J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Penguatan Risbang No. 60/E/KPT/2016

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
42(2):81-87, June 2017
DOI: 10.14710/jitaa.42.2.81-87

Urea treatment of rice straw at elevated temperature and pressure: Effects on fiber content, rumen fermentation and digestibility

A. Jayanegara, R. S. K. Ayinda and E. B. Laconi
Department of Nutrition and Feed Technology, Faculty of Animal Science,
Bogor Agricultural University, Bogor 16680 - Indonesia
Corresponding E-mail: anu_jayanegara@yahoo.com

Received September 28, 2016; Accepted January 21, 2017

ABSTRAK

Penelitian ini bertujuan untuk mengaplikasikan perlakuan urea pada jerami padi pada suhu dan tekanan tinggi (121ºC; 1,4 atm) serta pada waktu inkubasi yang singkat (30 atau 60 menit) dibandingkan dengan perlakuan urea konvensional (inkubasi selama 4 minggu). Perlakuan yang diujikan adalah: RS1: jerami padi tanpa perlakuan (kontrol), RS2: RS1+1% urea (tanpa inkubasi), RS3: RS1+1% urea (4 minggu inkubasi), RS4: RS2+autoklaf 30 menit, RS5: RS3+autoklaf 30 menit, RS6: RS2+autoklaf 60 menit, RS7: RS3+autoklaf 60 menit. Jerami padi perlakuan kemudian dianalisis kandungan seratnya dan diinkubasi secara in vitro. Data dianalisis menggunakan sidik ragam dan diuji lanjut menggunakan uji Duncan. Hasil menunjukkan bahwa perlakuan urea (RS3-RS7) tidak mengubah kandungan NDF dan ADF dari jerami padi dibandingkan dengan kontrol. Perlakuan urea menggunakan autoklaf selama 30 menit (RS4) meningkatkan secara nyata KcBK dan KcBO jerami padi sebesar 23,3% dan 25,6% dibandingkan dengan kontrol (P<0,05). Peningkatan waktu inkubasi jerami padi dalam autoklaf dari 30 menjadi 60 menit (RS6) tidak meningkatkan KcBK dan KcBO. Perlakuan tidak berpengaruh terhadap pH dan konsentrasi NH_3. Perlakuan urea (RS3-RS7) meningkatkan konsentrasi VFA dibandingkan dengan kontrol (P<0,05). Disimpulkan bahwa perlakuan urea menggunakan autoklaf selama 30 menit dapat menggantikan perlakuan urea secara konvensional dalam meningkatkan nilai nutrisi jerami padi.

Kata kunci: perlakuan urea, jerami padi, serat, kecernaan, rumen

ABSTRACT

The objective of this study was to apply urea treatment of rice straw at elevated temperature and pressure (121ºC; 1.4 atm) and at short treatment period (30 or 60 min) in comparison to conventional urea treatment (4 weeks incubation). Rice straw was subjected to the following treatments: RS1: untreated rice straw (control), RS2: RS1+1% urea (no incubation), RS3:RS1+1% urea (4 weeks incubation), RS4: RS2+autoclave 30 min, RS5: RS3+autoclave 30 min, RS6: RS2+autoclave 60 min, RS7: RS3+autoclave 60 min. The samples were analyzed for fiber contents and incubated *in vitro*. Data were analyzed using analysis of variance and Duncan's post-hoc test. Results showed that urea treatments (RS3-RS7) did not change NDF and ADF contents of rice straw in comparison to control. Urea treatment in autoclave for 30 min (RS4) increased rice straw IVDMD and IVOMD by 23.3% and 25.6% as compared to control, respectively (P<0.05). Extension of the incubation period to 60 min (RS6) did not improve further the IVDMD and IVOMD. Treatments did not change pH values and NH_3 concentrations. Urea treatment (RS3-RS7) increased total VFA concentration in comparison to control (P<0.05). It was concluded that urea treatment using autoclave for 30 min may replace conventional urea treatment to improve nutritional value of rice straw.

Keywords: urea treatment, rice straw, fiber, digestibility, rumen

INTRODUCTION

Rice is the main carbohydrate source consumed by Indonesian people and its position as the main staple food generally can not be replaced by any other carbohydrate sources such as corn, cassava, wheat, sorghum, etc. Because of its high demand in the country, rice cultivation dominates the use of arable land than other crops for food production (Tsujino *et al.*, 2016). Indonesia is the third biggest rice producer in the world after China and India (Sarnklong *et al.*, 2010) and, as a consequence of that, also produce high amounts of by-products such as rice bran, rice hull and rice straw. On the other hand, the growing of human population gives a pressure on utilizing land for even more food production and thus less land is available for animal feed production. Seasonal dry period may worsen the supply of cultivated forage production for animal feeding such as grasses and legumes. It is apparent that in the future animal production should rely on feeding by-products from food produced for human consumption (Devendra and Leng, 2011; Laconi and Jayanegara, 2015). Therefore, in the case of rice production, rice bran and rice straw are particularly important by-products to be used as animal feeds.

Rice straw has been traditionally used as animal feed in South, East and Southeast Asia countries, particularly for feeding of beef cattle and buffalo. Feeding sole rice straw for ruminants does not provide adequate nutrients for supporting high production level due to the fact that rice straw is low in protein, high in fiber, lignified components and silica, and low in digestibility (Van Soest, 2006). Further, rice straw is characterized by low degradation rate in the rumen, low rate of passage, and thus contributes to reduced feed intake (Sarnklong *et al.*, 2010). Such low quality roughage may contribute to a high emission of methane from the rumen due to more acetate formation at the expense of propionate (Jayanegara *et al.*, 2009; Jayanegara *et al*., 2013). A number of physical, chemical and biological treatments have been applied to improve nutritional quality of rice straw; these include chopping, grinding, alkali treatments (sodium hydroxide, calcium hydroxide), ammoniation or urea treatment, addition of fiber degrading enzymes and innocluation with white rot fungal species (Sarnklong *et al.*, 2010). With regard to urea treatment, it has been repeatedly shown that the treatment (at 2-6% DM addition level) increased rice straw digestibility by 2-100% than that of the untreated rice straw (summarized by Van Soest, 2006) and also improved productivity of animals (Gunun *et al.*, 2013). Urea treatment also adds substantial amount of nitrogen (crude protein) for microbial protein synthesis in the rumen in which this substance is naturally limited in rice straw (Polyorach and Wanapat, 2015).

Despite the benefit of urea treatment in improving nutritional quality of rice straw, its adoption rate by farmers in Indonesia to date is still low. Devendra (1997) has outlined some reasons why farmers do not apply the already developed and recommended methods for improving rice straw utilization, i.e. physical, socio-economic conditions and practical reasons. A drawback of urea treatment is that it needs approximately 3-7 weeks of treatment period before the rice straw can be fed to the animals (Elseed *et al.*, 2003; Yulistiani *et al.*, 2015). Apart from its time consuming, animals need to eat daily and applying such treatment means that the rice straw can not be fed at all during the treatment period. Any efforts to shorten the treatment period will therefore be valuable. In the present study, we aimed to apply urea treatment of rice straw at elevated temperature and pressure and at short treatmentperiod (30 or 60 min) by using an incubator (autoclave) in comparison to a conventional urea treatment (4 weeks incubation).

MATERIALS AND METHODS

Sample Preparation and Treatment

Rice straw was collected shortly after harvesting from paddy rice (Ciherang variety) field around Cangkurawok, Dramaga, Bogor. Rice straw was manually chopped (approximately 3 cm length), oven-dried at 60ºC for 24 h, and ground to pass a 1 mm screen by using a hammer mill. The ground rice straw sample (200 g each) was subjected to the following urea ($CO(NH_2)_2$) treatments:

RS1: untreated rice straw (control)
RS2: RS1 + 1% urea (no incubation)
RS3: RS1 + 1% urea (4 weeks incubation)
RS4: RS2 + autoclave 30 min.
RS5: RS3 + autoclave 30 min
RS6:RS2 + autoclave 60 min
RS7:RS3 + autoclave 60 min

Autoclave was used to generate high temperature and pressure, i.e. 121ºC and 1.4 atm, respectively. Urea was solubilized in water at 1:25

kg/l (equal to 4% v/v) prior to addition. Urea solution was sprayed to the ground rice straw (93.6% DM) to ensure homogenous mixture between both materials. Each treatment was conducted in three replicates.

Chemical Composition Determination

Rice straw samples receiving one of the seven treatments above were subjected to fiber determination, i.e. neutral detergent fiber (NDF) and acid detergent fiber (ADF) by following the procedure of Van Soest *et al.* (1991). With regard to NDF determination, an amount of 0.5 g sample was boiled in 100 ml neutral detergent solution for 1 h. The solution consisted of EDTA, sodium tetraborate, SDS, monoglycolether and sodium dihydrogenphosphate and distilled water. The analysis was performed without addition of heat stable amylase and sodium sulfite. Sample was then filtered on glass crucible (coarse porosity 1) and weighed. Similar procedure was applied for ADF determination, except that the solution used was acid detergent solution and consisted of CTAB, sulfuric acid and distilled water. Both NDF and ADF values were expressed exclusive of residual ash.

***In vitro* Rumen Fermentation**

In vitro rumen fermentation was performed according to Tilley and Terry (1963). Allocation of treatments into *in vitro* experimental units was following a randomized complete block design. Rumen inoculum was obtained from two fistulated Ongole Grade cattle at Biotechnology Research Center, Indonesian Academy of Sciences (Lembaga Ilmu Pengetahuan Indonesia, LIPI), Cibinong, Bogor. Incubation was conducted in three replicates and each treatment per run was represented by two fermentation tubes. In each run, an amount of 2 liter of McDougall buffer was prepared by mixing distilled water with the following chemicals: 19.6 g $NaHCO_3$, 7.42 g $Na_2HPO_4.7H_2O$, 1.14 g KCl, 0.94 g NaCl, 0.24 g $MgSO_4.7H_2O$ and 0.08 g $CaCl_2.2H_2O$. The buffer was then flushed with CO_2 to reach a final pH of 6.8. An amount of 0.5 g sample was inserted into 50 ml fermentation tube made from polyethylene. The tube was subsequently added with 40 ml McDougall buffer solution and 10 ml rumen fluid, and closed with a ventilated rubber cap. Fermentation was carried out in a shaker waterbath maintained at 39°C for 48 h. Fermentation was stopped by adding 2 drops of $HgCl_2$ and then centrifuged at 3,000 rpm for 15 min. Supernatant was taken for pH, total volatile fatty acid (VFA) and NH_3 analyses as described in Jayanegara *et al.* (2016a). The residue was added with 20 ml of 0.2% pepsin-HCl solution, incubated for another 48 h, and filtered through Whatman paper no. 41 under vacuum. It was oven-dried at 105°C for 8 h and ashed in a furnace at 600°C for 3 h to obtain DM and OM values of the residue, respectively. *In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were determined by subtracting DM and OM amounts in the residue, respectively, to their initial amounts prior to incubation. Two tubes per run with rumen fluid and buffer only but without sample substrate (blank) were incubated as described above, served as a correction factor to the DM and OM contents of the residuals.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) according to the following statistical model:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the observed value, μ is the overall mean, τ_i is the effect of different treatments, β_j is the effect of different blocks, and ε_{ij} is the random residual error. Significance of an effect of a particular parameter was stated at the probability level of $P<0.05$. Duncan's multiple range test was employed as the post-hoc test when the ANOVA result of a parameter showed significant difference among treatments. Statistical analysis was conducted by using SPSS software version 20.0.

RESULTS AND DISCUSSION

Addition of urea without incubation (RS2), conventional urea treatment (4 weeks incubation, RS3), urea treatment at elevated temperature and pressure for 30 min (RS4 and RS5) and 60 min (RS6 and RS7) did not cause any major changes on NDF and ADF contents of rice straw in comparison to those of the untreated rice straw or control (RS1, Table 1). Addition of urea without incubation (RS2) did not increase IVDMD and IVOMD of rice straw in comparison to control (Table 2). Conventional urea treatment (RS3) increased IVDMD and IVOMD of rice straw by 18.0% and 17.0% than those of control, respectively ($P<0.05$). Urea treatment at elevated temperature and pressure for 30 min (RS4)

increased rice straw IVDMD and IVOMD by 23.3% and 25.6% as compared to control, respectively (P<0.05). Extension of the incubation period to 60 min (RS6) did not improve further the IVDMD and IVOMD. Autoclave of RS3 substrate for 30 min (RS5) induced higher IVDMD and IVOMD (P<0.05), but it was not the case when performed for 60 min (RS7). Treatments did not change pH values and NH_3 concentrations of the incubation medium (Table 3). Addition of urea, either without incubation, with 4 weeks incubation, or incubated in autoclave for 30 or 60 min increased total VFA concentration in comparison to control (P<0.05).

Urea treatment is among the ammoniation technique in which the compound may release ammonia after being dissolved in water. Although ammonia is weaker alkali in comparison to, for instance, NaOH and thus less efficient in degrading fiber, it provides nitrogen which in turn may be converted to microbial protein and contributes to protein supply for animal production (Gunun *et al.*, 2016). Further, NaOH is less practical for small-holder farmers and may be overly expensive for the farmers to purchase. Ammonia itself can be absorbed into cell wall of rice straw and may break down the linkage between lignin and cellulose or hemicellulose (Van Soest, 2006; Sarnklong *et al.*, 2010). However, this is apparently not the case in the present experiment since NDF and ADF contents of rice straw did not decrease after 4 weeks incubation with urea solution. It seems that the addition level of 1% urea is not sufficient to break down lignocellulose complex. Therefore the level of urea for treatment needs to be increased for better effectivity in degrading fiber, provided that its upper limit does not cause any adverse or toxic effects to the animals. Despite the NDF and ADF contents of urea-treated rice straw were similar to those of control, its digestibility (both IVDMD and IVOMD) was improved. A plausible explanation is that urea treatment expands the cell wall or structural fiber of rice straw, enabling rumen microbes to attack, colonize and degrade the fiber component more easily. This is confirmed by an increase of VFA concentration after treating rice straw with urea solution since VFA is an end product of microbial metabolism in the rumen (Morvay *et al.*, 2011). Confirming the finding in the present experiment, such swelling of structural fiber due to urea or ammonia treatment had also been observed by other authors (Huang *et al.*, 2014; Mahmud-Ali and Bechtold,

Table 1. Neutral Detergent Fiber and Acid Detergent Fiber Contents of Rice Straw (% Dry Matter)

Treatment	Neutral Detergent Fiber	Acid Detergent Fiber
RS1	71.7	56.2
RS2	71.5	56.6
RS3	71.1	57.1
RS4	70.5	55.6
RS5	71.0	53.1
RS6	70.8	54.5
RS7	71.8	53.1

RS1: untreated rice straw (control); RS2: RS1 + 1% urea (no incubation); RS3: RS1 + 1% urea (4 weeks incubation); RS4: RS2 + autoclave 30 min; RS5: RS3 + autoclave 30 min; RS6: RS2 + autoclave 60 min; RS7: RS3 + autoclave 60 min.

Table 2. *In vitro* Dry Matter Digestibility (IVDMD) and *in vitro* Organic Matter Digestibility (IVOMD) of Rice Straw when Incubated in Buffered Rumen Fluid

Treatment	IVDMD (%)	IVOMD (%)
RS1	34.4^{a}	37.1^{a}
RS2	36.2^{a}	40.0^{ab}
RS3	40.6^{b}	43.4^{bc}
RS4	42.4^{b}	46.6^{c}
RS5	48.5^{c}	51.9^{d}
RS6	40.1^{b}	46.2^{c}
RS7	43.7^{b}	46.8^{c}
SEM	0.467	0.579
P-value	<0.001	<0.001

RS1: untreated rice straw (control); RS2: RS1 + 1% urea (no incubation); RS3: RS1 + 1% urea (4 weeks incubation);RS4: RS2 + autoclave 30 min; RS5: RS3 + autoclave 30 min; RS6: RS2 + autoclave 60 min; RS7: RS3 + autoclave 60 min.

Superscripts within the same column but different letters are significantly different at P<0.05.

Table 3. pH, Total Volatile Fatty Acid (VFA) and Ammonia Concentration of Rice Straw when Incubated in Buffered rumen Fluid *in vitro*

Treatment	pH	VFA (mM)	NH_3 (mM)
RS1	6.87	93.1[a]	6.55
RS2	6.87	164[d]	6.75
RS3	6.97	167[d]	7.43
RS4	6.87	130[bc]	7.29
RS5	6.93	119[bc]	6.59
RS6	6.90	116[b]	8.46
RS7	6.93	141[c]	7.13
SEM	0.025	2.902	0.192
P-value	0.415	<0.001	0.198

RS1: untreated rice straw (control); RS2: RS1 + 1% urea (no incubation); RS3: RS1 + 1% urea (4 weeks incubation); RS4: RS2 + autoclave 30 min; RS5: RS3 + autoclave 30 min; RS6: RS2 + autoclave 60 min; RS7: RS3 + autoclave 60 min.

Superscripts within the same column but different letters are significantly different at P<0.05

2015).

Urea treatment on rice straw in combination with high temperature and pressure shortened the treatment period to only 30 min and resulted in similar effectivity to that of conventional urea treatment (4 weeks incubation). High temperature and pressure induce the release of acetyl groups from hemicellulose structure, increase acidity of substrate, promote hemicellulose solubilization and then increase the digestibility of rice straw (Van Soest, 2006). On the other hand, high temperature may induce faster reaction between ammonia from urea and plant cell wall, resulting a considerably shorter incubation period for a similar magnitude of digestibility improvement as compared to control. A prolongation of incubation period in autoclave from 30 to 60 min did not change NDF and ADF contents as well as the digestibility of rice straw, suggesting that 30 min autoclave is sufficient for improving the nutritional value of rice straw. An increase of pressure may further shorten the period of ammonia treatment. For instance, steam pressure plus ammonia at 13-15 atm and 5-10 min incubation period improved *in vitro* total gas production by 27% in comparison to control (Weimer *et al.*, 2003). In another study, Bals *et al.* (2010) demonstrated that ammoniation of some forages at high pressure (200-400 psi, equal to 13.6-27.2 atm) in combination with high temperature (130-150^{o}C) for 15-30 min decreased significantly NDF concentration and NDF digestibility of the forages. Such use of extremely high pressure for ammonia or urea treatment (e.g., 10 atm or above), however, has a potential problem regarding safety issue during the process. Furthermore, although it has shown promising results in research, its applicability on pilot, farm or even commercial scale is still questionable and needs further investigation. Use of moderate pressure, i.e. 1-5 atm, is apparently much more realistic, applicable and practical on a larger scale.

Values of pH and NH_3 concentrations in the artificial rumen were unchanged by treatments. In *in vitro* batch system, buffering capacity of incubation medium is maintained as adequate as possible in order to keep relatively constant pH until the end of incubation (Getachew *et al.*, 1998; Rymer *et al.*, 2005). This is applied since in such system VFA production is accumulated, which is in contrast to *in vivo* condition where VFA is absorbed through rumen villi and gradually disappear from the rumen. Apart from that, fermentation of high fiber materials such as rice straw is less likely to cause a pH change since its fermentation rate is slow and thus its VFA production rate is also slow (Agbagla-Dohnani *et al.*, 2001). On the contrary, fermentation of low fiber materials (typical for grains or concentrates) results in a fast rate of VFA production and lactic

acid which may lower pH dramatically and leads to acidosis (Pourazad *et al.*, 2016). With regard to ruminal NH_3 concentration, it is an intermediate product of protein metabolism in the rumen. Higher extent or rate of protein degradation results in higher ruminal NH_3 concentration (Jayanegara *et al.*, 2016b). The substance is further used by microbes to synthesize their microbial protein or being absorbed through rumen villi (Sinclair *et al.*, 2014). Therefore NH_3 concentration in the rumen depends on different rate between protein degradation and microbial protein synthesis and/or absorption. Urea treatment, both conventional and using autoclave, did not increase ruminal NH_3 concentration. Such response is apparently due to the low concentration of CP present in rice straw (approximately 4% DM). Addition of 1% urea improved the CP content to almost 7% DM, but this level is still considered too low so that the NH_3 produced is immediately taken up by rumen microbes, resulting in a relatively low NH_3 concentration in the rumen *in vitro*.

CONCLUSION

Urea treatment at a level of 1% and incubated for 4 weeks improved rice straw digestibility although the treatment did not decrease its NDF and ADF contents. Urea treatment using autoclave for 30 min may replace such conventional urea treatment to improve nutritional value of rice straw. An extension of the incubation period in autoclave from 30 to 60 min did not improve further the digestibility of the material.

ACKNOWLEDGEMENT

This research is funded by Indonesian Ministry of Agriculture (Agency for Agricultural Research and Development) through "Kerjasama Kemitraan Penelitian dan Pengembangan Pertanian Nasional (KKP3N)" research grant.

REFERENCES

Agbagla-Dohnani, A., P. Noziere, G. Clement and M. Doreau. 2001. *In saccco* degradability, chemical and morphological composition of 15 varieties of European rice straw. Anim. Feed Sci. Technol. 94:15-27.

Bals, B., H. Murnen, M. Allen and B. Dale. 2010. Ammonia fiber expansion (AFEX) treatment of eleven different forages: improvements to fiber digestibility *in vitro*. Anim. Feed Sci. Technol. 155:147-155.

Devendra, C. 1997. Crop residues for feeding animals in Asia: technology development and adoption in crop/livestock systems. In: Renard, C. (Ed.). Crop Residuals in Sustainable Mixed Crop/livestock Farming System. CAB International, Wallingford, UK.

Devendra, C., and R.A. Leng. 2011. Feed resources for animals in Asia: issues, strategies for use, intensification and integration for increased productivity. Asian Australas. J. Anim. Sci. 24:303-321.

Elseed, F.A.M.A., J. Sekine, M. Hishinuma and K. Hamana. 2003. Effects of ammonia, urea plus calcium hydroxide and animal urine treatments on chemical composition and *in sacco* degradability of rice straw. Asian Australas. J. Anim. Sci. 16:368-373.

Getachew, G., M. Blummel, H.P.S. Makkar and K. Becker. 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Technol. 72:261-281.

Gunun, P., M. Wanapat and N. Anantasook. 2013. Rumen fermentation and performance of lactating dairy cows affected by physical forms and urea treatment of rice straw. Asian Australas. J. Anim. Sci. 26:1295-1303.

Gunun, N., M. Wanapat, P. Gunun, A. Cherdthong, P. Khejornsart and S. Kang. 2016. Effect of treating sugarcane bagasse with urea and calcium hydroxide on feed intake, digestibility, and rumen fermentation in beef cattle. Trop. Anim. Health Prod. 48:1123-1128.

Huang, S., Y.Z. Sun, Y. Xu and Z. Meng. 2014. Studies on influence of ammonia on properties of cellulose I-β based on molecular dynamics simulation. Acta Polym. Sin. 14:188-193.

Jayanegara, A., H.P.S. Makkar and K. Becker. 2009. Emisi metana dan fermentasi rumen *in vitro* ransum hay yang mengandung tanin murni pada konsentrasi rendah. Med. Pet. 32:184-194.

Jayanegara, A., S. Marquardt, E. Wina, M. Kreuzer and F. Leiber. 2013. *In vitro* indications for favourable non-additive effects on ruminal methane mitigation

between high-phenolic and high-quality forages. Br. J. Nutr. 109:615-622.

Jayanegara, A., S.P. Dewi, N. Laylli, E.B. Laconi, Nahrowi and M. Ridla. 2016a. Determination of cell wall protein from selected feedstuffs and its relationship with ruminal protein digestibility *in vitro*. Med. Pet. 39:134-140.

Jayanegara, A., S.P. Dewi and M. Ridla. 2016b. Nutrient content, protein fractionation and utilization of some beans as potential alternatives to soybean for ruminant feeding. Med. Pet. (accepted).

Laconi, E.B., and A. Jayanegara. 2015. Improving nutritional quality of cocoa pod (*Theobroma cacao*) through chemical and biological treatments for ruminant feeding: *in vitro* and *in vivo* evaluation. Asian Australas. J. Anim. Sci. 28:343-350.

Mahmud-Ali, A., and T. Bechtold. 2015. Aqueous thiocyanate-urea solution as a powerful non-alkaline swelling agent for cellulose fibres. Carbohyd. Polym. 116:124-130.

Morvay, Y., A. Bannink, J. France, E. Kebreab and J. Dijkstra. 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. J. Dairy Sci. 94:3063-3080.

Polyorach, S., and M. Wanapat. 2015. Improving the quality of rice straw by urea and calcium hydroxide on rumen ecology, microbial protein synthesis in beef cattle. J. Anim. Physiol. Anim. Nutr. 99:449-456.

Pourazad, P., R. Khiaosa-Ard, M. Qumar, S.U. Wetzels, F. Klevenhusen, B.U. Metzler-Zebeli and Q. Zebeli. 2016. Transient feeding of concentrate-rich diet increases the severity of subacute ruminal acidosis in dairy cattle. J. Anim. Sci. 94:726-738.

Rymer, C., J.A. Huntington, B.A. Williams and D.I. Givens. 2005. *In vitro* cumulative gas production techniques: history, methodological considerations and challenges. Anim. Feed Sci. Technol. 123-124:9-30.

Sarnklong, C., J.W. Coneja, W. Pellikaan and W.H. Hendriks. 2010. Utilization of rice straw and different treatments to improve its feed value for ruminants: a review. Asian Australas. J. Anim. Sci. 23:680-692.

Sinclair, K.D., P.C. Garnsworthy, G.E. Mann and L.A. Sinclair. 2014. Reducing dietary protein in dairy cow diets: implication for nitrogen utilization, milk production, welfare and fertility. Animal 8:262-274.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci. 18:104-111.

Tsujino, R., T. Yumoto, S. Kitamura, I. Djamaluddin and D. Darnaedi. 2016. History of forest loss and degradation in Indonesia. Land Use Policy57:335-347.

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Soest, P.J. 2006. Rice straw, the role of silica and treatments to improve quality. Anim. Feed Sci. Technol. 130:137-171.

Weimer, P.J., D.R. Mertens, Ponnampalam, B.F. Severin and B.E. Dale. 2003. FIBEX-treated rice straw as feed ingredient for lactating dairy cows. Anim. Feed Sci. Technol. 103:41-50.

Yulistiani, D., Z.A. Jelan, J.B. Liang, H. Yaakub and N. Abdullah. 2015. Effects of supplementation of mulberry (*Morus alba*) foliage and urea-rice bran as fermentable energy and protein sources in sheep fed urea-treated rice straw based diet. Asian Australas. J. Anim. Sci. 28:494-501.