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Effect of Phosphorus Supplementation to Ammoniated Rice Straw on Rumen Fermentability, Microbial Protein Synthesis and *in vitro* Nutrient Degradability

Research Article

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ABSTRACT

The effect of phosphorus supplementation to ammoniated rice straw was studied. The *in vitro* experiment was carried out following the first stage of Tilley and Terry method. The treatments consisting of four diets were A= 50% ammoniated rice straw + 50% concentrate (control), B= A + 0.2% phosphorus (P) supplement, C= A + 0.4% phosphorus (P) supplement, and D= A + 0.6% phosphorus (P) supplement of dry matter. Completely randomized design was used as the experimental design with differences among treatment means were examined using Duncan's multiple range test. Variables measured were total bacterial and cellulolytic bacterial population, cellulolytic enzyme activity, ammonia (NH_3) and volatile fatty acid (VFA) concentrations, as fermentability indicators and synthesized microbial protein, as well as degradability indicators including dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose. The results indicated that fermentability and degradability of diets consisting ammoniated rice straw with P supplementation were significantly higher than the control diet ($P<0.05$). It is showed that P supplementation is important for rumen fermentation and growth of rumen microbes. Overall supplementation of phosphorus at 0.4% of dry matter to ammoniated rice straw shown best results in terms of rumen fermentation, microbial protein synthesis and *in vitro* degradability.

KEY WORDS ammoniated rice straw, degradability, fermentability, microbial protein, phosphorus.

INTRODUCTION

Improving fiber use following treatment with alkalis (Van Soest, 2006) suggest that scope exists to derive more nutrients from fiber by microbial fermentation in the rumen. However, potentially degradable fiber may be transported from the rumen before fermentation could be complete. The extent of fermentation in the available time depends on the biomass of cellulolytic bacteria. Nutritive value of some straws and other by-product feeds can be improved by addition of urea and minerals such as phosphorus. Phosphorus is very important for normal rumen metabolism, reproduction, skeletal growth, and production (Knowlton and Herbein, 2002; Wu *et al*. 2003). Low intake of phosphorus occurs frequently in young stock and dry cows due to a lack of supplementation or concentrates feeding. Sometimes the problem may be due to poor availability of phosphorus sources. Most of the normal forages consumed by ruminants are little more than adequate with respect to their phosphorus (P) content.

Phosphorus (P) is generally the most costly mineral to supplement in animal diets. Chandler (1996) indicated that P accounts for more than 50% of the cost of typical vitamin-mineral mixes used on dairy farms. There are a strong interaction between the host animal and the rumen microorganisms with respect to P supply and utilization. Rumen microbes have specific phosphorus requirements to degrade the cell walls of feedstuffs. Also, rumen microbes need P to maintain metabolism and growth (Komisarczuk and Durand, 1991); and total P content of rumen microorganisms ranges from 2 to 6% of the dry matter (Valk and Sebek, 1999). The suggested lower concentration of P to maintain normal microbial growth in the rumen is 100 mg/L of ruminal fluid (Durand and Kawashima, 1980). For optimum plant cell degradation and microbial protein synthesis within the rumen, the available P should be at least 5 g/kg fermented organic matter, supplied via the diet and saliva (Komisarczuk and Durand, 1991). Microorganisms are largely dependent on dietary P for their P requirement and the host animal is affected first under a marginal P deficiency (Durand and Kawashima, 1980). There are many studies that report data on the P requirements of ruminal microbes. These data have mainly been obtained using batch culture systems (Milton and Ternouth, 1984) and using continuous culture techniques (Komisarczuk *et al*. 1987). Therefore, this study was conducted to examine the effects of P addition on *in vitro* fermentability, microbial protein synthesis and degradability of ammoniated rice straw.

MATERIALS AND METHODS

The present study was carried out at the Laboratory Ruminant Nutrition Faculty of Animal Science Andalas University and BFNM Laboratory of Animal Science IPB, Bogor, Indonesia.

The experimental diet consisted of 50% ammoniated rice straw and 50% concentrate. The rice straw was previously treated with 4% urea. Rice straw ammoniated and concentrate mixture (rice bran, coconut cake, NaCl and mineral mixture), were ground in mills to pass a 1 mm sieve prior to chemical analysis and *in vitro* fermentation. The chemical composition of rice straw ammoniated and concentrate mixture are 12.95% crude protein and 60.45% TDN. Four levels of phosphorus (P2O5) (0% (A), 0.2% (B), 0.4% (C), and 0.6% (D) concentration on dry matter) were added on the substrates, respectively. A completely randomized design was used as experimental design consisting of four treatments. *In vitro* fermentability and degradability of nutrients were determined using the first stage of the Tilley and Terry procedure (Tilley and Terry, 1969). Ruminal fluid was obtained from a cannulated steer. Fermentation tubes contained of 10 mL of ruminal fluid and 40 mL of McDougall buffer solution and 0.5 g samples were incubated in 100 mL polyethylene tubes in 39 ˚C in a shaken water bath for 48 hours. Treatments were replicated four times within an experiment and the experiment was repeated twice. Two fermentation tubes that did not contain diets were also incubated and used as blanks. Sample was taken from each fermentor for bacterial counting. Fermentation was terminated after 48 hours by injecting the tubes with 1 mL of $HgCl_2$.

Tubes were then centrifuged at 14000 x g for 15 min and the supernatant was collected and stored. Tubes with residue were dried at 60 ˚C for 48 hours and weighed and the data were used for degradability determination. These residues were also analyzed for their dry matter (DM), organic matter (OM) and nitrogen by using standardized procedures (AOAC, 1990), the NDF, ADF, and cellulose of residues were determined as per method of Goering and Van Soest (1970). Supernatants were used in order to determine NH_3 concentration (microdifusion Conway method), total VFA concentration (Gas chromatography) and rumen fluid pH. Total and cellulolytic bacterial populations were determined by methods described by Suryahadi (1990), cellulase enzyme activity and the amount of synthesised microbial protein was determined by methods described by Widyastuti (2005) and Gopar (1981), respectively. Data were analyzed by ANOVA using the GLM procedure. Differences between the control treatment and P supplementation treatment were analyzed by Duncan's multiple range test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Rumen fermentation and microbial protein synthesis

The perusal of data pertaining to effect of P supplementation on bacterial population and other variables of rumen fermentability is shown in Table 1. There was significant ($P<0.05$) decrease in ammonia nitrogen while that of total VFA concentration was increased due to supplementation of P. Although, not significant ($P>0.05$) cellulolytic enzyme activity, cellulolytic bacterial population and microbial protein synthesis tended to increase in phosphorus supplemented group than control. It was revealed that cellulolytic rumen bacteria were present in small population in liquid phase used as samples for counting cellulolytic bacterial numbers;most of cellulolytic bacteria may be attached to fibrous components in solid phase samples. The results also indicated that P plays important role in rumen microbial growth and cellulolytic enzyme activity due to which fiber degradation and total VFA concentration was increased. These results are in agreement with earlier works reported by Durand and Kawashima (1980).

Table 1 Effect of phosphorus supplementation on total and cellulolytic bacterial population and fermentation in the rumen

Variables	P Supplementation (% DM)			
	0	0.2	0.4	0.6
Total bacterial population (x10^{10} colony/mL)	29.67	13.50	26.50	39.19
Cellulolytic bacterial population (x10^{8} colony/mL)	18.83	21.67	24.3	31.67
Cellulolytic enzyme activity (U/mL)	1.42	2.17	1.72	1.64
Synthesised microbial protein (%/g)	0.19	0.18	0.21	0.13
N-NH_3 (mM)	11.09^{a}	10.02^{b}	9.25^{c}	8.80^{d}
Total VFA (mM)	88.75^{c}	98.12^{b}	106.87^{a}	111.87^{a}

Means within a row with different superscript are `significantly different (P<0.05).

Comparisons of the different levels of P supplementation showed that, 0.4% P was better to influence rumen fermentation positively. This finding was also reported by Petterson (2002) who suggested that the best P supplement to beef cattle was 0.4-0.5% on dry matter of low quality roughage.

In vitro degradability

The results pertaining effect of P supplementation to ammoniated rice straw on *in vitro* degradability is presented in Table 2.

Table 2 Effect of phosphorus supplementation on *in vitro* degradability of ammoniated rice straw (%)

Variables	P Supplementation (% DM)			
	0	0.2	0.4	0.6
DM degradability (%)	52.91^{c}	54.85^{bc}	57.66^{a}	60.79^{a}
OM degradability (%)	54.69^{c}	58.43^{b}	60.18^{a}	62.69^{a}
NDF degradability (%)	39.31^{b}	41.58^{b}	43.94^{a}	50.91^{a}
ADF degradability (%)	27.99^{c}	32.78^{bc}	37.59^{a}	40.30^{a}
Cellulose degradability (%)	29.47^{b}	33.04^{b}	38.74^{a}	41.61^{a}

DM: dry matter; OM: organic matter; ADF: acid detergent fibre; NDF: neutral detergent fibre.

The means within the same row with at least one common letter, do not have significant difference (P>0.01).

The addition of P at different levels affected significantly on all degradability variables (P<0.05). Control diet (A) had the lowest DM, OM, NDF, ADF and cellulose degradability (P<0.05). An increase in P supplementation from 0.2% (B) 0.4% (C) and 0.6% (D) increased the degradabilities of DM, OM, NDF, ADF and cellulose, and the increase in degradabilities of DM, OM, and fibrous fractions followed linear patterns with the levels of P supplementation. The results of the present study are in line with those of Valk and Sebek (1999), who pointed out that phosphorus supplementation, increased dry matter degradability in dairy animals. Weiss and Wyatt (2004) reported that when the dietary phosphorus was increased from 0.34 to 0.45% of dry matter in diet of cows, DMI increased from 12.4 to 30.5 kg/d. The present results in degradabilities of DM, OM and fibrous fraction of diets consisting of ammoniated RS and concentrate were in association with the increase in total VFA concentration as affected by P supplementation.

These indicates that P supplementation had promoted rumen bacterial growth and cellulolytic enzyme activities which increased fermentability and degradability of diets consisting of ammoniated rice straw and concentrate. This study has also shown that diets consisting of ammoniated rice straw were deficient in P, and P supplementation is important to improve fibre degradation of fibrous feedstuffs. The present results were in agreements with the results of earlier workers (Bravo *et al.* 2003; Durand and Kawashima, 1980). It was suggested that improvement in fibre degradation by P supplementation occurred through its specific stimulation on growth of rumen cellulolytic bacteria and anaerobic rumen fungi. The present study revealed that the best result was obtained by supplementing P at 0.4% on dry matter.

CONCLUSION

Supplementation of phosphorus to ration containing ammoniated rice straw and concentrate influenced rumen fermentability and nutrient degradability. The effects occurred through reduction in ammonia concentration, increase in total bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. The best level of P supplementation is obtained at a 0.4% of diet dry matter.

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