Indian J. Anim. Nutr. 2019. 36 (1): 30-34
doi: 10.5958/2231-6744.2019.00005.7

Indian Journal of
Animal Nutrition

Bioavailability of Major Minerals from Commonly Used Ruminants Feeds Using Dialyzability Technique

Abhilasha Singh*, Veena Mani, Bhawana Shukla, Anjila Kujur-, Suman Kapila[1]
Animal Nutrition Division, ICAR- National Dairy Research Institute, Karnal-132001, Haryana, India

ABSTRACT

Four feeds namely maize (*Zea mays*) grain, deoiled rice (*Oryza sativa*) bran, groundnut (*Arachis hypogaea*) cake and mustard (*Brassica campestris*) cake were used to assess the bioavailability of calcium, phosphorous, magnesium, sodium and potassium by dialyzability technique under *in vitro* conditions. For this, three Karan Fries calves kept on roughage based diet were used for collection of rumen liquor through stomach tube. *In vitro* dry matter digestibility (IVDMD) of different samples was estimated at three stages *i.e.* simulating ruminal, abomasal and intestinal conditions followed by determination of bioavailability of major minerals in the soluble fraction of intestinal stage sample through a 12 kD dialyzable membrane. Maize grain (69.91±1.11) possessed the highest (P<0.05) IVDMD value followed by mustard cake (68.51±1.087), groundnut cake (63.59±2.07) and deoiled rice bran (51.22±1.05) at the intestinal stage of digestion. Similarly, the highest (P<0.05) bioavailability of Ca, P and Mg (71.76±1.66, 87.57±0.37 and 85.05±0.12%, respectively) was found for maize grain followed by mustard cake, groundnut cake and deoiled rice bran, however, Na bioavailability was higher (98.10±0.10%) in case of groundnut cake and numerically similar values were obtained for K. Results suggested that *in vitro* method could be used for prediction of bioavailable minerals fraction from feeds, however, validation by actual *in vivo* experiment is required before accepting the bioavailability values while considering these for dietary mineral requirements.

Key words: Bioavailability, Dialyzability, In *vitro* digestion, IVDMD, Major minerals

INTRODUCTION

There is scanty information on bioavailability of minerals from different feed ingredients. Reliance on simplistic attributes such as solubility of the plant mineral in water or unphysiological extractants (*e.g.* citric acid) produces results having poor correlation with *in vivo* assessments of mineral bioavailability. Now a days, to assess the bioavailability of minerals, *in vitro* methods have gained importance because of accuracy of results, speed of analysis and relatively low costs. Solubility after the sequential simulation of gastric and intestinal digestion can give convincing values for ruminants. The accessibility of minerals to rumen microbes is equally important. Use of such *in vitro* techniques for prediction of availability can be made but account must be taken of possible interactions between feeds and results will always have to be validated *in vivo* (Suttle, 2010). Besides this, minerals in feeds and forages are associated with other compounds or trapped in the indigestible nutrient fractions resulting in slow release or making these unavailable for use. Therefore, mineral content can be determined chemically while bioavailability is difficult to be estimate (Evitayani *et al.,* 2006). Hence, this study was designed to investigate the bioavailability of major minerals (Ca, P, Mg, Na and K) present in mustard cake, groundnut cake, maize grain and deoiled rice bran under *in vitro* conditions.

MATERIALS AND METHODS

The samples of test feed *i.e.* maize grain, groundnut cake, mustard cake and deoiled rice bran were collected from feed mill of ICAR-National Dairy Research Institute, Karnal and dried in hot air oven at 80°C for 48 h and ground to pass through 1 mm sieve using Willey mill and stored in 200 mL capacity plastic bottles. These were analyzed for their proximate principles (AOAC, 2005) and fiber fractions (Van Soest *et al.,* 1991). Residues left after NDF and ADF determination were pooled separately for further analysis of NDF and ADF bound minerals.

Three stage *in vitro* experiment was conducted to determine DM digestibility of different feed ingredients. Ground samples were subjected to a

*Corresponding author: E-mail: abhilasha1495@gmail.com [1]Animal Biochemistry Division

simulated ruminal, abomasal and intestinal phase digestion by following the method of Tilley and Terry (1963) with certain modification as described by Calsamiglia and Stern (1995). Rumen liquor was collected from three male Karan Fries calves (Av. age= 12 month; BW=120 kg) using stomach tube. After collection of rumen liquor in pre warmed thermos flask, brought to laboratory and used as a source of rumen microbes. About 0.5 g of ground sample was taken for ruminal digestion to which 10 mL of fresh strained rumen liquor and 40 mL of McDougall buffer (McDougall, 1948) were added, CO_2 was passed through the contents for about 10 sec. and the stoppard flasks were incubated for 24 h at 38ºC with periodic shaking. Ruminal digestion was followed by addition of 2 mL of 6N HCl and 0.1 g pepsin enzyme (from porcine mucosa) and pH was then adjusted to 2.0 with additional HCl. After 24 h of incubation at 38ºC in abomasal followed by intestinal with addition of 0.5 mL of 1N NaOH and 13.5 mL of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin (pancreatic enzymes from porcine pancreas, P1750, Sigma-Aldrich). The contents were incubated at 38°C for 6 h. After incubation, the flasks were removed and centrifuged at 3000 rpm for 10 min. and supernatant was collected for further analysis.

In dialysis procedure, pre treatment of dialyzable membrane (flat width 25 mm, internal diameter 16 mm, molecular weight 12 kD, Sigma-Aldrich) was done by boiling it for 5 min. in Millipore water. The ready to use membranes were kept in 20% ethanol solution in refrigerator at 4ºC and rinsed several times with Millipore water. A known quantity of sample (10 mL) was taken from the supernatant collected in third stage of *in vitro* digestion and was filled in dialyzable membrane and tied with thread. Samples were kept in 1000 mL beaker containing 500 mL Millipore water for 24 h. The contents were continuously shaken using magnetic stirrer (Kaushik, 2013). The bioavailabilty of minerals was the amount of dialyzable mineral expressed as a percentage of total mineral in the feed sample (Hansen, 2008). Bioavailability of minerals was determined by estimating concentration of the mineral in dialysate.

The modified method of closed system of acid digestion of samples (EPA, 2001) was followed for digestion of samples for mineral estimation. The contents of Ca, Mg, Na, and K were analysed using atomic absorption spectrophotometer (Hitachi Model Z – 5000 with Zeeman correction). The P content was estimated by spectrophotometer (AOAC, 2005). The data were statistically analyzed using SPSS (2010).

Table 1. Chemical composition (% DM basis) and mineral profile of the feeds

Parameter	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Dry matter	91.52	91.62	91.76	90.97
Crude protein	37.70	45.45	9.81	16.60
Ether extract	5.90	6.12	4.22	1.10
Crude fibre	8.37	7.61	2.42	13.09
Nitrogen free extract	39.91	34.52	83.56	54.32
Ash	8.12	6.32	1.53	14.90
Neutral detergent fibre	29.50	18.12	11.70	41.10
Acid detergent fibre	20.62	14.32	2.80	25.50
Calcium	0.48	0.32	0.15	0.80
Phosphorous	1.58	0.74	0.41	1.90
Magnesium	0.63	0.51	0.34	1.14
Sodium	0.082	0.05	0.025	0.033
Potassium	1.84	1.25	0.78	1.25

Table 2. Mineral (% of total) found in NDF and ADF fraction of feeds

Parameter	Fibre fraction	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Calcium	NDF	3.63	3.79	3.23	6.52
	ADF	2.69	2.36	1.91	2.79
Phosphorous	NDF	2.135	2.417	2.432	5.52
	ADF	0.76	0.78	0.457	1.751
Magnesium	NDF	2.57	2.72	1.75	3.37
	ADF	1.13	1.19	0.83	1.12
Sodium	NDF	0.718	0.752	0.372	0.775
	ADF	0.274	0.282	0.153	0.313
Potassium	NDF	0.693	0.714	0.412	1.331
	ADF	0.355	0.321	0.166	0.51

(n=6, replicates per feed)

RESULTS AND DISCUSSION

The chemical composition of the four ingredients has been presented in Table 1. Deoiled rice bran had higher NDF (41.10%) followed by mustard cake (29.50%). Lowest values for NDF (11.70%) and ADF (2.30%) were observed in maize grain. The values are in agreement with those reported by ICAR (2013). The highest values for Ca (0.80%), P (1.90%) and Mg (1.14%) were found in DORB. The lowest levels of Ca (0.15%,), P (0.41%), Mg (0.34%), Na (0.025%) and K (0.78%) were found in maize grain. Kumar and Kaur (2007) reported that Ca and P contents in different concentrate ingredients varied from 0.11 to 0.67% and from 0.68 to 1.61%, respectively. The results of present study are comparable with the values given by ICAR (2013).

The maximum association of Ca with NDF and ADF fraction was found in DORB (6.52 and 2.79%). The association of all the minerals with fiber fractions were lowest in case of maize grain among all four feedstuffs (Table 2). Among all minerals, solubility of Ca was quite low. No information is available on minerals associated with NDF and ADF fractions in concentrate ingredients. However, reports are available indicating variation in association of minerals with cell wall constituents in forages. Serra *et al.* (1996) reported 0.7 Ca, 14.3 P, 1.9 Mg and 3.7% K associated with NDF and 0.2 Ca, 4.4 P, 0.7 Mg and 2.8% K with ADF fraction in same forages. Evitayani *et al.* (2006) showed that 27.5 Ca, 8.3 P and 18.1% Mg was associated with NDF and 7.8 Ca, 1.8 P and 2.2% Mg was associated with ADF in grasses.

There was increasing trend in digestibility (Table 3) when incubation proceeded from ruminal to intestinal stage in all feeds and a similar trends was also reported by Gupta *et al.* (2016) and Parihar *et al.* (2017). Significantly ($P<0.05$) higher IVDMD values were found in maize grain (66.52-72.10%) and lowest in deoiled rice bran (42.90-57.27%). The highest digestibility was 69.91% in maize grain. Digestibility of

Table 3. *In vitro* DM digestibility (%) estimated by three stage digestion technique

Feed	Ruminal stage	Up to abomasal stage	Up to intestinal stage
Mustard cake	$65.59^{Ca}\pm0.7$	$68.74^{Cab}\pm0.6$	$71.19^{Bb}\pm1.04$
Groundnut cake	$57.44^{Ba}\pm1.8$	$65.32^{Bb}\pm0.51$	$68.04^{Bb}\pm2.1$
Maize grain	$66.52^{Ca}\pm0.9$	$71.12^{Db}\pm0.7$	$72.10^{Cb}\pm0.03$
Deoiled rice bran	$42.90^{Aa}\pm0.64$	$53.49^{Ab}\pm0.24$	$57.27^{Ab}\pm1.7$

Figures with different superscripts in a row^{a,b} and in a column$^{A, B,C, D}$ differ significantly ($P<0.05$): (n= 9 replicate per feed)

Table 4. Bioavailability (%) of various major minerals from different feeds using dialyzability technique

Parameter	Mustard cake	Groundnut cake	Maize grain	Deoiled rice bran
Calcium	65.28[ab]±0.69	61.88[a]±1.23	71.76[c]±1.66	66.70[b]±1.03
Phosphorous	81.20[a]±0.13	82.73[b]±0.27	87.57[c]±0.37	81.22[a]±0.25
Magnesium	82.42[a]±0.06	84.34[b]±0.09	85.05[c]±0.12	82.49[a]±0.15
Sodium	97.99[b]±0.35	98.10[b]±0.10	95.55[a]±0.50	96.04[a]±0.36
Potassium	95.89±0.61	94.33±0.75	96.06±0.11	95.88±0.25

[a,b,c]Values with different superscripts in a row differ significantly (P<0.05); (n= 9 per feed)

mustard cake at ruminal, abomasal and intestinal stage of incubation was found to be 65.59±0.7, 68.74±0.6, 71.19±1.04% and the corresponding values were 57.44±1.8, 65.32±0.51, 68.04±2.1% for groundnut cake. Mondal *et al.* (2010) reported comparable values of DM digestibility in mustard cake (69.63%) and groundnut cake (60.88%) but it was lower for maize grain (50.3%).

The Ca bioavailability (%) was 61.88±1.23 for groundnut cake and 65.28±0.69 for mustard cake. Kumar *et al.* (2015) reported slightly lower values than the present values. Maize grain had higher (P<0.05) bioavailability values for Ca (71.76±1.66), P (87.57±0.37) and Mg (85.05±0.12%), respectively than those from mustard cake, groundnut cake and de oiled rice bran which might be due to the lower fibre content, lower association of these minerals with NDF, ADF and higher degradability of maize grain. Values for Na and K were quite high (94.33 to 98.10%) though significantly higher values were found for Na release for both the cakes but considering the extent of release these values seemed be of no practical relevance. Brown *et al.* (2004) reported similar pattern of ruminal release of K from corn and cotton seed meal and reported values of more than 80%. Ceresnakova *et al.* (2007) conducted *in sacco* study on some grasses such as hybrid rila, hybrid niva, grass silage and reported the disappearance of K up to 98 to 99%. Trinacty *et al.* (2000) observed total tract release of K to be 98.4% from lucerne hay using nylon capsule method. Bamikole (2009) studied the macro-mineral bioavailability in goats fed forages of nitrogen fertilized guinea grass and guinea grass verano stylo mixture and observed that K all forages was well utilized with no significant variations.

CONCLUSIONS

Though, *in vitro* method could be used for prediction of bioavailable minerals fraction from feeds, however, validation by actual feeding trial is required before accepting the bioavailability values while formulating rations as the present bioavailability values were much higher than those reported by NRC (2001) under practical situations.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-NDRI, Karnal, Haryana for providing financial support to carry out this study.

REFERENCES

AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemists. 18th edn. Virginia, USA.

Bamikole, M.A. and Babayemi, O.J. 2009. Macro mineral utilization in West African dwarf goats fed combinations of fertilized and unfertilized Guinea grass, Verano stylo and a concentrate mix. *Livestc. Res. Rural Dev.* 21 (9).

Brown, M.S., Drager, C.D., Cochran E.M. and Lauterbach, E.A. 2004. Ruminal mineral release from corn and cottonseed meal. *Beef Cattle Research in Texas*. pp 109-111.

Ceresnakova, Z., Flak, P., Polacikova, M. and Chrenkova, M. 2007. *In sacco* macromineral release from selected forages. *Czech. J. Anim. Sci*. 52: 175-182.

Calsamiglia, S. and Stern, M.D. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminant. *J. Anim. Sci.* 73: 1459-1465.

E.P.A. 2001. Method-1631. *Development of Digestion Procedures to Determine Mercury In Solid and Semisolid Samples.* Frontier Geosciences, available from EPA sample control centre DYN Crop I & ET, Alexandria, VA22304 (703-461-2100., SCC@ dyncorp.com.).

Evitayani, W.L., Fariani, A., Ichinohe, T., Hayashida, M., Razak, S.A.A. and Fujihara, T. 2006. Macro mineral distribution of forages in South Sumatra during rainy and dry seasons. *J. Food, Agric. Environ*. 4: 155-160.

Gupta, S., Tomar, S.K., Mani, V., Kumar, R. D., Parihar, D.S. and Kujur S.T.A. 2016. Bioaccessibility of copper, molybdenum and zinc from selected oilseed cakes using three stage *in vitro* technique. *Indian J. Anim. Nutr*. 33: 149-153.

Hansen, S.L. 2008. *Nutritional Interrelationships between Iron, Copper and Manganese in Domestic Livestock*. Ph.D. Thesis. North Carolina State University, North Carolina, USA.

ICAR. 2013. *Indian Feeds and Fodder*. Krishi Bhawan, New Delhi, India.

Kaushik, R. 2013. *Development and Evaluation of Vitamin and Calcium Fortified Milk*. Ph.D. thesis, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India.

Kumar, R.D., Kewalramani, N., Mani, V., Gupta, S. and Parihar, D.S. 2015. Bioaccessibility of calcium, phosphorous and magnesium from different oil seed cakes. *Indian J. Anim. Nutr*. 32: 457-459.

Kumar, R.S. and Kaur, H. 2007. Macro mineral status and dietary cation-anion difference value of some Indian feeds and fodders. *Anim. Nutr. Feed Technol*. 7: 111-117.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A. 2009. *Animal Nutrition.* 6th edn. Dorling Kindersley (India) Pvt. Ltd. pp 635-638.

McDougall, E.I. 1948. Studies on ruminant saliva. The compostion and output of sheep saliva. *Biochem.* 43: 99.

Mondal, G., Chopra, R.C. and Barman, K. 2010. Nitrogen fractions, digestion kinetics and in vitro digestibility of some ruminant feeds. *Indian J. Anim. Nutr*. 27: 25-30.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. edn. National Academy of Sciences. Washington, D.C., USA

Parihar, D.S., Mani, V., Kewalramani, N., Gupta, S., Kumar, D. and Kujur, S.T.A. 2017. Bioaccessibility of trace minerals from different oilseed cakes under *in vitro* conditions. *Indian J. Anim. Nutr*. 34: 70-73.

Serra, A.B., Serra, S.D., Ichinohe, T. and Fujihara, T. 1996. Amount and distribution of dietary minerals in selected philipins forages. *Am. J. Anim. Sci*. 9: 139-147.

SPSS. 2010. *Statistical Packages for Social Sciences*. Version 16, SPSS Inc., Illinois, USA.

Suttle, N.F. 2010. *Mineral Nutrition of Livestock*. 4th edn. CAB International, Oxfordshire, UK. p 27.

Tilley, J.M.A. and Terry, R.A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassland Soc.* 18: 104-111.

Trinacty, J., Simek, M., Loucka, R. and Takahashi, J. 2000. Total tract digestibility of alfalfa hay minerals evaluated by nylon capsule method. *Czech. J. Anim. Sci*. 45: 65-69.

Van Soest, P.J., Robertson, J.B. and Lewis, B. 1991. Methods for dietary fibre, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Received on 18-10-2018 and accepted on 21-02-2019