Importance of Phosphorus Supplementation in Improving Fermentability, Microbial Protein Synthesis and Degradability of Ammoniated Rice Straw

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ABSTRACT

Rumen microbes need minerals for supporting its growth and activity in fermenting and digesting feeds. However, the supply of minerals should match the amount of energy that is available for microbial growth and protein synthesis, and for fermentation. An experiment is carried out to study the importance of phosphorous (P) supplementation in rations containing ammoniated rice straw (RS) and concentrate on its fermentability, microbial protein synthesis and degradability. 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 (RS) + 50% concentrate (control), B = A + P supplement at 0.2%, C = A + P supplement at 0.4%, and D = A + P supplement at 0.6%. Completely randomized design was used as the experimental design with differences among treatment means were examined using Duncan multiple range test. Variables measured were ammonia (NH_3) and volatile fatty acid (VFA) concentrations, total bacterial and cellulolytic bacterial population, cellulolytic enzyme activity, as fermentability indicators and microbial protein synthesis, as well as degradability indicators including dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose. The results indicate that P supplementation at the levels of 0.2, 0.4 and 0.6% reduced ammonia concentration ($P<0.05$) and increased VFA concentration ($P<0.05$), but did not affect other variables. Degradabilities of DM, OM, NDF, ADF and cellulose were increased by P supplementation ($P< 0.05$). It is concluded that P supplementation is important for improving fermentability and degradability of rations containing ammoniated RS and concentrate. The improvement occurred through the increase in cellulolytic bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. The best level of P supplementation is 0.4% (DM basis).

Key words: ammoniated rice straw, phosphorous, fermentability, microbial protein synthesis, degradability

INTRODUCTION

The presence of microbes in the rumen has enabled ruminants to eat fibrous diets such as grasses, agricultural and agriculture industrial byproducts. Rice straw (RS) is one of fibrous feed that is usually used to substitute or to replace grasses; the use of RS becomes important when the availability of grasses is limited during summer. However, the animal production has not reached its optimum level when RS is given to the animals. This is due to limitations in nutrient content and digestibility of RS.

The use of RS can be improved by treatments such as physical (grinding), chemical (alkali or ammoniation) and biological (treatment with microbes producing fibrous degrading enzymes) treatments. Among these treatments, ammoniation with urea is the best chemical treatment. This is because the treatment was simple and easy with low cost, the treatment has also enabled to loose the lignocellulose bonds which increased fibre degradation by rumen microbes (Leng, 1981). The use of urea-ammoniated RS as animal feed has increased body weight gain and milk production, feed intake, dry matter (DM) and organic matter (OM) digestibilities (Promma *et al*., 1985; van Soest, 2006; Sundstol, 1991). However, the use of urea-ammoniated RS could not be used up to 100% level as this level has slowed down animal

growth rate (Zain *et al*., 2000; Zain and Jamarun, 2001).

To increase the use of urea-ammoniated RS, the important strategy is stimulating growth of rumen microbes. This is because of rumen microbes are important organisms that are capable of degrading or digesting fibrous feeds. The other reason is rumen microbial cells are important nitrogen sources for ruminants.

Rumen microbes need minerals for supporting its growth and activity in fermenting and digesting feeds, other than energy, protein, lipid, vitamin, etc. However, the supply of minerals should match the amount of energy that is available for microbial growth and protein synthesis, and for fermentation. Mineral supplementation is necessary, especially when high fibrous diets were given to the animals; these minerals are required for supporting microbial growth (Preston and Leng, 1987; Komisarczuk and Durand, 1991; Little, 1986). One of the minerals is phosphor (P). P is important as part of microbial cells, supporting microbial growth and enzyme activities, having roles in nutrient metabolism in animal, low in its content and availability in feed (Bravo *et al*., 2003; Rodehutscord *et al*., 2000; Little, 1986). P supplementation has increased growth and enzyme activity of cellulolytic bacteria which subsequently increased degradation fibrous fraction of feeds (Komisarczuk *et al*., 1987; Kennedy *et al*., 2000). This P mineral, then, must be used as supplement to improve utilization of ammoniated RS.

Therefore, this experiment is carried out to study the importance of phosphorous (P) supplementation in rations containing ammoniated rice straw (RS) and concentrate on its fermentability, microbial protein synthesis and degradability.

MATERIALS AND METHODS

Materials

Materials consisted of ammoniated rice straw (RS), concentrate, P_2O_5, rumen fluid, McDougall buffer solution, H_2SO_4 15% solution, HCl solution, $HgCl_2$ saturated solution, Na_2CO_3 saturated solution, boric acid solution, NaOH solution, solid brain heart infusion (BHI) medium, and dilution solution.

The experimental diet composed of 50% ammoniated RS and 50% concentrate, and this diet was used as a control diet (A). The rice straw was previously treated with 1.5% urea. Crude protein of diet was 10.16%. P_2O_5 was used as P source and added in diet with the levels were 0, 0.2, 0.4 and 0.6% (DM basis).

Experiment in Nutrient *In Vitro* Fermentability and Degradability

In vitro fermentability and degradability of nutrient were conducted in a batch culture system which followed the first stage of Tilley and Terry procedure (1969). Feed samples (5 g) were placed into a 100 ml polyethylene tube which was then mixed with anaerobic McDougall buffer solution (pH 6.8; 40 ml) and rumen fluids (10 ml) from a rumen cannulated steer. The tubes were then incubated in a shaker water bath at 39^{o}C for 48 h. Two fermentation tubes without sample diets were also incubated and used as blanks. Sample was taken from each fermentor for bacterial counting at the end of fermentation time. After 48 h, fermentation was terminated by injecting the tubes with $HgCl_2$ (1 ml). Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Supernatants were used to analyse NH_3 concentration (microdifusion Conway method), total VFA concentration (Gas chromatography) and rumen fluid pH. Total and cellulolytic bacterial population was determined by methods described in Suryahadi (1990), cellulase enzyme activity and microbial protein synthesis was, respectively, determined by methods described in Widyastuti (2004) and (Gopar, 1981). The residues were dried at 60 ^{o}C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, OM and N based on proximate analysis, the NDF, ADF, and cellulose of residues were determined by Goering and van Soest (1970) procedure.

Experimental Design and Data Analysis

The experimental design was a completely randomized design consisting of four treatments was used with four replications. Variables measured were fermentability indicators (total and cellulolytic bacterial population, cellulolytic enzyme activity, ammonia and total VFA concentration), synthesized microbial protein and degradability of dry matter (DM), organic matter (OM), and fibrous fractions (NDF, ADF and cellulose). ANOVA using the GLM procedure was used to analyse the data (Steel and Torrie, 1981). Differences between the control treatment

and P supplementation treatment were determined by Duncan multiple range test (DMRT) (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

In Vitro Fermentability and Microbial Protein Synthesis Study

Treatment effects were significant on ammonia and total VFA concentrations (P<0.05), but did not influence total and cellulolytic bacterial population, cellulolytic enzyme activity and microbial protein synthesis (Table 1).

Ammonia concentration was decreased with the increase levels of P supplement. However, the ammonia concentration was still in the normal range for microbial protein synthesis, i.e. 4-12 mM (Erwanto *et al*., 1993). The reduction in ammonia concentration was, presumably, due to the utilization of ammonia by the rumen microbes. The rumen microbes used the ammonia as nitrogen (N) source for stimulating its growths (Preston and Leng, 1987); however, the data in microbial protein synthesis was not in a line with the results in ammonia concentration. This indicates that P supplementation may affect growth of rumen microbes that degrade protein.

The increase in the levels of P supplementation had produced positive effects by increasing total VFA concentration. This result was in agreement with the results of Komisarczuk *et al*. (1987) indicating that VFA concentration could be increased by P supplementation. VFA is a product of feed fermentation in the rumen which is used by the ruminants as source of energy (McDonald *et al*., 2002). This experiment indicated that the availability of P in ammoniated rice straw (RS) based diet was not sufficient for supporting rumen microbes to ferment the ammoniated RS. P supplementation at the levels of 0.2 up to 0.6% was capable of increasing VFA concentration meaning that P is important mineral required by the rumen microbes for fermenting ammoniated RS. Therefore, P mineral needs to be added in ammoniated RS based diet.

Table 1. Effect of phosphorous supplementation on total and cellulolytic bacterial population and fermentation in the rumen (mean values)

Variables	Treatment[1]			
	A	B	C	D
N-NH_3 concentration (mM)	11.09[a]	10.02[b]	9.25[c]	8.80[d]
Total VFA cocnentration (mM)	88.75[c]	98.12[b]	106.87[a]	111.87[a]
Total bacterial population (x 10^7colony/ml)	29.67	13.50	26.50	39.19
Cellulolytic bacterial population (x 10^7 colony/ml)	18.83	21.67	24.30	31.67
Cellulolytic enzyme activity (unit/ml)	1.42	2.17	1.72	1.64
Microbial protein synthesis (%/g)	0.19	0.18	0.21	0.13

Note: [1]A = (ammoniated RS + concentrate 50%:50%) ration + P supplement 0%, B = A + P supplement 0.2%, C = A + P supplement 0.4% and D = A + P supplement 0.6%; P = phosphorous; Values within the same rows differ significantly at (P< 0.05).

Table 2. Effect of phosphorous supplemen-tation on *in vitro* degradability of ammoniated rice straw (mean values)

Variables	Treatment[1]			
	A	B	C	D
Dry matter degradability (%)	52.91[c]	54.85[bc]	57.66[ab]	60.79[a]
Organic matter degradability (%)	54.69[c]	58.43[b]	60.18[ab]	62.69[a]
NDF degradability (%)[2]	39.31[b]	41.58[b]	43.94[ab]	50.91[a]
ADF degradability (%)[2]	27.99[c]	32.78[bc]	37.59[ab]	40.30[a]
Cellulose degradability (%)[2]	29.47[b]	33.04[b]	38.74[a]	41.61[a]

Note: [1] A = (ammoniated RS + concentrate 50:50%) ration + P supplement 0%, B = A + P supplement 0.2%, C = A + P supplement 0.4% and D = A + P supplement 0.6%; P = phosphorous; Values within the same rows differ significantly at (P< 0.05).

The importance of P supplementation into ammoniated RS based diet can also be shown by the results in total and cellulolytic bacterial population although the effects were not significant. P supplementation at a level of 0.6% tended to increase total bacterial population. A linear increase in cellulolytic bacterial population was also observed with the increase in the levels of P supplement. Cellulolytic enzyme activity in rations containing ammoniated RS with P supplementations tended to be greater than that in control diet. These results indicate that P supplementation has stimulated the growth of rumen bacteria, especially the cellulolytic bacteria. The increase in cellulolytic bacteria was followed by the increase in cellulolytic enzyme activity. This caused an increased in fermentability of ammoniated RS based diet which was indicated by the increased in VFA concentrations.

The increase in VFA concentrations as a result of P supplementation did not increase microbial protein synthesis. This is because of the increase in VFA concentrations was not followed by the increase in ammonia concentrations. This indicates that P supplementation may support cellulolytic bacterial growth, but may depress proteolytic bacterial growth. As a consequence, there was a shift in the ratio of cellulolytic and proteolytic bacterial populations although there was no change in total bacterial population. This means that the ratio between energy source and N source was not in optimum level for microbial protein synthesis. As a result, a readily N source, such as urea, is still needed when P is added as supplement to ammoniated RS basal diet. Other possibility is in relation with the requirement of rumen microbes for minerals other than P, such as sulphur (S). The availability of minerals other than P may be limited to stimulate rumen microbial growth and protein synthesis in the present experiment. As a result, P supplementation needs to be combined with other minerals.

In Vitro Degradability

Table 2 indicates that P supplementations produced significant effects on degradabilities of dry matter (DM), organic matter (OM) and fribous components (NDF, ADF and cellulose). There was a linear increase in all nutrient degradability with an increase in the levels of P supplementation. P supplementation has increased cellulolytic bacterial populations and cellulolytic enzyme activities (Table 1). These have improved fermentability of ammoniated RS based diet by increasing total VFA concentration, and improved DM, OM and fibrous fraction degradabilities. The present result is in agreement with the result of Kennedy *et al*. (2000) who indicated P supplementation increased digestability of baggase (sugarcane byproduct) by 44%.

Although there was a linear increased in fermentability and degradability with the increase of P levels, there were no significant differences between P level at 0.4% and 0.6%. This means that P level at 0.4% (DM basis) is sufficient for improving fermentability and degradability of ammoniated RS based diet.

CONCLUSIONS

P supplementation is important for improving fermentability and degradability of rations containing ammoniated RS and concentrate. The improvement occurred through the increase in cellulolytic bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. The best level of P supplementation is 0.4% (DM basis). Further study is required to determine effects of N source or other minerals supplementation in combination with P supplementation for stimulating rumen microbial growth protein synthesis.

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