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Determination of Available Phosphorus in Compounded Cattle Feed using Citrate Buffer by ICP-OES

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ABSTRACT

A study was carried out to develop the test method for the determination of available phosphorus in compounded cattle feed manufactured. There are a few methods of determination of the available phosphorus, however these are lengthy, complex and cumbersome. Therefore, it was required to develop a fast and robust method of determination of available phosphorus. Cattle feed samples from different sources were extracted using citrate buffer of pH 3 and then available phosphorus was analyzed using ICP-OES after microwave digestion of the extract to determine available phosphorus. The study yielded promising and consistent results of available phosphorus in compounded cattle feed in terms of recovery of the analyte ranging from 96.5 to 109 %. The study showed that the proposed test method can be used by a laboratory to determine available phosphorus in compounded cattle feed with accuracy and precision.

KEYWORDS: Available Phosphorus, Citrate Buffer, Feed, ICP-OES, Phosphorus.

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INTRODUCTION

Phosphorus (P) is an essential macro mineral which is required in metabolism, growth, structural and reproductive processes in dairy animals.Its deficiency can lead to several problems such asdecrease in weight, lower P levels in blood, poor reproduction, and reduced production performance in ruminants (Singh et al., 2018). A major partof phosphorus in animal diet from plant sources is present in phytate form (Ravindran et al., 1994). Monogastric animals lack endogenous phytase enzyme so are unable to digest phytate P which is a major form of P storage in plants (Ravindran et al., 1994). In ruminant animals, phosphorus can only be absorbed from the gastrointestinal tract, if it is soluble (Breves and Schroder, 1991). In ruminants, P from water soluble inorganic sources is available for absorption, however the solubility of P from organic sources depends upon capability of the animal to convert organic to more acceptable organic form or inorganic form (Underwood and Suttle, 1999; Singh et al., 2018). Ruminal microbes secrete Phytase enzyme which can hydrolyze phytates to release inorganic P. In high producing ruminants, short duration of exposure of phytate molecules to the phytase from ruminal microorganisms may limit ruminal phytate degradation(Jarrett et al., 2014).Under certain circumstances like formaldehyde or heat treatment of seeds, there is decrease in the efficiency of phytase activity as phytate P becomes 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; Singh et al., 2018).Thus, inorganic P is more readily bioavailable than organic P. Supplemental P or mineral mixtures or inorganic salts of P, like di-calcium phosphate (DCP) are added to natural feed constituent to meet the P requirements (Prasad et al., 2015) so that there is no deficiencyof P in the diet of animals.

Animal nutritionists advocate to add 1-2% mineral mixture in cattle feed prepared as per IS 1664 to ensure the supply of major and micro minerals including P.The objective of keeping available P in the IS specification of compounded feeds for cattle is to ensure the addition of 1-2% mineral mixture.

Mineral mixture prepared as per IS 1664 contains 12% P which is contributed by calcium hydrogen phosphate (more commonly known as DCP). DCP contains 18% P which is almost 85% bioavailable (Haleet al., 2017; NRC, 2001) and is soluble in citrate buffer. Therefore, addition of 1-2% of mineral mixture would provide 0.10-0.20 % available P.

Thus, the determination of available phosphorus (AP) in animal feed is essential and is requirement of BIS standards of compounded cattle feed (IS 2052) and poultry feed (IS 1374). The already established methods of available phosphorus are either arduous, erroneous or time consuming. Therefore, development of a quick and robust method for determination of available phosphorus is imperative to meet the regulatory requirements and to support the feed industry.

The amount of P dissolved in citrate buffer solution at pH 3 is measured and expressed as available phosphorus. Thus, available phosphorus (inorganic phosphorus) could be effectively extracted using citrate buffer of pH ~3. The extract is then acid digested using nitric acid in a microwave digester to stabilize elements as an ionic solution and available phosphorus was estimated by using ICP-OES. The proposed method was applied to different cattle feed samples for determination of available phosphorus. Therefore, in this study it was attempted to develop a fast and accurate test method to determine available phosphorus in compounded cattle feed.

MATERIALS AND METHODS

Materials

Different compounded cattle feed samples (N=26) from both co-operatives and private sectors were takenfor the study. These samples were sent to CALF laboratory by customers to analyze available phosphorus in the feed samples. The samples were ground using mill grinder (Foss CT 293 Cyclotec, origin: Denmark) and allowed to pass through 1 mm sieve. All reagents, chemicals and consumables were of analytical grade and purchased from reputedIndian brands / foreign brands.

Methods

Reagent Preparation

0.1 M 1L Citric Acid solution: 19.212g of anhydrous citric acid was weighed accurately and was dissolved in deionized water and final volume was adjusted in 1000 mL volumetric flask.

0.1 M 100 mL Tri-Sodium Citrate solution: Accurately weighed 2.941g of tri-sodium citrate dehydrate was dissolved in deionized water and final volume was adjusted in a 100 mL volumetric flask.

Citrate Buffer preparation: Citrate buffer (pH"3.0) was prepared by mixing 465 mL of 0.1M citric acid and 35 mL of 0.1M tri-sodium citrate solutions and final volume was adjusted to 1L using deionized water in a volumetric flask.

Sample preparation for available phosphorus

About 2g of each sample was weighed accurately in a 250mL conical flask. 200mL of the prepared citrate buffer was added and the solution was manually shaken to dissolve clumps of feed. The solution was kept for 30 min. at room temperature and was then filtered through Whatman filter paper no. 40 into another 250mL conical flask.

The analysis was performed for 26 different samples on 10 different days along with a spiked sample for each set. Spiking was done with DCP ($CaHPO_4.2H_2O$; Molecular weight: 172) to check recovery of the analyte.

Microwave digestion

10 mL aliquot from the filtrate for available phosphorus was taken in microwave vessel. 0.2g of original cattle feed samples was taken in separate microwave vessels for total phosphorus estimation. 8 mL concentrated HNO_3 and 0.5mL H_2O_2 was added to the vessels. Then microwave digestion was carried out (Table 1) with method based on BS EN 15621:2017 using microwave digester systems (Anton Paar Multiwave Pro microwave reaction system, origin: Austria and CEM MARS 5 Express microwave digester, origin: USA). After digestion, the contents of the vessel was carefully transferred to a 100 mL volumetric flask and volume was adjusted using deionized water.

Table 1. Microwave digestion program steps of microwave digester

Steps	Temperature (˚C)	Time (min.)
Temperature ramp	100	15
Temperature hold	100	5
Temperature ramp	150	10
Temperature hold	150	5
Temperature ramp	200	15
Temperature hold	200	15
Cooling	70	25

ICP-OES analysis

The digested sampleswere analyzed for available phosphorus and total phosphorus using ICP-OES (Perkin Elmer Avio-500, origin: USA and Optima-8000, Origin: USA) instruments with method based on BS EN 15621:2017.

RESULTS AND DISCUSSION

Available Phosphorus (AP) and Total Phosphorus (TP)

Different samples were taken and total phosphorus was estimated by microwave digestion followed by analysis in ICP-OES. Total phosphorus includes both inorganic and organic phosphorus content. Available (inorganic) phosphorus being soluble in citrate buffer is effectively extracted using citrate buffer and filtered. Available phosphorus was then analyzed by ICP-OES after microwave digestion of the filtrate. Results of available phosphorus along with total phosphorus in the samples are given in Table 2. It can be observed that the found available phosphorus is 42-86% of total phosphorus. This indicates that the available inorganic phosphorus is less than the total phosphorus, i.e. some phosphorus is in other organic forms which includes phytate form. The diverse range of the % of AP of TP also shows the miscellaneous types of feed samples taken, therefore the method can be applied in various types of samples, implying the versatility of the method.

Table 2. Available Phosphorus and the % of Available Phosphorus in Total Phosphorus

Set No.	Sample No.	Available Phosphorus (AP) %	Total Phosphorus (TP) %	% AP of TP
I	a	0.7	0.99	70.7
	b	0.54	0.87	62.1
II	c	0.52	0.8	65.0
	d	0.77	1.02	75.4
III	e	1.01	1.25	80.8
	f	0.68	1.01	67.3
IV	g	0.34	0.67	50.7
	h	0.22	0.52	42.3
	i	0.84	1.04	80.6
	j	0.73	0.98	74.3
V	k	1.08	1.26	85.4
	l	1.14	1.40	81.2
	m	1.14	1.42	80.2
	n	0.83	1.10	75.3
VI	o	1.02	1.39	73.1
	p	1.24	1.47	83.9
VII	q	0.6	0.91	65.7
	r	0.54	0.77	69.6
VIII	s	1.04	1.42	73.2
	t	1.02	1.37	74.1
	u	0.8	1.17	68.3
IX	v	0.81	1.21	66.8
	w	1.07	1.37	77.5
	x	0.99	1.34	73.5
X	y	1.04	1.38	74.8
	z	0.49	0.95	51.1

From Table 2, the following can be inferred that the the total P in different feed samples ranged from 0.52 to 1.47%, which could be due to specific feed formulation and amount of mineral mixture used. The available P in different feed samples ranged from 0.22 to 1.24 which is 42.3 to 85.4 % of total P indicating the varied level of bioavailability of feed phosphorus. From the % of AP in TP, we can observe that the number of samples having low available P, i.e. <60% is 3. The number of samples having intermediate available P, i.e. 60% to 75% is 14. The number of samples having high available P, i.e. >75% is 9. The number of samples having low total P content which might be due to less quantity or no addition of mineral mixture. The number of samples having higher available P were having higher total P which can be due to the high quantity of mineral mixture addition.

Recovery of analyte

In each set, one sample was taken in duplicate and of them one was spiked with ~1 % P using DCP. Recovery of the analyte is given in Table 3. Recovery of spiked samples in each experimental set was found in the acceptable range of 100 ± 20 % (APVMA, 2004). This provides the confirmation of the effectiveness of the method to determine available phosphorus. It showed that the proposed methodology was able to extract available P and quantify that accurately.

Table 3. Recovery % of available phosphorus in spiked samples

Set No.	Sample No.	AP[1] % in Blank sample	Spike level (%)	AP[1] % found in Spiked sample	Recovered amount (%)	Recovery (%)
I	a	0.70	1.15	1.89	1.19	103.4
II	b	0.54	1.03	1.62	1.08	104.8
III	e	1.01	1.12	2.17	1.16	103.5
IV	f	0.68	1.13	1.88	1.2	105.8
V	i	0.84	1.13	2.06	1.22	107.9
VI	o	1.02	1.14	2.12	1.1	96.4
VII	r	0.54	1.1	1.71	1.17	106.3
VIII	t	1.02	1.08	2.16	1.14	105.5
IX	x	0.99	1.14	2.24	1.25	109.6
X	y	1.04	1.15	2.2	1.16	100.8

[1 – Available phosphorus]

CONCLUSION

Citrate Buffer method of determining available phosphorus yielded expected results and recovery of analyte in spiked samples was in the acceptable range of 100 ± 20%. Hence the proposed method of extraction with citrate buffer and then further analysis in ICP-OES is an effective and robust method for the analysis of available phosphorus in compounded cattle feed as per IS 2052.

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