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In vitro study of phosphorus supplementation (sodium orthophosphate, $NaH_2PO_4.2H_2O$) on degradability and fermentation of rice straw

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Abstract

Rice straw is the basal feed in some developing countries. Although its availability is abundant but nutritive value and digestibility is very poor. During the last five decades much research has been done to improve the quality of rice straw by using different treatments and supplements. But these treatments have only been partially successful. Therefore, this study was evaluated the effect of sodium orthophosphate ($NaH_2PO_4,2H_2O$) as a source of phosphorus supplements on utilization of rice straw and rumen fermentation. *In-vitro* dry matter and organic matter degradability were increased ($P<0.002$) in all supplemented phosphorus level than control. Dry matter degradability was higher (479 g/kg) with higher level of phosphorus (5.6, g/kg) at 48 hour. Similarly organic matter degradability was higher (438 g/kg) with higher level of phosphorus (5.6, g/kg) at 48 hour. For NH3-N concentration in rumen fluid the effect of phosphorus were decreased ($P<0.002$) than control but remained at normal range (230 mg/L) for proper rumen function. The effects of phosphorus were also decreased ($p<0.021$) for pH in rumen fluid. Lowest P^H (6.7) was observed at higher level of Phosphorus (5.6 g/kg) in rumen fluid. Sodium orthophosphate can be used as an alternative source of supplement which is not only the source of phosphorus but also act as a source of sodium which is an important mineral helped in maintaining the pH of rumen fluid and microbial growth. As rice straw is deficit in phosphorus, sodium orthophosphate improved its utilization and had an important role in ruminant digestion and metabolism.

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Introduction

Livestock especially ruminant animals are the major component of agriculture. Straws are poor livestock feed and rice straw is no exception. In some developing countries ruminant animals mostly depend on low quality forages such as rice straw. It contains about 80 percent of substances which are potentially digestible and are therefore sources of energy, but actual digestibility by ruminants is only 45 to 50 percent (Jackson, 1977).Rice straw is low in protein, fat, minerals, vitamins and other nutrients. The carbohydrate portion of rice straw is combined with some other compounds like lignin, silica, phenolic compounds and oxalate etc. These compounds have no digestibility as well as no nutritive value. Rice straw differs from other straws in having a higher content of silica (12–16 vs. 3–5 percent) and a lower content of lignin (6–7 vs. 10–12 percent) (Talapatra *et al.,* 1998). Feeding only rice straw does not provide enough nutrients to the ruminants for optimum production due to highly lignified material (Khan and Chaudhry, 2010). Phosphorus (P) which is known as master mineral is very much important to improve the utilization of low quality forages in ruminants. P is an essential nutrient for microbes (Bryant *et al.,* 1999). A significant linear response in the voluntary feed intake was found in calves supplemented with P (Kennedy *et al.*, 2000). P supplementation increased dry matter intake in dairy animals (Valk and Sebek, 1999). When the dietary P was increased from 0.34 to 0.45% of dry matter in diet of cows, dry matter intake increased from 12.4 to 30.5 kg per day (Weiss and Wyatt, 2004). If animals are provided poor quality roughages and by-products that are generally deficient in P, it is necessary to be supplemented with this mineral to meet their nutritional requirements (McDowell LR *et al.,* 1984). 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 by the diet and saliva (Komisarczuk-Bony and Durand, 1991). Microorganisms are largely dependent on dietary P for their requirement and the host animal is affected first under a marginal P deficiency (Durand and Kawashima, 1980). The level of P (0.02 to 0.16 percent) in rice straw is less than the level of about 0.3 percent that animals need for growth and normal fertility (Nath *et al.*, 1969). Keeping consideration this study was conducted to examine the effectiveness of sodium orthophosphate as a source of P on rice straw degradability.

Materials and methods

Experimental work plan

Two way analysis of variance in duplicate were used to assess the degradability and fermentation profiles of rice straw with sodium orthophosphate as a source of P at three levels (0, 2.8 and 5.6 g/kg) for each of the five different incubation times (0, 6, 12, 24 and 48h).

Proximate analyses of rice straw samples were performed following the methods of AOAC (2004) for determination of dry matter (DM), crude protein(CP), acid detergent fibre (ADF), ether extract (EE), individual Ca , individual P and ash/total mineral. Organic matter (OM) and nitrogen free extract (NFE) were calculated from chemical composition Table 1.

Preparation of buffer inoculums

The buffer solution was prepared as described by McDougall (1948)according to the formula for synthetic saliva with some modification (Khan and Chaudhry, 2010).Rumen fluid was collected from immediately after slaughtered a mature cow and fluid was transported to the laboratory by a flask (37^{o}C) for in vitro trial. The rumen fluid was mixed with pre warmed buffer at 1:3 (rumen fluid: buffer) ratio.

In vitro incubation

The incubations of rice straw were conducted in 50-ml centrifuge tubes each containing about 0.4 g of ground (1 mm) sample. Sodium orthophosphate was added according to experimental design. Then 40 ml of buffered rumen fluid were added to each tube. The tubes were sealed with rubber stoppers fitted with

pressure release narrow glass rod. Incubation was conducted at 38°C in a water bath(WB10, Germany). After 0, 6, 12, 24 and 48 h the tubes were collected from water bath and submerged in an ice box to stop further fermentation. pH of the buffered rumen fluid was measured immediately with a pH meter. The liquid and residue were separated by filtering with filter cloth.

The supernatantof the buffered rumen fluid (20 ml) was collected to determine ammonia concentration in rumen fluid and were acidified with 10 ml of 1 N HCl. Acidified sample were distillated and titrated (0.1N HCl) with kjeldahl apparatus (Germany). Residues were washed with distilled water and used to determine DM and OM degradability.

Statistical analyses

The *in vitro* data were analyzed by using ANOVA in General Linear model of Minitab (version 15) to compare degradability and NH3-N concentration of rice straw with different levels of P supplementation. Significant differences between means of different groups were compared by using the Tukey's test at $p > 0.05$.

Results

Chemical analysis of rice straw

The DM, CP, ADF, EE, NFE, ash, individual Ca and P were calculated on DM basis. The details chemical analysis of rice straw, used during the experimental period was shown in Table 1.

Table 1. Chemical composition (g/100g DM) of rice straw.

Feed items	DM (% of fresh weight)	% of DM							
		OM	CP	ADF	EE	NFE	Ash	Ca	P
Rice straw	92.8	92.3	3.54	35.3	1.16	52.2	7.68	0.21	0.07

In Vitro DM degradability of rice straw

The *in vitro* DM degradability was increased ($P<0.001$) with higher level of P and longer incubation time (Table 2). The result showed that the degradability of DM was increased in both supplemented group than the control. Although there was a tendency to increase the degradability in control group but the increasing rate was not same as supplemented level. The effect of degradability varied with the level of P and different incubation time. Higher degradability was found at supplemented level 5.6 at 48 hour.

Table 2. Dry matter degradability of rice straw (g/kg) at different level of phosphorus and incubation time.

Level of phosphorus(g/kg)	Time					SEM
	0 h	6 h	12 h	24 h	48 h	
0	147	152	166	168	181	25.6
2.80	185	298	366	382	388	32.9
5.60	199	337	384	447	479	5.96

Levelof phosphorus; $p<0.001$, Time; $p<0.001$.

In vitro OM degradability of rice straw

The result represented that the degradability of OM was increased ($P<0.001$) in P supplemented group than the control (Table 3).

The *in vitro* OM degradability was increased with higher level of P and longer incubation time. The effect of degradability varied with the level of P and time. Higher degradability was found at supplemented level 5.6 at 48 hour.

NH3-N concentration (mg/L) in rumen fluid with rice straw

The NH_3-N concentration in rumen fluid was decreased ($P<0.002$) in P supplemented group than control. NH3-N concentration in rumen fluid was

decreased with increased level of P (Fig. 1).The effect of P level onNH_3-N concentration was negative but within the acceptance range.

pH of rumen fluid with rice straw treated sodium orthophosphate

The pH of rumen fluid was decreased ($p<0.021$) in P supplemented group than control. pH of rumen fluid was decreased with increased level of P (Fig.2). The effect of P level on rumen pH was negative. The lowest P^H 6.7 was observed at higher level of P supplementation where as 7.1 in control group.

Table 3. Organic matter degradability of rice straw (g/kg) at different level of phosphorus and incubation time.

Level of phosphorus(g/kg)	Time					SEM
	0 h	6 h	12 h	24 h	48 h	
0	132	143	155	169	179	28.0
2.80	163	249	345	366	385	29.9
5.60	193	287	380	407	438	6.09

Levelof phosphorus; $p<0.001$, Time; $p<0.001$.

Discussion

Chemical analysis of rice straw

It was expected that rice straw should have higher ADF content and lower CP. The feed sample was first dried in sun so it contained more DM. In the present experiment the ash value of rice straw was unexpectedly lower than published value (Khan and Chaudhry, 2010). It was may be due to different in variety, soil quality and any other environmental effect. The estimated value of Ca and P were similar with Khan and Chaudhry, 2010.

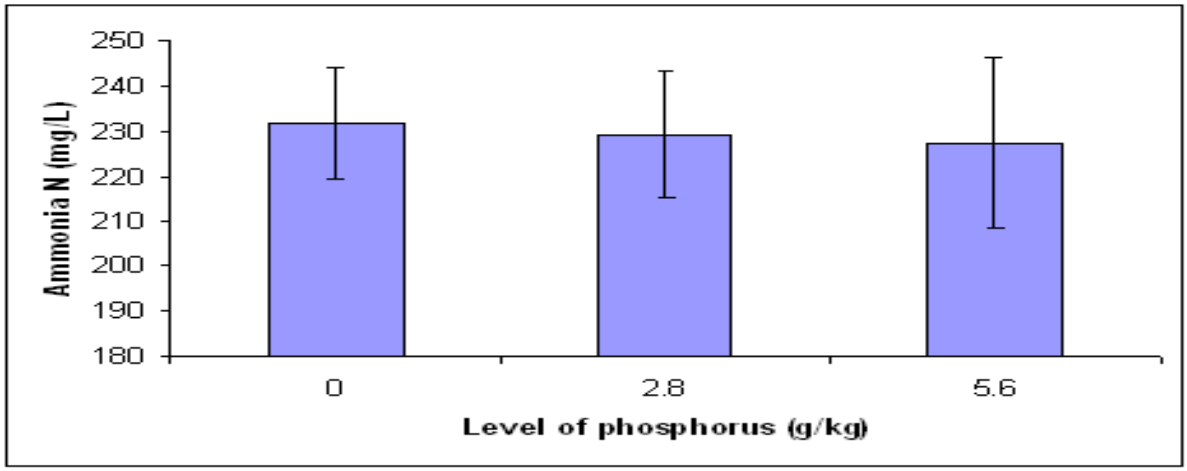

Fig. 1. NH_3-N concentration (mg/L) in rumen fluid at different level of phosphorus.

Effect of phosphorus supplementation on DM and OM degradability of rice straw

The DM and OM degradability of rice straw was increased with sodium orthophosphate due to the presence of P that rumen microbes need to maintain metabolism and growth. P supplementation is very important for rumen fermentation and growth of rumen micro bacteria (Mardiati *et al.,* 2010). Growth and replication of the rumen bacteria are dependent upon P supply (Patterson, 2002). We assumed that P might have effect to increase microbial activity and higher protein synthesis, which resulted higher OM and DM degradability with higher level of phosphorus. The DM and OM degradability of rice straw without any treatment was very low due to various ligno- cellulose bonds. Phosphorus is necessary for normal growth and function of rumen micro-organisms especially for cellulose digestion

that leads to increased DM and OM degradability (Harris *et al.,* 2003).Phosphorus is very important mineral for ruminal microbes that lead to increase DM and OM degradability (Bryant *et al.*,1999; Sutarpa *et al.*,2011). In addition our previous study on rice straw with triple super phosphate (TSP) for DM and OM degradability was increased (Mahfuz *et al.,* 2014). Nutritive value of some straws and other by-product feeds can be improved simply by adding urea and minerals as such as P (Mardiati *et al.*,2010). Zain *et al.* (2010) also reported that phosphorus supplementation increased bacterial population, total VFA concentration and degradability of ammoniated rice straw.

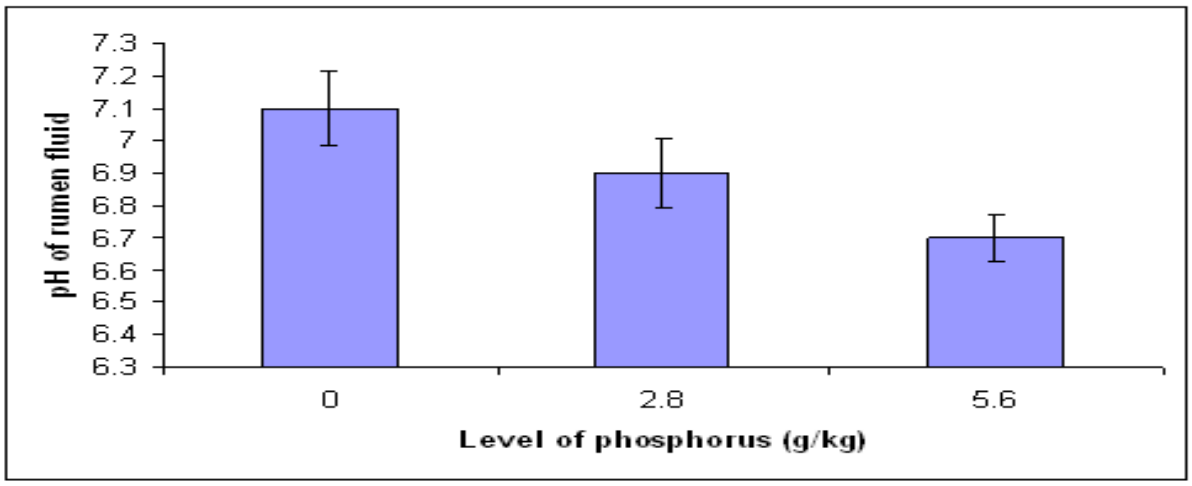

Fig. 2. PH of rumen fluid at different level of phosphorus.

Effectof phosphorus supplementation on NH3-N concentration (mg/L) in rumen fluid with rice straw

Gas volume is a good parameter to predict digestibility, fermentation of end-product and microbial protein synthesis of the substrate by rumen microbes in the in vitrosystem (Sommart *et al.*, 2000).

The optimum concentration of ammonia in rumen fluid varies widely, from 85 to over 300 mg/L. Maximum microbial growth was reported between 20 to100 mg/L ammonia (Pisulewski *et al.,* 1981). In the present study, rumen ammonia of cross breed cow was average 230 mg/L which was within the acceptable range to maintain rumen function. NH3-N concentration was slightly decreased with higher level of sodium orthophosphate. This was due to use of NH_3-N by rumen microbes for their own cell synthesize. NH_3-N, CO_2 and CH_4 in the rumen are used to synthesize microbial cells (Kampa *et al.,* 2006). Mardiati *et al.* (2010) also reported that addition of P supplement at diet had reduced ammonia concentration.

pH of rumen fluid with rice straw treated sodium orthophosphate

pH of rumen fluid generally remains 5 to 7 depending on the type of diet fed to animal. When low quality forages are provided to animal, pH may rise to 7.5 (McDonald *et al.,* 2002). The digestibility of feed depends on type of feed and pH (Calsamiglia *et al.*, 2008).pH of rumen fluid was decreased with higher level of sodium orthophosphate but within the acceptance ranged (around 6.7 to 6.9). The supplement (NaH_2PO_4, $2H_2O$) contains sodium and hydrogen that might helped to reduce pH at different level of P.

Conclusion

Rice straw is not only highly lignified but also deficient of nitrogen, phosphorus and other nutrients. This study has shown that P supplementation improved DM and OM degradability of rice straw by reducing ammonia and pH in rumen fluid. Further study is needed to determine the actual doses of sodium orthophosphate with rice straw. Finally it may be concluded that Sodium orthophosphate may be a

source of supplementation to improve the utilization of rice straw.

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References

AOAC. 2004.Official Methods for Analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC.

Bryant MP. 1999. Observation on the nutrition of Bacteriodes succinogenes-a ruminant cellulytic bacterium. Journal of Dairy Science **42,** 1831-1847.

Calsamiglia S, Cardozo PW, Ferret A, Bach A. 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. Journal of Animal Science **86,** 702-711. http://dx.doi.org10.2527/jas.2007-0146

Durand M, Kawashima R. 1980. Influence of minerals in rumen microbial digestion. In: Y. Ruckebusch and P. Thivend (eds.) Digestive Physiology and Metabolism in Ruminants. AVI Publ Co. Westport, CT. http://dx.doi.org/10.3168/jds.S00220302(94)77286-6

Harris B, Adams AL, Van Horn HH. 2003. Mineral Needs of Dairy Cattle. Animal Science Department, Institute of Food and Agricultural Sciences, University of Florida, Florida, USA. http://dx.doi.org/10.1016/j.smallrumres.2014.04.003

Jackson MG. 1977. Review article: The alkali treatment of straws. Anim. Feed Science and Technology. http://dx.doi.org10.1016/0377-8401(77)90013-X

Kampa S, Wanapat M. 2006. Influenceces of energy sources and level on ruminal fermentation and microbial protein synthesis in dairy stress, Pakistan Journal of Nutrition **5**, 294-300. http://dx.doi.org/10.3923/pjn.2006.294.300

Kennedy PM, Lowry JB, Conlan LI. 2000. Phosphate rather than surfactant accounts for the main contribution to enhanced fibre digestibility resulting from treatment with boiling neutral detergent. Animal Feed Science Technology **86,** 177-190. http://dx.doi.org/10.1016/S0377-8401(00)00164-4

Khan MMH, Chaudhry AS. 2010. Chemical composition of selected forages and spices and the effect of these spices on in vitro rumen degradability of some forages. Asian-Australas Journal of Animal Science **23**, 889–900. http://dx.doi.org/110.5713/ajas.2010.90442

Komisarczuk-Bony S, Durand M. 1991. Effects of minerals on microbial metabolism. Ed.J. P. Jouany. Institut National de la Recherche Agronomique (INRA), Paris. Rumen Microbial Metabolism and Ruminant Digestion, 179-198. http://dx.doi.org/10.1016/j.smallrumres.2005.02.014

Mahfuz SU, Chowdhury MR, Khan MMH, Baset AB. 2014. Effect of triple super phosphate supplementation on degradability of rice straw and ammonia nitrogen concentration. Small Ruminant Research. **120**, 15-19. http://dx.doi.org/10.1016/j.smallrumres.2014.04.003

Mardiati Z, Jamarun N, Tjakradidjaja AS. 2010. Phosphorus Supplementation of Ammoniated Rice Straw on Rumen Fermentability, Syntesised Microbial Protein and Degradability in Vitro.

International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering **4(5),** 357-359.
http://waset.org/publications/8898.

McDonald P, Edwards RA, Greenhalgh JF, Morgan CA. 2002. Animal Nutrition. Longman Group Limited, Harlow.

McDowell LR, Conrad JH, Ellis GL 1984. Mineral deficiencies and imbalances and their diagnosis. In: Symposium on Herbivore Nutrition in Sub-Tropics and Tropics-Problems and Prospects, Gilchrist FMC, Mackie RI, 67–88.
http://dx.doi.org/10.1007/s12011-010-8799-6

McDowell EI. 1948. Studies on rumen saliva. The composition and output of sheep[s] saliva. Institute of animal pathology, university of cabridge, 99-100.

Nath **K , S**ahai **K, K**ehar **ND.**1969. Effect of water washing, lime treatment and lime and calcium carbonate, supplementation on the nutritive value of paddy *(*oryza sativa) straw. Journal of Animal Science **28,** 383-385.
http://dx.doi.org/10.2527/jas1969.283383x

Patterson J. 2002. Defining the phosphorus requirements of cattle. Proc. 37th Pac NW Anim Nutr Conf. pp. 11-19.
http://wsare.usu.edu/

Pisulewski PM, Okorie AU, Buttery PJ, Haresign W, Lewis D. 1981. Ammonia concentration and protein synthesis in the rumen. Journal of the Science of Food and Agriculture **32,** 759-766.
http://dx.doi.org/ 10.1002/jsfa.2740320803

Sommart K, Parker D, Linson P. 2000. Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava rice straw and dried ruzi grass as substrate. Asian Australian Journal of Animal science **13**, 1084-1093.
https://doi.org/10.5713/ajas.2000.1084

Sutarpa INS, Suyandnya P, Partama IBG, Astawa PA. 2011. Effect of vitamin –mineral supplementation in commercial feed on the digestibility coefficient and rumen fermentation of Bali cattle. Journal of the Indonesian Tropical Animal Agriculture **36(1).**
http://dx.doi.org/10.14710/jitaa.36.1.69-74

Talapatra **SK, R**ay **SC, S**en **KC.** 1998. Calcium and phosphorus assimilation of ruminants on oxalate-rich diets. Journal of Agricultural Science **38,** 163.
https://doi.org/10.1017/S0021859600005426

Valk H, Sebek LBJ. 1999. Influence of long-term feeding of limited amounts of phosphorus on dry matter intake, milk production and body weight of dairy cows. Journal of Dairy Science **82,** 2157-2163.
http://dx.doi.org/10.3168/jds.S00220302(99)75459-7

Weiss WP, Wyatt DJ. 2004. Macro mineral digestion by lactating dairy cows: Estimating phosphorus excretion via manure. Journal of Dairy Science **87,** 2158-2166.
http://dx.doi.org/10.3168/jds.S00220302(04)70035-1

Zain M, Ninggrat R, Jamarun NAT. 2010. Effect of phosphorus supplementation of ammoniated rice straw on rumen fermentability, synthesised microbial protein and degradability in vitro. In: Advances in Animal Bioscience: Proceedings of the British Societyof Animal Science. Annual Conference, Belfast, Northern Ireland, UK, p. 210.
https://doi.org/10.1017/S2040470010003535