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Sheep Diet Enriched with Selenium Yeast: Investigating Its Relationship with Physiological Stress and Bibliometric Parameters

Short Communication

M.D. Mariezcurrena-Berasain[1], A. González-Nicanor[1], G. Velázquez Garduño[2], B. Ortíz López[3], E.D. Archundia-Velarde[2], A. Medina García[1] and M.A. Mariezcurrena-Berasain[4*]

[1] Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Instituto Literario 100, C.P. 50000, Estado de México, México
[2] Universidad Tecnológica del Valle de Toluca, Toluca, Estado de México, México
[3] Blvd. San Angel 3886, Mercado de Abastos, San Benito, 80260 Culiacán Rosales, Sinaloa, México
[4] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Instituto Literario 100, C.P. 50000, Estado de México, México

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*Correspondence E-mail: mariezcurrenaberasa@gmail.com

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ABSTRACT

The bibliometric analysis was conducted to determine the impact of enriched-selenium yeast supplementation in sheep diet. The objective of this paper is to evaluate the dietary organic selenium on metabolic stress as well as the blood metabolites in sheep. Thirty female Pelibüey and Dorper sheep were used during a fattening period (60 days) of an average age of 6-8 months old. The basal diet was corn, cookie ground, sorghum, distillers dried grain (DDG), oat, and molasses-based diet with 3.1 Mcal/kg weight and combined with 10.16% of crude protein. Animals were randomly divided into 3 groups (10 animals per group): Control group (T1, basic diet with no additive), T2, fed with the basal diet with a 0.3 mg Se/kg (*Saccharomyces cerevisiae*); and T3, fed with the basal diet with a Se 0.60 ppm. Experimental results were compared with the bibliometric indicators collected from 2010-2014 for a density-equalizing mapping. Data were retrieved from Scopus. Red blood cell count, hematocrit, hemoglobin, white blood cells, and red blood cell indices mean globular volume (VGM) and concentration hemoglobín globular mean (CHGM) were higher for treatment with a lower concentration of Se (T2, 0.3 ppm). A total of 3 countries contributed papers from the top 8 most cited papers about Selenium in the diet of sheep. All countries are from the developed world with a high degree of production and healed care. In conclusion, Supplementation with selenium-enriched yeast at a ratio of 0.3 ppm did not affect hematological variables and improved red cell life even before slaughter.

KEY WORDS Bibliometric, *Saccharomyces cerevisiae*, selenium, sheep.

INTRODUCTION

Over the years, a few volumes of works have been published in the field of the diet of sheep, however, the impact of a given paper on the specialty remains unknown. Sheep production in Mexico faces several challenges that are sometimes hampered by a lack of certain nutrients, and trace elements such as selenium and chromium.

The deficiency is mainly in sheep, it has been linked to various diseases, such as white muscle disease and suppression of the immune system. In addition, it can alter both the production and quality of the final product. Selenium is found in all cells and tissues, is considered a modifier element metabolism, and improves carcass quality, necessary for growth and fertility (Thomson, 2004; Libién-Jiménez *et al*. 2015; Velázquez-Garduño *et al*. 2015;

Bahena Culhuac *et al.* 2023). It is also considered an essential trace element for the promotion of human and animal health since it acts as a coenzyme in 15 dependent enzymes; Selenium, among which stands out its activity in the glutathione peroxidase, whose function is to reduce oxidative damage and establish intracellular redox state (Chauhan *et al.* 2015)

Use it in ruminant feed is particularly appropriate in the form of sodium selenite (Na_2SeO_3), but recent research has referred to the risk of their use for their high toxicity, particularly when it is not properly balanced or mixed in feed (Stoebe *et al.* 2015). In recent years research on the physiological role of selenium has been directed to organic forms, such as through selenium-enriched yeast and selenium methionine (Pedraza-Hernández *et al.* 2019; Elaref *et al.* 2020; Elghandour *et al.* 2024), said the tolerable maximum requirement is between 0.05 and 5.00 ppm, for that reason; is necessary to use products with a high degree of safety. Other studies have mentioned the effect of the addition of selenium in the diets of sheep and lambs on some blood parameters (Kachuee *et al.* 2019; Zarbalizadeh-Saed *et al.* 2020; Mariezcurrena-Berasain *et al.* 2022). However, the addition of selenium to the diet of animals can cause certain metabolic changes that may affect health and productivity. Therefore, it requires further studies involving blood parameters to analyze the metabolic pathway of the mineral to be supplemented in the diet of animals (Alimohamady *et al.* 2013). Thus, the main objective of this study was to evaluate the effect of organic selenium supplementation in the diet of sheep on the degree of physiological stress by measuring blood variables. In addition, other parameters were analyzed as cites, self-cites, and countries that most publish.

MATERIALS AND METHODS

Bibliometric study

The study was a bibliometric analysis of the Scopus Library database for the years 2010-2014. In 2010-2014, we used the subject category ‘selenium diet, stress and sheep ' to determine the 8 most cited articles of Organic Selenium in the diet. Scopus is a multidisciplinary bibliographic database of peer-reviewed research literature.

Experimental study

Thirty female Pelibüey and Dorper sheep were used during a fattening period (60 days). Sheep were an average age of 6-8 months old and healthy (27.75±3.37 kg and body condition 4), they were deworming with Ivermectina (2 µg/kg weight) and supplemented with A, D, and E Vitamins. The fattening period was from July to September 2013 at Agrovix Company S.P.R. of R.L., at Jocotitlan, Mexico State, Mexico. Animals were fed with a corn, cookie ground, sorghum, distillers dried grain (DDG), oat, and molasses-based diet with 3.1 Mcal/kg of DM and combined with 10.16% of crude protein, according to the NRC (1998). Sheep were randomly divided into 3 groups (10 animals per group). The control group (T1, basic diet no supplementation fed), and treatment 2 group (T2) were fed with the basic diet with a 0.3 mg Se/kg (*Saccharomyces cerevisiae* Selyeast 3000^{TM}, enriched yeast, LFA Lesaffre) supplementation, and treatment 3 group (T3), with Se 0.60 ppm, for 61 days. Food and water were administrated *ad libitum* to all the groups (mineral premix was free of selenium, Tables 5 and 8).

Blood samples were collected on the 7, 14, 21, 28, 60, and 61st day of fattening from the jugular vein (10 mL) into the vacuum tubes (Vacutainer®), with EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant. Samples were transported freeze immediately to the laboratory to determine the hematological analysis in whole blood. Afterwards, samples were centrifuged at 7, 000 rpm for 15 min to obtain the serum. Hematological analysis done included erythrocyte count, hematocrit, mean corpuscular (erythrocyte) volume (MCV), mean corpuscular hemoglobin concentration (MCHC), plasma protein, leukocyte count, lymphocyte percent and lymphocyte number. A blood smear was made to analyze the granulocytes, especially the neutrophils. Hemoglobin (Hb) concentration, g/dL was determinate by a spectrophotometry (Cobas Mira Plus Roche®, Table 6).

Statistical analyse

The results were statistically analyzed by an ANOVA and a main comparison by Tukey test ($P<0.05$) with the software SAS® Analytics (SAS, 2002).

RESULTS AND DISCUSSION

There were 7 different countries of origin for the highly cited articles. The United States had the largest number of articles with 25 and 70. The China published a total of 40 and 3 articles. Brazil published a total of 22 and 3. Australia, France, Italy, and Iran each had three or more articles (Table 1).

The literature on selenium diets from different countries increased almost fourfold in the last 5 years. The current study found that the number of diets and selenium totally articles published between 2010 and 2014 indicates an important rapid development in the field of diets and use of selenium. The United States had the greatest number of article publications.

However, the authors have published more than 10 articles (Table 2).

Table 1 The top 5 countries that publish the most about selenium diet, stress, and sheep

Selenium and diet and sheep	
United States	25
Iran	6
Australia	3
Brazil	3
China	3
Selenium and diet and stress	
United States	70
China	40
Brazil	22
France	21
Italy	20

Table 2 The number of the top 10 authors that publish the most about selenium diet and stress sheep

Authors	Title	No. of articles
Dahlen *et al.* (2022)	Selenium supplementation and pregnancy outcomes	19
Carlson *et al.* (2009)	Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes	19
Whittier and Yelich (2011)	Recognizing achievement of young scholars working to foster the discovery, sharing, and application of knowledge concerning the responsible use of animals to enhance human life and well-being	19
Vonnahme *et al.* (2013)	Thyroid hormones and cortisol concentrations in offspring are influenced by maternal supranutritional selenium and nutritional plane in sheep	18
Caton *et al.* (2014)	Biofortification of maternal diets with selenium: postnatal growth outcomes	14
Sefi *et al.* (2022)	Antioxidant role of selenium against maneb-induced cardiotoxicity in mice	15
Ben Amara *et al.* (2013)	Dimethoate induces kidney dysfunction, disrupts membrane-bound ATPases and confers cytotoxicity through DNA damage. Protective effects of vitamin E and selenium	14
Soliman (2015)	Dose-response of vitamin E and selenium injection on growth performance, physiological and immune responses of Ossimi lambs	13
Amara *et al.* (2012)	Dimethoate induced oxidative damage and histopathological changes in lung of adult rats: modulatory effects of selenium and/or vitamin E	11
Sefi *et al.* (2014)	Effect of selenium on methimazole-induced liver damage and oxidative stress in adult rats and their offspring	9

The institutions with which they are affiliated are the North Dakota State University from the United States and the University of Sfax from Tunisia (Table 3).

Geographically, Tunisia contains the eastern end of the Atlas Mountains and at the northern reaches the Sahara Desert. Citation analysis and content analysis are commonly used as bibliometric parameters.

Table 3 The number of the top 5 affiliation to publish the most in selenium diet, stress, and sheep

Selenium and det and sheep	
North Dakota State University	19
USDA Agricultural Research Service, Washington DC	6
Oregon State University	5
Linus Pauling Institute	5
University of Wyoming	4
Land O'Lakes Munson Lakes Nutrition	3
Selenium and diet and Stress	
University of Sfax	15
Universidade de Sao Paulo–USP	10
Northeast Agricultural University	10
University of Saskatchewan	6
Universidade Federal de Santa María	6

The United States had the highest mean impact factor of their publications when compared with other parts of the world, which likely reflects the relatively high research citations (Royle *et al.* 2013). The number of sites is 56 in the analyzed period (Table 4).

Table 4 The 9 papers most cited about selenium and diet and stress and sheep

Author	Title	Citas
Fairweather-Tait *et al.* (2010)	Selenium bioavailability: Current knowledge and future research requirements	478
Meyer *et al.* (2010)	Effects of plane of nutrition and selenium supply during gestation on ewe and neonatal offspring performance, body composition, and serum selenium	91
Meyer *et al.* (2011)	Nutritional plane and selenium supply during gestation affect yield and nutrient composition of colostrum and milk in primiparous ewes	110
Ripoll *et al.* (2011)	Use of dietary vitamin E and selenium (Se) to increase the shelf life of modified atmosphere packaged light lamb meat	210
Neville *et al.* (2010)	Ovine offspring growth and diet digestibility are influenced by maternal selenium supplementation and nutritional intake during pregnancy despite a common postnatal diet	55
Hall *et al.* (2012)	Organic and inorganic selenium: I. oral bioavailability in ewes	82
Hammer *et al.* (2011)	Effects of maternal selenium supply and plane of nutrition during gestation on passive transfer of immunity and health in neonatal lambs	59
Lekatz *et al.* (2010)	Cotyledonary responses to maternal selenium and dietary restriction may influence alterations in fetal weight and fetal liver glycogen in sheep	39
Fairweather-Tait *et al.* (2010)	Selenium bioavailability: Current knowledge and future research requirements	478

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A total of 3 countries contributed papers from the top 10 most cited papers about Selenium in the diet of sheep. All countries are from the developed world with a high degree of production and healthcare. The United States contributed 8 papers to the list, which is more than one the number of all the other combined countries.

Figure 1 shows the cite and self-cites of the analyzed subject matter where a decrease is observed in the year 2014 of the self cites, this parameter has been analyzed to observe the absolute value of the mentioned articles.

Figure 1 Cites and self cites of 10 papers most cited selenium and diet and stress and sheep

The results of this research elucidate how developments in this specialty have evolved. It becomes apparent which authors have made outstanding contributions in this area and led the way in terms of clinical research. On the other hand, in the experimental assays, all hematological parameters studied for the 3 groups were within the ranges reported for sheep (Kotepui *et al.* 2014). Once the comparison of means by Tukey test with α= 0.05, it was found that the values of the variables for red blood cell count, hematocrit, hemoglobin, white blood cells, and red blood cell indices VGM and CHGM were higher for treatment with lower concentration of Se (T2, 0.3 ppm), as shown in Table 2. While for segmented neutrophils the highest values were for the concentration of 0.6 ppm selenium (T3).

This agrees with that reported by Alimohamady *et al.* (2013), who reported no significant differences between hematological parameters for different doses of organic Se supplementation (0.2 to 0.4 ppm) in lambs (Alimohamady *et al.* 2013).

Thus, as has been found in pigs, lambs, and calves (Gunter *et al.* 2003; Vignola *et al.* 2009). Although some have reported that selenium supplementation in sheep, has hematopoietic effects related to its action to protect the cell membrane of erythrocytes and as an antioxidant in organelles, which has been linked to increased blood cells (erythrocytes and leukocytes), which is associated with increased immune response (Mousaie *et al.* 2014; Alhidary *et al.* 2015; Chauhan *et al.* 2015).

Erythrocytes, hemoglobin, hematocrit, and mean globular volume (VGM) rates and volumen corpuscular medio (VHGM) presented values very similar to those described by Gomez *et al.* (1992). In this study we found a slight increase in the concentration of red blood cells and hemoglobin in red blood cells its effect was already reported in the life of these cells after supplementation with selenium-enriched yeast (Libién-Jiménez *et al.* 2015; Mariezcurrena-Berasain *et al.* 2022).

As for the white and the plasma protein content series, only the latter showed differences, it presented a slight decrease for the group with higher selenium content (T3). In parallel, there was no difference between treatment 2 and the control group (Table 3), however, the values found in the 3 groups were within what was reported as normal for sheep (Thomson, 2004). As can be used to suggest that supplementation did not significantly alter the total protein concentration in sheep. The results found in this investigation for white blood cells values were within normal ranges for ovine (Kaneko *et al.* 1997).

Those, who agreed with that reported for other studies, where no effect between supplementation and control groups on hematological parameters was not found. It is suggested that supplementation is preferred with enriched yeast in sheep and does not alter physiological parameters and protection can be assumed in the life of red blood cells causing beneficial effects on the health of the animal, even before slaughter (Alimohamady *et al.* 2013). Regarding the evolution of the observed response, the results agree with who indicated that changes in hematology rise to concentration are low supplementation (Table 4).

Thus, in the present experiment, in the case of erythrocytes and VGM, significant differences began until the fourth week. Similarly, for hematocrit and hemoglobin, the differences were in the third week, which could have been submitted in response to the increased number of red blood cells (Grace and Knowles, 2012).

Finally, hematocrit, diminished in the last sampling before sacrifice (10 min before), is proposed as a blood indicator for stress caused by confinement and transport, as mentioned in other studies (Table 7; McDonald *et al.* 2014; Bahena Culhuac *et al.* 2023). Since the highest value was found in the last sampling at the end of fattening before transport, which in terms of handling, is the most stressful, as the time before slaughter. As these results were above those determined by Oyarce *et al.* (2002) (32.7±2.74%). Similarly, in the last sampling before sacrifice, the hematocrit value showed significant differences, so we can deduce that stress had an impact on that experiment.

Table 5 Analysis of the immunological parameters about the treatment with Selenium in the sheep diet

Treatment	Plasmatic protein (mg)	Leukocytes	Neutrophil Segment	Lymphocytes
T1	80.50[a]	9.31	41.66	53.48
T2	80.14[a]	11.59	41.98	51.55
T3	78.15[b]	9.04	42.18	51.74

T1: control group, basic diet with no additive); T2: fed with the basal diet with a 0.3 mg Se/kg (*Saccharomyces cerevisiae*) and T3: fed with the basal diet with a Se 0.60 ppm.

Table 6 Significance analysis of the hematological parameters of the treatments with selenium in the sheep diet

Variable	Variance	Coefficient of variation	Significant difference	Average/SD
Erythrocytes X10^6/Ul	1.55	13.04	1.24	9.56±1.24
Hematocrit (%)	19.07	10.7	4.36	40.77±4.4
Hemoglobin (g/cc)	213.19	10.76	14.6	135.60±14.7
Mean globular volume (%)	41.2	15	6.41	42.79±6.4
MCHC (g/100 mL)[2]	0.58	0.22	0.76	332.08±0.7
Plasma protein (g/100 mL)	19.92	5.6	4.46	79.62±4.5
Leucocytes (mil/mm³)	52.96	72.71	7.27	10.00±7.3

SD: standard division and MCHC: mean corpuscular hemoglobin concentration.

Table 7 Analysis of the hematological parameters of the samples taken in different times after transportation

Sample[1]	Erythrocytes X10^6/Ul	Hematocrit (%)	Hemoglobin (g/dL)	Mean globular volume (%)	Concentración hemoglobín globular mean (mg)
M1	10.21[a]	41.10[ab]	136.66[ab]	40.16[b]	332.00[ns]
M2	10.33[a]	41.23[ab]	137.16[ab]	39.80[b]	332.16[ns]
M3	10.49[a]	39.26[b]	130.50[b]	37.36[b]	331.83[ns]
M4	9.01[b]	40.26[b]	133.93[b]	44.56[a]	332.13[ns]
M5	8.93[b]	39.40[b]	131.00[b]	44.50[a]	332.00[ns]
M6	9.12[b]	42.07[a]	146.64[a]	47.85[a]	332.32[ns]
M7	8.73[b]	40.28[b]	133.96[b]	45.82[a]	332.14[ns]

[1] Time of sample at 0 (M1), 7 (M2), 14 (M3), 21 (M4), 28 (M5), 60 (M6), 61 (M7) of day of fattening.

Table 8 Analysis of the hematological parameters of each treatment

Treatment	Erythrocytes	Hematocrit	Hemoglobin	VGM[2]	CHGM
	X10^6/uL	(%)	(g/dL)	(%)	(%)
T1 (0 ppm)	9.57	40.42[ab]	134.41[ab]	42.2	331.98
T2 (0.35 ppm)	9.69	41.78[a]	139.00[a]	43.12	332.18
T3 (0.6 ppm)	9.39	40.07[b]	133.27[b]	43.06	332.07

T1: control group, basic diet with no additive); T2: fed with the basal diet with a 0.3 mg Se/kg (*Saccharomyces cerevisiae*) and T3: fed with the basal diet with a Se 0.60 ppm.
VGM: Mean globular volume and CHGM: Concentration hemoglobín globular mean.

Therefore, despite having significant differences in some of the variables of hematocrit, and hemoglobin, and no significant changes have been detected in erythrocytes, VGM and CHGM, leukocytes, neutrophils, and lymphocytes; selenium ranges used in this experiment did not cause values that come out of normal for this species, as established by Dominguez *et al.* (2009).

Combining doses of Cr and 0 to 0.3 mg of selenium yeast chelated, reported no damages in the production variables or carcass. Similarly, Kitchalong *et al.* (1995) compared with inorganic and organic sources of Se and found no changes in the carcass using doses of 0.3 to 0.45 mg/kg. Despite using higher doses than that proposed by the NRC which indicate that 0.4 ppm is an overdose and recommended by the FDA (NRC, 1998) are still below toxic levels.

CONCLUSION

Supplementation with selenium-enriched yeast at a ratio of 0.3 ppm did not affect hematological variables and improved red cell life even before slaughter. However, the absence of changes in stress indicators prevents the presentation of states of stress demonstrable through laboratory tests. Neither transportation nor sacrifice caused stress in the animals in the dosage mentioned.

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