

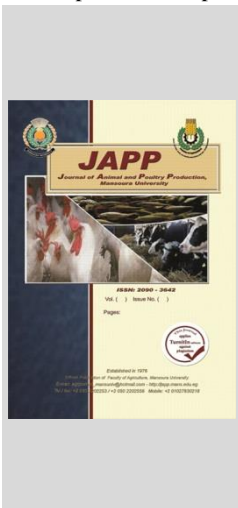
# Journal of Animal and Poultry Production

Journal homepage: [www.japp.mans.edu.eg](http://www.japp.mans.edu.eg)  
Available online at: [www.jappmu.journals.ekb.eg](http://www.jappmu.journals.ekb.eg)

## Effect of Long-Term Supplementation of Selenium on Wool Production and Quality in Sheep

Saudi, E. M.\* and R. M. Gheetas

Animal production department, Faculty of Agriculture, Al-Azhar University , Cairo, Egypt.



### ABSTRACT

Selenium is an essential trace element for wool growth and its characteristics especially during the exposure of animals to stress. Twenty adult, dry, non-pregnant ewes aged 3-4 years old ranging from 45 to 50 kg body weight, were chosen for the trial and divided into two equal groups, each of 10 ewes. The ewes were fed on a formed diet according to National Research Council (1985). The control group was fed on the formed diet without any supplements. The treated group was fed on the same diet plus 0.33gm selenoprotein (0.7 available selenium). The trial lasted for one year. The grease Fleece Weight (GFW), Clean Fleece Weight (CFW), Fibre Diameter (FD), Staple Length (STL), Fibre Length (FL), Staple Strength (SST), Elongation percentage (ELO%) and Glutathione Peroxidase enzyme (GPX) in the blood serum were measured to study the effect of long term supplementation of selenium on wool production and quality in sheep. GFW, CFW, STL and FL weren't affected significantly. The FD in tip portion was affected significantly  $P \leq 0.5$  by the addition of selenoprotein to the diet of the ewes. There was a significant difference between treated and untreated group in the sulphur content of the tip. The GPX was significantly higher in the treated group compared to control one. No significant difference between treated and untreated group in SST and elongation percentage. Addition of selenium to the diet of dry – non pregnant ewes didn't greatly affect wool production or quality in natural environmental conditions since there were no stresses on animals.

**Keywords:** Selenium, wool production, wool quality, wool characteristics

### INTRODUCTION

Selenium (Se) acts as the catalytic center in the active sites of several antioxidant enzymes and proteins such as glutathione peroxidase (GSH-Px). The GSH-PX function as an antioxidant during exposure to environmental stress. These antioxidant enzymes respond to oxidative stress by neutralizing and eliminating reactive oxygen species (ROS) (Pappas *et al.*, 2008).

Wool is very sensitive to selenium deficiency while Se supplementation significantly increased wool production (Wilkins & Kilgour, (1982), Hill *et al.* (1969) and Langland *et al.* (1991a, 1991b). The marginal selenium deficiency in reproducing sheep commonly causes reduced growth of wool, fibre diameter and affects lambs at birth and weaning (Masters *et al.*, 1993). So, the aim of this study was to study the effect of supplementing the diet of sheep with selenoprotein for one year on wool production and quality.

### MATERIALS AND METHODS

The present study was carried out at the **Sheep Research Farm** of the Animal Production Department, Faculty of Agriculture, Al-Azhar University. The Sheep chosen for the trial were all crossbreeds of the Ossimi breed.

**Experimental animals**

Twenty adult, dry, non-pregnant ewes, 3-4 years of age, with body weight ranging between 45 to 50kg, were chosen from the ewes flock. The animals were divided equally into 2 groups, each of 10 ewes, with nearly equal average age and live body weight. All animals were kept in semi open sheds having free access to shade and sun rays.

### Feeding regime

During the trial, all the animals were given a diet according to their average body weight, composed of a standard concentrate mixture (600 gm) plus 100 gm of Berseem hay (*Trifolium Alexandrinum*) plus 300 gm of chopped bean straw. This diet contained 1000 gm dry matter, 515 gm TDN and 100 gm CP, This diet covered the maintenance and production requirements according to NRC (1985). All groups received the basal diet which contained 0.044 mg selenium / kg dry matter.

### Treatments

The trial started from 30<sup>th</sup> May, 2013 and ended 30<sup>th</sup> May, 2014 (364 days wool growth period) and included 2 groups of ewes treated as follows:

- 1- Untreated group** received the basal diet only without any supplements ( **control** ) .
- 2-The treated group** was supplemented with 0.33 gm selenoprotein / kg dry matter/ head/day (0.7mg available selenium) as it was suggested by many authors.

### Collection of wool samples from the dams

The experimental ewes were shorn on May 29<sup>th</sup> 2013 and tattooed on the right mid side position 10×10cm<sup>2</sup> (100cm<sup>2</sup>). The tattooed areas were shaved to the skin, and left for one year to allow the wool to grow. On the 29<sup>th</sup> of May 2014, the ewes were shorn and the grease fleece weights were recorded, meanwhile the tattooed areas (100 cm<sup>2</sup>) were shaved to the skin and the shaved wool was collected in plastic sacks with all information recorded on a paper kept with the wool inside each sack.

\* Corresponding author.  
E-mail address: [dr\\_taher\\_nrc@yahoo.com](mailto:dr_taher_nrc@yahoo.com)  
DOI: 10.21608/jappmu.2020.95824

**Collection of blood samples from the ewes**

Blood samples were collected on 28<sup>th</sup> of May 2014 . Ten ml blood samples were collected from five ewes of each group to estimate Glutathione peroxidase activity (GPX) in blood serum.

Samples were taken via jugular venipuncture using 18 gauge needles and 10mL heparin vacutainers. Samples were centrifuged at 3,500 rpm for 15 minutes at 4oC. Plasma supernatant was frozen at -20oC for analysis. Unfortunately, analysis was not conducted at time of publication.

**Procedures:**

**Physical measurements of the wool ;**

The physical measurements included fibre diameter, staple length, the grease wool weight /100cm<sup>2</sup> and clean wool yield /100cm<sup>2</sup>. The grease fleece weight was recorded and the clean fleece weight was calculated on basis of the 100cm<sup>2</sup> samples at the end of the expeiment.

**Fibre diameter**

Fibres were cut into three parts (tip, middle and root portions). Each portion was measured on all fibres. Fibre diameter was measured for each portion separately using Nikon profile projector Model V-12 provided with a digital micrometer. Fibre diameter was measured on a screen and recorded on a computer sheet .The average fibre diameter in microns for each portion was then found together with the standard error of the mean.

**Staple length**

Staple length was measured on 20 staples from each sample using a centigraded ruler to the nearest 0.25 cm. The mean staple length was then calculated with the standard error for each treatment.

**Fibre length**

Fibre length was measured on 100 individual fibres from each sample using a centigraded ruler to the nearest 1 mm. The mean fibre length was then calculated with the standard error for each treatment.

**Grease wool weight per 100 cm<sup>2</sup>**

The grease wool weight shaved per 100 cm<sup>2</sup> of skin was weighed using a digital read –out balance to the nearest 0.01 gm.

**Clean wool yield per 100 cm<sup>2</sup>**

Clean wool yield per 100 cm<sup>2</sup> of shaved skin was found after extraction of grease by Soxhelt apparatus using ethyl ether b.p: 64 as a solvent .The extraction was done on samples previously freed from other contaminants. The clean wool yield was then calculated from the yield of 100 cm<sup>2</sup> samples as follows: Clean scoured yield = Weight of scoured and dried sample / Weight of greasy sample \* 100. The percentage increase in clean fleece weight was then calculated from the following equation: Percentage increase in clean fleece weight = the clean fleece weight - The clean fleece weight of the control group / Clean fleece weight of the control group \* 100.

The contaminants percentage was calculated from the following equation: The grease fleece weight - The clean fleece weight / Grease fleece weight \* 100.

**Grease fleece weight:**

Grease fleece weight was recorded at the time of sampling using top-pan balance to the nearest 50 grams. The yield was then calculated from the yield of 100 cm<sup>2</sup> shaved samples of skin. The daily wool growth rate was then

calculated by dividing the weight of wool growth rate by 364 days.

**Mechanical properties**

The mechanical properties included strength and elongation of the staple. Tensile strength was measured on representative specimens using INSTRON machines series 4460 controlled by a flexible and easy to operate software complying with all the current international standards. Wool samples were prepared so as sample length was 1 inch (25.4mm), with 0.5 cm thickness and sample weight of 200 mg. The clamp speed was (500 mm/ min.). Maximum, minimum and average forces (N), besides maximum elongation (mm/s) were computed by the tester.

**Glutathione peroxidase in Serum of the experimental ewes :**

On 28<sup>th</sup> of May , blood samples were collected from jugular vein of five ewes of each group to estimate Glutathione peroxidase enzyme activity (GPX) in their blood serum using Colorimetric Technique (Spectrophotometer Jenway 6300 U.K). The concentration of GPX was calculated according to the following equation (Pamuku and Yarim, 2001):

$$1\mu\text{u/ ml} = 1\mu\text{mol NADPH/min/ml.}$$

**Sulphur in wool of the experimental ewes**

Sulphur was estimated in tips and bases of five samples of each group using the Turbidimetric (Baso4) Method according to Zygmunt, M., (1986).

**Statistical analysis**

Data were analyzed using Independent two sample T-test of SPSS, (1999) (Statistical Product and Service Solutions).The following equation was used:

$$t = \frac{x_1 - x_2}{\sqrt{S^2 \left[ \frac{1}{n_1} + \frac{1}{n_2} \right]}}$$

Where ,

X<sub>1</sub> is the average of group 1 for each studied trait.

X<sub>2</sub> is the average of group 2 for each studied trait

N<sub>1</sub> is the number of the individuals of group 1

N<sub>2</sub> is the number of the individuals of group 2

**RESULTS AND DISCUSSION**

**Physical properties**

**Grease and Clean fleece weight (GFW),(CFW);**

Table (1) showed that grease and clean fleece weights did not differ significantly (P≤0.05) between groups, either treated or not treated. The mean GFW of untreated group was 1642.20±44.11 gm, while after treatment (supplementing the diet with 0.33 gm selenoprotein /1 Kg DM of diet) which contained (0.7 mg available selenium), the grease fleece weight recorded a mean of 1538.60±15.06gm, showing no significant difference between the two means.

**Table 1. Means ± standard errors of Grease fleece weight (GFW)(gm), Clean fleece weight (CFW)(gm) in two groups of dry ewes ;**

| Treatment       | GFW (gm)                   | CFW (gm)                   | C%    |
|-----------------|----------------------------|----------------------------|-------|
| Untreated group | 1642.20±44.11 <sup>a</sup> | 1365.90±36.58 <sup>a</sup> | 20.22 |
| Treated group   | 1538.60±15.06 <sup>a</sup> | 1250.50±12.04 <sup>a</sup> | 26.63 |

Means within columns with similar superscripts are not significantly different.

C%= Percentage of contaminants.

The clean fleece weight (CFW) in untreated group was (1365.90±36.58gm) vs. (1250.50±12.04 gm) compared to the supplemented one , the difference however

was not significant. The percentage of contaminants was 20.22 % in control group and 26.63% in treated group , both differences however were not significant since all the animals were in the same environment.

**Means and standard errors of Staple and Fibre length (cm) are shown in table (2);**

The untreated group recorded 11.53±0.35 cm, while the treated group recorded a non-significant increase in STL of 1.07 cm, and the mean STL was 12.60±0.24 cm. These results indicated that supplementing the diet with 0.7 mg selenium led to insignificant increase in staple length. Fibre length followed the same trend as staple length with the differences between treated and untreated animals being insignificant.

**Table 2. Means ± standard errors of Staple and Fibre length (STL),(FL) (cm) in two groups of dry ewes ;**

| Groups          | Staple Length(cm)       | Fibre Length(cm)        |
|-----------------|-------------------------|-------------------------|
| Untreated group | 11.53±0.35 <sup>a</sup> | 12.25±0.36 <sup>a</sup> |
| Treated group   | 12.60±0.24 <sup>a</sup> | 13.95±0.39 <sup>a</sup> |

Means within columns with similar superscripts are not significantly different.

**Table 3. Means ± standard errors of Fiber diameter (FD) (µm) in Base , Middle and Tip portions of wool fibers in two groups of dry ewes, supplemented and not supplemented with selenium;**

| Groups          | Base (FD)               | Middle (FD)             | Tip (FD)                | D= (B - T)% |
|-----------------|-------------------------|-------------------------|-------------------------|-------------|
| Untreated group | 31.32±1.25 <sup>a</sup> | 29.86±1.24 <sup>a</sup> | 28.15±0.66 <sup>b</sup> | 10.12       |
| Treated group   | 32.40±1.68 <sup>a</sup> | 31.28±1.73 <sup>a</sup> | 30.34±1.76 <sup>a</sup> | 6.36        |

D= (B - T) % = the percentage of difference between (base – tip).

Means within columns with different superscripts are significantly different (P≤0.05)

Results in table (3) showed that there was no significant difference between the treated (32.40±1.68 µm) and the untreated group(31.32±1.25 µm)at the base portion. This result indicated that addition of selenium in the form of selenoprotein to the diet of dry ewes have showed a slight insignificant effect on fibre diameter.

At the middle portion of the fibre, no significant differences were noticed between fibre diameters of the untreated group and the same group which was treated with selenium (31.28±1.73 µm and the untreated group 29.86±1.24 µm of dry ewes.

At the tip portion of the fibre, the difference between the treated (30.34±1.76 µm) and the untreated ewes (28.15±0.66 µm) in fibre diameter was not significant. It was also obvious from table (3) that there was a decrease in fibre diameter from base to tip being (10.12 µm) in the untreated group of ewes vs. (6.36 µm) in the treated group of ewes. Fibre diameter reduction was noticed although the treated group of ewes showed far less reduction in fibre diameter of the tip portion (2.06 µm / 6.36%) as compared to the untreated ewes which had an average reduction in fibre diameter of (3.17 µm /10.12%).

This result in table (3) indicated a gradual reduction in fibre diameter from base to tip of the fibre. This reduction however, was less in the treated group of ewes. This emphasized the effect of adding selenium to the diet of dry ewes on ameliorating the effect of environmental factors, eg, solar radiation and ambient temperature on the tip of fibres,

thus reducing the amount of degradation that might affect the fibre.

Reactive oxygen species (ROS) are released during processes such as respiration, apoptosis, cell signaling, and host defence, and cause tissue damage and pathogenesis when present at quantities above that which the cell can regulate (Novo &Parola 2008). They are also produced when photosensitisers are triggered by light (termed photo-oxidation) (Davies & Truscott 2001). Peptide residues that are particularly susceptible to oxidation include the sulfur-containing cysteine and methionine and aromatic residues such as tyrosine, tryptophan, phenylalanine, and histidine. The oxidation products of these amino acids include dihydroxyphenylalanine (Berlett & Stadtman 1997; Domingues *et al.* 2003; Žegota *et al.* 2005), which is precursor for pigment formation in wool.

**Table 4. Means ± standard errors of Fiber diameter (FD) (µm) in Base , Middle and Tip portions of wool fibers in two groups of dry ewes , supplemented and not supplemented with selenium;**

| Groups          | Base (FD)               | Middle (FD)             | Tip (FD)                | D= (B - T)% |
|-----------------|-------------------------|-------------------------|-------------------------|-------------|
| Untreated group | 31.32±1.25 <sup>a</sup> | 29.86±1.24 <sup>a</sup> | 28.15±0.66 <sup>b</sup> | 10.12       |
| Treated group   | 32.40±1.68 <sup>a</sup> | 31.28±1.73 <sup>a</sup> | 30.34±1.76 <sup>a</sup> | 6.36        |

D= (B - T) % = the percentage of difference between (base – tip).

Means within columns with different superscripts are significantly different (P≤0.05).

**Table 5. Means ± standard errors of Sulphur (S) (%) in Base and Tip portions of wool fibers in two groups of dry ewes ;**

| Groups          | Base %(S)              | Tip %(S)               | d. (B-T)% |
|-----------------|------------------------|------------------------|-----------|
| Untreated group | 4.14±0.28 <sup>a</sup> | 3.30±0.28 <sup>b</sup> | 0.84      |
| *Treated group  | 4.40±0.32 <sup>a</sup> | 3.98±0.18 <sup>a</sup> | 0.42      |

Means within columns with different superscripts are significantly different (P≤0.05).

**Glutathione Peroxidase; GPX (µu):**

It was clear from this table (6) that the ewes which received selenoprotein (selenomethionine) showed higher levels of glutathione activity 8.67±0.94 (µu/ml). Lower GPX level was recorded for the untreated group being 6.15±0.71(µu/ml) . It is well known that GPX is an enzyme naturally occurring sulphur amino acid tripeptide composed of L-cystiene, L-glutamic acid and glycine. Glutathione peroxidase has a serious role in the prevention and treatment of degenerative disease and act as anti-oxidant and deactivator of free radicals (Rotruck, 1973). Selenium is also an essential element for the production of GPX, it is therefore reasonable to believe that addition of selenoprotein to the diet of dry ewes caused a significant (P≤0.05) increase in the GPX activity which in turn would give some protection of the wool fibres from degradation .

**Table 6. Means ± standard errors of Glutathione Peroxidase (GPX) Enzyme level of activity (µU/ml) in blood plasma in two groups of dry ewes ;**

| Groups          | GPX(µU/ml)             |
|-----------------|------------------------|
| Untreated group | 6.15±0.71 <sup>c</sup> |
| Treated group   | 8.67±0.94 <sup>b</sup> |

Means within columns with different superscripts are significantly different (P≤0.05).

This result is in accordance with other results of this study specially the sulphur content of the fibre base and tip portions which showed the preservation of most of the sulphur content in tip portions of the fibres in the animals which received selenoprotein and were exposed to natural atmospheric conditions.

**Table (7) Staple strength (gm/tex) and Fibre elongation %:**

The results in table (7) showed that although staple strength was increased in treated dry ewes compared to the untreated group of dry ewes, yet the differences were not significant.

The treated group of ewes had a mean staple strength of (1.35±0.48 gm/tex) vs. the untreated group of dry ewes which recorded (1.13±0.24 gm/tex), the differences however, were not significant. On the other hand, elongation percentage which is correlated to the sulphur content of the fibre showed a slight non-significant reduction in elongation percentage in the treated group of ewes which were negatively parallel to the staple strength values. This was an expected result since the more strong the fibre is, the less elongation that is exhibited by the fibre. It is clear; however, that adding selenoprotein to the diet of dry ewes did not significantly affect the fibre strength but caused an insignificant reduction in fibre elongation percentage, to a degree that would not affect the fibre behavior during further steps of processing. The way in which fibre diameters change over the year of wool growth, hereafter termed fibre diameter profile (FDP), influences wool quality. The characteristics of this FDP have been shown to influence staple strength (Hansford and Kennedy 1988; Denney 1990; Peterson 1997). Hansford, (1994) reported that the way in which sheep interact with their environment varies between individual animals within and between groups. FDP can be used to examine these differences between sheep. Differences in the level of a long staple variation in fibre diameter have been observed between individual sheep and sire groups (Denney 1990), selection lines (Jackson and Downes 1979). The variation of fibre diameter along FDPs has been used as an indicator of their sensitivity to environmental change.

**Table 7. Means ± standard errors of staple strength (SST) (gm/tex) and Elongation (ELO) (%) in two groups of dry ewes;**

| Groups          | staple strength (SST)<br>(gm/tex) | Elongation (ELO)<br>(%) |
|-----------------|-----------------------------------|-------------------------|
| Untreated group | 1.13±0.24 <sup>a</sup>            | 16.05±1.58 <sup>a</sup> |
| Treated group   | 1.35±0.48 <sup>a</sup>            | 13.82±1.17 <sup>a</sup> |

Means within columns with similar superscripts are not significantly different.

**CONCLUSION**

Supplementing the diet of dry-non pregnant ewes with selenium for a long-time in normal environmental conditions had no great effects on wool production and quality since animals lived without suffering from environmental stresses.

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### تأثير الإمداد طويل الأمد بالسيلينيوم علي إنتاج الصوف ونوعيته في الأغنام الطاهر محمد سعودي و ربيع محمد غيطاس قسم الإنتاج الحيواني - كلية الزراعة - جامعة الأزهر بالقاهرة

يعتبر السيلينيوم عنصرا ضروريا لنمو الصوف وخصائصه خاصة أثناء تعرض الحيوانات للضغط. عشرون من النعاج الجافة غير الحوامل تراوحت أعمارها من ثلاث إلى أربع سنوات. وأوزانها تراوحت بين ٤٥ الي ٥٠ كيلو جرام تم اختيارهم للتجربة وقسموا إلي مجموعتين بكل مجموعة عشرة نعاج. تم تغذية النعاج علي عليقة مكونة طبقا لمقررات المجلس القومي الأمريكي (١٩٨٥). تم تغذية المجموعة المقارنة علي العليقة المكونة دون أية إضافات. تم تغذية المجموعة المعاملة علي العليقة المكونة بالإضافة الي ٠,٣٣ جرام من السيلينيوم و٠,٧ ملليجرام من السيلينيوم المتاح. استمرت التجربة لمدة عام كامل. تم قياس كل من وزن الصوف الخام ووزن الصوف النظيف وقطر الألياف وطول الخصلة والمتانة والإستطالة كما تم قياس مستوي إنزيم الجلوتاثيون بير أوكسيديز في سيرم الدم وذلك لدراسة أثر إضافة الإمداد طويل بالسيلينيوم علي إنتاج الصوف ونوعيته في الأغنام. لم تؤثر الإضافة معنويا علي كل من وزن الصوف الخام والنتيظف أو طول الخصلة وطول الألياف. كان هناك اختلافا معنويا بين المجموعة المقارنة والمعاملة عند مستوي معنوية ٥% وأضا كان هناك اختلافا في المحتوى من الكبريت في قمم الألياف. كان هناك اختلافا معنويا عند مستوي معنوية ٥% في مستوي إنزيم الجلوتاثيون بير أوكسيديز في سيرم الدم في المجموعة المعاملة. لم يكن هناك اختلافا معنويا بين المجموعتين في كل من المتانة أو الإستطالة. إضافة السيلينيوم لم تؤثر بدرجة كبيرة في هذه التجربة وذلك لأن الحيوانات كانت تعيش تحت الظروف الطبيعية ولم تعاني من أية ضغوط.