Effect of mulberry leaf pellet (MUP) supplementation on feed intake and rumen microbial population in beef cattle fed on rice straw

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Abstract

The experiment was conducted to investigate the effects of mulberry leaf pellet (MUP) on feed intake, and microbial population in beef cattle fed with rice straw. Four, ruminally fitulated crossbred (Brahman x Thai native) beef cattles with initial body weight of 420 ± 15 kg were randomly assigned according to a 4 x 4 Latin square design. The dietary treatments were supplementation with MUP at 0, 200, 400 and 600 g/d, respectively and rice straw fed ad *libitum.* The results showed that roughage intake and total dry matter intake (kg/d) were significantly higher when compared with control group (P<0.05). Ruminal temperature and pH were not significantly affected (P>0.05) by MUP supplementation. However, ruminal NH_3-N concentrations tended to be increased when supplementation with MUP. In addition, viable total bacteria in the rumen was enriched by MUP supplementation, especially at 600 g/d. Based on this study, it could be concluded that supplementation of MUP at 600 g/d improved DM intake, ruminal NH_3-N and rumen microbial population in beef cattle.

Keywords: Mulberry leaf pellet, microbial population, NH_3-N, beef cattle, rice straw

1. Introduction

Rice straw is the main crop-residue which farmers usually store for use as a ruminant feed in tropical areas, especially in Asia [1]. However, it is low in nutritive value and poor in digestibility. When animals are fed on rice straw, a supplementary strategy is necessary for optimal nutrient for microbial protein synthesis. Strategic supplementation for both carbohydrate and protein particularly non-protein nitrogen (NPN) needs to be undertaken [2]. Mulberry *(Morus alba*) trees are present in many regions of the world and are a potential source of protein for ruminant livestock [3]. Crude protein in mulberry leaves was 13.7 - 23.4% [5, 5, 6, 7, 8, and 9]. Digestible nutrients, net energy and protein fractions of mulberry fodder *(Morus alba)* were available for ruminants [10]. Moreover, protein especially with NPN (urea) in ruminants feeding for possible was increasing microbial protein synthesis [2]. Furthermore, urea can be used as protein source as degradable protein by microorganisms in the rumen. However, using mulberry leaf pellets as a protein supplementation for beef cattle in order to determine effect on rumen microorganism population had been not yet studied. Therefore, the objectives of this study were to investigate the effect of mulberry leaf pellet on feed intake, and microbial population in beef cattle fed on rice straw.

2. Materials and methods

Animals, treatments and experimental design: Four, ruminally fistulated crossbred (Brahman x Thai native) beef cattle with 420±15 kg of BW were randomly assigned according to a 4 x 4 Latin square design to investigate in this experiment. The dietary treatments were as follows: 0, 200, 400 and 600 g/h/d, respectively. MUP products were prepared according to Wanapat et al [11]: In brief, collecting mulberry leaves 120 – 150 day after regrowth and sun dried about 2 – 3 days. Mulberry leaves were then ground to pass 1mm screen using Cyclotech Mill, Tecator, Sweden, mixed mulberry leaf meal with urea, cassava starch, molasses, salt, mixed mineral and sulfur in ratio (Table 1). After mixed well all ingredients added water with ratio 0.8:1 (water and mixing meal, respectively); accordingly made pellets using pellets machine and then sun dried 22-27 hours. All animals were kept in individual pen an individual fed concentrate (14.2 % CP) at 0.5% of BW (DM), twice daily at 07.00 h and 16.00 h. Rice straw was fed to cattle *ad libitum.* The experiment was conducted for four periods, and each lasted for 21 d. During the first 14 d was a period for DM feed intakes measurements while during the last 7 d all cattle were moved to metabolism crates for urine collections. Chemical compositions of concentrates, rice straw and MUP are shown in Table 1.

Table 1: Ingredients and chemical compositions of concentrates, mulberry leaf pellets and rice straw.

Items	Ratio		
	Concentrates	Pellets	Rice straw
	--------%DM--------		
Ingredients			
Cassava chip	75.0	-	
Mulberry meal	-	82.0	
Cassava starch	-	0.5	
Rice bran	6.0	-	
Coconut meal	5.0	-	
Palm meal	6.5	-	
Urea	3.5	10.0	
Molasses	1.0	4.5	
Sulfur	1.0	1.0	
Mineral premix	1.0	1.0	
Salt	1.0	1.0	
Chemical composition (% of DM)			
DM	94.1	92.3	96.0
OM	92.5	88.2	86.2
Ash	7.5	11.8	13.8
CP	14.2	48.7	3.9
NDF	17.4	20.4	75.9
ADF	11.5	14.5	47.3
TDN (%)[1]	79.2		47.0

[1]Total digestible nutrients (TDN) was calculated from the digestibility values of nutrients: TDN% = %DCP + %DCF + 2.25 (%DEE) + % DNFE.

Sample collection and chemical analysis: Concentrate, rice straw were dried at 600C and were ground 1 mm screen using Cyclotech Mill (Tecator, Sweden) and then analyzed for DM, ether extract, ash and CP content [12], NDF, ADF and ADL [13]. Rumen fluid samples were collected at 0, 2, 4, and 6 h post-feeding on the last day of each period. Approximately 200 mL of rumen fluid was taken at each time at the end of each period. Rumen fluid samples were immediately measured for pH and temperature using a portable pH temperature meter (HANNA, instruments HI 8424 microcomputer, Singapore) and were filtered through four layers of cheesecloth. Rumen fluid samples were divided into two portions; one portion was used for NH_3-N analysis where 5 ml of 1M H_2SO_4 solution was added to 45 ml of rumen fluid. The mixture was centrifuged at 16,000 x g for 15 minutes and supernatant was stored at–200C prior to NH_3-N analysis. Second portion was taken immediately for culturing for identification of bacteria using the roll-tube technique [14].

Statistical analysis: Statistical analyses were performed using the GLM procedure of SAS [15]. Data were analyzed using the model $Y_{ijk}=\mu+M_i+A_j+P_k+\varepsilon_{ijk}$ where Y*ijk*, observation from animal, j, receiving diet i, in period k; μ, the overall mean; Mi, effect of MUP (i=1, 2, 3 and 4); A_j, the effect of animal (j = 1, 2, 3 and 4); P_k, the effect of period (k = 1, 2, 3 and 4); and ε_{ijk}, the residual effect. Difference between treatment means were determined by Duncan's New Multiple Rang Test (DMRT) [16] with P<0.05 were accepted as representing statistically significant differences.

3. Results and Discussion

Table 2: Effect of dietary treatment on dry matter feed intake.

Items	MUP supplementation, g/d				SEM
	0	200	400	600	
Rice straw DM intake					
kg/d	6.6[a]	6.8[b]	7.0[b]	7.3[c]	0.04
%BW	1.6[a]	1.6[ab]	1.7[bc]	1.7[c]	0.03
Concentrate DM intake					
kg/d	2.0	2.0	2.0	2.0	0.02
%BW	0.5	0.5	0.5	0.5	0.00
Total DM intake					
kg/d	8.6[a]	9.0[b]	9.4[c]	9.9[d]	0.04
%BW	2.1[a]	2.1[a]	2.3[b]	2.3[b]	0.11

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

Chemical composition of feed: The values for composition of feed ingredients were in Table 1. Concentrate, pellets and rice straw contained 14.2%, 48.7% and 3.9% CP, respectively.

Table 3: Effects of dietary treatment on ruminal pH, temperature, and NH_3-N.

Items	Levels of MUP supplementation, g/d				SEM
	0	200	400	600	
Ruminal parameters					
pH	6.3	6.3	6.3	6.3	0.03
NH_3-N, mg/dL	10.7[a]	13.6[ab]	16.6[bc]	18.5[c]	0.99

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

These nutritional values were expected to support normal performance of these experimental cattle. DM, OM, Ash, CP, NDF, ADF of rice straw was 96.0, 86.2, 13.8, 3.9, 75.9, and 47.3%, respectively. This study was similar to the report by Wanapat [17] that dry matter and protein content of rice straw were 96.7 and 3.3%. The dry matter contents of pellet was 92.3%, dry matter of the pellet were kept well and were similar to the work of many researches [18, 19, 20].

Effects of MUP supplementation on feed intake: Table 2 shows many dietary factors that may influence on dry matter intake in ruminants, such as physical characteristics, ingredients and nutrient composition. In this study dry matter intake was influenced by: MUP source or protein source. The results showed that dry matter intake of rice straw, and total intake were significantly higher (P<0.05) when increasing levels of MUP supplementation (6.6, 6.8, 7.0, 7.3; 8.6, 9.0, 9.4, 9.9 kg/d, respectively) while concentrate dry matter intake was similar. Dry matter intake of rice straw in this study was increased when MUP was supplementation. This result related with McCollum and Galyean [21] who reported that providing a protein supplement to ruminants consuming low-quality forage increased total dry matter intake [22, 23, 24, 25] and total intake was influenced of level of protein [26]. When additional protein is required to using cheaper protein sources or local protein feed resources can be a cost-effective way to add CP to beef cattle diets. In this study, use of MUP (mulberry leaves and urea) as protein source could be cheaper when compared with soybean meal.

Effects on ruminal pH, temperature and NH_3-N concentration: The effects of dietary treatment on ruminal pH, temperature, and NH_3-N are show in Table 3. There were no effects on ruminal pH and temperature, the averaged values were stable at pH 6.3 and temperature between 38.6 to 38.8. These levels were optimal for rumen fermentation. While Van Soest [27] reported that if pH values lower than 6.2 they were negative effects on rumen microbial fermentation by decreasing NDF and ADF digestibility with increasing time under suboptimal pH. Hoover [28] also reported that pH value between 5.0 to 5.5 were negative effects on fiber digestibility. NH_3-N concentration tended to be increased in the MUP diets with averages: 10.7, 13.6, 16.6, and 18.5 for non-supplementation and those supplementation with 200, 400, 600 g/h/d of MUP, respectively.

Table 4: Effect of dietary treatment on ruminal microbes and viable bacteria in beef cattle

Items	Levels of MUP supplementation, g/d				SEM
	0	200	400	600	
Viable bacteria, cfu/mL					
Total, x 10^9	2.9^a	2.7^a	3.5^b	4.1^c	0.11
Amylolytic, x 10^7	2.6^a	2.8^{ab}	3.0^b	3.0^b	0.09
Proteolytic, x 10^7	1.7^a	1.8^a	2.4^b	2.7^b	0.11
Cellulolytic, x 10^9	0.8^a	0.9^a	1.2^b	1.3^b	0.66

^{a,b,c}Means in the same row with different superscripts differ (P<0.05) cfu = Coloning forming unit

Notably, ruminal NH_3-N concentration was highest on diets treatment with supplementation MUP at 400 and 600 g/h/d as increasing of crude protein. Ruminal NH_3-N concentration was a major source of N for microbial protein synthesis [29] efficiency to increasing of microbial digest fiber, that could be relatively greater feed intake as shown in Table 2. However, NH_3-N concentrations with MUP diet was maintained at 13.61 to 18.51 mg/dL (10.7 to 13.6 mM) and this concentration were well above levels (3.57 mM) recommended to optimal ruminal digestion [30]. When intake increased it could increased levels of NH_3-N concentration had been reported [31, 32, 1]. During this period, all animals had increased feed intake, which could explain the high levels of rumen NH_3-N concentration observed.

Rumen microorganism population: Rumen microorganism population is presented in Table 4. MUP supplementation was affected on change of ruminal microbial population, which increased in amylolytic bacteria, proteolytic bacteria, and cellulolytic bacteria and significantly higher at 400 and 600 g/d supplementation of MUP. Similarly at on total viable bacteria was highest in dietary T3 and T4 due to suitable substrate for bacteria utilization in terms of protein source in MUP. Ruminal bacteria play importante role in the digestibility of organic matter and neutral detergent fiber thus total feed intake were highest when supplementation of MUP at 400 and 600 g/d (P<0.05) under in this study, total viable bacterial was increased and this result was in agreement with many researchers [34, 32, 20, 33] or increasing protein for ruminant fed with low quality of roughage increased amount of microbial bacteria [35, 36, 1].

4.Conclusion

Mulberry leaf pellet (MUP) could be used as protein source supplementation at 400- 600 g/h/d. The result revealed improvement of dry matter roughage intake, NH_3-N concentrations, viable total bacteria, amylolytic bacteria, proteolytic bacteria, and cellulolytic bacteria.

5.Acknowledgments

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6.References

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