

## Effects of dietary level of selenium and grain on digestive metabolism in lambs

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**ABSTRACT:** The objective was to evaluate the effect of different levels of selenium with two levels of grain corn in the diets on ruminal, postruminal, and total tract digestion of nutrients, ruminal fermentation characteristics, and selenium balance in lambs. A split-plot design was used in four periods with repeated Latin square using eight Suffolk × Dorset male lambs with four levels of selenium (sodium selenite, Na<sub>2</sub>SeO<sub>3</sub>), without adding selenium, 0.3, 0.6, and 0.9 mg of selenium/kg dry matter (DM) with 70 and 50% of corn grain in the diet. The four selenium levels affected organic matter as follows: starch, neutral detergent fibre, nitrogen and selenium excretion; selenium ruminal digestion; organic matter, neutral detergent fibre, nitrogen and selenium postruminal digestion; and organic matter, neutral detergent fibre, nitrogen and selenium total tract digestion ( $P < 0.05$ ). Starch and organic matter flow decreased with 0.6 and 0.9 mg of selenium with the 70% grain diet ( $P < 0.05$ ). Selenium flow increased linearly with selenium levels ( $P < 0.01$ ) with both levels of grain. Selenium supplementation increased linearly selenium concentration in ruminal fluid ( $P < 0.01$ ), but did not affect other ruminal characteristics. Also, selenium absorption and retention was increased by selenium concentrations ( $P < 0.01$ ). The apparent absorption of selenium increased (31%,  $P < 0.01$ ) with the 70% grain diet. In conclusion, the high content of nonstructural carbohydrates improved the availability of selenium and 0.9 mg of selenium/kg DM improved the absorption and availability of selenium.

**Keywords:** nutrient digestion; ruminal fermentation characteristics; balance selenium

Dietary requirement of selenium (Se) for sheep was 0.1–0.2 mg Se/kg DM and it was established based on glutathione peroxidase activity and protection against Se-dependent diseases (National Research Council, 1985). The requirement for growing lambs has been revised to 0.2–0.3 mg Se/kg DM with average daily gains ranging 200–350 g (National Research Council, 2007). This requirement is higher than the earlier one and was calculated using the factorial method considering an absorption coefficient of Se that varies with dietary concentrate

level (0.31 and 0.60 with forage- and grain-based diets, respectively), since the availability of Se is influenced by the grain level (Koenig et al., 1997).

Se requirements suggested for lambs take into account changes in ruminal fermentation and nutrient digestibility. Ruminal microorganisms transform dietary Se, such as sodium selenite, into Se forms of lower bioavailability (National Research Council, 1983) and incorporate Se into their bacterial mass (Whanger et al., 1970; Serra et al., 1994a) as selenoamino acids (Van Ryssen et al., 1989). These mi-

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crobal effects are the cause of the lower absorption of Se in ruminants compared with non-ruminant animals (Serra et al., 1994a). Se availability can affect the *in vitro* yield of volatile fatty acids (VFA) (Kim et al., 1997) and the *in vivo* yield of bacterial mass (Serra et al., 1994b), but studies with regard to the effect of Se on nutrient digestibility are few (Serra et al., 1994b; Hernández-Calva et al., 2007). Some effects on microbial activity and digestibility could be observed with a relatively high concentration of Se in the diet. This work was based on the hypothesis that requirement of Se in lambs is higher than that suggested by National Research Council (2007) and that the Se absorption is influenced by the ingredients, specifically the grain level, which can cause differences in the digestion process of dietary nutrients. Therefore, the aim of the present work was to evaluate the effect of supplemental Se, as sodium selenite, on ruminal fermentation, microbial population, and

nutrients digestibility in lambs fed diets containing two levels of grain.

## MATERIAL AND METHODS

### Animals

The trial was conducted using eight cross-bred (Suffolk × Dorset) male lambs ( $43.2 \pm 2.1$  kg, 11 months of age) fitted with cannulas in the rumen and proximal duodenum. Surgeries were performed two months prior to the start of the experiment. Lambs were housed in an indoor slotted-floor metabolism unit of Colegio de Postgraduados, Mexico. The lambs were placed into metabolic cages ( $0.62 \times 0.80$  m) 14 days before initiation of the trial, their feed and water consumption were registered. Prior to the trial, lambs were vaccinated for clostridial diseases (Ultrabac 8<sup>®</sup>; Smith

Table 1. Composition and content of nutrients in experimental diets fed to lambs<sup>a</sup>

Variable	70% grain				50% grain			
	C	0.3	0.6	mg Se/kg DM	C	0.3	0.6	0.9
<b>Ingredient composition (%)</b>								
Corn straw	20.0	20.0	20.0	20.0	38.8	38.8	38.8	38.8
Broken corn grain	69.5	69.5	69.5	69.5	50.0	50.0	50.0	50.0
Soybean meal	2.0	2.0	2.0	2.0	1.0	1.0	1.0	1.0
Corn gluten	5.3	5.3	5.3	5.3	7.0	7.0	7.0	7.0
Urea	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.0
Limestone/Ca orthophosphate <sup>b</sup>	1.8	1.8	1.8	1.8	1.7	1.7	1.7	1.7
Trace mineral salt <sup>c</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium selenite ( $\mu\text{g/kg DM}$ ) <sup>d</sup>	–	430	1090	1740	–	510	1170	1830
<b>Content of nutrients</b>								
Dry matter (%) <sup>e</sup>			91.41				92.32	
Organic matter (%) <sup>e</sup>			95.56				93.69	
Maintenance net energy (Mcal/kg) <sup>f</sup>			1.89				1.67	
Gain net energy (Mcal/kg) <sup>f</sup>			1.26				1.06	
Starch (%) <sup>e</sup>			53.98				39.54	
Crude protein (%) <sup>e</sup>			14.31				15.56	
Neutral detergent fibre (%) <sup>e</sup>			31.94				50.59	
Ca (%) <sup>f</sup>			0.80				0.71	
P (%) <sup>f</sup>			0.36				0.35	

C = control, less than 0.04 mg Se/kg contained in feed, DM = dry matter

<sup>a</sup>chromic oxide 0.40 g/kg was added as a digesta marker, <sup>b</sup>these sources were used to formulate in the diet: 0.7 Ca : 0.3 P, <sup>c</sup>trace mineral salt contained the following:  $\text{CoSO}_4$  0.68 g/kg,  $\text{CuSO}_4$  1.04 g/kg,  $\text{FeSO}_4$  3.57%, ZnO 1.24 g/kg,  $\text{MnSO}_4$  1.07 g/kg, KI 0.052 g/kg, NaCl 92.96 g/kg, <sup>d</sup>sodium selenite was mixed in stock premix with finely ground corn, <sup>e</sup>values obtained from laboratory analysis, <sup>f</sup>values obtained from tables (NRC, 2007)

KlineBeechman Animal Health, Inc., St. Joseph, USA) and treated for internal and external parasites (Ivomec Plus<sup>®</sup>; Merck, Rahway, USA). The care of animals during the surgery and experiment was in accordance with the guidelines of the Mexican Council on Animal Care (NOM-062-ZOO-1999).

## Diets and feeding

Experimental diets shown in Table 1 were formulated to keep the nutrition requirements for growing lambs (National Research Council, 2007). Two groups per four lambs were fed with 70 or 50% grain corn diets, and within each group, supplemental Se levels were: without Se (less than 0.04 mg Se/kg DM, contained in feed), 0.3, 0.6, and 0.9 mg Se/kg DM, as sodium selenite. Selenite was mixed in a stock premix with finely ground corn. Dry matter intake (DMI) was restricted to 2.34% of body weight to avoid residual material from diet, so all lambs were fed with 1010 g of basal diet, in two portions served at 7.00 and 19.00 h. Supplemental Se as an equivalent amount of corn was combined with the basal diet at the time of feeding.

## Sampling

The four experimental periods consisted of ten days of diet adjustment followed by four days of sampling. Basal diet was collected in each period to be analyzed. Duodenal and faecal samples were collected twice daily in each lamb, during the last four days in each period. Samples were taken as follows: day 1, 8.00 and 14.00; day 2, 9.30 and 15.30; day 3, 11.00 and 17.00; and day 4, 12.30 and 18.30 h. Individual samples consisted of 200 ml of duodenal fluid and 50 g (wet basis) of faecal material. Urine samples were collected twice daily at 7.00 and 18.00 in each experimental period. The collected urine was weighed and 10% sub-sample was stored in a plastic bag and frozen for later Se analysis. Ruminal fluid samples were obtained from each lamb on day 4 between 10.00 and 11.00 h (3–4 h after feeding), via ruminal cannula with a vacuum pump. Immediately following the collection, ruminal fluid pH was determined using a portable pH meter. Ruminal fluid samples were then strained through four layers of cheese cloth. Protozoa and bacteria were conserved by combining a 3 ml aliquot of ruminal fluid with 3

ml of formaldehyde (18% vol/vol), and refrigerated at 3°C for later counts. 20 ml of strained ruminal fluid were mixed with 5 ml of freshly prepared 25% (w/v) meta-phosphoric acid and then frozen (–20°C) for later analysis of VFA and ammonia nitrogen (N) concentrations. At the end of the trial, an equal amount of ruminal fluid was obtained from all lambs and composited for isolation of ruminal bacteria via differential centrifugation (Bergen et al., 1968) to be used in purine determination.

## Laboratory analysis

At the end of the trial, frozen samples of experimental diets and faeces were thawed overnight at room temperature and analyzed for DM by drying in an oven at 65°C for 48 h (AOAC, 2003). Dried diets and faeces samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, UK). Ground diets and faecal samples were pooled per lamb and analyzed for organic matter (OM) by ashing at 600°C for at least 8 h, Kjeldahl method for determination of nitrogen (AOAC, 2003), ammonia (N) (AOAC, 2003), neutral detergent fibre (NDF) (Weizhong and Udén, 1998), starch (Herrera-Saldana et al., 1990), purines (Zinn and Owens, 1986), VFA concentrations from ruminal fluid (gas chromatography) (Erwin et al., 1961), and chromium (atomic absorption spectrophotometry). Total bacterial and protozoa counts in ruminal fluid were obtained using the Petroff Hausser and the Neubauer Counting Chambers, respectively. Ten counts by sample were realized to count bacteria, while fifteen counts were realized to count protozoa.

Se was analyzed by atomic absorption spectrophotometry with hydride generation after an acid digestion by microwave, in the Development of Analytical Methods laboratory at FES-C, UNAM, Mexico. Approximately 1 g of sample, 10 ml of demineralized H<sub>2</sub>O (Millipore, Billerica, USA), 5 ml HNO<sub>3</sub>, and 2 ml H<sub>2</sub>O<sub>2</sub> (J.T. Baker, Mexico City, Mexico) were placed in teflon containers for 10 min and digested using a microwave oven (MAR 5, CEM, Falcon, USA). The containers were cooled to room temperature, their contents were placed in 50 ml volumetric matrices (each container was rinsed three times with HCl 7M), taken to the mark, and phophorlated with HCl 7M. Later, the samples were moved to labeled polyethylene containers and

the quantification of the total Se was determined from a calibration curve ranging 5–25 mg Se/l (selenium standard, High-Purity,  $1000 \pm 3 \mu\text{g/ml}$  in 2% of nitric acid, 99.99 purity, Scientific Company Selenium Powder) (Capelo et al., 2006).

Microbial organic matter (MOM) and nitrogen (MN) leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). OM fermented in the rumen was considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and MOM reaching the duodenum. Feed N entering the small intestine was considered equal to total N leaving the abomasum minus ammonia N and MN, thus including any endogenous contributions. Methane production was calculated based on the theoretical fermentation balance for observed molar distribution of VFA (Wolin, 1960).

## Experimental design and treatments

Experimental design was a split-plot design in four periods composed of two  $4 \times 4$  Latin squares (four lambs in each Latin design), where whole plots are dietary corn levels and subplots are supplemental Se levels. The statistical model for the trial was as follows:

$$Y_{ijkl} = \mu + C_i + L_{j(i)} + P_k + Se_l + CSe_{il} + \varepsilon_{ijkl}$$

where:

$Y_{ijkl}$  = response variable

$\mu$  = true mean effect

$C_i$  = corn level (whole plot)

$L_{j(i)}$  = lamb within corn level effect (whole plot error)

$P_k$  = period effect

$Se_l$  = selenium level effect

$\varepsilon_{ijkl}$  = residual error

Treatment effects were tested for linear and quadratic components by means of orthogonal polynomials (Statistical Analysis System, Version 8.0, 2000).

## RESULTS

### Nutrients digestion

Table 2 shows percentages of nutrient digestibility. With the 70% grain diet plus Se levels, the

ruminal digestion values for OM, starch, NDF, and feed N were higher ( $P < 0.05$ ), whereas 50% grain diet plus Se decreased ruminal digestion of OM and NDF ( $P < 0.05$ ). In general, there was a Se  $\times$  grain interaction in ruminal digestion of OM, starch, and NDF. Se levels with 70 and 50% of grain improved the availability of ruminal Se. Microbial efficiency was lower with the 70% grain diet plus supplemental Se, but it was improved with the 50% grain diet plus Se; specifically, microbial and N efficiency improved with the 50% grain diet plus 0.9 mg Se/kg DM. Postruminal digestion of OM, starch, NDF, and N was greater with the 70% grain vs. the 50% grain diet (72.5, 84.2, 71.6, 81.9% vs. 59, 72.7, 59.2, 75.5%;  $P < 0.05$ ). Specifically, the diet with 70% of grain plus 0.9 mg Se/kg DM did not affect the postruminal nutrient digestion. Only, 50% grain plus 0.9 mg Se/kg DM improved significantly postruminal digestion of OM, NDF, and N.

The digestion of nutrients in the total gastrointestinal tract was higher with 70 than 50% grain in the diet (OM: 88.2 vs. 78.6; starch: 97.2 vs. 93.7; NDF: 88.7 vs. 84.2; N: 84.8 vs. 79.2; Se: 86.1 vs. 73.6,  $P < 0.05$ ). Besides, only the higher Se level (0.9 mg Se/kg DM) with 50% grain enhanced total nutrient digestion.

### Ruminal pH and fermentation

Ruminal fermentation characteristics of lambs are shown in Table 3. Se levels increased linearly ( $P < 0.05$ ) the concentration of Se in ruminal liquid, but there was no effect on ruminal pH or VFA concentrations. Concentrations of protozoa, bacteria, and ammonia N were not affected by the concentration of Se and dietary grain affected ruminal pH and total VFA, since the higher concentration of acetic acid occurred with 50% grain and 70% grain increased propionic acid and decreased methane production.

### Selenium balance

Absorption of Se from the gut and excreted in urine was proportional to the concentration of supplemental Se ( $P < 0.01$ ) (Table 4). With the 70% of grain diet, Se absorption increased ( $P < 0.01$ ) by 31% in response to Se level in the diet and the amount of retained Se increased quadratically

Table 2. Influence of supplemental selenium (%) on digestibility in lambs fed with 70 or 50% grain diets

Variable	70% grain					50% grain				
	mg Se/kg DM									
	C	0.3	0.6	0.9	SEM	C	0.3	0.6	0.9	SEM
<b>Ruminal digestion (%)</b>										
OM <sup>4</sup>	47.5	57.7	58.9	58.6	5.2	51.2	43.8	48.4	40.3	4.5
Starch <sup>4,5</sup>	75.6	81.0	83.7	84.9	3.1	78.5	74.3	77.0	74.5	2.7
NDF <sup>4,7</sup>	47.9	62.6	62.9	61.0	4.6	64.7	59.0	60.9	55.1	4.0
Feed N	64.3	70.7	70.9	70.9	3.6	67.4	60.0	70.7	62.2	3.1
Se <sup>1,7,8</sup>	12.6	52.6	65.0	61.0	5.8	14.6	42.5	54.3	54.0	5.1
Microbial efficiency <sup>9</sup>	34.6	23.7	21.2	25.0	7.9	28.0	35.3	32.4	46.6	6.9
N efficiency <sup>10</sup>	1.01	0.83	0.81	0.85	0.08	0.81	0.92	0.80	0.96	0.07
<b>Post ruminal digestion (% of that leaving abomasum)</b>										
OM <sup>2,3,4</sup>	78.8	69.1	68.8	73.5	4.3	51.8	58.6	54.7	70.6	3.8
Starch <sup>3,6</sup>	89.3	84.1	80.7	82.7	3.0	71.3	71.6	69.0	78.9	2.6
NDF <sup>2,3,4</sup>	78.7	68.3	67.6	71.9	4.2	53.1	59.3	54.0	70.6	3.7
N <sup>2,3,4</sup>	86.6	79.0	79.0	83.0	2.7	72.0	75.8	72.1	82.3	2.5
Se <sup>1,3,7,8</sup>	85.0	68.4	55.3	68.6	5.1	64.3	43.8	38.8	60.2	4.5
<b>Total tract digestion (% of intake)</b>										
OM <sup>2</sup>	89.2	86.7	87.2	89.8	2.8	78.2	76.3	77.1	83.1	2.4
Starch <sup>8</sup>	97.5	96.9	96.9	97.6	1.1	94.1	92.6	93.2	94.8	0.9
NDF <sup>2,8</sup>	89.5	87.9	88.0	89.6	2.2	84.4	82.8	82.2	87.4	2.0
N <sup>1</sup>	86.6	82.4	83.3	86.9	2.3	79.3	77.8	78.6	83.9	2.0
Se <sup>1,4,8</sup>	85.6	85.6	84.2	89.0	3.7	71.5	67.7	72.4	82.8	3.2

C = control, DM = dry matter, OM = organic matter, NDF = neutral detergent fibre

<sup>1</sup>Se main effect,  $P < 0.01$ , <sup>2</sup>Se main effect,  $P < 0.05$ , <sup>3</sup>grain main effect,  $P < 0.01$ , <sup>4</sup>Se  $\times$  grain effect,  $P < 0.05$ , <sup>5</sup>Se linear effect 70% grain,  $P < 0.01$ , <sup>6</sup>Se linear effect 50% grain,  $P < 0.05$ , <sup>7</sup>Se quadratic effect 70% grain,  $P < 0.01$ , <sup>8</sup>Se quadratic effect 50% grain,  $P < 0.01$ , <sup>9</sup>g of microbial nitrogen/kg of OM fermented, <sup>10</sup>nonammonia N leaving the abomasum/N intake

( $P < 0.05$ ) in response to Se levels in both grain diets ( $P < 0.05$ ). The average urinary Se was 64% of intake for all treatments.

## DISCUSSION

### Nutrients digestion

Total nutrients digestion was not affected with 70% grain and levels of Se, this result was not associated with microbial efficiency, but rather may be due to better availability of Se it was associated with the amount of starch and fibre content in both experimental diets. These results suggest that the increase in nonstructural carbohydrates improves the utilization of Se, while diets with less starch plus higher Se supplemented (0.9 mg Se/kg DM) improved

the digestion of OM, NDF, and N; specifically, Se availability was improved with the higher level of Se. These results are also in agreement with our previous findings that the digestibility of nutrients and the utilization of supplemental Se were higher than those of control animals (Hernández-Calva et al., 2007). Besides, Wang et al. (2009) and Shi et al. (2011) reported an improvement in total digestibility of nutrients and NDF due to Se addition from 0.15 to 0.45 mg Se/kg DM. However, Serra et al. (1994b) showed that addition of 0.2 mg Se had no effect on NDF digestibility in sheep.

### Ruminal fermentation

Se at doses around 0.3 mg Se/kg DM in diet increased (more than by 14%) the molar concentra-

Table 3. Influence of supplemental selenium on ruminal fermentation characteristics in lambs fed with 70 or 50% grain diets

Variable	70% grain					50% grain				
	mg Se/kg DM									
	C	0.3	0.6	0.9	SEM	C	0.3	0.6	0.9	SEM
Ruminal Se (ng/ml) <sup>1,4,5</sup>	5.9	9.3	10.6	13.8	1.84	8.6	8.2	14.6	15.5	1.59
Ruminal pH <sup>2</sup>	5.6	5.4	5.5	5.6	0.10	6.2	6.0	6.2	6.2	0.09
Ammonia N (mg/dl)	16.6	15.7	25.2	20.1	3.81	22.8	22.5	21.5	23.7	3.32
Methane production <sup>2,6</sup>	0.51	0.52	0.53	0.52	0.02	0.62	0.60	0.60	0.62	0.02
<b>Ruminal microorganisms</b>										
Protozoa (cells × 10 <sup>5</sup> ) <sup>3</sup>	8.8	8.0	7.7	7.4	1.90	5.0	7.0	5.5	3.8	1.69
Total ciliated protozoa (%)	1.5	0.6	0.4	2.8	0.57	2.0	1.5	1.6	2.4	0.53
Bacteria (cells × 10 <sup>10</sup> )	2.3	2.1	1.9	2.3	0.41	2.0	2.6	2.4	2.1	0.36
<b>Ruminal VFA (mol/100 mmol)</b>										
Acetate <sup>2</sup>	53.3	55.1	57.5	56.0	1.24	65.9	64.2	64.4	66.2	1.07
Propionate <sup>2</sup>	24.6	24.6	25.6	25.7	1.79	19.0	20.7	20.3	18.7	1.55
Butyrate <sup>3</sup>	22.1	20.3	16.9	18.3	1.01	15.1	15.1	15.3	15.1	1.05
Acetate : propionate ratio <sup>2</sup>	2.4	2.3	2.3	2.3	0.21	3.5	3.2	3.2	3.6	0.19

DM = dry matter, VFA = volatile fatty acids

<sup>1</sup>Se main effect,  $P < 0.01$ , <sup>2</sup>grain main effect,  $P < 0.01$ , <sup>3</sup>grain main effect,  $P < 0.05$ , <sup>4</sup>Se linear effect 50% grain,  $P < 0.01$ , <sup>5</sup>Se linear effect 70% grain,  $P < 0.01$ , <sup>6</sup>methane mol/mol glucose equivalent fermented

tion of butyric acid (Kim et al., 1997; Naziroglu et al., 1997; Hernández-Calva et al., 2007). The same way, other authors have reported that the production of acetic, propionic, butyric acids, and total VFA was increased by Se supplementation (Hidiroglou and Lessard, 1976; Wang et al.,

2009), and by vitamin E or Se/vitamin E supplementation (Naziroglu et al., 1997). The increased VFA concentrations with Se supplementation suggest a greater ruminal microbial fermentation rate (Wang et al., 2009). In other studies, ammonium (Naziroglu and Aksakal, 1997) or methane

Table 4. Influence of supplemental selenium on selenium balance in lambs fed with 70 or 50% grain diets

Variable	70% grain					50% grain				
	mg Se/kg DM									
	C	0.3	0.6	0.9	SEM	C	0.3	0.6	0.9	SEM
<b>Absorption</b>										
µg/day <sup>1,2,5,6</sup>	263.3	430.7	690.3	1005.0	25.3	152.9	284.5	528.5	858.5	21.9
Se intake (%) <sup>1,3,7</sup>	85.6	85.6	84.2	89.0	3.7	71.5	67.7	72.4	82.8	3.2
Urine Se										
µg/day <sup>1,3,6,7</sup>	59.9	78.3	141.3	262.9	21.6	18.3	51.6	90.3	89.7	15.3
Se intake (%) <sup>3</sup>	20.7	15.9	17.6	24.9	3.0	9.7	12.6	13.1	8.9	2.1
Total Se output (%) <sup>2,8</sup>	58.4	52.2	54.3	69.7	5.8	26.2	30.0	32.9	34.3	4.3
<b>Retention</b>										
µg/day <sup>1,4,6,7</sup>	190.7	339.8	547.5	716.6	40.5	130.2	230.6	427.8	764.0	28.7
Se intake (%) <sup>4,7</sup>	64.7	70.0	67.4	63.7	5.8	61.2	54.9	58.8	73.8	4.1
Se absorption (%) <sup>4,7,8</sup>	75.3	81.5	79.0	71.1	4.1	84.9	80.8	80.8	88.9	2.9

C = control, DM = dry matter

<sup>1</sup>Se main effect,  $P < 0.01$ , <sup>2</sup>grain main effect,  $P < 0.01$ , <sup>3</sup>grain main effect,  $P < 0.05$ , <sup>4</sup>Se × grain effect,  $P < 0.01$ , <sup>5</sup>Se linear effect 50% grain,  $P < 0.01$ , <sup>6</sup>Se linear effect 70% grain,  $P < 0.01$ , <sup>7</sup>Se quadratic effect 50% grain,  $P < 0.01$ , <sup>8</sup>Se quadratic effect 70% grain,  $P < 0.05$

(Cruz-Monterrosa et al., 2011) concentration did not change, although Se-yeast reduced ammonia N content (Liu et al., 2007; Wang et al., 2009). Ruminal bacteria and ruminal protozoa were not affected in our experiment but, in general, Se availability in the rumen facilitates its use by the ruminal microorganisms (Hidiroglou et al., 1968). Ruminal bacteria can transform Se to soluble forms of cysteine to selenocysteine and insoluble forms such as elemental Se and metal selenide (Serra et al., 1994b; Van Ryssen and Schroeder, 2003); however, the proportions and the importance of the microelement are not known for rumen bacteria. Other authors observed a greater proportion of ciliated protozoa in response to Se supplementation (Nazirolu and Aksakal, 1997; Mihaliková et al., 2005). Apparently, ruminal microbes can capture the available Se and reduce the salts of Se such as sodium selenate or selenite to elemental Se, or incorporate it as seleno-aminoacids (Hudson and Glenn, 1984). Koenig et al. (1997) and Van Ryssen et al. (1998) noted that at higher levels of dietary concentrate, bacteria isolated from the rumen of sheep had incorporated more Se. Both reports attributed this response to the greater rumen microbial activity in the more nutrient-dense concentrate diet. Koenig et al. (1997) suggested that the diet effect on rumen pH (6.44 vs. 5.41) and the population of rumen microbes might be reflected in the bacterial Se profile. Specifically, *Prevotella* is known to reduce Se to elemental form (Se<sup>0</sup>), while *Selenomonas ruminantium*, an organism predominating in the concentrate-fed rumen, incorporates Se into selenoamino acids. So, in this study, supplementary Se did not cause dose Se-dependent differences on ruminal pH and microbes population with higher-concentrate diet (70% grain), maybe due to the chronic acidosis caused by easily fermentable carbohydrates, which caused changes on normal fermentative population (Owens et al., 1998).

### Selenium balance

Total digestive tract absorption of Se ranged 68–89% of Se intake in our study, which is higher than the 67–73% range for timothy hay diets reported by Serra et al. (1994a). The absorption of Se depends on the chemical form of Se (Koenig et al., 1997) and on the composition of the diet. Harrison and Conrad (1984), using the balance

method, found Se availability to range 17–50% of intake in non-lactating dairy cows given a variety of diets. In our experiment, the absorption and retention of Se in urine were high, which is a marked diet effect, since the high content of non-structural carbohydrates improved the availability of Se. Van Ryssen et al. (1998) compared Sel-Plex organic Se and selenite in sheep fed either 55 or 75% concentrate diets and found that Se contents in liver, kidney, heart, and muscle were higher in animals fed the concentrate diets. Most of the inorganic Se not used immediately in the liver for selenoprotein synthesis is quickly excreted via the urine. However, in our study, Se balance was affected by the levels of Se and grain. The amount of Se recommended by the National Research Council (2007) was tripled in our experiment, improving the availability, absorption, and digestibility of Se itself as well as of other nutrients. The amount of Se did not cause toxicity problems, and the maximum tolerable amount is 5 mg Se/kg DM (National Research Council, 2007).

Ruminal Se with 50% grain was higher than 70% of grain in the diet while Se absorption and retention was lower and there were no differences in both groups. Mainly, Se consumed in foods and supplements exists in a number of organic and inorganic forms, as previously discussed. In ruminants, microbial digestion in the rumen and reticulum precedes digestion in the abomasum and small intestine (National Research Council, 2000). Low absorption of Se in ruminants is believed to result from reduction of dietary Se to insoluble forms such as elemental Se or selenides in the rumen environment. However, it is speculated that diets high in concentrate increase the availability of Se in rumen, and, consequently, increase its absorption. There is no evidence that Se is absorbed in the wall of the rumen, but high levels of vitamin A or carotenoids may antagonize vitamin E in the small intestine (Irving, 1958) and there is an association between the availability of selenium and vitamin E in blood; both associated with oxidative stress (Sadeghian et al., 2012).

### CONCLUSION

Feeding Se in lambs above the current recommendation level of the National Research Council (2007) was enhanced with maximum doses of 0.9 mg Se/kg DM. Additionally, the results of the

present study suggest that the absorption and availability of Se responded to the amount of dietary grain fed to lambs, whereas addition of Se, as sodium selenite, improved Se absorption and availability.

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