



# Phosphorus Deficiency Influences Rumen Microbial Activity: Review

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## Abstract

Phosphorus (P) is the second major element requires after calcium in the diet. It is a key mineral in respect of animal nutrition but at the same time one of the main environmental contaminants from livestock production. However, decreasing dietary P concentrations below requirements impairs the health and productivity of dairy cows. Eighty percent of the P is found in the skeleton with its major role as a constituent of bones and teeth. The remainder of P is widely distributed throughout the body in combination with proteins and fats and as inorganic salts. P is essential in the transfer and utilization of energy and it remains present in every living cell in the nucleic acid. Calcium (Ca) and P are closely associated with each other in body metabolism. Adequate Ca and P nutrition depends on three factors: a sufficient supply of both nutrients, with a suitable ratio between them, and the presence of vitamin D for proper absorption into the body. Deficiency of P leads to "pica" including reduced appetite, retarded growth, low milk yield, and impaired fertility. Recently calved cows may become recumbent and display post-parturient hemoglobinuria. A detailed discussion about the effect of P deficiency on rumen microbial activity is presented in the article.

**Keywords:** *In Vitro*; *In Vivo*; Phosphorus Deficiency; Rumen Fermentation; Ruminants

## Introduction

Phosphorus is a key mineral in animal nutrition but at the same time one of the main environmental contaminants from livestock production [1]. Among the mineral inadequacies, phosphorus deficiency is most widespread and affects livestock production and health in many parts of the world [2-7]. Extreme P deficiency in ruminants leading to clinical bone disorders is relatively rare under practical conditions, while moderate to marginal deficiency is undoubtedly very widespread, particularly in tropical countries. The two clinical disorders most often reported as a suspected result of moderately deficient P intake are depressed feed intake

and reduced fertility [8]. The depressive effect on feed intake may be at least in part, a primary result of impaired rumen function [8,9].

Salivary phosphorus plays a very important role in the phosphorus nutrition of ruminants. Phosphorus exists in saliva largely in the highly available form of inorganic salts. It has two important functions, to act as a buffer against large depression in pH resulting from the production of organic acids and to provide adequate P for the rumen-reticular microbes. Recycling P through saliva in the rumen is much more (about 3-4 times) than the daily dietary P intake. The concentration of available phosphorus in rumen

fluid between 3-4 mmol L<sup>-1</sup> is considered adequate for microbial growth and fermentation [8,10]. P concentration below 3 mmol L<sup>-1</sup> is expected in case of long-term reduction of dietary phosphorus intake, for example when rations composed of unconventional feedstuffs or based on poor quality roughages such as straw and other crop residues [11,12]. Ruminal microbes have specific requirements for P for fermentation and growth [13].

Salivary glands of cattle can concentrate plasma inorganic P 3 to 8 fold depending on salivary flow rate and plasma P concentration [14]. A study indicated that 300 kg steer may secrete about 20g P day<sup>-1</sup> on an adequate P diet and 10g P day<sup>-1</sup> on a P deficient diet. It is observed that the amount of P secreted in the saliva is influenced both by salivary flow rate and P intake. If feed intake is kept constant and P intake varies, the concentration of P in the saliva is directly proportional to P intake. If feed intake is increased, it increases salivary flow rate and thus the total salivary P secretion without affecting salivary P concentration [15]. According to Kincaid, et al. [16], a minimal dietary P level of 2.8–4.0 g kg<sup>-1</sup> of digestible OM has been suggested to meet the requirements of ruminal microbes for N assimilation and cellulose fermentation. Cellulolytic bacteria were consistently reported to have higher P requirements than amylolytic bacteria [17,18]. Therefore, ruminal fiber degradation may be impaired with low dietary P supply [13,19].

Phosphorus in addition to its role in skeletal and soft tissues is essential for the maintenance and metabolic activity of the rumen microbial population. In ruminants, microbial phytase activity in the rumen plays an important role to degrade phytate and increase phytate-P availability for microbes and the host [20-22]. Nevertheless, degradation of phytate-P may not be complete in the rumen, especially under feeding conditions that decrease rumen fermentation as well as reduce ruminal retention time due to a short particle size of forages, and high feed intake levels [23,24].

Rumen microbial populations produce phytase, but it has been suggested that passage rate [23], grain type [25,26], processing method [27,28], and dietary concentration of Ca [29] may reduce the ability of ruminal phytases to cleave phosphate (PO<sub>4</sub>) from phytate.

Harder, et al. [19] reported an increased total bacterial abundance and improved fermentation profile with the treatment of barley grain with 5% lactic acid (LA) in a P deficient diet [3.1 g P kg<sup>-1</sup> dry matter (DM)], using rumen simulation technique. These results suggest that such LA treatment of grain increased P availability, thereby replacing readily available inorganic P supplementation to meet microbial P needs. Low P intake was shown to reduce the amount of salivary P and as a consequence reduce the soluble

P concentration in the rumen [9], which in turn may affect the synthetic and degradative ruminal processes [8]. There is a highly positive correlation exist between P levels in saliva vs. rumen fluid and between plasma vs. saliva. Rumen microbial population requires a large supply of available phosphorus. The total phosphorus content of rumen microbes may range from 2 to 6% on a dry weight basis [30,31]. It is necessary for carbohydrate fermentation and is a constituent of microbial nucleic acids (the P content is 10.03% in DNA and 9.64% in RNA), phospholipids (teichoic acid contains up to 4% organic P in the cell wall of Gram +ve bacteria, and such as phospholipids which occur mainly in cytoplasmic and outer membranes of Gram-ve bacteria) and phosphorylated co-enzymes from the B-complex group (flavin phosphates, pyridoxal phosphate, and thiamine pyrophosphate) inorganic polyphosphates are stored by microorganisms and used as a source of P for ATP synthesis. Inorganic phosphate contained in rumen contributes to buffering acids originating from the fermentative process in the rumen [10]. The lack of inorganic P appeared to suppress protein breakdown in the rumen causing a decrease in the concentration of ruminal ammonia and branched-chain fatty acids [1].

Sometimes secondary factors may be associated with P deficiency like high dietary iron (Fe) content, especially ferrous Fe as present in drinking water may form insoluble complexes with PO<sub>4</sub> which in turn could reduce P absorption. In cows, however, this negative effect on P digestibility could not be confirmed with ferrous lactate infused abomasally up to 1,250 mg of Fe day<sup>-1</sup>. [22]. This might be caused by the acidic rumen environment, and the large fluid volume leading to lower concentrations, and hence solubilization of otherwise most insoluble Ferro-phosphate complexes. This would require further investigation and it is not known to what extent complex formation plays an important role in ruminant nutrition as in the nutrition of mono-gastric. Other dietary factors (inorganic P fraction in total dietary P, neutral detergent fiber (NDF) content, fermentable organic matter (OM) content, roughage proportion of the diet) were considered in the simulation study performed by Dijkstra, et al. [32] but appeared to be relatively unimportant in explaining variation in P digestibility and P excretion. The adverse ratio of calcium and phosphorus also remained a cause create phosphorus deficiency [33,34].

### Effect of P Deficiency on Rumen Microbial Activity

Reports of various studies on the effect of P deficiency on rumen microbial activity are limited and summarized below:

- **In Vivo Studies**

The effect of P deficiency on rumen digestion and protein synthesis has not always shown clear-cut results in *in-vivo* studies because of large variations in the response of the

host in terms of feed intake and endogenous P return.

Witt, et al. [35] studied the effect of dietary P on ruminal digestion in adult steers fed on low (0.12%) and adequate (0.23%) P diets and reported lower ruminal P concentration with low P diets as compared to high P diet (208 mg vs. 398 mg L<sup>-1</sup>), ruminal P, dry matter disappearance rate and cellulose digestion were unchanged due to low P intake. They further suggested that to maintain digestion in the rumen, adult ruminants animals may recycle endogenous P via saliva to the rumen and secretions through the ruminal wall to maintain P near 200 mg L<sup>-1</sup> even when P intake was temporarily or seasonally low.

Milton, et al. [36] created three treatments in sheep fed on a high Ca-low P diet by infusing saline or Phosphate solution into abomasum with or without the diversion of parotid saliva, three treatments were low ruminal, low blood (148 mg inorganic P (Pi) L<sup>-1</sup>, 2.8 mg Pi L<sup>-1</sup>), high ruminal, high blood (314 mg Pi L<sup>-1</sup>, 4.7 mg Pi L<sup>-1</sup>) and low ruminal, high blood (70 mg Pi L<sup>-1</sup>, 4.4 mg Pi L<sup>-1</sup>) P concentrations and observed that there was no difference between treatments in organic matter digestibility, the digestibility of neutral detergent fiber fraction on both low ruminal treatments was 5% lower than on the high ruminal treatment. In an experiment with sheep fed a semi-purified diet containing urea but free from P, total P concentration in rumen fluid fell to 2.6 mmol L<sup>-1</sup> (80mg L<sup>-1</sup>), the digestibility of organic matter decreased from 82 to 72 % and CP digestibility from 65 to 58%. The nitrogen balances became negative (-2.36 g day<sup>-1</sup> vs. + 1.05 g day<sup>-1</sup>, with P supplemented group). As urea was the only nitrogen source, it was suggested that microbial synthesis of protein from non - protein nitrogen was lower in the rumen due to P insufficiency [37]. Durand, et al. [38] observed that non-ammonia nitrogen flows from the rumen of lamb fed on a semi-purified diet containing 1.2g P per kg OM digestibility decreased by 15% compared to a diet containing 1.5 or 4.6 g P per kg OM digestibility. Holler, et al. [11] demonstrated that straw-based diet providing 29% of total nitrogen (N) as urea N and low in P (1.3 g P day<sup>-1</sup>) in sheep depressed ruminal fluid total P by 70 % (5.65 to 1.71 mmol L<sup>-1</sup>). Ammonia concentration in ruminal fluid increased from 3.6 to 7.51 mmol L<sup>-1</sup>, whereas total volatile fatty acids (TVFA) concentration reduced from 125 to 100 mmol L<sup>-1</sup>. Molar proportions of VFA appeared to be less affected by P deficiency though there was a tendency towards higher propionate production at the expense of both acetate and butyrate. It was suggested that dietary P depletion led to reduced microbial growth and activity in the rumen.

Breves, et al. [39] indicated that microbial nitrogen pool and turnover were significantly reduced from 11000 to 8100 mg and 590 to 430 mg N h<sup>-1</sup>, respectively in the rumen of depleted sheep fed P deficient semi-synthetic diet providing

1.05g P day<sup>-1</sup>. These results are the following findings of Breves, et al. [40] that measured the flow of microbial nitrogen to the proximal duodenum in P depletion and repletion and confirmed the depressive effect of P depletion on microbial protein synthesis in the rumen of sheep. Holler, et al. [41] fed sheep with a low P pelleted straw-based diet containing urea which provided 25% of total N content and showed that when P content of rumen liquor fell to 3 mmol L<sup>-1</sup> then there was a defined shift in metabolisms such as fall in the concentration of VFA, rise in a molar proportion of propionic acid and ammonia or a reduction in the activity of microflora accompanied by reduced synthesis of microbial protein. Lessmann, et al. [42] and Breves, et al. [43] also showed the depressive effect on P depletion (compared to P repletion) on microbial protein synthesis. Petri, et al. [44] observed that P deficiency in lactating goats did not significantly affect rumen fluid kinetics. It caused a significant increase in pH and a reduction in the size of the rumen ammonia pool and its outflow rate. Digestibility of OM as well as the efficiency of microbial yield was reduced from 34.1 to 13.7 g N day<sup>-1</sup>. The transfer of N from microbial origin to milk protein decreased from 5.3 to 2.7 g day<sup>-1</sup>, whereas secretion of N in milk protein not originating from rumen microbes remained unchanged.

Jain, et al. [45] studied the effect of dietary P inadequacy on rumen microbial activity in growing calves fed on the urea supplemented straw-based diet. Results of *in vivo* rumen studies indicated that Pi levels in rumen liquor were significantly reduced (28.86 vs. 15.99mg 100mL<sup>-1</sup>) during P inadequacy which led to increases in strained rumen liquor (SRL) ammonical nitrogen (NH<sub>3</sub> - N) (12.47 vs. 17.64 mg 100mL<sup>-1</sup>), decreased total N (94.48 vs. 45.22 mg 100mL<sup>-1</sup>). Whereas no change in pH and TVFA concentration was observed, the values for the acidic buffering capacity of SRL were significantly higher with a decrease in alkaline buffering capacity during P deficiency. The above *in vivo* rumen studies were extrapolated in the *in - vitro* system by further decreasing Pi levels in fermentation vessels through modifying the P composition of infused artificial saliva. Different Pi levels created were 55.21, 24.82, 12.99, 8.05, 3.13 and 0.98mg 100mL<sup>-1</sup> of fermentation medium. Thus, concluded that dietary P inadequacy (1.08 to 1.28 g P kg<sup>-1</sup> DM) markedly reduced Pi concentration in plasma, saliva, and rumen liquor and produced disturbances in rumen microbial activity. The level of 12.99 mg Pi 100 mL<sup>-1</sup> in an *in- vitro* medium was adequate for optimum synthetic and degradative activity of rumen microbes.

In a study by Perez, et al. [46] effect of solubility of phosphorus was also been observed on the phosphorus kinetics and rumen fermentation activity in dairy goats.

Jain, et al. [33] reported an adverse ratio of Ca: P (39:1) in the ration of advanced pregnant buffaloes fed on a gram

straw-based diet, which remained a cause of hemoglobinuria due to deficiency of phosphorus.

Long, et al. [47] reported that steers fed 0.10% P had decreased ( $P < 0.01$ ) DMI and total fecal output, but increased ( $P < 0.01$ ) apparent DM digestibility compared with steers fed 0.30% P. Although N intake and retention were not affected by treatment, steers fed the 0.10% P diet tended ( $P = 0.10$ ) to absorb more N compared with steers fed 0.30% P; and, steers fed the 0.10% P diets excreted more N in the urine ( $P = 0.02$ ) and less N in the feces ( $P < 0.01$ ) compared with steers fed the 0.30% P diets. Steers fed the 0.10% P diets also consumed 70.1% less ( $P < 0.01$ ) total P each day, and excreted 51.9% less ( $P < 0.01$ ) P in feces and 94.6% less P in the urine ( $P < 0.01$ ) compared with steers fed 0.30% P. Excretion of water-soluble P in the feces was greater ( $P < 0.01$ ) on a  $\text{g day}^{-1}$  basis for steers fed 0.30% P when compared with steers fed 0.10% P. However, the proportion of total fecal P excreted as water-soluble P increased ( $P < 0.05$ ) by 23.0% in steers fed 0.10% P compared with steers fed 0.30% P. Blood P concentration was positively correlated ( $r = 0.60$ ;  $P < 0.01$ ) to urinary P concentration when steers were fed 0.10% P; however, when steers were fed 0.30% P, there was no correlation ( $r = 0.36$ ;  $P = 0.16$ ) between blood and urine P.

Mudgal, et al. [34] also concluded that feeding of lentil (*Lens culinaris*) straw (having ratio of Ca to P, 10:1) alone in growing kids was unable to sustain their growth performance.

In a recent study, Kohler, et al. [48] reported that the concentration of inorganic P in saliva, rumen, and abomasal fluid was significantly decreased with the P restricted feeding in sheep. In addition, there was a trend for increased rumen butyrate and decreased abomasal ammoniacal nitrogen concentration in the P restricted group. Furthermore, rumen ammoniacal nitrogen and butyrate were positively correlated with each other and with rumen inorganic P concentration in the P restricted group.

#### • **In Vitro Studies**

In vitro work, using short-time batch culture techniques with pure cultures of rumen bacteria has shown the essentiality of P for growth [17] and its effect on growth rates and yield [31]. The work by Komisarczuk, et al. [49] who cultured *Bacteroides Succinogens*, a major rumen cellulolytic bacteria in a P-limited medium showed a significant depression in growth, ATP concentrations, and endoglucanase specific activity as compared to P supplemented medium. Further work using suspensions of mixed rumen microorganisms (frequently P depleted) has shown the beneficial effects of additional P concerning increased cellulolytic activity [50,51] and increased nitrogen utilization [38].

A minimum requirement of  $20\text{mg Pi L}^{-1}$  in the medium for a pure culture of cellulolytic bacteria [17] and 20 to  $80\text{ mg Pi L}^{-1}$  in the medium for mixed culture [10,51,52] have been suggested for optimum rumen microbial activity.

However, the above studies were made using short-term batch culture techniques and only examined the effect of P on one or at the most two products of microbial fermentation and synthesis and did not take into account the adaptation of microbes to the deficiency. Therefore, a series of the assay using either semi-continuous (Rusitec) or entirely continuous culture systems were employed to examine the effect of P on the principal parameters associated with degradative and synthetic processes and to ascertain more precisely the minimum requirements for each of these processes, apart from physiological interactions with the host.

Komisarczuk, et al. [53] showed the effect of progressive reduction in the P concentration on rumen microbial activity in a continuous culture technique. The artificial saliva containing 120, 80, 40, and  $0\text{ mg Pi L}^{-1}$  was used. They noticed a significant increase in pH and ammonia, when Pi content in the vessel fell to  $4\text{ mg L}^{-1}$ , whereas TVFA concentration did not show any significant change until Pi concentration was  $< 1\text{mg L}^{-1}$ . ATP concentration on the other hand significantly reduced for all vessels when Pi concentration was  $< 48\text{mg L}^{-1}$ . It was shown that although the microbial population can survive conditions of quite a severe Pi depletion, a marked effect on microbial activity was encountered when Pi concentration was less than  $50\text{mg L}^{-1}$ . In another study, Komisarczuk, et al. [54] observed that levels of P lower than  $3\text{g kg}^{-1}$  organic matter fermented in semi-continuous culture technique drastically reduced degradative activity. Cellulose digestion was much more affected than hemicellulose degradation and VFA production. Further  $^{15}\text{N}$  – specific enriched data showed that P deficiency reduced the incorporation of bacterial-N coming from  $\text{NH}_3\text{-N}$  in both liquid and solid phase associated bacteria. Komisarczuk, et al. [55] studied the effect of P levels on ATP and VFA production in rumen content in an *in vitro* continuous culture system and observed that VFA production decreased between 40 and  $0\text{ mg P L}^{-1}$ . A significant difference in ATP concentration was reported at 80, 40 and  $0\text{ mg P L}^{-1}$ . It was suggested that ATP seems to be more sensitive to P inadequacy than VFA.

Durand, et al. [56] showed that infusion of artificial saliva supplemented with P ( $120\text{ mg L}^{-1}$ ) in the fermentors containing 15 g ammoniated straw  $\text{bag}^{-1}$  improved significantly fiber digestion, VFA, and gas production but the protein in the effluent was not much affected. The results emphasized the role of P in fiber digestion.

Komisarczuk, et al. [57] demonstrated the effect of

P deficiency on bacterial protein synthesis and chemical composition and ATP concentrations in solid and liquid phases of fermentors using the rumen simulation technique (Rusitec). The results indicated that the total N content of the bacterial population associated with the liquid phase was significantly lower and  $^{15}\text{N}$  enrichment was reduced in both microbial populations by the lack of P. The P deficiency induced a marked decrease in microbial protein synthesis in both phases (overall by 45%). Microbial yield decline by 5g N  $\text{kg}^{-1}$  OM fermentation in P deficient condition. Ammonia N was increased ( $30\text{mg day}^{-1}$ ) but ureolytic activity was not affected. ATP concentrations were greatly reduced in both phases. However, P deficiency did not affect protozoal numbers. A similar low microbial yield was also observed by Durand, et al. [58] during P deficiency.

Komisarczuk, et al. [59] used a continuous culture technique to study the P requirement of rumen microbes and infused solution of artificial saliva containing 120, 80, 40, and 0 mg Pi  $\text{L}^{-1}$  into reaction vessels resulting in respectively 48, 28, 4, and  $<1\text{mg Pi L}^{-1}$  in the vessels. Results indicated that the concentration of protozoa was not significantly affected by Pi concentration. Reduction in Pi concentration to 4 and  $<1\text{ mg L}^{-1}$  resulted in a significant reduction in VFA accompanied by a rise in pH, but there was no significant effect on molar proportions of VFA until Pi level falls to  $< 1\text{ mg L}^{-1}$ .  $\text{NH}_3\text{-N}$  values were also increased with the lowest value of Pi. Cellulose and hemicellulose digestion was significantly reduced by decreasing the Pi levels, but there was no depression in starch digestion indicating that cellulolytic bacteria were extremely sensitive to P deficiency. Similar sensitivity was also observed by Durand, et al. [56]. The amount of microbial  $\text{-N}$  synthesized daily averaged 0.48g and was maintained with Pi concentration down to 4 mg  $\text{L}^{-1}$ . There was, however, a significant reduction to 0.26 g with Pi  $<1\text{mg L}^{-1}$ . The efficiency of microbial protein synthesis was variable. It was estimated that the minimum Pi concentration required in rumen fluid *in-vivo* to maintain maximum degradative and synthetic activities was 75 to 100 mg  $\text{L}^{-1}$  and that the overall P requirement of the microbes was of the order of 5.1g  $\text{kg}^{-1}$  digested organic matter intake.

Jain, et al. [45] reported that pH and  $\text{NH}_3\text{-N}$  values increased significantly by decreasing the Pi levels in *in-vitro* system. Total-N and microbial protein fell markedly when Pi levels were below 12.99 and 8.05 mg  $100\text{mL}^{-1}$ , respectively in the fermentation medium. TVFA concentration remained unchanged however the tendency of TVFA concentration after Pi levels below 12.99 mg  $100\text{m L}^{-1}$  was towards decreasing side. There was no significant difference among treatments in acetate and propionate, while butyrate at the lowest Pi level i.e. 0.98mg  $100\text{mL}^{-1}$  was significantly lower than remaining treatments. There was a significant difference among treatments for *in-vitro* DM and OM digestibility.

Gunn, et al. [60] studied the effect of phosphorus deficiency upon ruminal microbial activity and nitrogen balance in lambs and observed that microbial N flow was reduced by 45%, and the flow of microbial N was not related to N balance. This experiment clearly shows that P deficiency causes depression in ruminal microbial protein production regardless of the food intake of the lambs.

The study of Ceresnakovai, et al. [61] shows that the *in sacco* method is not an objective one for the determination of the kinetics of phosphorus release in the rumen from forages due to rebinding of phosphorus with the nylon-bag residues from the rumen environment.

In a study, Zain, et al. [62] also concluded that P supplementation is important to improve the fermentability and degradability of rations containing ammoniated rice straw and concentrate.

Zulkarnaini, et al. [63] showed that P supplementation is important for rumen fermentation and growth of rumen microbes. They also concluded that overall supplementation of phosphorus at 0.4% of dry matter to ammoniated rice straw shown the best results in terms of rumen fermentation, microbial protein synthesis, and *in vitro* degradability.

### Microbial P Requirement

The microbial requirements for P are largely determined by the incorporation of P into the microbial matter. Since this is a function of microbial growth, microbial P requirements for microbial protein synthesis vary according to the actual availability of energy and N. The mean ratio of P: N ( $\text{g g}^{-1}$ ) in mixed bacteria has been reported 0.188 [10] and 0.145 [64]. Rumen bacteria incorporate approximately 1.25 g N  $\text{MJ}^{-1}$  metabolizable energy (ME) consumed in the diet [65] and on this basis, it may be calculated that they incorporate approximately 0.26 g P and 0.18 g P  $\text{MJ}^{-1}$  ME, respectively for P: N ratios 0.188 and 0.145. These P: N ratios result in respective estimates of microbial P requirements, 2.8 g P and 3.7 g P per kg OM digestibility [13]. Available P: ME intake ratio for optimum microbial growth in the rumen was 0.22 to 0.25 [8], while this ratio in some temperate diet components (i.e. sugar beet pulp for which some samples had a P: ME ratio of only 0.07) and many samples of tropical forages (guinea grass, molasses grass, elephant grass, pangola grass, jaragua grass, Guatemala grass, native Brazilian grass, Townsville stylo, etc.) was found to be quite low, maybe less than 0.12 or in some cases as low as 0.03. Therefore, long-term feeding on such feeds may result in disturbances in rumen function. The concentration of P in rumen varies between 30 to 40 mg% and it is mainly in inorganic form and originates from hydrolysis of an organic compound from intake with saliva. The presence of water-soluble phosphates in ruminal ingesta

is reported to be essential for the maintenance of normal flora. Thus it is necessary to supplement the P deficient diet with water-soluble phosphates to maintain satisfactory ruminal digestion [66].

Mc Dowell, et al. [67] indicated that 0.18 to 0.70% P in the ration of beef cattle for growing and fattening steers and heifers, 0.31 to 0.40% P in lactating dairy cows, and 0.16 to 0.37% P in sheep and goats was adequate.

In a study, Zain, et al. [62] also concluded that the most effective level of P supplementation is 0.4% (as compared to the 0.2 and 0.6%) of dry matter when the rations containing ammoniated rice straw and concentrate.

In an article on Agricultural Science [68] 0.46% P, (DM basis) level was predicted as the best-suited level in cow's diet by considering all kinds of experimental indexes and the regression analysis.

Naseer, et al. [69] found that the cumulative volume of gas production increased with increasing the level of phosphorus (0, 0.037, 0.074, and 0.111 of 1 g DM). Total gas produced at 72 h of incubation was higher for concentrate and hay (H) + concentrate (C). The highest value of individual VFA for H+C was at level 2 of treatment and for concentrate at level 4, while the addition of different levels of P has decreased the value of individual VFA for hay. No significant effects were observed on pH, NH<sub>3</sub>-N levels, or truly dry and organic matter digestibility with P addition. The specific activity of amylase and carboxymethyl cellulose of sonicated the contents of the bottle after incubation time was significantly increased at level 2 for H, C, or C+H [70].

## Conclusion

Reports of various *in-vivo* and *in-vitro* studies on the effect of P deficiency on rumen microbial activity indicated that P deficiency in rumen increases NH<sub>3</sub>-N and pH, whereas TVFA, microbial protein, ATP and cellulose, and hemicellulose digestion were reduced significantly without affecting the starch digestion, indicating that cellulolytic bacteria are extremely sensitive to P deficiency. The microbial synthesis of protein from NPN was lower in the rumen due to P insufficiency. Dietary P depletion led to reduced microbial growth and activity in the rumen. Therefore attention should be given to adequate P nutrition.

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