of bio-ammoniated rice straw bales

In general, bale sizes affected *a*, *b*, and *c* fractions as well as the gas production of bio-ammoniated rice straw (P<0.05; Table 2). The *a* fraction of the T15, T25, and T35 were at -0.79, 0.26, and 0.82 mL/100 mg DM, respectively (Table 2). These indicate that there was an increase of *a* fraction (P<0.05) with the increasing of the bale size. Microorganisms in probiotic produce cellulose and hemicellulose enzymes that degrade cell walls to simpler and more soluble carbohydrates thus increased the amount of soluble carbohydrate or a fraction of feedstuffs. Agus *et al*. (2007) reported that the increase of *a* fraction (soluble fraction) in bio-ammoniated rice straw was a result of the breakdown of cellulose and hemicellulose by microbes in probiotic during fermentation process. Besides that, the increase in the CP content of bio-ammoniated rice straw bales with larger size (Table 1) also play an important role in increasing *a* fraction due to protein is one component of soluble fraction.

The lowest *b* fraction was shown by the T35, then followed by those in the T25 and T15 bales (17.6, 18.2, and 20.2 mL/100 mg DM, respectively; P<0.05; Table 2). The low *b* fraction of the T35 was correlated with the increasing cell walls degradation due to increasing activity of probiotic microorganisms in the bales. Since *b* fraction refers to insoluble fraction of feed, such as cellulose and hemicellulose, then increasing cell walls degradation resulted in low *b* fraction. This is supported by CF content data of the T35 that was lower (P<0.05) than those in the T15 and T25 (18.8 vs. 24.1 and 22.1, respectively; Table 1).

The *c* fraction of the T35 was greater than those in the T15 and T25 (0.019 vs. 0.012 and 0.014mL/h, respectively; P<0.05; Table 2). The greater gas production rate (*c* fraction) in the T35 was due to it slow CF content compared to the other treatments, which leads the feedstuffs are easier to be bio-ammoniated in the rumen. Davies *et al*. (1999) reported that gas production rate is determined by the ability of microbes to ferment feed; easier and more fermentable substrates lead to faster gas production; thus the gas production cumulatively would be more.

The T35 had the greatest gas production compared with the other treatments (13.8 vs. 11.4 and 11.5, respectively; P<0.05; Table 2). This high gas production was caused by anaerobic conditions that was easily achieved on the T35, thus anaerobic microbes of probiotic were developed at the most optimal rate. A greater gas production can be achieved when microbial activity rumen was optimal (Menke and Steingass, 1988) that reflected the quality of feedstuffs. The optimal development of the anaerobic microbes caused increased the decomposition of cell wall components into simpler and soluble components, thus the CF content of the T35 was lower than the others (Table 1). The existence of a soluble material such as hexoses will affect the amount of VFA production, which in turn will also affect the amount of gas produced (Blümmel and Ørskov, 1993). In addition, gas production of baled bio-ammoniated rice straw was greater compared with bio-ammoniated rice straw without baling (12.2 vs. 11.9 mL/100 mg DM). This indicates that baling process could increase gas production in bio-ammoniated rice straw, which is reflected an increasing in the digestibility of feedstuffs.

Conclusions

Based on the results of this study, it can be concluded that larger size of bio-ammoniated rice straw bale increased CP but decreased CF contents of bio-ammoniated rice straw. Bales with larger size also increased the *a* and *c* fractions as well as *in vitro* gas production, while the *b* fraction was decreased.

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