

## Influence of organic or inorganic selenium in diets on *in situ* dry matter degradability and ruminal kinetics in sheep

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**ABSTRACT.** This study assessed the *in situ* dry matter (DM) degradability and ruminal kinetics of the solid and liquid fractions of diets with two selenium sources (inorganic and organic), using three Pelibuey lambs ( $45 \pm 2$  kg) with permanent rumen cannulae. Two treatments were used: basal diet plus inorganic selenium (IS), and basal diet plus organic selenium Sel-Plex 50<sup>2</sup> (OS). The statistical analysis used the model of two compartments and two independent rates (G4G1) with non-linear regression (PROC NLIN of SAS) and mean comparison. The results showed no differences ( $P > .05$ ) for DM degradability, however the degradation parameters ( $a$ ,  $b$  and  $a+b$ ) were different ( $P < .05$ ) in favor of IS. In contrast, the degradation rate ( $c$ ) was higher for OS. The solid fraction kinetics showed differences ( $P < .05$ ) in slow ( $k_1$ ) and fast ( $k_2$ ) flow rates; also there were statistical differences in fecal matter (FM) output favoring OS, but no differences ( $P > .05$ ) were observed in mean retention time in slow (MRTC1) and fast (MRTC2) compartments or in total mean retention time (TMRT). The kinetics of the liquid fraction showed differences ( $P < .05$ ) in all parameters in favor of OS. In conclusion, the selenium source modifies ruminal parameters, which were better for organic selenium.

Key word: *In situ*, degradability, kinetics, selenium source, sheep.

## Influencia del selenio orgánico o inorgánico en dietas sobre la degradabilidad *in situ* de la material seca y cinética ruminal en ovinos

**RESUMEN.** El presente estudio valoró la degradabilidad *in situ* de la material seca (MS) y cinética ruminal de las fracciones sólida y líquida de dietas con dos fuentes de selenio (inorgánico y orgánico), usando tres ovinos Pelibuey ( $45 \pm 2$  kg) con cánulas permanentes de rumen. Se usaron dos tratamientos: dieta basal mas selenio inorgánico (SI), y dieta basal mas selenio orgánico Sel-Plex 50<sup>2</sup> (SO). Para el análisis estadístico se utilizó el modelo de dos compartimentos y dos tasas independientes (G4G1) con regresión no lineal (PROC NLIN de SAS) y comparación de medias. Los resultados no mostraron diferencia ( $P > .05$ ) para la degradabilidad de MS, sin embargo los parámetros de degradación ( $a$ ,  $b$  y  $a+b$ ) fueron diferentes ( $P < .05$ ) en favor de SI. En contraste, la tasa de degradación ( $c$ ) fue mayor para SO. La cinética de la fracción sólida mostró diferencias ( $P < .05$ ) en las tasas de pasaje lenta ( $k_1$ ) y rápida ( $k_2$ ); también se encontraron diferencias estadísticas en la salida de material fecal (MF) en favor de SO, pero no se observaron diferencias ( $P > .05$ ) en el tiempo medio de retención en los compartimentos lento (TMRC1) y rápido (TMRC2). La cinética de la fracción líquida mostró diferencias ( $P < .05$ ) en todos los parámetros en favor de SO. En conclusión, La fuente de selenio modifica los parámetros ruminales, los cuales fueron mejores para selenio orgánico.

Palabras clave: *In situ*, degradabilidad, cinética, fuente de selenio, ovinos.

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## Introduction

Nutrient digestibility is one of the most important aspects to consider in ruminant nutrition; thus, the knowledge of liquid and solid ruminal kinetics is essential; this also is important for the sources of selenium supplementation, which is a subject of research because selenium is an essential element in animal nutrition. Edens (2002) pointed out that there are two sources for supplementing selenium in animal diets: inorganic (sodium selenite and selenate) and organic (Sel-Plex 50<sup>2</sup>). However, organic compounds are potentially better sources of selenium for all animal species, because of their higher bioavailability compared to inorganic selenium

sources (McDowell, 1997; Wolfram, 1999), and because selenium's metabolic pathway in the animal body depends on its chemical structure (Cai *et al.*, 1995).

At present there is considerable information on selenium for serum levels, tissues concentrations in organs, weight gain and dairy cattle, but relevant information is necessary on its effects in the animal's gastro-intestinal tract; specially in small ruminants. The objective of the present study was to compare organic *vs.* inorganic selenium sources on *in situ* dry matter degradability and ruminal kinetics of the solid and liquid fractions of a typical diet in sheep.

## Materials and methods

### Location

The research was carried out at Facultad de Zootecnia de la Universidad Autónoma de Chihuahua, located in Chihuahua, México.

### Animals and treatments

Three rams of  $45 \pm 2$  kg live weight were used, equipped with permanent ruminal cannulae of 10 cm in diameter. The *in situ* dry matter degradability and ruminal kinetics were evaluated, comparing the solid and liquid fractions of a typical diet used for lactating sheep (Table 1). Two treatments were used: basal diet plus inorganic selenium (IS), and basal diet plus organic selenium Sel-Plex 50<sup>2</sup> (OS). The animals were housed in individual pens during the experimental period, and they were fed *ad libitum*, offering feed twice a day (8:00 a.m. and 3:00 p.m.). Water was also given *ad libitum*. There were 2 experimental

periods, with 17 d each, with 14 d for adaptation and 3 for sample collection.

### *In situ* ruminal dry matter degradability

For the rumen incubation, nylon bags (5 x 10 cm) with rounded corners were used, with a 53- $\mu$ m mesh. Each bag contained 8 g of feed sample, which had a particle size of 2 mm. The bags were tied with a 20-cm nylon cord to a metal weight to ensure that the samples were immersed in the ruminal ventral sac. The incubation times were: 0, 6, 9, 12, 18, 24, 48 and 72 h. When the bags were removed from the rumen they were placed in cold water (4°C) to stop microbial activity, after which the bags were washed with tap water at low pressure until clear water came out of the bag. Later, the bags were placed in an air-forced stove for 48 h, and then they were weighed.

The ruminal dry matter disappearance in each

Table 1. Ingredients and proximate analysis (%DM) of the diet used in the present experiment.

Ingredients	Diets		Fraction	Proximate analysis	
	IS	OS		IS	OS
Rolled corn	18.15	18.15	DM	91.50	91.80
Oat bran	18.48	18.48	ASH	7.50	7.50
Alfalfa hay	57.44	57.44	CP	15.30	14.60
Soybean meal	3.03	3.03	ADF	28.30	28.30
Molasses	2.46	2.46	NDF	58.90	62.80
Common salt	0.10	0.10			
Mineral premix	0.34	0.34			
Sel-Plex 50 <sup>2</sup>	--	0.20			

IS = Mineral premix with inorganic selenium (Superbayphos<sup>2</sup>: P,Ca,Fe,Mg,Cu,Zn,Co,I,Se)

OS = Mineral premix with organic selenium (Super-Min<sup>2</sup>: P,Ca,Cl,Mg,Na,S,K,Mn,Cu,Co,I + Sel-Plex 50: organic selenium)

DM: Dry Matter, CP: Crude Protein, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber

treatment was estimated using Ørskov and McDonald's (1979) model  $P = a + b(1 - e^{-ct})$ , where:

a = soluble fraction.

b = degradable fraction.

c = degradation rate by hour.

a + b = Potential degradability.

t = time.

The effective degradability (ED) of dry matter was estimated from the ruminal degradability parameters. Ruminal turnover constants (k) were assumed of 2, 4, 6 and 8% per hour (Singh *et al.*, 1989); using the equation:  $ED = a + (b \cdot c) / (c + k)$  of Ørskov and McDonald (1979).

Statistical procedures. To fit the model parameters, the program NEWAY EXCEL version 5.0 for WINDOWS<sup>®</sup> (Chen, 1997) was used. The information was processed and the means were compared using SAS (1998).

#### *Solid fraction ruminal kinetics*

To evaluate the kinetics of the solid fraction the alfalfa was labeled with ytterbium (Yb) according to the procedures of Prigge and Varga cited by Galyean (1997). The rams' rumen was infused with 30 g of labeled alfalfa, before the morning feeding, and the fecal samples were collected from each animal at 8, 16, 24, 36, 48, 60 and 72 h after the infusion (Ferreiro, 1990). The Yb extraction was performed according to the methodology of Hart and Polan (1984) and its quantification was carried out in an atomic absorption spectrophotometer, using a nitrous oxide flame (Ellis *et al.*, 1982).

The kinetics were estimated considering the two-compartment model of Ellis *et al.* (1979) based on the exponential disappearance of a single dose of the marker, with the objective of testing whether the selenium source affects the duration time of solid

particles in the first compartment. In addition to the original model (Vd.-exponential or G1G1), three other models were run with the gamma function  $n=2$  to  $n=4$  in the first compartment (G2G1 to G4G1) using the models and programs of Ellis and coworkers according to Moore *et al.* (1992), using the PROC NLIN (Marquardt method) of SAS (1998); the sums of the squared errors (SSE) were compared in the four models, and the G4G1 model was selected because it had the lowest SSE.

#### *Liquid fraction ruminal kinetics*

To evaluate the liquid fraction kinetics, the liquid marker Co-EDTA was used, which was prepared after Prigge and Varga (cited by Galyean, 1997). A volume of 20 ml of the marker was infused directly in the rumen 24 h after Yb infusion, and samples of ruminal liquid were collected from each animal at 4, 8, 12 and 24 h, according to the procedures described by Galyean (1997) and Ferreiro (1990). The samples were centrifuged at 3000 rpm during 15 min to eliminate the sediment, and the Co was measured using an atomic absorption spectrophotometer with an air-acetylene flame. The equations described by Galyean (1997) were used to estimate the kinetics of the liquid fraction.

Statistical Analysis. Data processing for both fractions included means comparisons for detecting statistical differences among treatments using SAS (1998).

Chemical analyses. The dry matter (DM) content was measured using 5 g of sample at 105°C. Nitrogen was determined with the Kjeldahl methodology (AOAC, 1990); the acid detergent fiber (ADF) and the neutral detergent fiber (NDF) were measured according to the methodology of Goering and Van Soest (1970).

## Results and discussion

#### *Dry matter ruminal degradability parameters*

The adjusted values of the rumen degradation constants are shown in Table 2; the soluble fraction (a) differed ( $P < .05$ ) between treatments; considering that at time zero no microbial action has occurred, then the differences could be due to lack of homogeneity in mesh size of the bags, or in the surface area proportion of the bags (Mehrez and Ørskov, 1977; Van Hellen and Ellis, 1977; Playne *et al.*, 1978).

In the degradable fraction (b) no differences ( $P > .05$ ) were observed among treatments; however the rate of degradation per hour (c) was different ( $P < .05$ ) between treatments, where the OS ration was 44% higher than IS. The potentially degradable fraction (a+b) showed differences ( $P < .05$ ) where OS was 5.3% lower than IS.

This indicates that the selenium source affected microbial action, and because of this, the ruminal degradation parameters. This is in agreement with Woods *et al.* (2003) who used different diets.

The results obtained for fractions a, a+b and c are higher than those obtained by Rivera (1999) studying grasses with different cut intervals; and the results of the present trial are lower than those reported by Aguilera (2002) who studied basal diets with oat bran treated with urea and enzyme additives. This indicates that selenium source affects the degradability and thus the degradability.

#### *Solid fraction ruminal kinetics*

The results of the parameters for solid fraction are shown in Table 3. There were no differences  $P < .05$

Table 2. Adjusted Parameters of the *in situ* ruminal degradability

Parameter	Treatment		SEM
	IS	OS	
Soluble fraction (a), %	29.48 <sup>a</sup>	28.47 <sup>b</sup>	0.2883
Degradable fraction (b), %	28.53 <sup>a</sup>	26.48 <sup>a</sup>	1.0218
Degradation rate by hour (c), %	0.034 <sup>a</sup>	0.049 <sup>b</sup>	0.0037
Potential degradability (a+b), %	58.01 <sup>a</sup>	54.94 <sup>b</sup>	0.8282
Effective degradability			
2 %	46.70 <sup>a</sup>	46.27 <sup>a</sup>	0.5301
4 %	41.60 <sup>a</sup>	41.73 <sup>a</sup>	0.5301
6 %	38.67 <sup>a</sup>	38.97 <sup>a</sup>	0.5301
8 %	36.83 <sup>a</sup>	37.03 <sup>a</sup>	0.5301

SEM = Standard Error of the Mean

<sup>abc</sup>Means within the same row with different literal, differ (P<.05)

between treatments for Yb concentration at time zero (A), which indicates that the Yb was administered in the same quantity in the 3 sheep during the 2 sampling periods; for the slow flow rate or time-dependent ruminal flow rate ( $k_1$ ) there was a group difference (P<.05) where OS had a flow rate 9.8% higher than IS. The same effect was observed in post-ruminal fast flow, independent of the time for the models G2G1, G3G1 y G4G1 ( $K_2$ ), where OS had a flow rate 4.6% higher than IS. This means that organic selenium increases flow rates in both compartments, and this could result in a DM feed consumption increment (Feng *et al.*, 1996). However, the lag time

represented by the first appearance of the marker in feces (? , h) was not different (P>.05). The mean retention times of both the slow compartment (MRTC<sub>1</sub>) and fast compartment (MRTC<sub>2</sub>) were not different (P>.05).

The total mean retention time (TMRT), did not differ (P>.05) among treatments; nevertheless fecal-output (FO) dry matter differed (P<.05) between treatments, because the OS group showed 17.4% more FO than IS; this indicates that animals fed organic selenium probably consumed more than those fed inorganic selenium.

The data analysis of Table 3 shows that the effect

Table 3. Ruminal kinetics of the solid fraction<sup>1</sup>.

Parameter <sup>2</sup>	Treatment	
	IS	OS
A, Mg/g	0.553 ± 0.035 <sup>a</sup>	0.521 ± 0.038 <sup>a</sup>
$K_1$ , h <sup>-1</sup>	0.041 ± 0.002 <sup>a</sup>	0.045 ± 0.002 <sup>b</sup>
$K_2$ , h <sup>-1</sup>	0.391 ± 0.011 <sup>a</sup>	0.409 ± 0.005 <sup>b</sup>
?, h	12.12 ± 0.195 <sup>a</sup>	11.70 ± 0.201 <sup>a</sup>
MRTC <sub>1</sub> , h	24.51 ± 1.19 <sup>a</sup>	22.23 ± 1.05 <sup>a</sup>
MRTC <sub>2</sub> , h	10.24 ± 0.291 <sup>a</sup>	9.80 ± 0.121 <sup>a</sup>
MRTT, h	46.87 ± 1.137 <sup>a</sup>	43.72 ± 0.794 <sup>a</sup>
SF, g	269.55 ± 5.034 <sup>a</sup>	316.37 ± 8.656 <sup>b</sup>

A = Yb concentration at time zero;  $K_1$  = Low flow rate;  $K_2$  = Fast flow rate; ? = Passed time from Yb doses to first show up in feces; MRTC<sub>1</sub> = Mean retention time in low compartment; MRTC<sub>2</sub> = Mean retention time in fast compartment; TMRT = total mean retention time in the tract; SF = Dry matter of fecal output.

<sup>1</sup>Estimated through no lineal regression (PROC NLIN of SAS, 1998), using the two compartment model, and two independent rates (G4G1) (Moore *et al.*, 1992).

<sup>2</sup>Mean by treatment ± Standard error.

<sup>a, b</sup> Means in the same row with different superscript, differ (P<.05).

of OS is manifested by increased flow rates in both compartments, and by the low retention time and exposure to digestion of feed particles in the entire tract; this would stimulate a higher flow rate and turnover in liquid ruminal kinetics which would benefit ruminal microorganism growth (Owens and Isaacson, 1977).

#### Liquid fraction ruminal kinetics

There were differences ( $P < .05$ ) between treatments (Table 4) in all variables of the liquid fraction ruminal kinetics, and there was also a clear and close relation from a physiological point of view between the fractional dilution rate ( $K_{df}$ ), volume, liquid flow rate ( $K_f$ ) and ruminal liquid turnover. However, Owens and Goetsch (1986) pointed that the ruminal volume and the  $k_{df}$  should vary inversely.

With respect to  $K_f$ , the present results show that

OS produced 50% more flow and 114.6% more volume than IS; this means that if the volume increases; the  $K_f$  also should increase; otherwise there would be a liquid accumulation. It is possible that ruminal liquid increases as a consequence of increased ruminal DM. González (1996) discussed that when feed remains in the rumen for a longer time, it may increase its distention, stimulating the ruminal liquid production.

In the ruminal turnover, it can be noted that the OS group showed 40.3% more time than IS, which is beneficial to ruminal microorganism growth. Owens and Isaacson (1977) pointed out that an increase in turnover is convenient in terms of microbial growth efficiency, because it improves non-protein nitrogen usage, and reduces the fermentation rate of feed that can be digested.

Table 4. Ruminal Kinetics of the liquid fraction<sup>1</sup>.

Parameter <sup>2</sup>	Treatment	
	IS	OS
Liquid fractional dilution rate ( $K_{df}$ ), %/h	13.20 ± 0.90 <sup>a</sup>	9.30 ± 0.30 <sup>b</sup>
Liquid ruminal volume, l	1.43 ± 0.03 <sup>a</sup>	3.07 ± 0.47 <sup>b</sup>
Liquid flow rate ( $K_f$ ), l/h	0.20 ± 0.00 <sup>a</sup>	0.30 ± 0.06 <sup>b</sup>
Ruminal fluid turnover time, h	7.70 ± 0.55 <sup>a</sup>	10.80 ± 0.35 <sup>b</sup>

<sup>1</sup>Estimated according to Galyean (1997)

<sup>2</sup>Mean by treatment ± Standard error

<sup>a, b</sup>Means in the same row with different superscript, differ ( $P < .05$ ).

## Conclusions

The results obtained in the present research show that organic selenium increases the flow rate of the solid fraction in both the slow and fast compartments; this produces a higher liquid turnover, and because

of this, higher feed intake, which is beneficial to the animals, because the maintenance requirements are the same, but protein utilization is non linear, and this could improve the productive efficiency.

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