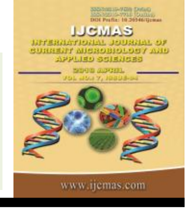
Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 3518-3530

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com



Review Article https://doi.org/10.20546/ijcmas.2018.704.397

Phosphorus Nutrition in Dairy Animals: A Review

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A B S T R A C T

Keywords

Phosphorus nutrition, Dairy animals

Article Info

Accepted:
28 March 2018
Available Online:
10 April 2018

Phosphorus (P) has a key role in production and reproduction performance of dairy animals. However, feeding of high levels of dietary P has no additional benefits to dairy animals but this practice generally leads to more excretion of P, which in turn causes environmental pollution as P accelerate eutrophication of surface waters. A practicable and cost-effective approach to managing P level in the diet of dairy animals is required to minimize excretion of P in manure without impairing animal performance. Therefore, to access the requirement of P supplementation to dairy animals in Punjab various reports of P status in soils, feed and fodders were reviewed and its supply to the dairy animals were accessed. From this study, it can be concluded that the soil and plants of various districts of Punjab are deficit in P and there is a need of P supplementation either in the form of mineral mixture (60-100 g/d) or any other P source in the diet of dairy animals.

Introduction

Phosphorus (P) was discovered by Hennig Brand in 1669 through urine distillation and it's Greek meaning is "bringer of light or light bearer". P occurs abundantly in nature (0.08-0.12 % weight in earth crust) both as in compound and elemental form. The compound forms of P are available as a) organic compounds and b) as inorganic salts. The inorganic P occurs as phosphate while organic P occurs in plants as phytic acid, phospholipid, nucleic acid etc (Mcdowell and Sharpley, 2003).

P is one of the macro mineral found in every cell of the body and is used in several body processes including critical energy pathways (ATP), cell signalling and synthesis of cell membranes, RNA, DNA and bone (Hill *et al.*, 2008). It is hard to think of any physiological function which would not involve directly or indirectly phosphoric acid compound therefore P is now widely acclaimed as master mineral. Moreover, it is second most abundant mineral in the body after calcium. Approximately 80 % of the total P in cattle body is in the bone and teeth as hydroxyapatite and remaining is distributed in soft tissue and body fluid (NRC, 2001). Furthermore, P is third most costly nutrient of all the diet supplements and accounts for more than 50% of the cost of typical vitamin-mineral mixes used on dairy farm (Chandler, 1996). Therefore, the aim of present review is to (1) broaden the knowledge about P, its metabolism and importance in dairy animals (2) comprehensively evaluate the efficacy of P supplementation on performance and/or health in ruminants by comparing the outcomes of studies with P supplementation in dairy animals (3) summarise the current status of P content in soils and plants of various regions in Punjab and to access the P requirement of Punjab dairy animals.

Role of P in ruminants

P level in blood

Whole blood contains 350-450 mg/L and most of which present in the cells. The element occurs in variety of forms (inorganic and organic P), mostly organic form is found. The inorganic form of P ranges from 4 to 8 mg/dL (about 10% is bound to serum protein and 50-60 % in ionized form) for dairy cattle with slight higher value for young animals. The intercellular P concentration in cattle is about 78 mg/dL and whole blood contains 6-8 time more P than plasma as higher concentration of P is present in erythrocytes (NRC, 2001). The organic P in plasma is mainly a constituent of phospholipids. All body P is in a dynamic state of turnover with a half life of about 1.4 hour for blood cell and 1.97 hour for brain. Moreover, plasma is not a reliable indicator of P status because of the quick compensatory reaction on P repletion (Valk *et al.,* 2000).

P in saliva

In ruminants the salivary glands are the major site for endogenous inorganic phosphorus (Pi) secretion into the gastrointestinal tract (GIT). Salivary Pi has 2 major functions: 1) acts as a buffer in the rumen, 2) provides adequate P for rumen microbes (Cohen, 1990). Salivary secretions of P constitute about 80% of the endogenous P recycled to the gastrointestinal tract (the balance is mainly from bile), depending on dry matter intake (DMI), usually combined with P intake, and the fiber content of the diet (Care, 1994). The daily secretion rate between 5 and 10 g Pi in sheep and between 30 and 60 g Pi in cows is achieved by both the high salivary flow-rate and the ability of the salivary glands to concentrate Pi 3-8 folds in comparison with plasma Pi (Breves and Schroder, 1991; Karn, 2001).

P in rumen and rumen micobugs

Rumen microbes have a P requirement apart from the animal's requirement which must be met for optimum rumen microbial activities to occur (Breves and Schroder, 1991; Powell *et al.,* 2002). Rumen microbes obtain P from the P released during fermentation of feed in the rumen and from salivary secretions. The concentration of P in the rumen contents was reported to be 200-600 mg/L and in this context Durand and Kawashima (1980) suggested the maximum P requirement for ruminal microbes is satisfied when the diet contains 4 g P/Kg digestible organic matter. This is equivalent to $<0.30\%$ dietary P. P is mainly of the inorganic variety which originates from the hydrolysis of organic compound present in diets and from salivary secretions. While, rumen microbes concentrate P mainly in organic form as nucleotides (Komisarczuk, 1985). Rumen microbes appear to be an intermediary source of P for the host animal. Salivary P incorporated by the microbes requires further digestive solubilisation in order to be used by the host animal and would be less available than soluble P (Pfeffer, 1995) or even partly unavailable for the animal (Challa and Braithwaite, 1988; Rodehutscord *et al.,* 2000). It was observed that rumen microbes had higher P requirement than host animal, therefore, care should be taken to meet the microbes P requirement (Preston and Fander, 1964; Vitti *et al.,* 1988). Recently, Ramirez-

Prez and Meschy (2005) proposed that 7 g P/kg fermented organic matter was required for normal rumen function. In another study by Durand and Komisarczuk (1988) reported that available P (from dietary sources and salivary recycling) within the rumen should be at least 5 g/kg of organic matter digested to optimize degradation of cell walls. If a feed is low in its P content then salivary P secretion also get lowered which ultimately affect the performance of microorganism in the rumen. However, maximum microbial degradative and synthetic activities can be maintained if ruminal inorganic P levels are at least 75-100 mg/L (Komisarczuk *et al.,* 1987). Moreover, when cattle were fed 0.12 % dietary P, ruminal fluid concentration was over 200 mg P/L, considerably greater than the 20 to 80 mg of P/L needed for maximum cellulose digestion under *in vitro* conditions (Chicco *et al.,* 1965). This concentration of P in rumen is achieved usually in cattle by salivary recycling of P and from diets adequate to meet the animal's requirement.

Sources for P

Contents of P in crops and forages are highly variable and depend on soil fertility, plant species, stage of maturity, climate and fertilization (Underwood, 1981). Temperate grasses contain more P than tropical grasses; and more mature forage has lower P contents, with the lowest P contents measured in summer (Minson, 1990; Valk *et al.,* 2000). P content of crop and forage decline markedly with advancing maturity and season affects the concentration of P more in legumes than in grass, while, it was reported that during hay and silage making loss of P occur generally (Coates *et al.,* 1990). P an essential element for plant growth and occurs in the plant in either an inorganic form (orthophosphate and pyrophosphate) or organic form (phospholipids, phosphosugars, adenosine diphosphate, adenosine triphosphate, nucleic acids polymers, and phytate). Very little or low amounts of P is located in roots and leaves. In seeds predominant form of P is phytates which occurs as storage form for both phosphates and inositol (Ravinderan *et al.,* 1995). Of the total P present in the cereal grains, 60-80% occurs in conjugation form as phytate i.e. the salts of phytic acid (an anion form of phytic acid). O'dell *et al.,* (1976) reported that 90 % of phytate P in maize is found in the germ portion of the kernel. On the other hand the aleurone layer of wheat and rice seeds is the richest source of phytate P. Phytin contains 28.2 % P and constitutes between 1-3% by weight of many cereal and oil seeds (Nelson, 1967: Cheryan, 1980). Tyagi *et al.,* (1998) reported that wheat contains 0.27 % phytate that accounts for 61% of the total P present in it. Moreover, Paik (2000) found that proportion of phytate P varies from 11.9% in tapioca to 83.1% in wheat bran.

The average concentration of P in grain is 3.5-4.5 g/kg DM which is 3-4 times higher than straw. Among grains soluble and insoluble phytate comprises 50-70%, phospholipid, phosphoprotein, nucleic acid 20-30% and mineral phosphate 8-12%. Introduction of P fertilizer in soil increases its concentration in plants. The level of P among various feeds and fodders along with other dietary supplemental sources of P have been presented in Table 1 and 2.

P metabolism in dairy animals

P in feed can either of inorganic origin like orthophosphate, di and tri substituted inorganic phosphate or of organic origin like phytate phospholipid, phosphoprotein etc. In case of dairy animals salivary secretions also act as a major source of P and its concentration in cattle saliva is 370-720 mg/L (Clark *et al.,* 1953). Inorganic P sources which are water soluble are available for absorption in ruminant, while the solubility of P in organic compound depends upon the ability of e animal to convert organic into inorganic form or more acceptable organic form (Underwood and Suttle, 1999). In rumen, micobes secrete phytase enzymes which hydrolysed the phytate P and released inorganic P. The organic P which is not hydrolysed in rumen becomes soluble in low pH of abomasums (Breves and Schroder, 1991; Care, 1994). Under certain circumstances like formaldehyde or heat treatment of seeds, there is decrease in the efficiency of phytase activity as phytate P become inaccessible to phytase or there is saturation of rumen phytase by large amounts of dietary phytate P (Pfeffer, 1995; Konishi *et al.,* 1999; Park *et al.,* 1999).

Generally, absorption of ingested P depends upon its solubility at the point of contact with the absorbing membranes (Mcdowell, 1992). It is primarily absorbed in the small intestine mainly in duodenum and jejunum (Reinhardt *et al.,* 1988; Care, 1994), but the relationship between P intake and P absorption is still inconclusive whether it is inverse relationship or curvilinear (Morse *et al.,* 1992). Furthermore, only a small amount of P is absorbed from rumen, omasum and abomasums which still need to be researched thoroughly (Breves and Schroder, 1991).

Absorption of P occurs via two district mechanisms

Vitamin D dependent active transport system operates mainly when animals are fed low dietary P.

Passive absorption occurs when large amount of absorbable P is consumed.

The excess of inorganic phosphate formed in the process of the exchange or liberated during resorption of bone tissue is excreted from the body via the kidney. In poultry, rabbit, small lab animals and young ruminants (during first two weeks of their life), P is eliminated mainly through the kidney i.e through urine. In Pig it is eliminated to equal extent through urine and faeces while in adult ruminant all most all P excretion occur through faeces (Betteridge *et al.,* 1986). Recently, Alvarez-Fuentes *et al.,* (2016) reported that on average, lactating dairy cows excreted 58% of ingested P in feces and 0.44% in urine, and secreted 40% in milk. Whereas, at constant P intake, cows with higher milk production excreted less P than cows with lower milk production. Fecal excretion of P contains three fraction 1) P of dietary origin unavailable for absorption or not absorbed 2) P of endogenous origin that inevitably has to be excreted under actual nutritional and physiological conditions 3) P of endogenous origin which is excreted to maintain homeostasis (NRC, 2001). Therefore, the study of P and efficiency of its utilization in dairy animals is not simple because of enormous amount of P is secreted endogenously in the saliva which sometimes surpasses the total amount of endogenous P excreted in faeces (Kleiber *et al.,* 1951).

Role of P in milk production and reproduction

P required for milk production comprises a major fraction of the total P requirements of a modern dairy cow (NRC, 2001). The P requirement according to various feed evaluation systems of a 600-kg nonpregnant cow producing 30 kg of milk/d varies between 59 and 85 g/d (Valk *et al.,* 2000). Recently, Klope *et al.,* (2013) reported that milk P (g/d) is inherently related to milk yield and probably milk yield will be higher at higher intake levels of P. As per NRC (2001), the average milk P concentration is 0.9 g of P/kg of milk and for lactating dairy cow range of P is from 0.30 to 0.40 % of the diet DM, whereas, for dry cows about 0.22 to 0.26 %

dietary P is sufficient to meet their requirements. Similarly, results of Wu *et al.*, (2000) indicated that dietary P at 0.38 to 0.40 % is sufficient for high producing cows, whereas an apparent digestibility of P of 40 % or less might be an indicator of excessive intake of P.

P has been most commonly associated with decreased reproductive performance in dairy cows. Natural deficiencies of P were reported to cause subnormal fertility, depressed or irregular oestrus and delayed conception in cattle (Morrow, 1969). But, there is no evidence that suggests feeding P in excess of NRC (2001) requirements improves reproductive performance. Recently, Lopez *et al.*, (2002) reported that feeding P in excess of NRC (2001) recommendations has no effect on reproductive performance in lactating cows. In this study a total of 267 Holstein cows were randomly assigned at calving to a control diet containing 0.37 % P (dry basis) or to a treatment diet containing 0.57 % P. Results (Table 3) of this study revealed that feeding of higher P had no effect on any of the observed reproductive parameters and no effect on milk production or milk composition. But, still higher level of P are being fed to animals may be because of 1) Lack of scientific literature providing the absolute minimum P requirement of lactating dairy animals to support moderate to high levels of milk production 2) Aggressive marketing of P supplements.

P status in Punjab

Soil and plant status

Most of the soil in Punjab is deficient in P (Brar, 1979) and rich in molybdenum (Mo) content (Nayyar *et al.*, 1980). Both these elements P and Mo interact in antagonistic manner, so fodder raised on such soil produces P deficiency in plants and therefore in animals (Dhillon *et al.*, 1972). Moreover, high aluminium (Al) and iron (Fe) contents of soil also bring out P deficiency by forming insoluble non-absorbable P-complexes (Mcdowell *et al.*, 1992) and diet containing high Al reduced P bioavailability significantly (Crowe *et al.*, 1990). Motsara (2002) reported that in Punjab 29, 49 and 22% of soil samples are low, medium and high in P content, respectively. It was also observed that the P deficiency in green fodder was comparatively higher in the summer season (21.2%) than in winter season (13.4%) (Sikhtajinder, 2012). The available P content (Kg/hectare) in soil, straw, green fodder and concentrate ingredients in different zone of Punjab have been illustrated in Table 4 and 5, respectively.

A study conducted by Sikhtajinder (2002) reported that 66.67, 55.56, 18.18 and 11.11 % samples of bajra, maize, berseem and oat of Ludhiana, Sangrur and Fatehgarh Sahib district, respectively were deficient in P. Whereas, the level of P in maize, bajra, berseem fodder was found to be 2500, 1200 and 2800 ppm, respectively (Gupta and Ahuja, 1998). However, in Bathinda district the overall mean P level in fodder was 0.31% (Sashitola, 2011) which was well above the critical limit i.e., 0.25% (Mcdowell, 2003). P level in green and dry fodder of Mansa and Ferozepur district of Punjab (Sikhtajinder, 2012) has been provided in Table 6.

Animal status

Among all districts of Punjab, high incidences of hypophosphatemia were recorded in cows (71.4%) as compared to buffaloes (41%) of Ferozepur district (Randhawa, 2007; Bhandari *et al.*, 2015). Overall deficiency of Pi in CB cows of Ludhiana, Sangrur and Fatehgarh Sahib district was 31.82, 31.43 ad 50.0 %, respectively, while, in buffaloes it was found to be 25.00, 32.05 and 41.17 % deficit, respectively.

Requirement of P

Ingredients	Amount fed(Kg)	DM (Kg)	Protein (Kg)	TDN (Kg)	P (g)
Maize	40	8	0.8	4.8	16
Wheat straw	0.55	0.5	0.01	0.2	0.04
Concentrate	4	3.5	0.7	3	31.5
Total		12	1.5	8	47
Requirement		12	1.4	7.2	48
Extra		-	0.1	0.8	-

Table.1 P Status of dry and green forages on DM basis

Sr. No.	Feed stuff	P (%)	Sr. No.	Feed stuff	P (%)
1	Maize stover	0.16	2	Sorghum stover	0.12
3	Rice straw	0.09	4	Wheat straw	0.06
5	Urd straw	0.18	6	Ground nut Straw	0.17
7	Gram straw	0.14	8	Cowpea green	0.25
9	Lucerne green	0.31	10	Guar green	0.24
11	Oat green	0.29	12	Bajra green	0.29
13	Barley grain	0.37	14	Maize grain	0.31
15	Wheat bran	0.91	16	Oat grain	0.36
17	Bajra grain	0.34	18	Sorghum grain	0.28
19	Guar Chuni	0.58	20	Mustard Cake	1.18
21	Sesame cake	1.09	22	Cotton seed cake	0.49
23	Linseed cake	0.52			

Source (Garg *et al.*, 2005)

Table.2 Dietary supplements of P

Sr. No.	Name	Ca%	P%
1	Dicalcium phosphate	23	18
2	Monocalcium phosphate	16.4	21.6
3	Steamed bone meal	31.5	14.2
4	Tricalcium phosphate	38	19.5
5	Sodium tripolyphosphate	--	25
6	Deflourinated phospahte	32	16.2
7	Low fluorine phosphate	36	14
8	Soft phosphate	17.2	9.1
9	Phosphoric acid	---	31.6
10	Ammonium phosphate	0.52	20.6

Source (Garg *et al.*, 2005)

Table.3 Characteristics of estrous behaviour for lactating cows fed diets containing 0.37 % or 0.57 % P

Characteristics	0.37 % P	0.57 % P
Days to first natural estrous	68	67
Days to first service	89	90
Conception rate at first AI	39.4	42.0
Overall conception rate at 30 d	34.3	38.0
Pregnancies lost (30 to 60 d)	15.2	16.2
Pregnancies lost after 60 d	6.0	5.4
Days open	112	116
Services/conception	2.9	2.6
Anovulatory condition %	29.9 (40/134)	27.1 (36/133)

(Lopez *et al.*, 2002)

Table.4 Status of available P content (Kg/hectare) in soil of Punjab

P Catagories with range	Mean available P	Percent area of state
Low (<12)	5.11	36.6
Medium (12-22)	16.47	17.8
High (22-50)	32.58	19.6
Very High (>50)	108.89	26.2

(Sharma *et al.*, 2011)

Table.5 P status in straw, green fodder and concentrate ingredients in different zone of Punjab

Sr. NO.	Area/zone	Straw	Green fodder	Concentrate ingredients
		P %	P%	P%
1	South –west zone (ferozepur, mansa, Muktsar)[1]	0.08	0.47	0.78
2.	Central plain zone (Amritsar, Ludhiana, Patiala)[2]	0.10	0.43	0.77
3	Sub mountain region[3] (Ropar, Gurdaspur)	0.09	0.30	0.57

([1] and [2] Garg *et al.*, 2007a and 2007b, [3]Bhandari *et al.*, 2007)

Table.6 Level of P in green and dry fodder of Mansa and Ferozepur district of Punjab

Mineral	Critical level	Green fodder overall deficiency/excess (%)		Dry fodder overall deficiency/excess (%)	
		Mansa	Ferozepur	Mansa	Ferozepur
Phosphorus	0.25%	28.3 low	6.5 low	100 low	100 low
Molybdenum	3ppm	41.5 excess	29.5 excess	98.4 excess	98.3 excess

Table.7 P requirements (g/d) for maintenance of cattle and buffaloes

BW (Kg)	P (g/d)	BW (Kg)	P (g/d)	BW (Kg)	P (g/d)
200	4	400	8	600	12
250	5	450	9	650	13
300	6	500	10	700	14
350	7	550	11	750	15
400	8	600	12	800	16

Table.8 Percentage of farmers supplementing mineral mixture to their animals in various districts of Punjab

Sr. No.	Zone of Punjab	District selected	% Farmer using MM for their animals	References
1.	Sub-mountainous undulating zone	Nawan Sahar Hoshiarpur	2	Hundal *et al.*, (2007)
2.	Central Plain zone	Ludhiana Kapurthala	25 14.3	Kaushal *et al.*, (2007)
3	Flood prone bait zone	Tarntaran Jalandhar	5 28.8	Wadhwa *et al.*, (2007a)
4	Undulating plain zone	Hoshiarpur Gurdaspur	25 2	Kaur *et al.*, (2007)
5	Western plain	Ferozepur Moga	2.5 9.6	Bakshi *et al.*, (2007)
6	**Western zone**	**Bathinda Mansa**	**10.6 2.5**	Wadhwa *et al.*, (2007b)

The mean plasma Pi level in cattle and buffaloes of Muktsar and Bathinda district was 5.28 and 5.54 mg/dL, 4.70 and 4.86 mg/dL, respectively (Sashitola, 2011) which was above the critical level of 4.5 mg/dL (Mcdowell, 2003). The overall incidence of subclinical hypophosphatemia in CB cows and buffaloes was 35.11 and 23.86 %, respectively in both the districts. Whereas, the prevalence of hypophosphatemia in central district of Punjab was 29.8 and 26.7 % in CB cows and buffaloes, respectively (Singh, 1999).

Deficiency symptoms of P

Of all the minerals deficiencies in cattle, the deficiency of P is the most common one on a global level (Mcdowell *et al.,* 1992). Peg-leg, pica and bone chewing are observed or when soil is known to be uniformly P deficient. Diagnosis of sub-clinical deficiency associated with reduced cattle production is generally both more difficult and more important. When plasma level is less than 2mg/dL, then deficiency occur. Symptoms of P deficiency include energy deficiency-reproductive problems, pica/ depraved appetite/allotriophagia, osteophagia, sarcophagia, rupturing of achillus tendon in young bulls and haemoglobinurea.

Requirement of P

As per ICAR (2013) the P requirements of cattle and buffaloes for maintenance are represented in Table 7. In case of growing young and adult cattle/buffaloes the P requirements are 9 and 6 g/Kg BW gain. Similarly, for one Kg milk production there is need of 1.8 g P by both lactating cattle and buffaloes.

So, for a cow of 400 Kg BW having 10 Kg milk yields, the P requirements can be calculated as follow:

Requirements of 400 kg cow: DMI= 12 Kg; TDN= 7.2 Kg; CP = 1.4 Kg; and P = 0.40% DM is below:

So, from above example it has been concluded that if farmers are feeding concentrate mixture having mineral mixture 2 % (Mineral mixture contains P= 12%) to their animals then there is no need of supplementing additional P source to the dairy animals. Supplementation of P is also necessary as bioavailability of P from plant sources has been reported to be low due to phytic acid-P (McDowell, 1992). Moreover, Singh *et al.*, (2005) found deficiency of P and other minerals in dairy animals of Punjab yielding 10 Kg of milk per day and suggested mineral supplementation in different agro-climatic zones of Punjab. The percentage of farmers supplementing mineral mixture to animals in various districts of Punjab is presented in Table 8. Therefore, in Punjab there is a need of supplementation of P either by supplementing P sources or mineral mixture (60-100 g/d) to fulfil the P requirement of dairy animals.

Optimizing dairy production with the aim to meet the nutritional requirements of dairy animals precisely and minimize the loss of undesired end products to the environment requires promotion of feeding balance ration in the form of total mixed ration under field conditions. The farmers which prepared their concentrate mixture without addition of mineral mixture have to supplement either mineral mixture to their animals @ of 60-100 g/day [depending upon P content of mineral mixture (9 or 12 %)] or any other P supplements in the diet. Recently, P has received attention for environmental reasons, such as depletion of finite inorganic P sources (e.g., dicalcium phosphate) and pollution of ponds and streams causing eutrophication. Therefore, optimizing P intake and understanding the factors affecting P utilization will have environmental and economic benefits.

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How to cite this article:

Jaswinder Singh, J.S. Hundal, A. Sharma, Udeybir Singh, A.P.S. Sethi and Parminder Singh. 2018. Phosphorus Nutrition in Dairy Animals: A Review. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 3518-3530. doi: https://doi.org/10.20546/ijcmas.2018.704.397