FEED DIGESTIBILITY AND RUMEN CHARACTERISTICS BY *IN VITRO* TECHNIQUE OF SWAMP BUFFALOES FED TOTAL MIXED FIBER SILAGE SUPPLEMENTED WITH SOLUBLE CARBOHYDRATE AND PROTEIN

Afnur Imsya[1,*], Yuanita Windusari[2] and Riswandi[1]

ABSTRACT

The objective of this work was to evaluate the effects of soluble carbohydrate and protein supplementation on feed digestibility and rumen characteristics of swamp buffaloes fed total mixed fiber (TMF) silage by using an *in vitro* technique. A completely randomized design with 3 treatments (soluble carbohydrate (SCH) and protein ratio) and 6 replicates was used. Treatments consisted of total mixed fiber silage rations with SCH: protein ratio of 300 g : 300 g (1 : 1) (A1), SCH : protein ratio of 600 g : 300 g (2 : 1) (A2), and SCH : protein ratio of 900 g : 300 g (3 : 1) (A3). Measurements were taken on nutrient digestibility rates (dry matter, organic matter, crude protein, and crude fiber), fiber digestibility rates (NDF, ADF, cellulose, and hemicellulose), and rumen fermentation condition (concentrations of RAN, total VFA, acetate, propionate, butyrate, and methane and pH). Results showed that significant effects ($P<0.05$) of treatments were found on nutrient digestibility rates (dry matter, organic matter, crude protein, crude fiber, ADF, cellulose, and hemicellulose), rumen fermentation conditions (concentrations of RAN, total VFA, acetate, propionate, butyrate, and CH_4). Meanwhile, no effects ($P>0.05$) of treatments were found on NDF digestibility and pH levels. It was concluded that TMF silage ration supplemented with SCH and protein ratio of 3 : 1 (900 g : 300 g) gave the best results in terms of nutrient digestibility rates and rumen fermentation condition of swamp buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, total mixed fiber, silage, soluble carbohydrate (SCH), digestibility, swamp buffalo

INTRODUCTION

The most common feeding problem faced by swamp buffaloes is low quality and availability of roughages. Utilization of agricultural wastes such as rice straws and oil palm fronds and swamp vegetations as well as the application of ensiling technology can be the solution for this problem. In the previous study, a mixture of fiber sources known as total mixed fiber (TMF) composing 20% *Kumpai Tembaga* grass, 20% rice straws, and 20% Oil Palm fronds was developed. This composition had digestibility rates of 36.32% for dry matter, 35.96% for organic matter, and 17.86% for NDF, 10.84 mM $N\text{-}NH_3$, 12.39 mM acetic acid, 4.39 mM propionic acid, 4.36 mM butiric acid, and 6.91 mM methane gas production *in vitro* (Imsya *et*

[1]Animal Science Department, Faculty of Agriculture, Sriwijaya University, South Sumatra, Indonesia, *E-mail:aimsya@yahoo.com
[2]Biology Science Department, Faculty of Mathematics and Natural Science, Sriwijaya University, South Sumatra, Indonesia

al., 2016). The application of ensiling technology to TMF is a solution to ensure continuous feed availability. Imsya *et al.* (2018) found that TMF silage made with sodium diacetate additive could be stored within 45 day incubation time without significant effect on its nutritional value. This TMF silage contained 10.59% protein and 68.65% NDF. Feed of agricultural waste origins often contains protein in an amount lower than required by rumen microbes to degrade fiber. Rumen microbes require 70 to 80 g CP/kg of dry matter (Lazzarini *et al*., 2009; Figueiras *et al*. 2010).

Supplementation can be done to improve feed fiber digestibility. Supplementation of nitrogen compound to low quality roughages is very important to do (Dettman *et al*., 2010; Souza *et al*., 2010). However, it might bring negative effect on intake of low quality roughage as a result of increased competition for essential nutrients between fibrolytic and non fibrolytic microbes (Souza *et al*., 2010; Mlay *et al*., 2010; Costa *et al*., 2008). Several previous studies showed that there was a significant relation between protein supplementation and energy supplementation in order to avoid competition between fibrolytic and non fibrolytic microbes in fiber digestion (Lazarinni *et al*., 2016; Detmann *et al*., 2010). Information about the best ratio of protein and energy supplementation for swamp buffaloes fed TMF silage as a basal diet is not yet available. Therefore, this study was aimed at assessing, by an *in vitro* technique, the effects of soluble carbohydrate and protein source ratio on nutrient digestibility and rumen condition characteristics of swamp buffaloes fed TMF silage basal diet.

MATERIALS AND METHODS

The research using an *in vitro* technique, the rumen fluid from 3 years old rumen fistulated swamp buffalo bulls with a body weight of 350±5 kg. The treatments according to a completely randomized design with 3 treatments and 4 replicates. Treatments consisted of 3 rations namely: P_1 : 300 g SCH and 300 g protein (1 : 1), P_2 : 600 g SCH and 300 g protein (2 : 1) and P_3 : 900 g SCH and 300 g protein (3 : 1). In each treatment, corn and soybean meals were used as SCH and protein sources, respectively. Each ration was supplemented with 1.5 x Ca, P, and S minerals (NRC, 2000). By using an *in vitro* technique, measurements were taken on nutrient digestibility (dry matter, organic matter, crude protein, and crude fiber), fiber digestibility (NDF, SDF, cellulose, hemicelluloses), and rumen fermentation conditions (concentrations of RAN, total VFA, acetate, propionate, butyrate, and CH_4 and rumen pH). Nutrient contents of feed used in treatment rations are presented in Table 1.

Production of TMF silage

TMF was formulated by using 20% *kumpai tembaga* grass + 20% oil palm frond + 20% rice straw (Imsya *et al*., 2016). Silage production was done based on the result of previous study using sodium diacetate (SDA) of 7 g/kg fresh TMF. Sodium diacetate was evenly sprayed onto TMF ingredients before all ingredients were put into plastic bags. The plastic bags were closely tied and the mixtures were incubated for 45 days.

Sample and analysis

Feed digestibility and rumen condition characteristics were determined by using an in vitro technique of Tilley and Terry (1963). For this purpose, McDougal solution as an artificial saliva buffer and 0.2% pepsin solution as an indicator were prepared. Rumen liquid obtained from a slaughter house was filtered by using a four-layer cheese cloth. Ten milliliters of rumen liquid was put into an incubation tube and 40 ml buffer solution, macro and micro mineral solution, reduction solution, and resazurin were added in (Goering and Van Soest, 1970). Into this tube containing 50 ml solution, 1 g sample was added in and CO_2 gas was flowed for 30 minutes before the tube was closed and incubated for 24, 48, and 72 h. In the end of each incubation time, 2 drops of $HgCl_2$ were added into the tube to kill the microbes. After all incubation times were reached, the media were centrifuged at 4000 rpm for 10 minutes to separate the residue and the supernatant. Fifty milliliters of 0.2% pepsin solution was added into the residue and the mixture was incubated for 48 h before it was filtered by using Whatman No.41 filter paper. The filtered residue was dried in an oven at 60°C for 48 h before it was used for nutrient content analysis and nutrient digestibility determination. The supernatant was used for determination of rumen condition characteristics (concentration of N-NH3 and VFA, SCFA).

RESULTS AND DISCUSSION

Effects of soluble carbohydrate (SCH) and protein ratio on *in vitro* nutrient digestibility of swamp buffaloes fed TMF silage basal diets

It was found that soluble carbohydrate (SCH) and protein ratio gave significant effects (P<0.05) on digestibility of nutrients (dry matter, organic matter, crude protein, crude fiber) and digestibility of fiber (ADF, cellulose, hemicellulose) but not (P>0.05) on NDF digestibility of swamp buffaloes fed TMF silage basal diets as shown in Table 1.

There were increased dry matter and organic matter digestibility with an increase in SCH and protein ratio up to 3 : 1 (P<0.05). With regard to dry matter, its digestibility with SCH: protein ratio of 3 : 1 was 11.63 and 7.14% higher than those with SCH : protein ratio of 1 : 1 and 2 : 1, respectively. Similarly, organic matter digestibility was found to increase (P<0.05) with SCH and protein supplementation. SCH and protein supplementation in the ratio of 3 : 1 resulted in higher organic matter digestibility than those in SCH and protein ratio of 2 : 1 and 1 : 1. SCH and protein supplementation was also found to significantly increase (P<0.05) crude protein and crude fiber digestibility. Increased SCH and protein balance gave a linear increase in crude protein and crude fiber digestibility. The ratio of SCH and protein supplementation significantly (P<0.05) gave linear increases in ADF and cellulose digestibility but not (P>0.05) in NDF digestibility. ADF and cellulose digestibility in SCH : protein ratio of 3 : 1 was found to be higher by 15.10 and 70%, respectively than those in SCH : protein ratio of 1 : 1. Hemicellulose digestibility in SCH : protein ratio of 1 : 1 was not different (P>0.05) from that in SCH : protein ratio of 3 : 1. However, hemicellulose digestibility in SCH : protein ratio of 2 : 1 was found to be significantly lower (P<0.05) than those in the other SCH : protein ratios. In general, SCH and protein supplementation gave positive effects on the digestibility rate of crude fiber and the other nutrients including dry matter, organic matter, and crude protein in swamp buffaloes fed TMF silage basal diets.

Supplementation is required by ruminant animals consuming low quality roughages. Protein supplementation, for example, is very potential as a substrate for fermentation in rumen, particularly for the growth of fibrolytic bacteria which in turn will lead to improved feed intake and fiber digestibility (Leng, 1990; Detmann *et al*, 2009; Souza *et al*., 2010). Increased digestibility which was found with increased SCH and protein supplementation showed that there was a direct relation between SCH and protein availability and improvement of rumen condition for nutrient metabolism by microbes. This was indicated by the finding that rumen ammonia nitrogen (RAN) concentration did not decrease as SCH and protein balances increased. Lazzarini *et al*. (2016) pointed that there was an interaction between starch and nitrogen supplementation in their utilization in rumen. Starch supplementation would result in better microbe assimilation. As this was followed by simultaneous nitrogen supplementation, RAN concentration was lowered. This lowered RAN concentration was not found in animals receiving only nitrogen supplementation. Nitrogen supplementation followed by non fibrous carbohydrate (NFC) supplementation gave the best results in nutrient digestibility in rumen (Souza *et al*., 2010). Soluble carbohydrate and protein supplementation in different ratios did not give significant effects on NDF digestibility but the tendency of digestibility reduction was found. This was in line with the results of a study conducted by Marcia *et al*. (2017) who found that the ratio of starch and protein supplementation did not give significant effects on NDF digestibility in cattle fed low quality roughages. Several studies conducted in tropical areas showed that there was a decrease in NDF digestibility with starch supplementation in rations (Lazaarini *et al*., 2016; Souza *et al*., 2010).

This could happen as a result of reflection of the fermentation process which occurred as the work of two groups of bacteria in the rumen, namely SCH digester bacteria and fibrolytic bacteria. The availability of SCH supplement in rations creates a phenomenon known as a carbohydrate effect in which there are an increased competition for essential nutrients between two groups of microbe species and a reduced utilization of insoluble fiber by fibrolytic microbes with lower competitive capacity (Lazzarinni *et al*., 2016; Arroquy *et al*., 2005; Carvalho *et al*., 2011). In addition, a decreased fiber digestibility which was found with starch supplementation in rations formulated with low quality roughages might be caused by increasing domination of non fibrolytic bacterial population reducing the activity of fibrolytic bacteria (Carvalho *et al*., 2011; Mould *et al*., 1983). In this study, no effect of supplementation on fiber digestibility was found as supplementation of SCH and protein was done simultaneously. Simultaneous supplementation of carbohydrate and protein reduced microbial competition for substrate and reduced or avoided destructing effects on fiber degradation (Souza *et al*., 2010; Lazzarini, 2016).Nutrition, particularly carbohydrate and protein, balance plays an important role in metabolism process, especially digestion, in rumen. Marcia *et al*. (2017) suggested that energy and protein balance was one with the most important role in metabolic mechanism. This is understandable as compared to excess protein availability, excess energy availability may result in more inconvenience in animal's body as a result of excess body heat production. Similarly, compared to excess available carbohydrate, excess available protein may result in more inconvenience in animal's body as a result of excess nitrogen catabolism process (Poppi *et al*., 1995; Illius *et al*., 1996).

Effects of soluble carbohydrate (SCH) and protein balance on *in vitro* rumen fermentation condition in swamp buffaloes fed TMF silage basal diets

Concentrations of N-NH3, total VFA, VFA partial, pH, and methane gas production are indicators of rumen fermentation condition. Further, rumen fermentation condition indicates whether the metabolism in rumen runs well or not. Rumen fermentation condition of swamp buffaloes fed TMF silage basal diets with carbohydrate and protein supplementation in this study is listed in Table 5.

It was shown that the ratio of soluble carbohydrate and protein supplementation in swamp buffaloes fed TMF basal diets gave significant effects ($P<0.05$) on concentrations of rumen ammonia nitrogen (RAN), total VFA, acetate, propionate, and butyrate and methane gas production but not ($P>0.05$) on rumen pH. Increased soluble carbohydrate and protein balance resulted in increased concentrations of RAN, acetate, propionate, butyrate, and methane gas (CH4). Butyrate concentrations in the ratio of soluble carbohydrate and protein supplementation of 2 : 1 and 3 : 1 were not different ($P>0.05$) but they were different ($P<0.05$) from that in the ratio of soluble carbohydrate and protein supplementation of 1 : 1. RAN concentration value is a reflection of balanced synthesis of rumen microbe protein which is often related to the proportion of fermented carbohydrate and the availability of ammonia from protein degradation cycle (Bpdine *et al*, 2000). RAN concentration was found to be increased as the balance of SCH and protein supplementation increased. This increase was suspected to relate to increased crude protein and organic matter digestibility in rumen with SCH and protein supplementation. This might be caused by the fact that RAN was a result of feed protein degradation so that when protein digestibility increased, RAN concentration also increased. Degradation of protein from feed or non protein nitrogen (NPN) resulted in RAN in rumen. RAN concentration in rumen is a description of the ability of rumen microbes in degrading protein contained in ration (Muhtaruddin *et al*., 2006; Prihandono, 2001). Results of this study were different from those of Lazzarini *et al*. (2016) who found no interaction between starch and protein supplementation and RAN concentration. However, they found that starch supplementation lowered RAN concentration while protein supplementation increased it. Souza *et al*. (2010) suggested that nitrogen supplementation followed by supplementation of energy from non fibrous carbohydrate gave the best result of RAN concentration in low quality roughages in tropical area condition. RAN concentration was found to significantly decrease with increasing balance of starch and protein supplementation in cattle fed hay (Marcia *et al*., 2017). A work of Arroquy *et al*. (2004) showed a linear increase of RAN concentration following supplementation of nitrogen in the form of urea and supplementation of non fibrous carbohydrate in the form of dextrose.Increased SCH and protein supplementation balance resulted in increased total VFA concentration.

This increase of VFA concentration was related to increases in crude fiber, ADF, and cellulose digestibility. However, NDF digestibility did not change significantly as VFA was the final product of fiber digestibility in ration. Carbohydrate components in ration including cellulose, hemicellulose, starch, and pectin are digested to result in VFA as a final product (Puastuti, 2005). Pentose is the main result of hemicellulose degradation in rumen. Hemicellulase hydrolyzes

Table 1. Nutrient contents of feeds used in treatment rations (%).

No.	Feed	Crude protein	Crude fiber	TDN
1.	Rice bran	11.2	18.5	65
2.	Ground corn	10.82	2.61	83
3.	Tofu waste	11.6	7.79	70
4.	Urea	261	0	0
5.	TMF silage	13.87	31.48	62.78

Source: Results of analysis at laboratory of animal nutrition and feed, Sriwijaya University (2018).

Table 2. Feeds used as concentrate ingredients and their nutrient contents (%).

No.	Feed	Use	Crude Protein	Crude Fiber	TDN
1	Rice bran	24.75	2.77	4.57	16.08
2	Ground corn	39	4.21	1.01	32.37
3	Tofu waste	35	4.06	2.72	24.50
4	Urea	1.25	3.26	0	0
Total		100	14.31	8.31	72.95

Source: Calculated by using data listed in Table 1 with the use of feed in concentrate.

Table 3. Nutrient contents of rations (%).

Feed	Use	Crude Protein	Crude Fiber	TDN
TMF Silage	60	8.32	18.88	37.66
Concentrate	40	5.72	3.32	29.18
Total	100	14.04	22.21	66.85

Note: Calculated based on data listed in Table 1 and 2.

Table 4. Nutrient digestibility in swamp buffaloes fed TMF silage basal diets with SCH and protein supplementation.

Nutrient Digestibility (%)	SCH : Protein Ratio		
	1 : 1	2 : 1	3 : 1
Dry matter	65.57a	68.32b	73.20c
Organic matter	64.13a	66.49b	71.72c
Crude protein	49.37a	51.61b	57.32c
Crude fiber	42.61a	45.96b	47.79c
NDF	65.15	64.77	61.32
ADF	34.55a	37.23b	39.77c
Cellulose	14.25a	16.39b	24.34c
Hemicellulose	38.12b	25.20a	36.54b

Note: different superscripts in the same row indicate significantly different treatment effect ($P<0.05$).

Table 5. Effects of the ratio of soluble carbohydrate and protein supplementation on rumen fermentation condition of swamp buffaloes fed TMF basal diets.

Rumen fermentation condition	Treatment SCH : Protein ratio		
	1 : 1	2 : 1	3 : 1
RAN	9.48a	10.66b	11.82c
Total VFA	79.52	119.44	139.41c
Acetate	13.26a	20.38b	22.34b
Propionate	3.79a	5.47b	5.5b
Butyrate	1.61a	2.83b	2.55b
Methane gas	8.39a	13.63b	14.56b
pH	6.8	6.8	6.7

Note: different superscripts in the same row indicates significantly different treatment effects ($P<0.05$).

hemicellulose into xylose and uronic acid. Uronic acid can also be produced from degradation of pectin by pectinase and polygalacturonidase. The second stage is intracellular metabolism of simple sugar into pyruvic acid by rumen microbes. Pyruvic acid is further transformed into VFA (McDonald *et al*., 2002). Microbe activities in fiber degradation in rumen produce VFA (Opera *et al*., 1975). VFA is the final product of carbohydrate fermentation and the main source of energy for ruminant animals (Parakkasi, 1999). Results of this study were supported by Bodine *et al.* (2000) who suggested that supplementation of degradable intake protein (DIP) did not give significant effects on VFA concentration in male cattle fed hay basal diets without supplementation of energy source such as corn.

Supplementation of SCH and protein in different ratios to TMF silage basal diets also gave significant effects on the concentrations of partial Volatile Fatty Acids (VFAs) consisting of acetate, propionate, butyrate, and any other acids produced in rumen as the final products of microbe fermentation. In this study, concentrations of partial VFA, particularly acetate and propionate increased. This was in line with the results of a work by Bodine *et al.* (2000) who found increased concentrations of organic acids (acetate and propionate) in rumen following the supplementation of degradable protein intake (DIP) and corn to hay basal diets. Meanwhile, Arroquy *et al.* (2004) found increased concentrations of organic acids including acetate, propionate, and butyrate in rumen as a response to supplementation of urea and non fibrous carbohydrate (NFC) in the form of starch. Concentration of organic acids in rumen was proven to be affected by supplementation of different types of carbohydrate. High concentrations of acetate, propionate, and butyrate were found in supplementation of dextrose but not starch. Increased butyrate concentration and decreased acetate concentration in the final part occurred because acetic and butyric acids are synthesized from acetyl-COA precursor (Arraquoy *et al*., 2004; Heldt *et al*., 1999). Increased concentrations of acetic and propionic acids led to increased production of methane gas. This occurred as in the metabolism process of crude fiber into acetate and butyrate, H2 gas, which was the precursor of methane gas production, was produced. Fiber fermentation which resulted in acetate as the final product was closely related to methane gas production. Production of acetic and propionic acids had profound effects on methane gas production which was also closely related to production of acetic and butyric acids. Meanwhile, production of propionic acid was not followed by production of H2 and CO2 (Church, 2002).

No changes were found in ruminal pH indicating that buffer condition in rumen could still be maintained following the supplementation of SCH and protein in different ratios. A study by Marcia *et al.* (2017) showed that supplementation of starch and protein in rations did not make ruminal pH change. Bodine *et al.* (2000) found no interaction effects of urea and corn supplementation on rumen pH. In addition, supplementation of dextrose, starch, and DIP was found to maintain rumen pH in a normal range for rumen metabolism of about 6.5 to 7 (Aqqurroy *et al*., 2004).

CONCLUSION

It was concluded that the best ratio of SCH and protein supplementation in swamp buffaloes fed TMF silage basal diets was 3 : 1 (900 g : 300 g). SCH and protein supplementation at 3 : 1 ratio increased digestibility of nutrients including dry matter, organic matter, crude protein, crude fiber, ADF, cellulose, and hemicellulose. It also improved rumen fermentation condition by increasing concentrations of RAN, total VFA, and partial VFA (acetate, propionate, and butyrate).

Suggestions

It was suggested that the utilization of TMF silage basal diets in swamp buffaloes be supplemented with SCH and protein in the ratio of 3:1. Further *in vivo* studies to assess the effects of this supplementation on the performance of swamp buffaloes are recommended.

REFERENCES

Arroquy, J.I., R.C. Cochran, M. Villareal, T.A. Wickersham, D.A. Llewellyn, E.C. Titgemeyer, T.G. Nagaraja, D.E. Johnson and D. Gnad. 2004. Effect of level of rumen degradable protein and type of supplemental non-fiber carbohydrate on intake and digestion of low-quality grass hay by beef cattle. *Anim. Feed Sci. Tech*., **115**: 83-99.

Arroquy, J.I., R.C. Cochran, T.G. Nagaraja, E.C. Titgemeyer and D.E. Johnson. 2005. Effect of types of non-fiber carbohydrate on in vitro forage fiber digestion of low-quality grass hay. *Anim. Feed Sci. Tech*., **120**: 93-106.

Arora, S.P. 1989. *Digestion of Microbes in Ruminant Animals. In* Muwarni, R. (ed.) Gajah Mada University Press, Yogyakarta, Indonesia.

Bodine, T.N., H.T. Purvis, C.J. Ackerman and C.L. Goad. 2000. Effects of supplementing hay with corn and soybean meal on intake, digestion, and ruminal measurements by beef steers. *J. Anim. Sci*., **78**: 3144-3154.

Carvalho, I.P.C., E. Detmann and H.C. Mantovani. 2011. Growth and antimicrobial activity of lactic acid bacteria from rumen fluid according to energy or nitrogen source. *Rev. Bras. Zootecn*., **40**: 1260-1265.

Church, D.C. 2002. *Digestive Physiology and Nutrition of Ruminants*, 11th ed. Oxford Press, Portland, USA.

Costa, V.A.C., E. Detmann, S.V. Filho, M.F. Paulino, L.T. Henriques and H.C. Mantovani. 2008. *In vitro* degradation of low-quality tropical forage neutral detergent fiber according to protein and (or) carbohydrates supplementation. *Rev. Bras. Zootecn*., **37**: 494-503.

Detmann, E., M.F. Paulino, H.C. Mantovani, S. de C.V. Filho, C.B. Sampaio, M.A. de Souza. I. Lazzarini and K.S.C. Detmann. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using *Michaelis-Menten* kinetics. *Livest Sci*., **126**: 136-146.

Detmann, E., M.F. Paulino and S.C.V. Filho. 2010. Otimização do uso de recursos forrageiros basais, *In Simpósio de Produção de Gado de Corte*, Viçosa, Brazil.

Figueiras, J.F., E. Detmann, M.F. Paulino, T.N.P. Valente. S.V. Filho and I. Lazzarini. 2010. Intake and digestibility in cattle under grazing supplemented with nitrogenous compounds during dry season. *Rev. Bras. Zootecn*., **39**(6): 1303-1301.

Goering, H.K. and P.J. Van Soest. 1970. *Forage Fiber Analysis (Apparatus Reagents, Procedures and Some Applications).* Agriculture Handbook. United States

Department of Agriculture, Washington D.C., USA.

Heldt, J.S., R.C. Cochran, C.P. Mathis, B.C. Woods, K.C. Olson, E.C. Titgemeyer, T.G. Nagaraja, E.S. Vanzant and D.E. Johnson. 1999. Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low-quality tallgrass-prairie hay by beef steers. *J. Anim. Sci*., **77**: 2846-2854.

Imsya, A. and F. Muhakka dan Yosi. 2016. Use of swamp grass and agricultural waste as materials for total mixed fiber (TMF) in rations and its effect on methane gas production and production efficiency of beef cattle. *Pakistan Journal of Nutrition*, 1**5**(4): 342-346.

Illius, A.W. and N.S. Jessop. 1996. Metabolic constraints on voluntary intake in ruminants. *J. Anim. Sci*., **74**: 3052-3062.

Lazzarini, I., E. Detmann, C.B. Sampaio, M.F. Paulino. S. de C.V. Filho, M.A. de Souza and F.A. Oliveira. 2010. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Rev. Bras. Zootecn*., **38**: 2021-2030.

Lazzarini, I., E. Detmann, S. de C.V. Filho, M.F. Paulino, E.D. Batista, L.M. de A. Rufino, W.L.D. and Reis. M. de O. Franco. 2016. Nutritional performance of cattle grazing during rainy season with nitrogen and starch supplementation. *Asian Austral. J. Anim*., **29**: 1120-1128.

Leng, R.A. 1990. Factors affecting the utilization of "poor-quality" forages by ruminants particularly under tropical conditions. *Nutr. Res. Rev*., **3**: 277-303.

Franco, M. de O., E. Detmann, S. de C.V. Filho, E.D. Batista, L.M. de A. Rufino, M.M. Barbosa and A.R. Lopes. 2017. Intake, digestibility, and rumen and metabolic characteristics of cattle fed low-quality tropical forage and supplemented with nitrogen and different levels of starch. *Asian Austral. J. Anim*., **30**: 797-803.

McDonald, P., A.R. Hendenon and S.J.E. Hercn. 2002. *The Biochemistry of Silage*, 2nd ed. Chalcombe Publications, Cenlerbury, UK.

Mlay, P.S., A.E. Pereka, S. Balthazary, E.J.C. Phiri, J. Madsen, T. Hvelplund and M.R. Weisbjerg. 2007. *In situ* degradation of poor quality hay in the rumens of mature heifers as influenced by sugar, starch and nitrogen supplements and an ionic feed additive. *Tanzania Veterinary Journal*, **24**: 23-37.

Mould, F.L., E.R. Ørskov and S.O. Mann. 1983. Associative effects of mixed feeds. I. effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages. *Anim. Feed Sci. Techn*., **10**: 15-30.

Muhtarudin. 2003. Preparation and use of Zn-Proteinate in rations to improve the value of wheat bran biodiesel and optimization of bioprocess in the digestion of goats. *Journal of Applied Agricultural Research*, **3**(5): 385-393.

National Research Council. 2000. *National Research Council Nutrient Requirements of Beef Cattle*. National Academy of Science, Washington D.C., USA.

Parakkasi, A. 1999. *Ruminant Nutrition and Feed Science*. University of Indonesia Press, Jakarta, Indonesia.

Prihandono, R. 2001. *Effect of bioplus probiotic supplementation, lysine zn and lemuru oil (Sardinella longiceps) on feed use rate and* *rumen fermentation production of sheep, essay*. Faculty of Animal Husbandry, Bogor Agricultural University, Bogor, Indonesia.

Poppi, D.P. and S.R. McLennan. 1995. Protein and energy utilization by ruminants at pasture. *J. Anim. Sci*., **73**: 278-290.

Puastuti, W. 2005. *Measurement of protein ration quality and its relevance to nitrogen retention and sheep growth*. Bogor Agricultural University, Bogor, Indinesia.

Souza, M.A., E. Detmann, M.F. Paulino, C.B. Sampaio, I. Lazzarini and S. de C.V. Filho. 2010. Intake, digestibility and rumen dynamics of neutral detergent fibre in cattle fed low-quality tropical forage and supplemented with nitrogen and/or starch. *Trop. Anim. Health Prod*., **42**: 1299-1310.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassland Soc*., **18**(2): 104-111.

Van Soest, PJ. 1994. *Nutritional Ecology of the Ruminant*, 2nd ed. Cornell University Press, Ithaca, New York, USA.