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EFFECT OF VARYING DIETARY LEVELS OF CALCIUM AND PHOSPHORUS ON APPARENT DIGESTIBILITY AND RETENTION OF MACRO AND MICRO-MINERALS IN LACTATING SAHIWAL COWS

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ABSTRACT

Overfeeding or underfeeding of minerals to the animals may affect health of dairy animals and cost of production. The objective of this study was to investigate the impact of varying dietary levels of Ca and P on apparent digestibility and retention of Ca, P, Mg and Zn. For this purpose, 36 second parity Sahiwal cows at peak lactation stage averaging 70±10 days in milk (DIM) were offered one of six different treatments of varying levels of Ca and P (NCP = Normal Ca and P, HP = 40% higher P, LC = 40% lower Ca, LCHP = 40% lower Ca and 40% higher P, HC =40% higher Ca, or HCHP = 40% higher Ca and P) following a Completely Randomized Design. Dietary Ca and P levels and their ratios did not affect ($P>0.05$) daily milk yield. Feeding higher than recommended Ca levels (HC, HCLP, HCHP) resulted in a significant decrease ($P < 0.05$) in apparent Ca digestibility and retention and a significant decrease ($P < 0.05$) in apparent Zn digestibility. Feeding higher than recommended P resulted in a significant decrease ($P < 0.05$) in apparent P digestibility, irrespective of dietary Ca levels. Dietary P levels did not ($P > 0.05$) affect apparent Zn digestibility. A decrease ($P < 0.05$) in apparent digestibility of Mg was observed in treatment groups HC and HCHP.

Keywords: Calcium, phosphorus, magnesium, varying levels, apparent digestibility.

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INTRODUCTION

For optimal performance of dairy animals, appropriate supply of minerals in diet needs to be considered (Kronqvist, 2011). Diet should be formulated to decrease excretion of valuable nutrients particularly minerals in feces and urine which interferes with underground and surface water quality (Morse *et al*., 1992; Tamminga, 1992; Underwood and Suttle, 1999) and also contributes in environmental pollution (Chapuis-Lardy *et al*., 2004; Knowlton *et al*., 2005).

Together Ca and P make up to 70% of the total minerals in the body. About 80-85% of Ca, P and Mg are stored in bones in the form of salts. Bone ash comprises of about 36% Ca and 17% P and a large part of which is in dynamic state (Harris Jr *et al*., 2003). Increase in dietary levels of one mineral may affect digestibility and excretion of others (Khorasani *et al*., 1997). In the body, Ca is regulated by homeostatic mechanisms involving 1, 25-dihydroxyvitamin D, PTH and calcitonin (McDowell, 2003). Source of P, P intake, Ca: P ratio, gut pH, physiological stage, any parasitic infestation and the inclusion levels of Ca, Fe, Al, Mn, K, Mg and fat are all factors that impact on the absorption of P (Horst, 1986; Harris Jr *et al*., 2003). An inverse relationship exists between P intake and absorption. A high Ca: P ratio may also decrease P absorption (Schneider *et al*., 1985) thereby decreasing P availability (Field *et al*., 1983). The availability of P depends on the chemical form of the P and other animal factors such as age, physiological stage of the animal and nutritional status (McDowell and Arthington, 2005).

Excessive intake of Ca may interfere with the absorption and metabolism of other minerals. While Ca may not have direct effect on Zn absorption, it can decrease Zn absorption in the presence of phytate (Oberleas *et al*., 1996). Other than Zn and Selenium, little information is available on effects of dietary Ca levels on other trace elements absorption in dairy cows (Harrison and Conrad; 1984; Alfaro *et al*., 1987). At higher dietary Ca levels, a negative interaction with Zn absorption is possible. Alteration in dietary Ca and P levels as well as their ratios has been found to have interactions with absorption of other macro and micro-minerals. Dietary P may have different effects on Ca, P, Mg and Zn metabolism depending on dietary Ca levels (Kronqvist, 2011). In certain conditions, high dietary Ca and P may interfere with Zn metabolism (Pond and Wallace, 1986; NRC, 2001). In general, a ratio of 1:1 to 2:1 is recommended for Ca and P in ruminant diet (McDowell, 1992). Some studies have shown that Ca: P is not critical unless it is greater than 7:1 or less than 1:1 (Martz *et al*., 1999).

The aim of this study was to investigate the effects of dietary levels of Ca and P on apparent digestibility and retention of Ca, P, Mg and Zn in lactating Sahiwal cattle. We hypothesized that an increase in dietary Ca and P levels will impact the apparent digestibility and retention of the minerals.

MATERIALS AND METHODS

This experiment was conducted at Livestock Experiment Station Khizerabad, district Sargodha. Laboratory analyses were undertaken at the Department of Animal Nutrition, UVAS, Lahore. Thirty-six, second parity Sahiwal cows at peak lactation stage averaging 70±10 days in milk (DIM) were offered six different dietary treatments (NCP, HP, LC, LCHP, HC and HCHP) with varying levels of Ca and P following a completely randomized design. A basal diet was formulated to contain 0.28% P on a DM basis. Except for variation in Ca and P levels, all diets were formulated according to the recommendations prescribed by NRC (1989) and offered to the animals in the form of a total mixed ration (TMR). The dietary Ca and P levels of the experimental diets are presented in Table 1.1., while details of the TMR formula and nutrient profile are presented in Table 1.2.

Table 1.1. Calcium and phosphorus contents of the experimental rations.

Treatment	Ca	P	Ca : P ratio
NCP	NRC recommended level	NRC recommended level	1.2 : 1.0
HP	NRC recommended level	40% high than NRC recommendation	1.2 : 1.1
LC	40% lower than NRC recommendations	NRC recommended level	1.0 : 1.0
LCHP	40% lower than NRC recommendations	40% higher than NRC recommendations	1.0 : 1.1
HC	40% higher than NRC recommendations	NRC recommended level	1.4 : 1.0
HCHP	40% higher than NRC recommendations	40% higher than NRC recommendations	1.4 : 1.1

Table 1.2. Composition of the basal diet and its associated nutrient profile (DM basis).

Ingredients	Inclusion level (%)
Wheat straw	32.80
Maize grains	20.00
Soybean meal	16.36
Maize oil cake	16.00
Rice tips	6.00
Molasses	7.00
Vegetable oil	0.84
Vitamin-mineral premix*	0.50
Sodium bicarbonate	0.50
Chemical composition	
Dry matter (%)	88.77
Crude protein (%)	12.8
Crude fiber %	17.11
Metabolizable energy (Mcal/kg)	2.41
Neutral detergent fiber (%)	20.24
Acid detergent fiber (%)	32.72
Calcium (%)	0.26 (40% lower than NRC)
Phosphorus (%)	0.28
Magnesium (%)	0.22
Sodium (%)	0.19
Potassium (%)	0.90
Zinc (mg/kg)	38.00
Copper (mg/kg)	10.80
Selenium (mg/kg)	0.38

*Vitamin-mineral premix contained 17.70% Mg; 3.60% K; 24.64% Na; 4.22% Cl; 16.42 mg/kg of Co; 1,642 mg/kg of Cu; 4,960 mg/kg of Fe; 122.64 mg/kg of I; 6,040 mg/kg of Mn; 45.37 mg/kg of Se; 5,560 mg/kg of Zn; 670,960 IU/kg of vitamin A; 213,680 IU/kg of vitamin D; 4,480 IU/kg of vitamin E.

Feeding regimen and management*:* Animals were placed individually in concreted floor pens to collect feces and urine separately. Experimental animals were fed twice daily (09:00 and 16.00 h) at the rate of 90% of voluntary intake of the adjustment period (Yunus *et al*., 2004). During the experiment all the animals were managed under uniform conditions. *Ad libitum* fresh and clean water was provided daily to the animals. Daily feed intake was recorded for each animal by subtracting orts from feed offered before the morning feeding. The cows were milked twice daily and milk production was recorded for each milking.

Before the start of the trial, the animals were treated with commercial anti-parasites medicine (Dectomax; Pfizer Animal Health) for controlling of internal and external parasites. All of the cows had been previously vaccinated against foot and mouth disease and hemorrhagic septicemia (manufactured by Veterinary Research Institute Lahore).

Sample collection and processing: Total orts were collected daily while samples of the TMR (1 kg) were collected on a weekly basis and then stored at -20 °C for subsequent laboratory analysis. Following an acclimatization period of 21 days, feces and urine were collected for 5 days. Each day and at 4 hours intervals, feces were collected for individual animals, weighed, thoroughly mixed and a sub-sample (10% by weight) was taken and stored in plastic bags at -20 °C for subsequent analysis of minerals content.

On day 20 of the adaptation period a sterile Foley urinary catheter was inserted into the urethra of each animal to enable collection of urine. The urinary catheter emptied into a collection vessel and at 4 hour interval, the amount of urine void was measured and acidified to pH lower than 2 (22 mL of 6N HCl/L of urine), sub-sampled (10% by volume) and stored at -20C° for further analysis. Milk samples (120 ml each) were collected at 10 consecutive milkings and stored at -20 °C.

Laboratory analysis*:* The fecal samples from individual animals were thawed at room temperature and combined to make one composite sample per animal. The composite fecal samples and feed samples (orts and TMR) were dried to constant weight at 60ºC in a hot air oven. For DM analysis a separate sample was dried at 100ºC to constant weight. After drying, the samples were ground to pass a 1 mm screen (Willey mill; Arthur H. Thomas Co., Philadelphia, PA).

Proximate composition of feed and orts samples was analyzed following standard procedures according to the Association of Official Analytical Chemists (AOAC, 2000). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined according to Van Soest *et al*. (1991). The gross energy of the TMR was determined using the bomb calorimetric method.

The feed, orts, feces, urine and milk samples were analyzed for P (AOAC, 2000), Ca, Mg (Atomic Absorption Spectrophotometer) and Zn (Inductively Coupled Plasma Optical Emission Spectrometry) concentrations (AOAC, 2000).

Calculations: Apparent digestibility was calculated as (mineral intake - mineral in faeces) / mineral intake) x 100. Apparent retention was calculated as (mineral intake - mineral in faeces – mineral in urine - mineral in milk) / mineral intake) x 100.

Statistical analysis: The data thus collected on minerals intake, apparent digestibility and apparent retention were statistically analyzed using ANOVA (Steel *et al*., 1997). Means were compared for significance of difference using Duncan Multiple Range Test (Duncan, 1955). Differences were declared significant at $P<0.05$.

RESULTS

Dry matter digestibility: As shown in Table 1.3, there was no effect ($P > 0.05$) of dietary treatment on apparent digestibility of DM.

Milk Yield: Dietary Ca and P levels and their ratios did not affect ($P > 0.05$) daily milk yield (Table 1.3).

Intake, apparent digestibility and apparent retention of minerals

Calcium: Dietary Ca levels had a significant ($P < 0.05$) effect on its apparent digestibility and retention (Table 1.3). Groups HC and HCHP had lower ($P < 0.05$) apparent Ca digestibility than all other groups.
Groups LC and LCHP were in negative Ca balance as indicated by the negative values for apparent Ca retention (Table 1.3). All of the other groups were in positive Ca balance. There was no significant difference ($P > 0.05$) in apparent Ca retention between those groups that were in positive Ca balance.

Phosphorus: Increased dietary P levels decreased ($P < 0.05$) apparent P digestibility irrespective of dietary Ca levels.

Magnesium: Increasing dietary Ca levels (Groups HC and HCP) decreased ($P < 0.05$) Mg apparent digestibility and apparent retention, with animals fed these diets being in negative Mg balance (Table 1.3). Animals fed the LC diet had the highest ($P < 0.05$) apparent Mg digestibility. There was no significant difference ($P > 0.05$) in apparent Mg retention between the groups in positive Mg balance.

Zinc: Increasing dietary Ca levels (Groups HC and HCP) decreased ($P < 0.05$) both Zn apparent digestibility and apparent retention, with animals fed these diets being in negative Zn balance (Table 1.3). There was no significant difference ($P>0.05$) in apparent Zn retention between those groups that were in positive Zn balance.

Table 1.3. Average (±SD) dry matter digestibility, and intake, apparent digestibility and apparent retention of calcium, phosphorus, magnesium and zinc in lactating Sahiwal cow fed different dietary levels of calcium and phosphorus.

Parameter	Dietary treatment groups						Interactions
	NCP	HP	LC	LCHP	HC	HCHP	
Dry matter digestibility (%)	65.3 ± 0.72	64.8±0.58	65.51±0.87	66.18±0.90	64.92 ± 0.65	65.33 ±0.99	
Milk yield (liters/day)	6.22 ± 0.16	6.00 ± 0.31	6.1 ± 0.34	5.87 ± 0.58	6.3 ± 0.36	6.27 ± 0.15	
Calcium							
Intake (g/d)	42.3 ± 0.87	43.0 ± 0.75	25.8 ± 0.33	25.4 ±0.48	61.3 ± 1.25	61.8 ±1.58	
Apparent digestibility (% of intake)	$25.1^{a} \pm 0.91$	$23.3^{a} \pm 1.09$	$23.4^{a} \pm 1.20$	$22.1^{a} \pm 0.83$	$17.9^{b} \pm 0.76$	$17.2^{b} \pm 0.85$	Ca, P; ns
Apparent retention (% of intake)	$5.0^{c} \pm 0.86$	$4.2^{bc} \pm 1.52$	$-1.9^{a} \pm 0.51$	$-2.2^{a} \pm 1.11$	$2.2^{bc} \pm 0.57$	$1.7^{b} \pm 0.51$	Ca, P; ns
Phosphorus							
Intake (g/d)	27.6 ± 0.56	39.0 ± 0.68	27.7 ± 0.36	38.2 ± 0.72	28.6 ±0.58	40.2 ± 1.02	Ca, P; ns
Apparent digestibility (% of intake)	$35.3^{b} \pm 0.41$	$30.1^{a} \pm 0.50$	$34.5^{b} \pm 0.47$	$29.9^{a} \pm 0.91$	$35.7^{b} \pm 0.53$	$28.3^{a} \pm 0.82$	Ca, P; ns
Apparent retention (% of intake)	12.1 ± 0.55	10.7 ± 0.92	12.7 ± 0.97	10.5 ± 1.12	13.3 ± 1.53	9.8 ± 1.03	Ca, P, ns
Magnesium							
Intake (g/d)	21.7 ± 0.44	22.0 ± 0.39	21.8 ± 0.28	21.5 ± 0.41	22.5 ± 0.46	22.7 ± 0.58	Ca, P; ns
Apparent digestibility (% of intake)	$35.1^{bc} \pm 0.73$	$34.3^{b} \pm 0.75$	$36.5^{bc} \pm 0.95$	$37.80^{c} \pm 1.08$	$28.8^{a} \pm 0.97$	$28.1^{a} \pm 0.74$	Ca, P; ns
Apparent retention (% of intake)	$8.5^{bc} \pm 1.20$	$7.5^{b} \pm 0.58$	$11.5^{cd} \pm 0.70$	$13.4^{d} \pm 1.26$	$-3.1^{a} \pm 1.29$	$-4.8^{a} \pm 1.33$	Ca, P; ns
Zinc							
Intake (mg/d)	374.1 ± 7.64	379.9 ± 6.65	376.4 ± 4.85	371.8 ± 7.02	388.2 ± 7.91	391.2 ± 9.99	
Apparent digestibility (% of intake)	$18.4^{b} \pm 0.86$	$18.8^{b} \pm 1.22$	$19.3^{b} \pm 0.86$	$20.2^{b} \pm 0.85$	$10.3^{a} \pm 1.28$	$10.3^{a} \pm 1.05$	Ca, P; ns
Apparent retention (% of intake)	$3.8^{b} \pm 0.75$	$4.2^{b} \pm 1.24$	$5.0^{b} \pm 0.63$	$5.2^{b} \pm 1.26$	$-4.9^{a} \pm 1.53$	$-5.4^{a} \pm 1.07$	Ca, P; ns

Means in the same row followed by different superscript letters differed ($P < 0.05$). Ca, P = interaction between Ca and P. ns = non-significant at $P < 0.05$.

DISCUSSION

Dry matter digestibility: Dry matter digestibility did not differ between the experimental rations. The levels of dietary Ca and P as well as the ratio between these two minerals did not impact on DM digestibility (Table 1.3). Similar findings were reported by Moreiva *et al* (2009) by conducting trial on transition cows. These cows were offered high (0.64%) and low (0.46%) Ca levels in their rations along with high (0.47%) and low (0.38%) P on DM basis. At all levels of Ca and P, DM intake remained unaffected. Similar results were obtained by Patino *et al* (2013) in their study involving lambs. Increasing P intake did not affect DM digestibility or OM digestibility, only mineral matter digestibility was negatively affected. Similarly, Begum *et al*. (2010) found no effect on DM digestibility with feeding Ca and P at 80, 100 and 120% of the NRC recommendations to the Nili Ravi buffaloes.

Milk production: Neither Ca or P level nor the ratio impacted on milk yield. Puggaard *et al*. (2014) found that decreasing P levels below 0.23% reduced milk production in early lactation; whereas, decreasing dietary P from 0.34% to 0.28% did not impact either DMI or milk production during the whole lactation period. However, during their trial at week 12, treatment group fed 0.23% dietary P was shifted to the high P diet (0.34%) because of high number of health issues. It was suggested that P mobilized from the bones was sufficient to meet the P requirements during mid and late lactation but not early lactation. Similar to the present study, Belyea *et al* (1976) found that feeding lower dietary Ca (0.25%) for 8 weeks did not affect the milk production of Holstein cows. Wang *et al* (2014) offered 0.57% (high), 0.47% (medium) and 0.37% (NRC recommended) levels of P to multiparous Holstein cattle. Even for high yielding cows lowering P (0.57 to 0.37%) did not cause any reduction in milk yield but decreased P excretion, which is environmentally beneficial. Morse *et al*. (1992) observed no effect of different dietary P levels (0.33, 0.43 and 0.54%) and Ca: P ratios between 1.1:1 and 2.9:1 on 3.5% fat corrected milk yield in Holstein cows.

Partially in agreement with the present study, Begum *et al*, (2010) reported increased milk production at 120% NRC recommended Ca and P levels (as compared to 80% and 100%) in Nili-Ravi buffaloes. No effect was observed between the groups fed 80% and 100% of NRC recommended levels. Individual effects of dietary Ca and P were not studied in their experiment. In contrast, Kincaid *et al*. (1981) observed increased milk production at higher (0.55% vs 0.30%) dietary P levels. Similarly, Wu *et al*. (2001) offered diets containing three P levels (0.31, 0.39 and 0.47%) on DM basis to multiparous Holstein cows and found that 0.31% appeared to be borderline deficient level for high yielding cows. This level was also less than NRC recommendations. Valk and Sebek (1999) reported a decrease in milk yield at lower dietary P levels (0.24% vs 0.37% and 0.57%) This difference in response could be attributed to the difference in daily milk yield as in the current study, animals used were low yielder (producing 5-6 L/d). Supplying dietary Ca and P at lower levels for a short-term period likely initiated the mobilization of these minerals from bones to meet the physiological needs.

Calcium and Phosphorus (Levels and ratios): Decline in Ca digestibility and retention were directly related to increasing levels of Ca, irrespective of dietary P levels (Table 1.3). Our data support the original hypothesis of the study. The experimental diets had similar nutrient composition except with different Ca and P concentrations (Table 1.2). The Ca: P ratio varied (1 : 1 to 1.4 : 1.1) but remained within the critical limits >7:1 or < 1:1 (ARC, 1980; Miller 1983). This is in agreement with Taylor *et al*. (2009) who reported a linear effect of dietary Ca levels (high 1.03%; medium 0.78%; and low: 0.52%) on fecal Ca excretion in lactating Holstein cows. More Ca was excreted by cows consuming more dietary Ca (195.40, 151.6 and 79.1 g/cow/d in high, medium and low dietary Ca groups, respectively). These findings also supported results of the present study that requirements of P in lactating cows are independent of dietary Ca concentrations. Verdaris and Evans (1976) evaluated effect of dietary Ca on minerals balance in Holstein cows and found that Ca digestibility and retention (percent of intake) were higher with low dietary Ca. Similarly, Graces and Evans (1971) reported depression in Ca absorption with high dietary levels in growing cattle.

With regards to Ca retention and digestibility it is not only the Ca concentration in diet that matters but also the Ca:P ratio. Moreira *et al* (2009) fed 0.64% (HCa) and 0.46 % (LCa) Ca in their rations along with 0.47% (HP) and 0.38% (LP) P on DM basis. Results clearly indicated that Ca and P interaction impacted on Ca digestibility as well as apparent absorption. Higher dietary levels of P also negatively affect the absorption of Ca (Tanaka and DeLuca, 1973). Similar results were found by Neil *et al*. (2015) who conducted trial on beef cattle. They reported if forage P content supply was ample for grazing cattle, increasing P supplementation could result in more P excretion without additional benefits to growth or nutrient digestibility. Greater excretion of P can have negative environmental impacts. This not only causes accelerated growth of algae or other aquatic plants but nutrient management issue forcing producers to overcome increased P excretion rate. Increasing the level of dietary P, decreased P digestibility in the present study, irrespective of the dietary Ca levels and Ca: P ratios. Reduction in P absorption at high P intake is usual (Guyton *et al*., 2003).

The small intestine is the major site for Ca absorption (Schröder and Breves, 2006); however, higher

Ca concentrations in the rumen result in considerable absorption of Ca prior to the abomasum (Khorasani *et al*., 1997; Schröder and Breves, 2006). Small intestine, especially the duodenum and jejunum are considered as major sites for P absorption (Care, 1994; Khorasani *et al*., 1997); however, small amounts of P are also absorbed from the rumen, omasum and abomasum.

Along with vitamin D (1, 25-$(OH)_2$ vitamin D), parathyroid hormones (PTH) and calcitonin perform major roles in Ca homeostasis (Tsujikawa *et al*., 2003; Goff, 2006). Under circumstances of lower intake (than requirements), Ca deposited in bones and soft tissues returns to the extracellular pool to maintain normal Ca levels (Littledike and Goff, 1987). In the current study, the short term feeding of lower Ca levels (0.26%) might have activated the same homeostatic mechanism to fulfill the physiological needs of the cows. Phosphorus absorption is affected by multiple factors including total P intake, source of P, the Ca: P ratio, and dietary Ca, Mg and fat levels (Ekelund, *et al*., 2003). Phosphorus absorption occurs via active and passive pathways. Vitamin D operates the active absorption when feed is low in P, whereas, passive absorption is takes control when higher amounts of P are fed (NRC, 2001).

Unlike non-ruminants, P homeostasis in ruminants is maintained through salivary recycling and endogenous fecal excretion (Karn, 2001; NRC, 2001). There is an inverse relation between amount of P fed and its absorption (Braithwaite, 1983; Klop *et al*., 2013). Schneider *et al*. (1985) found that when animals were offered P deficient diets, high levels of Ca reduced the absorption of P, due to reduction in rumen P solubility and also due to less P availability in the lower GIT. Taylor *et al*. (2009) determined the effect of dietary Ca on minerals balance in lactating cows. Similar to the findings of the present study, no effect of dietary Ca levels (High: 1.03%, Medium 0.78% and Low: 0.52%) on P balance as found in lactating Holstein cows at 140 DIM. It was concluded that P requirements of lactating cows could be considered independent of dietary Ca levels. It was further described that this difference might be due to the fact that within experiments, DMI and milk yields were similar among treatments, whereas, in their data set, P intake was different in different experiments due to variation in DMI. Ruminants can tolerate a wide range of ratios of both the minerals but depends upon adequacy in supply of Ca and P along vitamin D. When low P is fed to the ruminants, its homeostasis is maintained by reducing salivary P flow and increased absorption from small intestine (Coates and Ternouth, 1992).

Wang *et al*. (2014) studied effect of different levels of dietary P on its excretion in multiparous Holstein cows and found that decreasing dietary P from 0.57% to 0.37%, significantly ($P < 0.05$) reduced its urinary and faecal excretion. A decrease in apparent digestibility of P with increase in dietary P has been reported in various studies (Morse *et al*., 1992; Wu *et al*., 2000 and Knowlton and Herbein, 2002) in dairy cows. In contrast, Feng *et al*. (2015) reported lowest P digestibility in steers fed 0.15% dietary P. Similarly, Geisert *et al*. (2010) reported very low P digestibility in steers fed with 0.12% dietary P. Ternouth (1990) explained that slight reduction of P digestibility at very low dietary P levels can be attributed to the compromised microbial P requirements. In the current study the lowest dietary P level was 0.28% which is much higher than the levels used in these studies. At this dietary level, microbial P requirements might be fulfilled.

Field *et al*. (1983) studied the effect of dietary Ca and P on P metabolism in sheep and reported reduction in P absorption with higher dietary Ca concentrations. Similarly, Verdaris and Evans (1976) reported that absorption and retention of P (percent of intake) was higher with low dietary Ca and lower with high dietary Ca (0.2 vs 2.1% dietary Ca). These findings are also contradictory to the findings of the present study. This difference may be attributed to the reason that variation in dietary Ca (0.26%, 0.43% and 0.60% of DM) and P (0.28 % and 0.39% of DM) levels in the present study were higher than those used in these studies. Other factors like difference in nature of sample, physiological stage, breed, feeding pattern and experimental conditions can also impact on absorption and retention of these minerals.

Magnesium: Increasing dietary Ca levels (HC and HCHP) decreased ($P < 0.05$) apparent digestibility of Mg in the lactating cows. These findings support the hypothesis. It was postulated that increasing dietary Ca would impact the Mg absorption; however, data on absorption of inorganic component of diet and their availability are inconsistent (Khorasani and Armstrong, 1992). Magnesium metabolism can be influenced by alterations in Ca and P levels in the diet (Littledike and Goff, 1987). Magnesium absorption is not dependent upon the requirements of the cow. Under normal circumstances, normal levels are maintained in the body by regulating its excretion through urine. There are no labile reserves of Mg within the body; dietary Mg appears to be only significant once Mg enters the extracellular pool (Ram *et al*., 1998). While considering the interrelationship of Ca, P and Mg, it is fundamental to note that a very high concentration of these minerals is ossified in bones and at the time of need, these can be mobilized (Khorasani *et al*., 1997). The major site for Mg absorption in adult animals is the rumen (Martens and Schweigel, 2000) where it is taken up by both potential-dependent and potential-independent mechanisms (Martens, 2018). Calcium and Mg competes with one another for the same binding sites. Kronqvist (2011) found that for non-lactating cows during the dry period, increased dietary levels of Ca (13.6 g/kg) decreased digestibility of Mg compared to lower dietary Ca levels (4.9 and 9.3g/kg). Care (1994) also reported that higher dietary Ca levels decreased Mg absorption in cows. Lower bioavailability of Mg in response to higher dietary Ca levels was also reported by Alfaro *et al*. (1987). Verdaris and Evans (1976) reported Mg absorption and retention (percent of intake) was higher in Holstein cows fed on diets low in Ca (0.2% vs 2.1%). In contrast, Weiss and Wyatt (2004) reported no effect of dietary Ca and P levels on Mg digestibility. Results of Chicco *et al*. (1973) are also in line with the present study. They investigated the nutritional interrelationships of dietary Ca, P and Mg in sheep and found very little effect of P but significant effect of high dietary Ca (0.78%) in reducing Mg in bone and blood plasma. Calcium and Mg have been considered as absorption inhibiters of each other (EFSA, 2006). Increased dietary levels of Ca and P also decrease Mg absorption by the formation of insoluble Ca-Mg-P complexes in the intestines (Brink *et al*., 1992). There is no hormonal system that directly regulates Mg absorption (Charlton and Armstrong, 1989; Urdaz *et al*., 2003). Circulating levels of PTH, 1, 25 $(OH)_2$ D and calcitonin are determined by plasma Ca concentrations. Magnesium concentration is affected by these hormones secondarily (Reinhard *et al*., 1988). Along with the rumen pH, dietary Mg level, solubility level of Mg in the rumen, dietary K (Weiss and Wyatt, 2004) and Ca (Schonewille *et al*., 1997) affect Mg absorption.

Zinc: Different chelating materials like phytate can affect Zn absorption particularly in monogastric animals but in most instances, the factors that disturb Zn requirements of large animals are not clear (Blackmon *et al*, 1967). Mostly, Zn deficiency for ruminants can be noticed under field conditions if forage Zn contents range from 19-83 ppm (Miller, 1970). In the present study, treatments with higher dietary Ca significantly ($P < 0.05$) decreased Zn apparent digestibility. Feeding excessive quantities of Ca may decrease absorption of Zn in dairy cows (NRC, 2001). In studies with sheep, Suttle and Field (1970) found increased ($P < 0.5$) Zn excretion when dietary Ca was increased from 1.0 to 2.0%. Perry *et al*. (1968) reported similar observations that by doubling the dietary Ca of beef cattle, hair Zn content was significantly ($P < 0.5$) decreased. Pond and Wallace (1986) also observed that plasma Zn of ewes fed with high Ca diet was decreased significantly ($P < 0.5$). Higher levels of dietary Ca in the presence of dietary phytic acid source may decrease Zn absorption in monogastric animals (O'Dell *et al*., 1958). Kincaid (1979) found that unless fed with high dietary Ca (0.43 and 0.6%), apparent digestibility of Zn remained between 18-20%. Similar observations (12-22%) have been reported in adult cattle in previous studies (Miller and Cragle, 1965; Hansard *et al*., 1968). Available data suggests that there may be unknown organic factors present in common feedstuffs which can interfere with the absorption of zinc in ruminants and other species (NRC 2001).

In the present study dietary levels of P did not affect apparent Zn digestibility. These findings are contrary to our hypothesis. Similar results were found by Laflamme *et al*. (1985) who studied the effect of low and normal dietary P concentrations on Zn metabolism in calves. Neathery *et al*. (1990) reported increased apparent digestibility of Zn in Holstein calves fed with lower P levels. The interaction mechanism between dietary Zn and P to relative Zn absorption is not clearly understood. Miller (1981) described that in cows Zn absorption may increase as a consequence of active homeostatic means with low P diets. It was further reported little effect of dietary Ca on Zn digestibility. Plasma Zn concentration is the most widely used indicator for Zn status in animals. When plasma Zn concentration drastically reduces (within 24 to 36 h), it causes a decline in feed intake (Spais and Papasteriadis, 1974); however, in present study, feed intake remained unaffected and Zn levels remained at the required level and no Zn deficiency was noticed. In animals, Zn deficiency may be manifested in alteration in taste perception, accompanied by damage to the tongue epithelium (Anke *et al*., 1994) but no such effects were noticed in present study.

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