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EFFECT OF DIETARY SELENIUM LEVELS ON SOME SKIN CHARACTERISTICS IN BARKI SHEEP

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ABSTRACT

This experiment was carried out using in Ras Suder Experimental Station, south Sinai governorate twelve weaned Barki ram lambs WITH average LBW of 13.81 ±1.60 and 4 month of age. The present work was designed to throw some lights on the histological responses and the histochemical changes in the skin of Barki lambs in relation to different selenium levels in diets. The histological measurements involved the wool follicle dimensions (external and internal diameters, wall thickness and fibre diameter). Histochemical measurements dealt with the total contents of carbohydrates and proteins in the wool follicles.

Histological examination showed that selenium supplementation to the sheep diet at a level of 0.7 ppm significantly ($p<0.05$) increased the secondary follicle dimensions as compared to level of 1.4 ppm or control group. Values of fibre diameters significantly ($p<0.05$) higher 8.16 µm in the fibres produced from the secondary follicles as compared to 5.57 and 5.35 µm in 0.7ppm and control groups, respectively. Carbohydrate concentration was in the outer root sheath of the primary and secondary follicles markedly ($p<0.05$) increased by the usage of 0.7 ppm supplementation level of selenium. However, protein concentration increased ($p<0.05$) by increasing level of selenium to 1.4 ppm only.

It could be concluded that good selenium supplementation with adequate antioxidants is important in order to improve wool follicle characteristics.

Keywords: Sheep, skin, histology, histochemistry, selenium, wool follicles.

INTRODUCTION

Minerals can influence wool growth by affecting feed intake and altering rumen function, hence the supply of nutrients flowing from the rumen or by directly disrupting metabolism within the sheep. The wool matrix contains significant quantities of selenium which possibly alter follicle function and wool growth directly.

Selenium is widely distributed throughout the body and is primarily fixed as highly labile proteins, body reserves of selenium are rapidly depleted when animals are switched from seleniferous or selenium-adequate diets to low-selenium diets. Selenium concentration in tissues tends to reflect dietary sources of selenium, which are in the form of organic compounds rather than inorganic substance such as selenate or selenite. Selenium concentration in hair appears to be correlated with dietary intake (Grace and Watkinson, 1988).

Generally, diets containing 0.1 – 0.3 ppm selenium will provide adequate selenium for various animals. Whereas, concentration of 5 to 10

growing plants. This is because the antagonistic relationship between water irrigation and salinity selenium available in the soil (Mikkelsen *et al.*, 1988 and Sadek, Laila, 1995). Compared with non-ruminants, the absorption of selenium is much lower in ruminant animals (Jerry, 2003).

Selenium supplementation was reported to increase wool growth (Langlands *et al.*, 1991a and 1991b and Whelan *et al.* 1994). Wool production is more susceptible to selenium deficiency in growing ewes than in matures (Wilkins *et al.*, 1982). In this respect, reduction in wool growth due to selenium deficiency was independent on reduction in feed intake. Fry *et al.* (1996) stated an increase in wool length and fiber diameter in response to selenium supplementation. One of the principal methods for increasing selenium intake by livestock are using selenium fertilizer as a foliar and / or soaking plant seeds in selenium solution which resulted in an increase of selenium plant content and also elevated crop productivity (Mbagwu, 1983).

This study aimed to investigate the effect of two dietary levels of selenium supplementation on the histology and chemical composition of some skin traits in Barki sheep raised in Sinai Peninsula.

MATERIALS AND METHODS

The present study was carried out at Ras Suder Experimental Station, south Sinai Governorate (180 km north eastern of Cairo) belonging to the Desert Research Center. Three areas at the station were cultivated with alfalfa (*Medicago sativa*) seeds, the first area was served as control without any treatment (G1), while the second and third areas were cultivated with alfalfa seeds treated by soaking in 0.7 ppm and 1.4 ppm selenium as sodium selenate (Na_2 Se O_4) solution, respectively. After the growth of alfalfa (30 days after plant germination), 0.1 ppm selenium as sodium selenate solution was sprayed on plants as a foliar fertilizer. The second cut of alfalfa plants was harvested, air dried, chopped into small particles (2 -5 cm) and stored for animal feeding.

Twelve weaned Barki ram lambs (4 months of age and averaged 13.81±1.60 kg body weight) were assigned to three groups (4 of each) and were fed on alfalfa harvested from the three respective areas. Animals were healthy and clinically free from internal and external parasites and kept in semi open pens covered with asbestos sheets. Fresh water was available twice daily.

Skin samples were collected from the mid side region. For histological study, skin samples were immediately fixed in Calcium formol (Barker, 1958). Specimens were dehydrated in ethyle alcohol, cleared in benzene and embedded in paraffin wax. Sections of 6-8 µm in thickness were prepared and stained with Haematoxylin and Eosin according to Drury and Wallington (1980). The histological measurements included the external and internal diameters of both primary and secondary wool follicles and wall thickness were calculated. Wool fiber diameters were measured by using an image analyzer (LEICA, Q 500 MC) with lens 40/065.

For histochemical investigation of the wool follicles, Periodic acid Shiff's reaction (Mc-Manus and Coson, 1950) was used for detecting carbohydrates as red colour and mercury bromophenol blue (Pearse, 1968) was used for the demonstration of general proteins as represent by the blue colour.

Quantitative histochemistry covers a varity of techniques in which a histochemical reaction is microscopically quantified by optical means. Skin sections representing different levels of selenium supplementation and stained for general proteins and carbohydrates were subjected to cytophotometric measurement using (LEICA, Q 500 MC). The optical density values were considered to represent the concentration of intracellular material according to lambert low (Pears, 1980).

Data were statistically analyzed using one way analysis of variance utilizing General Linear model (GLM) of SAS (2002) and differences between means were tested using Duncan's multiple range test.

RESULTS AND DISCUSSION

The histological examination of Barki sheep skin revealed that it composed of two main layers, the epidermis and dermis. The epidermal layer contained five strata of squamuse epithelial cells, while the dermal layer embraced the wool follicle groups and their associated glands, the sweat and sebaceous glands (Fig, 1). The wool follicle population in the skin was made up of trio groups. Each trio group had three primary follicles and a variable number of secondary follicles.

The use of selenium fertilizer either at 0.7 or 1.4 ppm showed non significant effect on the dimensions of the primary wool follicles, except for medulla thickness. Whereas, secondary wool follicles dimensions were significantly ($P<0.05$) affected, except for fiber diameter (Table 1).

External diameter of the secondary follicles significantly ($P<0.05$) increased to 15.57 in 0.7 ppm group as compared to 13.7 and 13.3 µm in 1.4 ppm and control ones, respectively. The internal diameter of the secondary follicles showed the same trend, being 8.36, 7.16 and 6.90 for 0.7, 1.4 ppm and control groups, respectively. However, the wall thickness of the secondary follicles showed significant ($P<0.05$) increase in 0.7 ppm group (7.22 µm) and did not differ significantly in 1.4 ppm (6.11 µm) from that in the control group (6.40 µm, Table 1).

Selenium is incorporated into molecules of an enzyme called glutathione peroxidase. This enzyme had a vital role in protection of cell membranes against undesirable reactions with soluble peroxidase (Antioxidant). Good selenium nutrition is of key importance for antioxidant defense as well as efficient energy metabolism.

Selenium deficiency reduces wool growth without a reduction in feed intake, while the exact mechanisms involved are not known. Many of selenoprotiens have key metabolic roles as antioxidants and affect the redox status of cells. Uncontrol 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

peroxides of hydrogen and lipids. Oxidative stress causes gene repression through modulation of transcription factors. Such changes may induce temporary growth arrest and lengthening of the cell cycle in the follicle.

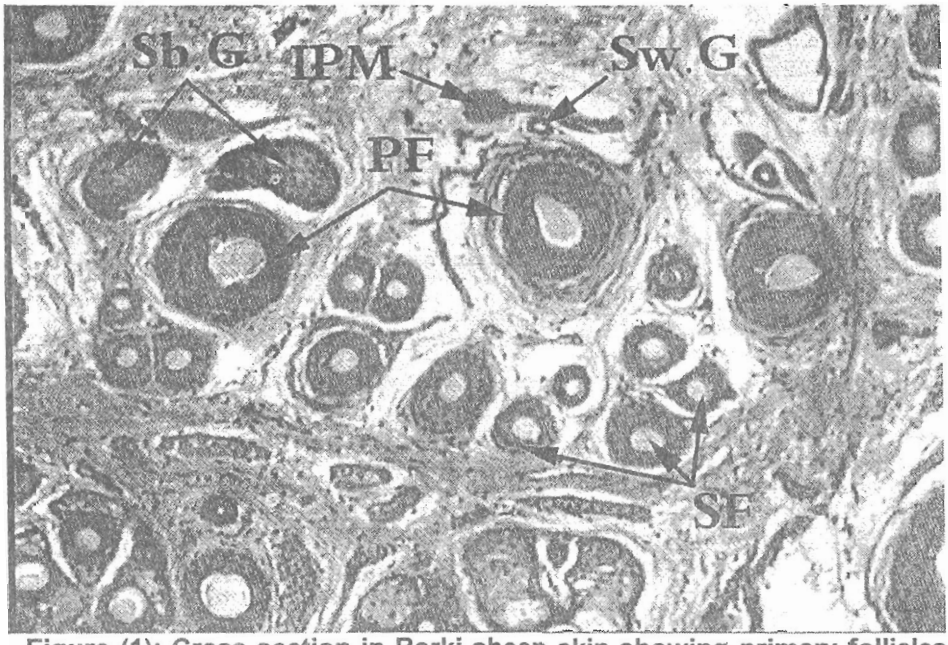

Figure (1): Cross section in Barki sheep skin showing primary follicles (PF), secondary follicles (SF), sebaceous glands (Sb.G), sweat gland (Sw.G) and irrector *pili muscle* (IPM).

It is of interest to note that both selenium supplementations showed no significant effect on the diameter of fibers produced by either primary or secondary follicles. Also, medulla thickness of the secondary follicles showed significant ($P<0.05$) decrease only in 0.7 ppm group as compared to the control group (Table 1).

Table (1): Histometric characteristics of primary and secondary follicles in skin of sheep fed different selenium levels.

Measurement (µm)		Selenium levels		
		Control	1.4 ppm	0.7 ppm
Primary follicle	External diameter	26.99±0.891	26.71± 0.913	26.63±0.925
	Internal diameter	19.33± 2.404	17.89± 2.463	17.04± 2.495
	Wall thickness	9.94±2.318	7.69±2.375	7.30±2.405
	Fiber diameter	15.33 ± 0.708	14.58 ± 0.726	14.06 ± 0.735
	Medulla thickness	10.19 ± 0.823^{a}	9.23 ± 0.843^{ab}	7.50 ± 0.854^{b}
Secondary follicle	External diameter	13.30±0.424^{b}	13.27±0.424^{b}	15.57±0.424^{a}
	Internal diameter	6.90±0.272^{b}	7.16±0.272^{b}	8.36±0.272^{a}
	Wall thickness	6.40 ± 0.305^{ab}	6.11 ±0.305^{b}	7.22± 0.305^{a}
	Fiber diameter	5.35 ± 1.167	5.57 ± 1.167	8.16 ± 1.167

Means with different letters in the same row are different significantly at $P < 0.05$.

In secondary follicles, the mean values of fiber diameters insignificantly increased to 8.16 µm in 0.7ppm than in 1.4 µm and control groups, being 5.57 and 5.35 µm, respectively (Table 1).

Selenium is important in sulfur amino acid synthesis. Sulfur amino acids protect animals against several diseases associated with low intakes of selenium. This protection is believed to be due to the antioxidant activity of selenium. Therefore, the sulfur amino acids, methionine and cystine can spare selenium through there antioxidant role. Fry, *et al.* (1996) found that selenium supplemented sheep had greater fiber diameter over summer – autumn seasons selenium supplementation. They also added that supplementation of vitamin E to deficient sheep, which developed symptoms of nutritional myopathy, had no effect on wool quality and quantity in contrast to the increase in wool length and fiber diameter which occurred in response to selenium supplementation in the same animal. In the same way, Whelan *et al.* (1994) found that selenium treatment significantly increased wool product without incurring penalty from increased fiber diameter in contrast to the result of Langlands *et al.* (1991a).

Histochemically, the total carbohydrate content in different skin structures of different selenium supplementation levels was similar as presented in Table (2) and Figure (2).

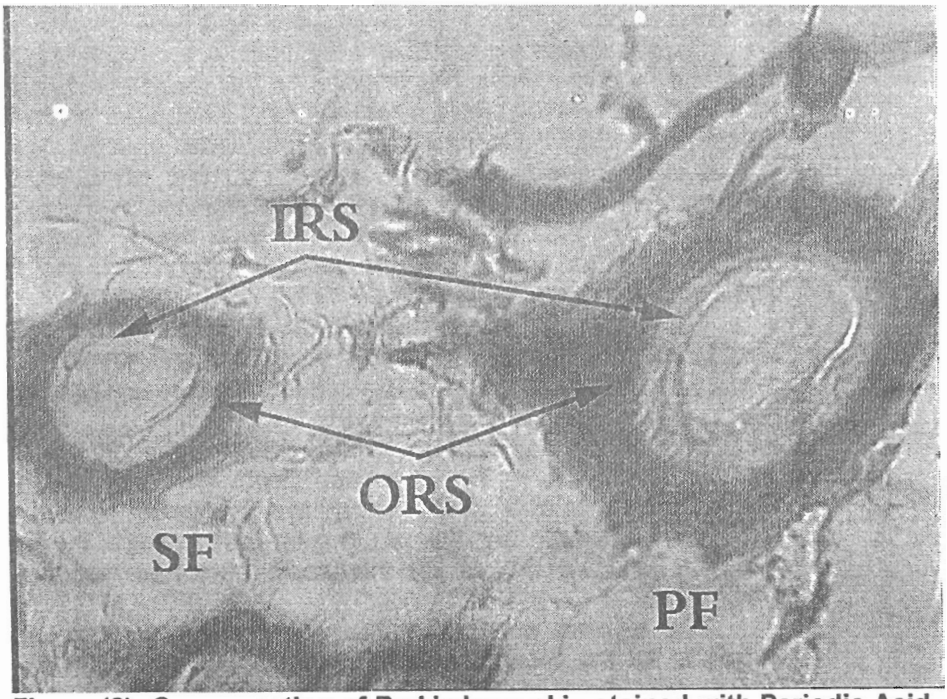

Figure (2): Cross section of Barki sheep skin stained with Periodic Acid-Schiff (P.A.S) method showing inner root sheath (IRS) and outer root sheath (ORS) of both primary (PF) and secondary (SF) follicles.

Table (2): Concentration of total carbohydrates in primary and secondary follicle structures (outer root sheath and inner root sheath) expressed as percentages of optical density values.

Experimental group	Primary Follicles		Secondary Follicles	
	Outer	Inner	Outer	Inner
Control	51.47±0.741^{b}	45.82±0.726	53.53±0.858^{b}	48.20±1.271
1.4 ppm	50.35±0.750^{b}	44.17±0.735	51.58±0.858^{b}	47.92±1.271
0.7 ppm	53.63±0.760^{a}	44.85±0.744	57.19±859^{a}	49.63±1.281

Means with different letters in the same column are differed significantly at P<0.05.

Generally, carbohydrates were more concentrated in the outer root sheath than in the inner root sheath of both primary and secondary follicles (Fig. 2). This was clear from the strong reaction of PAS in the outer root sheath of both primary and secondary follicles. Similar findings were observed in the skin of Kashmir sheep (Tej Sharma, 1982) and were mentioned also by Parmar, *et al.* (1988).

The carbohydrates are considered as a sign of follicle activity as reported by Montagna (1956), who assumed that the carbohydrates in outer root sheath was the source for protein synthesis during fiber growth.

Results shown in Table (2) indicated a significant ($p<0.05$) increase in the amount of the total carbohydrates in the outer root sheath of the primary and secondary follicles of 0.7 ppm group as compared to 1.4 ppm and control groups. On the other hand, supplementation with the two selenium levels had no significant effect on the carbohydrate content in the inner root sheath of the primary and secondary follicles.

Carbohydrates are oxidized in the body to provide energy which is very important for wool growth. The explanation that carbohydrate frees protein for wool production which would otherwise have been used to provide energy is probably too simple. Carbohydrate is also needed in order that amino acids can be utilized and for mitosis to take place in the follicle bulb. Selenium is required for the activation of the enzyme glutathione peroxidase which is an antioxidant that quenches hydroperoxides "high energy" oxygen – containing molecules that are produced during the metabolism of fats and carbohydrates that are highly toxic to cells. (Duane, 1987).

The distribution of general proteins as demonstrated by bromophenol blue stain in the inner and outer root sheaths of both primary and secondary wool follicles in the different supplemented selenium level groups was similar as represented in Table (3) and Figure (3).

In both primary and secondary follicles, the concentration of total proteins was higher in the outer root sheath than in the inner root sheath (Table 3). Parmar *et al.* (1988) stated that the protein content was larger in the outer root sheath than that of the inner root sheath of the skin follicle and this was probably due to the increased protein synthesis in the cellular proliferation.

The ultimate protein condition determining the rate of wool growth seems to be the concentration of essential amino acids in the tissue fluids around the follicle, and whereas the supply of amino acids to the animal is

determined by the amount and kind of protein. The synthetic potential of the proliferating cells of the follicle blub is high relative to that of other tissues.

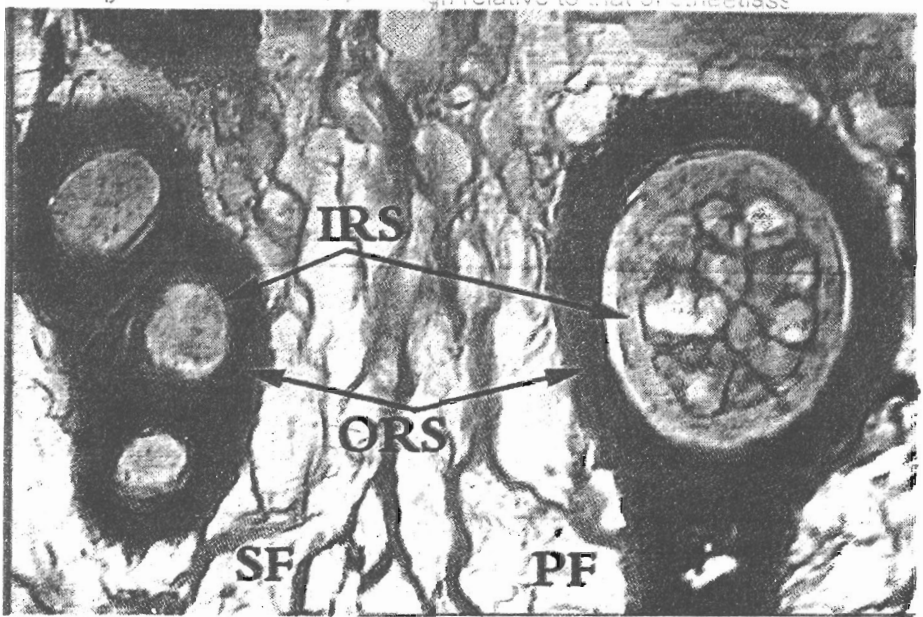

Figure (3): Cross section of Barki sheep skin stained with bromophenol blue showing inner root sheath (IRS) and outer root sheath (ORS) of both Primary (PF) and secondary (SF) follicles.

Table (3): Concentration of total proteins in primary and secondary follicle structures (outer root sheath, inner root sheath and fiber) expressed as percentages of optical density values.

Selenium levels	Outer		Inner	
	Primary follicles	Secondary follicles	Primary Follicles	Secondary follicles
Control	60.57±0.760	60.62±0.544	59.62 ±0.718[b]	56.38±0.942
1.4 ppm	61.37 ±0.680	61.62±0.471	61.79±0.642[a]	56.80±0.816
0.7 ppm	61.45 ±0.689	61.70±+471	59.06±0.650[b]	58.44±0.816

Means with different letters in the same column are differed significantly at P<0.05.

In the present study, supplementation of different selenium levels (0.7 and 1.4 ppm) led to insignificant increase in the concentration of proteins in the outer root sheath of both primary and secondary follicles and inner root sheath of secondary follicles. However, the inner root sheath of the primary follicles showed a significant increase in 1.4 ppm group compared to 0.7 ppm and control groups, being 61.79, 59.06 and 59.62%, respectively.

Differing dietary intakes of selenium have been associated with a range of biochemical functions. Many of these biochemical responses can be associated with changes in expression and activity of proteins that contain selenium as selenocysteine at the active site (Arthur and Beckett, 2000).

At physiological pH the selenol residue of selenocysteine is very reactive; consequently it can facilitate many biochemical reactions which involve oxidation and reduction of the selenium. The biochemical reactions catalyzed by mammalian selenoproteins fall into three broad categories, namely antioxidant defense systems, thyroid hormone metabolism and redox control of cell reactions.

Selenium is the most important biological antioxidant which is an essential component of the enzyme glutathione peroxides. This enzyme is a major intracellular antioxidant that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to nontoxic compounds (Duane,1987).

The more detailed studies of Langlands *et al.* (1994) showed that a dietary deficiency of selenium could limit wool growth. On the other hand, the effects of selenium and/or iodine supplementation on the growth rate, mohair production and plasma thyroid hormone concentrations in Angora kids were studied by Wichtel *et al.* (1996). They found that supplementary selenium but not iodine enhanced live weight gain during the final month of the experiment and added that neither selenium nor iodine supplement influenced mohair production.

These studies are not just academic issues but issue of major economic importance. Sheep producers in developed countries increasingly face declining "terms of trade", that is, reduced returns in the face of increasing costs. A worldwide reaction to this has been to seek to produce higher value wool, meat and dairy products, while also reducing the cost and the environmental risks of sheep production systems. At the same time, increasing percapita incomes in both developed and especially developing nations are already increasing the demand for more and better meat and fiber products.

It could be concluded that good selenium supplementation with adequate antioxidants is important in order to improve wool follicle characteristics.

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تاثير مستويات مختلفة من السلينيوم فى العليقة على بعض صفات الجلد فى الاغنام البرقى

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اجريت الدراسة بمحطة بحوث راس سدر والتابعة لمركز بحوث الصحراء بمحافظة جنوب سيناء على ١٢ ذكر برقى عمر ٤ شهور ومتوسط وزن ١٣,٨١ ± ١,٦٠ كجم بهدف دراسة تاثير مستويات مختلفة من السيلينيوم (٠,٧ ، ١,٤ جزء فى المليون) فى العليقة على بعض صفات الجلد فى الاغنام البرقى ، وتم اخذ عينات جلد من جميع الحيوانات لفحصها هستولوجيا وهستوكميائيا.

اوضحت الدراسة الهستولوجية ان اضافة السيلينيوم بمستوى ٠,٧ جزء فى المليون كان لة تاثير معنوى على ابعاد الحويصلات الثانوية مقارنة بالمستوى ١,٤ جزء فى المليون او مجموعة الكنترول. وكذلك زاد قطر الالياف المنتجة من الحويصلات الثانوية تحت نفس المستوى بمقدار ٨,١٦ ميكرومتر.

تاثر تركيز الكربوهيدرات بمستوى السيلينيوم ٠,٧ جزء فى المليون فى غمد الجذر الخارجى فى كل من الحويصلات الاولية والثانوية بينما كان هناك زيادة طفيفة فى تركيز البروتين فى مستويي السلينيوم.

تخلص الدراسة الى ان الاضافة المناسبة من السيلينيوم كاحد مضادات الاكسدة تعمل على حماية الانسجة من الضرر ومن ثم انتاج صوف عالى القيمة.