Effect of supplementing the diet of pregnant ewes with selenoprotein on wool production of the dams and their lambs.

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Abstract

A trial was carried out on 40 pregnant crossbred ewes aged 3-4 years and weighed 45-50kg.The animals were divided equally into 4groups, each of 10 ewes and fed on a formed diet according to **NRC (1985)** during pregnancy and the first month of lactation . The 1st group was fed on the formed diet without any supplements; meanwhile the 2nd ,3rd and 4th groups were fed on the formed diet+0.14, 0.23 and 0.33 gm selenoprotein / kg dry matter (0.3, 0.5 and 0.7mg available selenium) respectively in order to study the effect of adding different levels of selenium to the diet of pregnant ewes on wool production and quality in 184 days wool growth period and the number of primaries, secondaries and S/p ratio of their lambs at 7 days old. The results indicated no significant effect of the different levels of selenium supplement on grease fleece weight, fibre diameter ,staple strength and elongation. Addition of the levels of selenium (0.5 and 0.7mg se/ kg dry matter) to the diet of pregnant ewes increased significantly (**P≤0.01**) staple length and clean fleece weight (**P≤0.05**). The level of glutathione peroxidase enzyme (**GSH-Px**) increased in blood serum as a result of supplementing the diet with selenoprotein . The number of primary wool follicles in the lambs was not affected by adding selenoprotein to the diet of their mothers. Maternal diet supplemented with selenoprotein significantly increased the number of secondaries and S/p ratio (**P≤0.01**).

Key words: selenoprotein supplement – wool production – S/p ratio .

Introduction

It is well-known that the main factors affecting wool production (quantity and quality) in sheep are the density and ratio of secondary to primary wool follicles (**Butler, 1982**).In the feotus, primary wool follicles initiate and complete development during mid to late pregnancy, whereas secondary wool follicles start to initiate 10 days after the initiation of the first primary and continue during late gestation, with the maximum rate of maturation occurring from 1 to 3 weeks after birth (**Short, 1955; Hardy and Lyne, 1956**). Because development of wool follicles occurs during fetal life, maternal nutrition can affect wool follicles and wool production of the offspring (**Narayan, 1960; Schinckel and Short, 1961; Lyne, 1964**). Selenium (Se) is an essential trace element that has diverse biological functions for proper health of animals and human,especially during pregnancy.Maternal selenium affects performance and health of the progeny because all nutrients required by the developing foetus are transferred by the dam via the placenta, the colostrum and the milk (**Hamal *et al.*, 2006**). Selenium acts as the catalytic center in the active sites of several antioxidant enzymes and proteins such as glutathione peroxidase (GSH-Px) (**Rotruck *et al.*, 1973**] and (**Salvatore *et al.*, 1996**). **Pappas *et al.*, (2008**) reported that GSH-PX function as antioxidant during embryonic development. These antioxidant enzymes respond to oxidative stress by neutralizing and eliminating reactive oxygen species (ROS), (**Surai,2006**).Glutathione peroxidase enzyme is involved in differentiation of embryonic tissues through its effect as an antioxidant in the fetal skin causing low ROS, which can regulate IGF1 (Insulin like growth factor 1) and IGF-1R (Insulin like growth factor 1 receptor) , thus improves the fetal hair follicle development (**Moghadaszadeh and Beggs, 2006**). In addition , it has been established by (**Corbett, 2000**) that there can be substantial

reductions in wool growth rate during both the latter half of pregnancy and early lactation. Overall, reproduction usually reduces annual fleece growth of ewes by 10 to 14%; the greatest reduction being for ewes rearing twins. Consequently, the present study was carried out to evaluate the effect of supplementing the diet of pregnant ewes with selenoprotein on wool production of the dams and their lambs.

Materials and Methods

The present study was carried out on 40 adult dry pregnant crossbred ewes,3-4 years old ,with body weight ranging between 45 to 50kg. The animals were divided equally into 4 groups ,each of 10 ewes, with almost equal average age and live body weight. All animals were kept in semi open sheds having free access to shade and sun rays. During the first 15 weeks of gestation, all animals were given maintenance diet according to their average body weight, composed of a standard concentrate mixture (600 gm) plus 100 gm of Berseem hay (Trifolium alexandrinum) and 300 gm of chopped bean straw .This diet contained 1000 gm dry matter, 515 gm TDN and 100 gm CP. During the last 5 weeks of gestation, the diet contained 1400 gm dry matter, 880 gm TDN and 196gm CP **NRC(1985)** modified requirements. The addition of selenoprotein to the diet was started after the first month of gestation. All groups received the basal diet which contained 0.044 mg selenium / kg dry matter. The control group received the basal diet only. The 1st treated group was supplemented with 0.14 gm/ head /day of selenoprotein (0.3mg available selenium);the 2nd group: was supplemented with 0.23 gm/head/day of selenoprotein(0.5mg available selenium) and the 3rd group: was supplemented with 0.33 gm/head/day of selenoprotein (0.7mg available selenium). The trial lasted for 184days from August 25th (2012) to 25th 0f February(2013). At day 121 of gestation, blood samples were collected from five ewes of each group to

estimate Glutathion peroxidase Enzyme (GPX) in serum using Colorimetric Technique (spectrophotometer Jenway 6300 U.K) . The concentration of GPX was calculated according to the following equation : 1μu/ ml = 1μmol (nicotinamide adenine dinucleotide phosphate-oxidase) NADPH/min/ml **(Pamuku and Yarim, 2001).**One week post lambing , six single female lambs were chosen randomly from each group. Skin biopsies were taken from the mid-right side position of the lambs and immediately fixed in 10 percent neutral buffered formalin solution. The specimens were then processed through serial steps for histological examinations according to **(Luna, 1968)** and **(Mallory, 2010)** .The secondary and primary follicles were counted per square millimetre in all fields of the slides using a light microscope. The animals were shorn at the first day of the experiment and by the end of six months wool growth period. The experimental ewes were tattooed on the right mid side (100 cm^2) , then the tattooed areas 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. Ewes were then shorn and grease fleece weight was recorded.

Physical measurements: The physical measurements included, fibre diameter(FD), staple length (STL) ,grease and clean fleece weights (GFW and CFW). Fibre diameter was measured using a Nikon profile projector Model V-12 provided with a digital micrometer. Fibre diameter was measured on 40 samples representing 4 treatments and the results were recorded automatically .The average fibre diameter in microns was then calculated together with the standard deviation and coefficient of variability . Staple length was measured using a centigrade ruler. The mean staple length was then calculated with the standard error for each treatment. Grease fleece (184 days wool growth period) weight was recorded at the

time of sampling. The yield was then calculated from the yield of $100cm^2$ samples.

Mechanical properties: The mechanical measurements included, strength and elongation of the staple. Tensile strength was measured on representative specimens using INSTRON series 4460 controlled by a flexible and easy to operate soft ware 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 0.2 gm . The clamp speed was (500 mm/ min.). Maximum, minimum and average force (N),besides maximum elongation (mm/s)were computed.

Statistical analysis: SPSS(statistical packaged and service solutions) Program was used in data analysis (**SPSS, 1999).** One-way analysis of variance was used to test the effect of different levels of selenoprotein on wool quantity and quality in pregnant ewes and their lambs. The following equation was used $x_{ij}= \mu+\alpha_i+e_{ij}$ where x_{ij} is the observed value of the trait , μ is the grand mean of the trait , α_i is the effect of selenoprotein and eij is the experimental error. Comparison among means was followed using Duncan's new multiple range test , **Duncan (1955)**.

Results and Discussion:

1- Selenoprotein in the diet of pregnant dams and its effect on their wool production :

A- Physical properties:

Results in table (1) showed that the addition of different levels of selenoprotein to the diet of pregnant ewes (0.14 , 0.23 and 0.33 g selenoprotein / kg dry matter) did not affect grease fleece weight significantly.The grease fleece weights of the selenoprotein treated ewes

(groups 1,2,3) were (**450.32 ± 25.80**, **451.20 ± 23.22** and **453.06 ± 24.10 gm**) respectively and (**445.27 ± 19.06**) for the control group.

Results in table (1) showed that there were significant differences (**P≤0.05**) among treatments further in clean fleece weights, (**353.40±15.38 , 364.35 ± 8.88** and **366.96± 12.76 gm**) respectively relative to the control group (323.66±7.25). The increase in CFW in the treated groups relative to the control group was **9.19% , 12.57%** and **13.38 %** respectively. The percentage of contaminants in control group **27.31** ,which was higher than those in the selenoprotein treated groups (**21.52% , 19.25%** and **19.00%**) , respectively for groups 1,2 and 3.

Table (1) Means ± standard errors of GFW(gm), CFW(gm), percentage increase in CFW and contaminants (%) (184 days wool growth period):

Treatment	GFW(gm)	CFW(gm)	Percentage increase in CFW relative to control	Contaminants relative to control (%)
Control	445.27 ± 19.06^{a}	323.66±7.25 ^{b*}	-	27.31
Group 1	450.32 ± 25.80^{a}	353.40± 8.88^{ab*}	9.19	21.52
Group 2	451.20 ± 23.22^{a}	364.35±15.38^{a*}	12.57	19.25
Group 3	453.06 ± 24.10^{a}	366.96± 12.76^{a*}	13.38	19.00
Overall mean	450.00 ± 11.14	352.095± 6.18	-	-

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

GFW: grease fleece weight and **CFW**: clean fleece weight.

*: significant means

Results in table (2) showed that the addition of different levels of selenoprotein to the diet of pregnant ewes increased staple length significantly (P≤0.01) .The staple lengths of the selenoprotein treated

groups were (3.85 ± 0.37 , 4.42±0.17 and 4.75 ± 0.35 cm), respectively compared to (3.00 ± 0.28cm) for the control group. It was clear that staple length increased significantly by increasing the level of selenoprotein to the diet of pregnant ewes .Results in table (2) showed that there were no significant differences among treatments in fibre diameter (P≤0.647). It was noticed that fibre diameter showed slight insignificant increase with the increase of selenoprotein level in the diet

Table(2) Means ± standard errors of staple length (cm) and fibre diameter (μm) as affected by selenoprotein level in the diet of treated dams.

Treatment	STL(cm)	FD(μm)
Control	3.00 ± 0.30 ^{b**}	27.90 ± 2.30^{a}
Group 1	3.85± 0.37 ^{ab**}	29.80 ±1.60^{a}
Group 2	4.42±0.17 ^{a**}	30.40 ±2.00^{a}
Group 3	4.75 ± 0.35 ^{a**}	31.40 ±1.80^{a}
Overall mean	4.00 ± 0.18	29.90 ± 1.00

Means within columns with different superscripts are significantly different(P≤0.01).

Morel and Barouki, (1999) clarified that selenoproteins have a key metabolic role as antioxidants and affect the redox status of the cells. Uncontrolled peroxidation during severe selenium deficiency causes necrosis due to oxidative damage to cellular macromolecules. A lesser

deficiency may result in a milder oxidative stress caused by increased concentrations of hydrogen peroxides and lipids. Oxidative stress causes gene repression through modulation of transcription factors. Such changes may induce temporary growth arrest and lengthening of the cell proliferation cycle in the wool follicle. **Pietro *et.al.*(2010),** showed that selenium supplementation to the diet of weaned merino lambs had a positive influence on GFW without significant increase in FD . This is extremely important for fine wool producers since price penalties are received for a higher FD. Wool is very sensitive to selenium deficiency and Se supplementation significantly increased wool production , **(Hill *et al.* 1969 ;Wilkins & Kilgour, 1982 and Langlands *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). Paganoni *et al.* (2000)** , showed that the increase in yield with Methionine or canola meal feeding was caused by a decrease in wax. **Sherlock *et al.* (2001)**found that infusion with cysteine decreased non –fibre material and increased yield in Romney ewes. Similar changes had been observed by **Masters and Mata (1996)**, when they added methionine to the diet or through feeding canola meal.

B- Mechanical properties:

Results in table (3) showed that there were no significant differences among groups in SST ($P \leq 0.815$) ,although there was a tendency for SST to increase with the increase in the level of selenoprotein in the diet of treated groups. Staple elongation percentage slightly increased by adding selenoprotein to the diet of pregnant ewes, but did not reach significance . The staple elongation of the selenoprotein treated groups were (17.52±3.58%, 21.27±5.08% and 25.48±6.05%) compared to control group (15.94±4.87%).

Table(3) Means ± standard errors of staple strength (gm / tex) and elongation (%) in four groups of pregnant ewes.

Treatment	Staple strength (SST) (gm / tex)	Elongation (ELO) (%)
Control	0.11±0.04	15.94±4.87
Group 1	0.13±0.04	17.52±3.58
Group 2	0.16±0.04	21.27±5.08
Group 3	0.17±0.05	25.48±6.05
Overall mean	0.14±0.02	20.05±2.46

Means within columns are not significantly different.

The trend of staple elongation was parallel to that of staple strength. Organic Se sources supply the element to molecules containing Se, but selenomethionine is also deposited in body proteins by substituting methionine in protein molecules. Consequently, the Se concentration in tissues and body fluids is higher when Se is fed in the organic form compared to the inorganic form **(Beilstein and Whanger, 1986; Van Ryssen *et al.*, 1989).** The strength of wool fibers is influenced markedly by the nutrient supply via effects on fiber diameter and changes in intrinsic strength **(Reis,*et al.* 1992). Helal (2008)** also found that the use of ground date stones as a source for methionine and cysteine tended to increase staple strength compared with yellow corn . **Helal,(2005)** reported that elongation of wool staples tended to increase with the increase of wool methionine content. The present findings indicated that the insignificant increase in SST with the increase in the level of selenoprotein in the diet of treated groups was parallel to the insignificant increase in fibre diameter.

c- Glutathion Peroxidase Enzyme (μU/ml) of the pregnant ewes :

Results in table (4) showed that **Glutathion Peroxidase Enzyme (GPX)** level in the serum of pregnant ewes was increased in groups 1 and 3 of the

selenoprotein treated ewes as compared to the control group . GPX level in the control group ranged between 0.32 and 1.12. In 0.14 g selenoprotein treated group , the GPX level ranged between 1.28 and 3.37 (μU/ml). The level in 0.23 g selenoprotein treated group was between 0.64 to 1.12 (μ/ml) and in the 0.33 g selenoprotein treated group , it ranged between 1.6 and 4.18 (μU/ml). The concentration of selenium (Se) in whole blood, plasma or serum and the liver of animals, as well as glutathione peroxidase (GSH-Px) activity in the erythrocytes, is widely used by diagnostic laboratories to predict the Se status of animals **(Gerloff, 1992)**.In the present study, the highest levels of GPX were noticed in groups 1 and 3 of the selenoprotein treated groups. The higher expression of GSH-Px in skin indicated that maternal supplementation of selenoprotein in the diet could improve antioxidant status by reducing the level of ROS, which can act as an intracellular signal **(Thannickal and Fanburg, 2000).**

Table (4) :Random samples of glutathione peroxidase enzyme level (μU/ml) of control and treated sheep

Control	0.14 gm sep.	0.23 gm sep.	0.33 gm sep.
0.64	1.28	0.96	2.41
1.12	3.37	1.12	4.18
0.32	1.28	0.8	1.6
0.64	1.28	0.64	1.44
0.64	1.6	0.8	1.6

2- The effect of adding selenoprotein to the diet of pregnant ewes on the number of primaries , secondaries and s/p ratio of their lambs.

No significant differences were found among groups in the number of primary follicles per mm^2 as shown in table (5) . The numbers of primaries were (49.12 ± 4.14 , 45.00 ± 0.70 , 43.62 ± 3.68 and 52.62± 3.26) for the control,0.14 , 0.23 and 0.33 g selenoprotein treated groups, respectively .The number of secondary wool follicles per 1 mm^2 was increased ($P < 0.01$)in Selenoprotein treated groups, being 46.31 ± 4.16, 63.75± 1.45 and51.12± 4.38 ,respectively for treated groups 1,2 and 3 as compared to the control one (26.37 ±5.57).

Table (5) Mean ± standard errors of primaries per 1mm^2 , secondaries per 1mm^2 and secondary / primary follicles (s/p ratio) in the skin of the lambs born to selenoprotein treated dams.

Treatment	Primaries	Secondaries	S/ P ratio
Control	49.12 ± 4.14 ^{a}	26.37 ±5.57 ^{c**}	0.54 ± 0.11 ^{c**}
Group 1	45.00 ± 0.70 ^{a}	46.31 ± 4.16 ^{b**}	0.71 ± 0.04 ^{bc**}
Group 2	43.62 ± 3.68 ^{a}	63.75± 1.45 ^{a**}	1.49±0.14 ^{a**}
Group 3	52.62± 3.26 ^{a}	51.12± 4.38 ^{ab**}	0.96 ± 0.05 ^{b**}
Overall mean	47.59 ± 1.71	46.89± 3.94	0.93 ± 0.41

Means within columns with different superscripts are significantly different($P < 0.01$).

Thomas,(2002), indicated that there is either a limited number of follicle inductive cells in the skin (e.g. stem cells) or that the molecular signals required to initiate follicle formation are developmentally regulated and active only during foetal development. The number of secondary hair follicles was less dependent on genetic factors than primary follicles **(Purvis and Jeffery, 2007**). Secondary follicle initiation and branching is adversely affected by maternal nutrition and physiological stresses including high ambient temperature and maternal hypoxia late in pregnancy **(Schinckel, 1955; Short, 1955).** They added also that the secondary follicles initiated late in pregnancy having a lower fibre diameter ,the consequences of poor herd management during this time (day 70 to birth)led to decrease wool production and increase the average fibre diameter .The ratio of secondary to primary hair follicle appeared to be affected by maternal Selenoprotein addition to the diet. It was 0.71 ± 0.04, 1.49±0.14, 0.96 ± 0.05 , 0.54 ± 0.11 for 014 , 0.23 , 0.33 g selenoprotein treated groups

and the control, respectively. **Xiaoying *et.al* (2011)** showed that supplementation of the diet of Cashmere pregnant ewes with 0.5 nano-selenium resulted in non significant difference in the number of primary hair follicles. However, the number of secondary hair follicles and the secondary to primary follicle (S/P) ratio was significantly higher in selenium group than those in the control group, indicating that the Nano-Se supplement could promote the development of secondary follicles. They also suggested that maternal dietary supplements of Nano-Se during gestation could be used to improve the hair follicle development and promote growth development in Cashmere goat fetuses.

Conclusion

Addition of the different levels of selenium (0.5 and 0.7mg se/ kg dry matter) to the diet of pregnant ewes increased (**P≤0.01**) staple length and clean fleece weight (**P≤0.05**).There was no significant effect of the addition of the different levels of selenium on grease fleece weight, fibre diameter ,staple strength and elongation ,although there was a tendency of FD,SST and elongation to be increased with the increase of selenoprotein in the diet of dams. The level of glutathione peroxidase enzyme (**GSH-Px**) increased in serum as a result of supplementing the diet with selenoprotein, being greater in 0.7 mg supplemented group. The number of primary wool follicles in the lambs was not affected by adding selenoprotein to the diet of their mothers. Maternal nutrition with selenoprotein supplement led to increase the number of secondaries and S/p ratio (**P≤0.01**).It is therefore reasonable to conclude that adding selenoprotein to the diet of pregnant dams can affect wool production of them and S/P ratio of their lambs.

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تأثير إضافة السلينوبروتين إلي عليقة النعاج الحوامل على إنتاج الصوف من الأمهات وحملانها

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الملخص العربي

أجريت هذه التجربة على عدد أربعين من النعاج الحوامل الخليطة والتي تراوحت أعمارها بين ثلاث و أربع سنوات وتراوحت أوزانها بين خمسة وأربعين و خمسين كيلو جراما .قسمت هذه الحيوانات بالتساوي إلى أربع مجموعات بكل مجموعة عشرة نعاج . غذيت هذه الحيوانات على عليقة مكونة طبقا لمقررات المجلس القومي الأمريكي (١٩٨٥م) أثناء فترة الحمل والشهر ألأول من الرضاعة .غذيت المجموعة الأولي علي هذه العليقة المكونة بدون أية إضافات .المجموعة الثانية غذيت على نفس العليقة بالاضافة إلى ٠,١٤ جرام سلينوبروتين لكل كيلو جرام مادة جافة (٠,٣ ملليجرام من السلينيوم المتاح) .المجموعة الثالثة غذيت على ٠,٢٣ جرام سلينوبروتين لكل كيلو جرام من المادة الجافة (٠,٥ملليجرام من السلينيوم المتاح)بجانب العليقة. أما المجموعة الرابعة غذيت بجانب العليقة على ٠,٣٣ جرام سلينوبروتين لكل كيلو جرام من المادة الجافة (٠,٧ملليجرام من السلينيوم المتاح) بهدف دراسة تأثير إضافة مستويات مختلفة من السلينوبروتين إلى عليقة النعاج الحوامل على إنتاج الصوف ونوعيته من الأمهات في فترة نمو قدرها ١٨٤ يوم بالاضافة إلى دراسة تأثير الإضافة على عدد الحويصلات الاوليه والثانوية ونسبة الحويصلات الثانوية إلى الأولية من الأبناء في عمر أسبوع . النتائج أظهرت عدم وجود فروق معنوية لتلك الإضافة على كل من محصول الصوف الخام وقطر الألياف والمتانة والاستطالة . إضافة المستويات المختلفة من السلينيوم إلى عليقة النعاج الحوامل (٠,٥ و ٠,٧ مجم لكل كيلو جرام من المادة الجافة) أدى إلى زيادة معنوية في طول الألياف عند مستوى معنوية اقل من ٠,٠١ وزيادة معنوية في محصول الصوف النظيف عند مستوى معنوية اقل من ٠,٠٥ .لوحظ أيضا ارتفاع ملحوظ في مستوى إنزيم الجلوتاثيون بيراوكسيديز في السيرم كنتيجة لإمداد العليقة بالسلينوبروتين .لم يتأثر عدد الحويصلات الأولية في الحملان كنتيجة لإضافة السلينوبروتين إلى علائق الأمهات . علائق الأمهات المدعمة بالسلينوبروتين أدت إلى زيادة معنوية في عدد الحويصلات الثانوية ونسبة الحويصلات الثانوية إلى الاوليه عند مستوى معنوية اقل من ٠,٠١.