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Technical note

Effect of selenium source on productive behavior, serum and muscle selenium content, and serum level of albumin, α-, β- and ∂-globulins in Pelibuey sheep

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Abstract:

The objective was to compare the effects of sodium selenite (SS) and Sel-Plex® (SP) on dry matter intake (DMI), daily weight gain (DWG), feed conversion (FC), carcass yield, Se in serum, muscle, albumin, and globulins in Pelibuey lambs. Fifty (50) animals (LW=23.0 ± 1.1 kg; 5 to 6 mo) were stratified and randomly assigned to one of five treatments (n=10): 1) Basal diet, C); 2) C + 0.30 mg kg^{-1} DM of SS, 30SS; 3) C + 0.90 mg kg^{-1} DM of SS, 90SS; 4) C + 0.30 mg kg^{-1} DM of SP, 30SP; and 5) C + 0.90 mg kg^{-1} DM of SP, 90SP. There was no effect (P>0.05) on DMI; while 90SP and 30SS showed higher DWGs (293 and 260 *vs*

245, 243, and 230 g d^{-1}; $P<0.05$) compared to the other treatments. FC was better for 90SP and C. Final LWs, carcass yields and dorsal fat were not affected ($P>0.05$). In the *Longissimus dorsi*, 30SP increased ($P<0.05$) Se with respect to 90SP and C and was similar with 30SS and 90SS. There was no effect ($P>0.05$) on the *Gluteus maximus* and *Musculus deltoideus*. Albumin was higher with 30SP and 90SS; while α-globulin was higher with 30SS and 90SP. In conclusion, 0.90 mg of SP improved DWG and FC. Selenite and SP increased Se in serum up to 0.30, and it decreased with 0.90 mg per kilogram of SP. In the *Longissimus dorsi*, Se was improved in 30SP with respect to 90SP and C and was not similar to 90SS and 30SS. The organic Se of 90SP improved the level of albumin and α-globulins.

Keywords: Selenium sources, Weight gain, Carcasses, Albumin, α-globulin, *Longissimus dorsi et lumborum*, Growing sheep.

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Selenium (Se) is essential in the antioxidant defense system in animals and humans. Among its functions are to serve as part of the enzyme glutathione peroxidase (GSH-Px), which destroys free radicals in the cytoplasm and protects tissues from oxidative stress[1]. Se has been studied for its functions in the immune system and DNA protection[2]. On the other hand, deficiency of the mineral is associated with diseases such as white muscle, and repression of immunity in lambs. The negative effects of the deficiency are explained by the relationship between the mineral and the hormones produced by the thyroid[3,4].

Sodium selenite (Na_2SeO_3) has been the preferred source of inorganic Se in ruminant feeding. However, with the appearance of new sources of organic Se[5], the question as to which is better arose. Most of the mineral is found as GSH-PX and selenoproteins that are produced in the liver and distributed in serum. However, there are not many reports of the relationships between dietary Se and serum concentrations of albumin, α-, ß- and ∂-globulins in sheep.

Selenite is mainly used for the formation of selenoenzymes and differs from organic forms of greater availability than Se + yeasts[6,7,8]. Others have shown that it is possible to increase the Se content of meat with Sel-Plex®[9,10,11] compared to SS. Both forms increase in animal tissues, improving the consumption of Se by humans who ingest meat from animals supplemented with Se.

Based on the above, the objective was to study the effects of supplementation of the source of Se on feed intake, changes in body weight, feed efficiency, carcass weights, selenium in serum, muscles, albumin and globulins of growing Pelibuey lambs.

The study was carried out in the sheep module of the Experimental Farm of the Chapingo Autonomous University, in Chapingo, Mexico (98° 29´23´´N and 98° 53´27´´ W); at 2,250 m altitude with temperate subhumid climate. The temperature varies from 12 to 18 °C, with 645 mm of annual precipitation, distributed from July to September[12].

The study used 50 newly weaned Pelibuey lambs (LW=23 ± 1.1 kg; five to six months old), which were stratified and randomly assigned to one of five treatments (n=10): 1) basal diet (BD, C); 2) C+0.30 mg of Se kg^{-1} DM, of SS, 30SS; 3) C+0.90 mg of Se kg^{-1} DM, of SS, 90SS; 4) C+0.30 mg of Se kg^{-1} DM (Sel-plex™ OSe; Alltech, Inc., Nicholasville, KY), of SP, 30SP; and 5) C+0.90 mg of Se kg^{-1} DM, of SP, 90SP. The diet was formulated according to the recommendations of the NRC[10]. In the final composition, diet C contained 0.1 mg of Se per kg of DM, while 03SS and 03SP contained 0.4 mg. In the same sense, diets 09SS and 09SP contained 1.0 mg of Se per kg of DM (Table 1).

Table 1: Ingredients and nutritional composition of the experimental diet supplied to fattening Pelibuey lambs that received 0.30 or 0.90 mg kg^{-1} of dry matter of sodium selenite (SS) or Sel-Plex® (SP) during 56 d of confinement

Composition	Percentage of inclusion, (g kg^{-1})
Rolled corn	300.0
Ground corn	290.0
Corn stover	140.0
Soybean hulls	80.0
Soybean meal	60.0
Molasses	50.0
Corn gluten	44.0
Mineral mixture[a]	15.0
Calcium carbonate	10.0
Urea	5.0
Common salt	5.0
Bypass fat	1.0
Chemical composition	

Dry matter[1], %	87.0
Metabolizable energy[2], Mcal/kg DM	2.80
Crude protein[1], %	16.00
Rumen undegradable protein[2], %	6.00
Crude fiber[1], %	10.00
Ether extract[1], %	3.30
Ash[1], %	5.80
Vitamin A[1], IU/kg	1.50
Vitamin E[1], IU/kg	16.70
Selenium[1], mg/kg^{-1} DM	0.10

[1]Determined in the laboratories of the Chapingo Autonomous University. 56230. Chapingo, State of Mexico. [2]NRC, (2007). [a]Mineral mixture: Ca, 24%; Cl, 12%; Na, 8%; P, 3%; Mg, 2%; S, 0.5%; K, 0.5%; Zn, 5000 mg kg^{-1}; Mn, 4000 mg kg^{-1}; Fe, 2000 mg kg^{-1}; I, 100 mg kg^{-1}; Co, 60 mg kg^{-1}; Cr, 5 mg kg^{-1}; Vitamin A, 500000 IU kg^{-1}; Vitamin D, 300000 IU kg^{-1}; Vitamin E, 1000 IU kg^{-1}. Mezcla mineral-engorda® (Servicios Especializados Profesionales; Chapingo, Mexico).

The lambs received feed twice a day. Fifty (50) percent of the feed offered was served at 0700 h and the rest at 1500 h. These animals were trained to eat in individual Calan door-type feeders (American Calan, Inc.; Northwood, NH, US), equipped with a container of approximately 15 kg. Feed was offered *ad libitum* (15 % more than the previous day's intake). The portion was weighed, recorded and deposited in the feeders. Uneaten feed was removed, weighed and recorded. For each animal, a sample of the consumed feed and the non-consumed feed was obtained weekly.

The total DM was determined in an oven at 100 ºC for 24 h and incinerated in a muffle at 500 ºC to quantify the OM and ash content. DM intake was estimated by multiplying daily feed intake by its DM content.

The NDF and ADF contents of the diets were quantified following the procedures of Goering and Van Soest[(13)]; while the protein was obtained by Kjeldahl[(14)]. Changes in live weight (LW) were recorded weekly and used to calculate DWG. The lambs were slaughtered at the end of the fattening period following official slaughter procedures[(15)].

The carcass yield, expressed as a percentage, was calculated as the proportion of the weight of the hot carcass, divided by the LW and multiplied by 100. The weight of the cold carcass

was obtained 24-30 h after cold storage at approximately 2 ± 2 ºC. Carcass yield was recalculated and reported as cold carcass yield.

The research protocols and management procedures were carried out following the Official Mexican Standard (NOM-051-ZOO-195). During mobilization, the animals were treated in accordance with the standard NOM-024-ZOO-195.

Every 14 d, a blood sample, approximately 10 ml, was taken by puncture of the jugular vein, in vacutainer tubes without anticoagulant (Beckton-Dickinson, Franklin Lakes, NJ). The samples were kept in the environment for 60 min in order for them to coagulate and then they were refrigerated. The blood was centrifuged at 1,000 xg for 25 min at 4 ºC. The serum was stored in vials at -20 ºC and later sent to the laboratories of the Faculty of Veterinary Medicine and Zootechnics of UNAM, for the determination of albumin, α-, ß-, ∂-globulin and Se. The globulins were determined by the procedures of Connell *et al*[16]; while the Se in serum was quantified with a spectrofluorometer (Perkin Elmer model LS30), following the procedures of Tamari *et al*[17].

After 48 h of slaughter, the *Longissimus dorsi*, *Gluteus maximus* and *Musculus deltoideus* muscles were removed from each carcass according to the procedures of Covenin[18]. From each muscle and carcass, three cuts approximately 2.54 cm thick were obtained and packed individually. All cuts were frozen at -30 ºC and stored at -20 ºC until the corresponding analyses. After thawing, the thickness of the dorsal fat layer between the 12th and 13th rib was taken[18].

The meat samples were partially thawed at 4 ºC (to avoid fluid loss). Subsequently, the visible adipose tissue was removed, mixed with a Black and Decker™ food processor (Model HC3061, New Britain, CT, USA), packed in bags (Whirl-Pak Bags, Nasco, Fort Atkinson, WI), and stored at -20 ºC until the final analyses. Se was quantified with an atomic absorption spectrophotometer (SpectrAA 220®, New Britain, CT, USA), following the procedures of the manufacturers.

Data were analyzed using the statistical package SAS[19] (version 9.2, SAS Institute Inc., Cary, NC, US). DMI, LW changes, and FC were analyzed with the Mixed procedure of SAS in a completely randomized design with measures repeated over time[19]. The model included fixed effects of treatment, week and the treatment×week interaction. The random effect of animal was nested in treatments and was taken as the repeated term. The statistical model is described below, after removing the double or triple interactions that were not significant:

$\mathbf{Y_{ijkl}} = \mu + T_i + T_j + T \times T_{ij} + L_{k(i)} + E_{ijkl}$

Where:
$\mathbf{Y_{ikjl}}$ is the response variable, μ is the overall mean;
$\mathbf{T_i}$ is the fixed effect of treatment$_i$ (i = 1, 2, ..., 5);
$\mathbf{T_j}$ is the fixed effect of time (j = 1, 2, ..., 4);
$\mathbf{T \times T_{ij}}$ is the fixed effect of the interaction of i-th treatment$_i$ and j-th time$_j$;
$\mathbf{Lk_{(i)}}$ is the random effect of the animal;
$\mathbf{E_{ijkl}}$ is the random effect of experimental error.

A model similar to the previous one was used to study the levels of albumin, α-, ß- and ∂-globulins. Se concentrations in muscles were analyzed with Proc Mixed in a completely randomized design with a classification criterion[19]. The results were declared significant where it was observed that $P<0.05$. When differences between treatments were detected, the means were compared with the Tukey procedure with $\alpha=0.05$. The covariance structure that produced the lowest Akaike[20] criteria was that of composite symmetry in all the variables studied, except for Se levels in muscles, which better adapted to the autoregressive of order (1).

Table 2 shows the results obtained for DMI, DWG and FC of growing lambs supplemented with SS and SP for 56 d. The supplementation with Se did not influence ($P>0.05$) the DMI of the sheep. This may be because the addition of Se increases the digestibility of OM, NDF and N in the total tract and possibly facilitates the absorption of the mineral in the abomasum. However, it was not enough to increase the DMI.

Table 2: Mean values of feed intake, daily weight gain, feed conversion, live weight, carcass yield and dorsal fat of fattening Pelibuey sheep supplemented with 0.30 and 0.90 mg of selenium

Variable	Treatments (Selenium, mg kg^{-1} MS)					EE[1]	*P*[2]		
		Sodium selenite		Sel-Plex™					
	Control	0.30	0.90	0.30	0.90		Treat. (T)	Time	T x Time
Feed intake, kg DM d^{-1}	1.10	1.28	1.29	1.26	1.29	0.10	0.99	0.01	0.44
Daily weight gain, kg d^{-1}	0.230^{b}	0.243^{b}	0.245^{b}	0.260^{a}	0.293^{a}	0.04	0.01	0.02	0.33
Feed conversion, kg	4.78^{b}	5.26^{a}	5.26^{a}	4.48^{b}	4.40^{b}	0.19	0.03	0.36	0.25
Final live weight, kg	42.5	38.50	39.10	39.00	39.50	1.40	0.81	0.41	0.32
Hot carcass yield, %	53.20	54.10	54.10	52.50	52.80	0.98	0.69	0.25	0.11
Cold carcass yield, %	52.20	53.10	53.00	51.50	51.40	0.99	0.32	0.50	0.20
Dorsal fat, mm	2.20	2.70	2.30	2.50	1.99	0.32	0.80	0.23	012

[1] Standard error; [2]Probability (P<0.05); [abc] Values in the same row with distinct literal are different (P<0.05).

The results obtained in this study agree with Alimohamady *et al*[4], who observed an improvement in the digestibility of dietary components. Other studies reported different results. Domínguez-Vara *et al*[21] observed no differences in DWG and feed conversion in Rambouillet lambs fed 0.30 mg of Se per kg^{-1} of DM of SS compared to the control. The non-difference was attributed to the state of Se and its low availability in the diets.

In the present study, supplementation with 0.30 and 0.90 mg kg^{-1} DM of SS or SP impacted DWG. This may be due to an improvement in feed digestibility. On the contrary, the superiority in feed conversion with 90SP, possibly, is explained because the Se from SP has been shown to have a higher bioavailability with respect to SS[9,21].

Table 2 shows the final LWs, the weights of the hot and cold carcasses, and the thickness of the dorsal fat layer of sheep fed SS and SP. There was no effect of the level and source of Se (P>0.05) on the aforementioned variables. The lack of effect is explained by the similarity in the DMI of the animals. As is known, the LW of animals depends on feed intake and in the present case, this intake was similar between treatments, although the FC was different.

Vignola *et al*[6] indicated that supplementation with 0.3 and 0.9 mg of Se from SS or SP did not affect the *Longissimus dorsi* area, dorsal fat thickness and the weights of hot and cold carcasses of growing lambs. The loss of effect was attributed to the similar DMI among the different sources of the mineral.

As shown in Table 3, the effect of the treatments influenced ($P \leq 0.05$) the content of Se in the blood serum. As the Se in the diet increased, the concentration in blood serum increased and then it showed a decreasing return with 0.9 mg of Se kg^{-1} DM.

Table 3: Mean values of serum concentrations of selenium, albumin, α-, β- and ∂-globulins, *Longissimus dorsi et lumborum*, *Gluteus maximus* and *Musculus deltoideus* of fattening Pelibuey sheep supplemented with 0.30 and 0.90 mg kg^{-1} DM of selenium from sodium selenite or Sel-Plex™ for 56 days in confinement.

Variable	Treatments, mg kg^{-1}					CI[1]	SEM[2]	*P*[3]
	Control	Sodium selenite		Sel-Plex™				
		0.3	0.9	0.3	0.9			
Blood serum concentrations, mg L^{-1}								
Selenium	0.05[c]	0.08[a]	0.08[a]	0.09[a]	0.07[b]	0.08-0.50[4]	0.001	0
Albumin	45.91[b]	46.66[b]	49.91[a]	52.09[a]	48.09[b]	24.0-30.0[5]	1.51	0
α-globulin	12.16[c]	14.44[a]	12.51[c]	12.62[c]	13.13[b]		1.14	0
β-globulin	13.45	14.98	13.65	13.61	18.83		2.03	0.7
∂-globulin	23.4	25.24	24.38	24.62	23.65		1.28	0.4
Muscle concentration, µ/100 g								
Longissimus dorsi et lumborum	17.30[b]	20.50[ab]	20.22[ab]	23.82[a]	17.90[b]	9.0-40.00[4]	2.8	0
Gluteus maximus	14.37	15.32	17.62	23.72	19.7		4.75	0.4
Musculus deltoideus	15	24.57	29.82	31.32	20.3		4.77	0.3

[1]Concentration interval. [2]Standard error of the means. [3]Probability, ($P<0.05$). [4]Puls[22]; [5]Kaneko *et al*[24].
[a,b,c]Values in the same row with different literal are different ($P<0.05$).

According to Puls[22], adequate levels of Se in blood serum of growing sheep are 0.08 to 0.50 mg L^{-1}, in order to balance internal homeostasis. In the present study, all treatments were within the indicated range, except for C and 30SS, which showed concentrations below 0.07 mg L^{-1}.

As shown in Table 3, supplementation with different levels of SS or SP influenced ($P<0.05$) the Se level in the *Longissimus dorsi*, with no apparent effects ($P>0.05$) in the *Gluteus maximus* and *Musculus deltoideus*. The Se in skeletal muscle increases as the diet is richer in the mineral. Based on the report by Puls[22], the observed values of Se in the muscles are within the appropriate range for animals of the same characteristics as in the present study. The highest response to supplementation was 30SP. Perhaps due to the greater availability of the mineral to be incorporated into tissues, however, with the highest level, its response tends to decrease.

The results obtained in the present study agree with others previously published. Juniper *et al*[9] indicated that 0.35 mg of SS or SP increased the mineral in the muscles, in a manner dependent on the diets of finishing bovines. The difference between sources was remarkable. The Se of SS produced 0.31 mg of Se, while that of SP yielded 0.46 mg in the *Longissimus dorsi*, which are similar to those observed in the present study.

The levels of albumin, α-, ß- and ∂-globulins in the blood serum of sheep are presented in Table 3. The levels and sources of Se only increased ($P<0.05$) albumin and α-globulins. On the contrary, they did not affect the levels of ß- and ∂-globulins. Serum albumin originates in hepatocytes, from where it passes into the bloodstream (which represents approximately 13 % of the total protein produced by the liver)[23].

In the present study, the increased presence of albumin in lambs that consumed 90SS and 30SP was not due to the increase in the synthetic capacity of hepatic albumin. Nor was it because the animals had a high capacity for synthesis. The difference is explained because Se, as an antioxidant, improves the activity of hepatocytes, so perhaps it improved overall protein production.

Based on the report by Kaneko *et al*[24], the albumin concentration obtained in the present study was approximately twice the minimum recommended levels, and in all cases, it exceeded the maximum indicated. The maximum level was reached with 30SP and subsequently, it tended to decrease. The values observed in the present study are consistent with those observed by De Paula Silva *et al*[25] with several sheep breeds created in tropical conditions.

Immunoglobulins act as membrane receptors on β lymphocytes and are used by the immune system to identify and neutralize viruses and bacteria[26]. In the present study, the highest concentration of α-globulins was found in lambs that consumed 30SS and 90SP. This behavior was related to the antioxidant capacity of the mineral included in the diet.

In conclusion, the level of 0.90 mg of Sel-Plex™ improved DWG and FC. Selenite and SP increased serum Se in 30SS, 90SS and 30SP and it decreased with 0.90 mg per kg of SP. In the *Longissimus dorsi*, Se was improved in 30SP with respect to 90SP and C and was similar to 90SS and 30SS. The organic Se of 90SP improved the level of albumin and α-globulins.

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