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Research Article

In vitro Utilization of Gradedly Ammonized TMF (Total Mkxed Fiber) and Soluble Carbohydrate (SCH) and Protein Balance Supplementation in Rations of Pampangan Swamp Buffaloes

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Abstract | This study was aimed at obtaining the most appropriate balance of soluble carbohydrate (SCH) and protein supplementation to optimize the in vitro utilization of gradedly ammonized total mixed fiber (TMF) originating from agricultural wastes and swamp grasses in rations of Pampangan swamp buffaloes. In this study, optimization was done on TMF ammonization by using 12% graded level of urea (2, 4, and 6%). A completely randomized design with 3 treatments and 6 replicates was used. Treatments consisted of 3 levels of SCH and protein (P) balances, namely no supplementation (A0), 600 g SCH and 300 g P (A1), and 900 g SCH and 300 g P (A2), Measurements were taken on digestibility of nutrients including dry matter, organic matter, crude protein, fiber, NDF, and ADF and characteristics of rumen condition including RUN concentration, total VFA concentration, partial VFA concentration, rumen pH, and population of rumen microbes. Results showed that supplementation of SCH and protein balances gave significant effects (P< 0.05) on digestibility of nutrients, fiber fractions, and characteristics of rumen condition in swamp buffaloes fed basal ration of gradedly ammonized TMF. Increased SCH and protein supplementation in ration improved digestibility (dry matter, organic matter, NDF, ADF cellluoce) and concentration of RAN and total VFA It was concluded that SCH and protein balance of 3:1(900g SCH and 300g protein) was the best SCH and protein supplementation in rations of swamp buffaloes fed basal ration of gradedly ammonized TMF

Keywords | Gradedly Ammonization, Soluble Carbohidrate (SCH), Suplemmentation, *Total Mixed Fiber* (TMF), Swamp Buffalo.

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INTRODUCTION

Pampangan buffalo is a potential germplasm in South Sumatera Province. This swamp buffalo is found plenty in some areas including Pampangan, Pangkalan Lampam, and Jejawi Districts in Ogan Komering Ilir Regency and Rambutan District in Banyuasin Regency. This buffalo is characterized by its black-colored skin and hair, big head, long earlobe, short and backward curled horn, well developed and symmetrical udder, oblong-shaped back body part, docile temperament, and relative resistance to diseases. This buffalo seeks its feeds in swamp areas. There are four varieties of Pampangan swamp buffalo including black, red, mottle, and *lampung* (Windusari, et al, 2015).

With regard to the development of pampangan buffalo, the following problems are identified. The availability of natural roughage fluctuates. In rainy season, it is plenty while in dry season the growth of grasses and other roughages is constrained leading to inadequate feed supply for the animals. In addition, in dry season, some tidal swamp areas are used for rice farming. Nutritive values of rations given to swamp buffaloes are lower than required as the roughage they eat is of low quality and no concentrate supplementation is given. Feed technology including feed ensilage and ammonization is a solution that can be implemented to solve the problems.

Results of a previous study showed that the utilization of ammonized TMF as basal feed for beef cattle increased dry matter and organic matter digestibility by 13.38 and 16.37%, respectively, body weight gain by 0.47 kg/head/day, and feed efficiency by 7.4% (Imsya et al., 2015) However, the feed efficiency and body weight gain were not yet optimal. Another study showed that the ratio of soluble carbohydrate (SCH) and protein supplementation balance in a TMF silage-based diet of 3:1 was the best which gave increases in digestibility of dry matter by 11.63%, organic matter by 16.37%, fiber (ADF) by 15.10% in rumen, and best effects on rumen condition characteristics (Imsya et al, 2019).

Based on the above notion, a study on the optimization of the utilization of ammonized TMF through SCH and protein supplementation needs to be conducted in order to improve the production of pampangan buffaloes through the improvement of roughage sources and feed processing technology. This study was aimed at obtaining the most appropriate balance of soluble carbohydrate (SCH) and protein supplementation to optimize the in vitro utilization of gradedly ammonized total mixed fiber (TMF) originating from agricultural wastes and swamp grasses in rations of Pampangan swamp buffaloes.

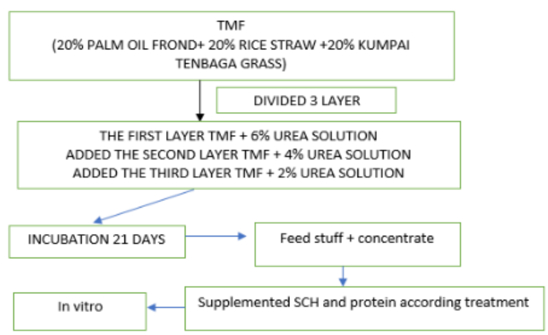
MATERIALS AND METHODS

This experiment was conducted in a completely randomized design with 3 treatments of SCH and protein (P) supplementation balances and 6 replications. Treatments consisted of no supplementation (A0), 600 g SCH and 300 g P (A1), and 900 g SCH and 300 g P (A2). Treatment rations were supplemented with concentrate made from ground corn, ricebran, tofu waste, salt, and urea.Complete rations consisted of roughage (60%) and concentrate (40%) (Table 1 and 2). Measurements were taken on digestibility of nutrients including dry matter, organic matter, crude protein, fiber, NDF, and ADF and characteristics of rumen condition including RUN concentration, total VFA concentration, partial VFA concentration, rumen pH, and population of rumen microbes in vitro

Gradedly Ammonized TMF

In this stage, ammonization of TMF consisting of swamp grasses and agricultural and plantation wastes was conducted. TMF was formulated from 20% kumpai tembaga grass, 20% oil palm frond, and 20% rice straw (Imsya, et al., 2016). Ammonization was done by spreading urea solution 1 liter/kg dry matter. Roughage components of TMF were chopped in 3-5 cm chops size before they were wilted in an open space for 24 hours. Wilted roughage components were then mixed with the other TMF components and a urea solution was prepared by diluting urea in water within the ratio of 1:1 w/l. Urea solution was sprayed over TMF components in a graded way. The urea used in each grade was 6%, 4% and 2%. All sprayed TMF components were then put into plastic bags which were tightly tied up and were incubated for 21 days. After the ammonization process was done, treatment rations for pampangan buffaloes weighed 150 kg were formulated and supplemented with SCH and protein in accordance to the intended ratio. Source of SCH is corn flour and source of protein is soybean flour. Nutrient digestibility, rumen condition characteristics, and microbe population were assessed in vitro by using a Tilley and Terry (1963) method.

The Graph of Methode and Treatment



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 fluid was taken from a 2.5-year old male swamp buffalo prior to feeding time in the morning. To obtain the fluid from the rumen, a stomach tube method was applied by using a vacuum pump, host, nylon sieve, thermos bottle, Erlenmeyer flask, and thermometer. The buffalo was fed swamp grass (*bento* grass) and concentrate (70:30). 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).

Table 1: Nutrition Composition of Feed Stuff (%)

No	Feed Stuff	Dry Matter	Crude Protein	Crude Fiber	TDN
1	Rice Bran	85,50	11,2	18,5	65
2	Ground Corn	88,00	10,82	2,61	83
3	Tofu Waste	88,35	11,6	7,79	70
4	Urea***	0	261	0	0
5	Gradedly Ommonized TMF	24,31	9,66	32,52	40,53

Source: Imsya *et al.* (2019).

Table 2: Feeds Used as Concentrate Ingredients and Their Nutrient Contents (%)

No.	Feed Stuff	Use	Dry Matter	Crude Protein	Crude Fiber	TDN
1.	Rice Bran	24,75	21,40	2,77	4,57	16,08
2.	Ground corn	39	34,32	4,21	1,01	32,37
3.	Tofu Waste	35	30,92	4,06	2,72	24,50
4.	Urea	1,25	0	3,26	0	0
Total		100	86,64	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	Dry Matter	Crude Protein	Crude Fiber	TDN
Gradedly Amonized TMF	60	14,58	5,79	19,51	24,31
Concentrate	40	34,65	5,72	3,32	29,18
Total	100	49,23	11,51	22,83	53,49

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

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 hours. 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 hours before it was filtered by using Whatman No.41 filter paper. The filtered residue was dried in an oven at 60°C for 48 hours 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)

Data Analysis

Data were subjected to an analysis of variance and a Duncan Multiple Range Test (SPSS 13.0 program).

RESULTS AND DISCUSSION

Increased SCH and protein supplementation in rations was found to significantly ($P<0.05$) improved digestibility of dry matter, organic matter, and crude protein of rations in a basal diet of gradedly ammonized TMF in swamp buffaloes (Table 4). SCH and protein supplementation balance of 3:1 was found to increase ($P<0.05$) digestibility of dry matter by 23.5%, organic matter by 26.23%, and crude protein by 28.60%. Accordingly, SCH and protein supplementation balance of 2:1 was found to increase ($P<0.05$) digestibility of dry matter, organic matter, and crude protein by 12.88, 12.96, and 17.09%, respectively. Nutrient digestibility was also found to increase significantly ($P<0.05$) in gradedly ammonized TMF basal rations with SCH and protein supplementation balance of 2:1. Digestibility of dry matter, organic matter, and crude protein increased by 9.41, 11.75, and 9.83%, respectively. Increases in SCH and protein balance gave linear improvement in the digestibility of dry matter, organic matter, and crude protein of rations of buffaloes fed gradedly ammonized TMF basal diets.

Increased digestibility ($P<0.05$) was also found in fiber fractions including NDF, ADF, and cellulose. SCH and protein supplementation balance of 3:1 improved NDF, ADF, and cellulose digestibility by 24.09, 10.97, and 36.51%, respectively. Similarly, SCH and protein supplementation balance of 2:1.was found to significantly increase ($P<0.05$) digestibility of fiber fractions. Increased digestibility by 11.09, 6.39, and 11.51% was found in NDF, ADF, and cellulose, respectively.

Soluble Carbohidrate and protein supplementation bal

Table 4: Digestibility of Nutrients and Fiber Fractions in Swamp Buffaloes Given SCH and Protein Supplementation and Basal Diet of Degradedly Ammonized TMF

Nutrient Digestibility (%)	Treatment imbangan SCH : Protein		
	Without Supplementation	2:1	3:1
Dry Matter	71,35[a]	78,07[b]	88,13[c]
Organic Matter	68,77[a]	76,85[b]	86,81[c]
Crude Protein	35,69[a]	39,20[b]	45,90[c]
NDF	32,92[a]	36,57[b]	40,85[c]
ADF	36,91[a]	39,27[b]	40,96[b]
Celullosa	27,52[a]	30,69[b]	37,57[c]

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

Table 5: *In vitro* rumen fermentation condition in buffaloes fed gradedly ammonized TMF basal diets with SCH and protein supplementation

Rumen Fermentation Condition	Treatment ratio SCH : Protein		
	Without Supplementation	2:1	3:1
RAN	12,82a	14,47b	15,36c
Total VFA	89,29a	105,98b	129,45c
Acetat	14,55a	17,39b	17,48b
Propionate	4,52	4,43	4,24
Butirat	4,07a	2,79b	2,92b
pH	6,8	6,8	6,7
Total bacteria	3,98x10^9a	4,14x10^9b	5,06x10^9c
Celullolitic Bacteria	2x10^9	2,3x10^9	2,8x10^9
Amilolitic Bacteria	1,2x10^9a	1,4x10^9b	1,8x10^9c

Note : different superscripts on the same row indicates significantly different treatment effect ($P < 0.05$)

ance in rations of buffaloes improved digestibility of nutrients by in vitro. Increases in nutrient digestibility in rations supplemented with carbohydrate and protein could be attributed to the effect of this supplement on the passage rate of fiber particles in rumen (Marjorrie A, et al., 2010; Imsya et al., 2019). Low quality feed needs supplementation for its optimal digestibility and nitrogen supplementation is an important support animals fed with low quality tropical roughages (Paulino et al., 2008; Wickersham et al, 2008) as the growth of rumen microbes, particularly fiber digester microbes, depends significantly on protein availability in rumen (Detmann, et al., 2009). Supply of additional protein compounds to animals feeding on low quality roughages would stimulate the growth of fibrolytic bacteria leading to increased feed intake and digestibility (Detmann, et al., 2009; Russell et al., 2001) as protein and carbohydrate are potential substrates for ruminal fermentation (Leng, 1990; Souza et al., 2010).

Supplementation of SCH and protein by the ratio of 3:1 to gradedly ammonized TMF basal diets resulted in improved digestibility of dry matter, organic matter, and cellulose by 73.20, 71.72, and 24.34%, respectively. This improved digestibility might be caused by the fact that ammonization of basal diet loosens up lignocellulose bonds making it easier for the enzymes to penetrate into the cells. In addition, SCH and protein supply improves rumen condition for nutrient metabolism by rumen microbes. Furthermore, there is an interaction of starch and nitrogen utilization by which nitrogen supply will induce a better assimilation of starch by rumen microbes. Souza et al. (2010) animals treated with SCH and protein supplementation indicated lower RAN concentration than animals treated with nitrogen supplementation only, it was also found that supplementation of non-fibrous carbohydrate (NFC) followed by nitrogen supplementation gave the best nutrient digestibility in rumen.

Nutrient metabolism in rumen for the amino acid formation and microbial crude protein production is affected by the balance of nutrients, particularly carbohydrate as energy source and protein as nitrogen source. This is in line with the notion that energy and protein balance plays an important role in rumen metabolic mechanism. This condition occurs on the basis of discomfort the animals feel when energy is in excess compared to the amount of available protein. Excess energy in the body which is not balanced with protein availability leads to increased body heat production. Similarly, excess protein availability results in discomfort in the animal body as nitrogen catabolism process occurs excessively (Poppi and McLennan, (1995); Illius and Jessop, 1996).

Fermentation condition in rumen which is an indicator of rumen metabolism is reflected in the concentration of rumen ammonia nitrogen (RAN), total VFA, partial VFA, rumen pH, and methane gas production. Rumen fermentation condition of swamp buffaloes feeding on TMF silage basal diet supplemented with SCH balance in this study is listed in Table 5. It was shown in this study that SCH balance supplementation gave significant effects ($P<0.05$) on the concentration of RAN, total VFA, acetate, and butyrate but not ($P>0.05$) on rumen pH and propionate concentration.

RAN and total VFA concentration was also found to increase with TMF silage basal diet supplemented with SCH and protein balance. Concentration of acetate and butyrate acids was found to increase but this increase was not affected by the improvement of SCH and protein ratio from 2:1 to 3:1. No significant effect of SCH and protein balance ($P>0.05$) was also found on propionate acid concentration. Population of total rumen microbes and amylolytic bacteria was significantly increased ($P<0.05$) but that of cellulolytic bacteria was not different (Table 4).

RAN concentration is a portray of the existence of balanced rumen microbial protein synthesis as there is a proportional relation between ammonia resulted from protein degradation and the amount of VFA as a result of carbohydrate metabolism (Bodine et al., 2000). Increases in SCH and protein balance in gradedly ammonized TMF basal diet resulted in increased RAN concentration by 19.81% (non supplementation vs. 3:1 ratio). RAN is an indicator of nitrogen availability in rumen (Detmann et al., 2014). Increased RAN concentration indicates increased digestibility of rumen crude protein and organic matter and this phenomenon was found with increased SCH and protein supplementation balance in this study (Table 5). Results of this study were in line with those of (Bodine et al., 2000) who found a quadratic increase in rumen ammonia concentration with the supplementation of protein in the form of degradable intake protein (DIP) and corn as the source of carbohydrate. RAN in rumen is principally a product of ration protein degradation or digestion; therefore, there is a linear correlation between protein digestibility and RAN concentration in rumen. In addition, as RAN resulted from protein degradation is metabolized by rumen microbes, RAN concentration describes microbial activity in protein metabolism in rumen (Muhtaruddin, 2003; Prihandonno, 2001). RAN concentration was also found to increase with SCH and protein supplementation at the ratio of 3:1 into basal diets containing TMF silage made by using sodium acetate (SDA) additive (Imsya et al., 2019).

However, results of this study were in contrast with those of (Lazzarini et al., 2016) who found that although protein supplementation increased RAN concentration but there was no interaction between starch and protein supplementation and RAN concentration. Supplementation of nitrogen followed with supplementation of energy from non fibrous carbohydrate gave the best result for RAN concentration with low quality roughages of tropical regions (Souza et al., 2010). However, a significant decrease in RAN concentration was observed in cattle fed hays supplemented with starch and protein balance (Marcia et al., 2017) Results of a study by (Arroquy et al., 2004) showed a linear increased RAN concentration with supplementation of urea as a nitrogen source and dextrose (non fibrous carbohydrate).

In this study, increased supplementation of SCH and protein balance into gradedly ammonized TMF basal diets resulted in significantly increased total VFA concentration ($P<0.05$). This increased total VFA concentration was related to fibrous fraction digestibility which was also found to increase [Table 3]. VFA concentration in rumen is an indicator of fiber digestibility as VFA is the final product of fiber metabolism. Cellulose and hemicellulose enzymes hydrolyze structural carbohydrates and fibers into xylose, glucose, and uronic acid. Further, these simple sugars are metabolized by rumen microbes into pyruvic acid and VFA, eventually. In ruminants, carbohydrate components including cellulose, hemicellulose, starch, and pectin are digested by rumen microbes to produce VFAs which are further used as the main energy source for the host animal (Puastuti, 2005; Parrakkasi, 1999; Heldt, et al., 1999). Results of this study supported those found by (Bodine et al., 2000) who found positive effects of degradable intake protein (DIP) supplementation in hay basal diets on VFA concentration when the supplementation was accompanied with the supplementation of corn as a source of energy.

The partial VFAs consisted of acetate, propionate, and butyrate. In this study, increased SCH and protein balance in gradedly ammonized TMF basal diets resulted in increased acetate concentration. Yet, butyrate concentration was found to be lowered and no difference was found in propionate concentration. These findings were in line with those of (Bodine et al., 2000) who found increased organic acid (acetate and propionate) concentration with the supplementation of degradable intake protein (DIP) and corn into hay basal diets. Meanwhile, in another study by (Arroquy et al., 2004), supplementation of starch (non fibrous carbohydrate) and urea resulted in increased concentration of organic acids including acetate, propionate, and butyrate. It was proven that the concentration of organic acids in rumen was affected by the types of carbohydrate supplemented. Higher concentration of acetate, propionate, butyrate, and lactate was resulted from the supplementation of dextrose than from that of starch. Increased butyrate and decreased acetate concentration was attributed to the fact that these organic acids are synthesized from acetyl-CoA precursor (Arroquy et al., 2004; Parker et al., 1999). Similar results in total VFA and partial VFA (acetate, propionate, butyrate) concentration were obtained from the supplementation of SCH and protein balance of 3:1 into TMF silage basal diets containing sodium acetate (SDA) additive (Imsya et al., 2019).

Significant effects of supplementation of SCH and protein balance into gradedly ammonized TMF basal diets were found on the population of total rumen bacteria and total amylolytic bacteria but not on the population of cellulolytic bacteria. Increased SCH and protein balance in rations gave significant effects (P<0.05) on the increment of total bacteria and amylolytic bacteria population. This increment in bacterial population could be attributed to the availability of simple carbohydrate (SCH) as the energy source for amylolytic bacteria. Supplementation of carbohydrate and protein in rations increases production of rumen microbial protein and feed energy which eventually avoids competition between fibrolytic and non fibrolytic bacteria (Detmann et al.,, 2010; Lazzarini et al.,, 2016).

However, no differences in ruminal pH were observed indicating that the buffer condition in rumen was well maintained. Similarly, no changes in rumen pH were found in rations supplemented with starch and protein (Marcia et al.,, 2017). Results of other studies showed that supplementation of urea and corn did not affect rumen pH (Bodine et al.,, 2000) and supplementation of dextrose, starch, and DIP did not change the average rumen pH and maintained rumen pH at the level of 6.5-7.

CONCLUSIONS AND RECOMMENDATIONS

The utilization of gradedly ammonized TMF as basal diet which was supplemented with soluble carbohydrate (SCH) and protein balance of 3:1 ratio in swamp buffaloes resulted in the best nutrient digestibility, fiber fraction, and rumen condition characteristics.

The digestibility rates were found to be 88.13% for dry matter, 86.81% for organic matter, 45.90% for crude protein, 40.85% for NDF, 40.96% for ADF, and 37.57% for cellulose.

Rumen condition characteristics showed that RAN concentration was 15.3 mM, VFA 129.45 mM, acetate 17.48 mM, propionate 4.24 mM, and butyrate 2.29 Mm

Degraded ammonization of TMF should be conducted by using 6% (4% and 2%) urea to produce the best nutritive values for animals.

The utilization of degradedly ammonized TMF in rations of swamp buffaloes should be accompanied with supplementation of SCH and protein balance of 3:1 in order to produce optimal nutrient digestibility rates and rumen condition characteristics.

Further studies on the effects of utilization of degradedly ammonized TMF in rations supplemented with SCH and protein balance of 3:1 on the performance of physiological condition of swamp buffaloes.

CONFLICT OF INTEREST

we declare that in this research there is no conflict of interests.

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AUTHORS CONTRIBUTION

All the authors have contributed equally in term of giving their technical knowledge to frame the article.

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