EFFECTS OF ZINC, SELENIUM AND VITAMIN E INJECTIONS ON THE CONCENTRATION OF FATTY ACIDS, CONJUGATED LINOLEIC ACID (CLA) ISOMERS AND CHOLESTEROL IN OSSIMI EWES BLOOD AND MILK

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SUMMARY

The present study was carried out at the Experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena, Egypt during the period from June to October 2010. The study was conducted on 30 Ossimi ewes divided randomly into two groups each of 15 animals: Group 1 is control (G^1) and group 2 is experimental group (G^2) . On thirty of day before lambing, then on day[1] , days[21] and days[42] post lambing all ewes from group (G^2) were given intramuscularly 5 ml 0.1% Na_2SeO_4, 10 ml 10% $ZnSO_4$ and 250 mg vitamin E. Fifty days post lambing the concentration of CLA in milk sample (0.66 of the fatty aced sum) was found higher ($P<0.05$) in G^2. However, cholesterol content was lower in (G^2) than in (G^1) (10.7 vs/14.22 mg/100 ml). The differences in blood plasma cholesterol were significant ($P<0.01$), the figures were 3.78 and 3.01 mmo1/l, respectively, while the high density lipoprotein (HDL rose up in G2 by 15.47% as compared in (G^1) . However the tri acid glycerol trended oppositely, which to 18.52% G^2. It could be concluded that the pre- and post-partum intramuscular injections of Se, Zn and vitamin E improved the lipid profile of milk.

Keywords: zinc, selenium, vitamin E, Polyunsaturated fatty acids (PUFA), Conjugated Linolic Acid (CLA) isomers, cholesterol, ewes milk

INTRODUCTION

The quality of milk fat has been extensively studied in relation to the type of feed, primarily in cows and ewes. Special attention is given to fatty acids that could play a positive role for human health, such as butyric acid, oleic acid, C18 to C22 polyunsaturated fatty acids (PUFA) Conjugated Linoleic Acid (CLA) (Chilliard *et al.,* 2001). Isomers of Conjugated Linoleic Acid (CLA) found in ruminants' milk and meats are products of incomplete biohydrogenation of unsaturated fatty acids by rumen bacteria *Butyrivibrio fibrisolvens (*Kepler *et al*., 1966). CLA refers to a mixture of positional and geometric isomers of octadecadienoic (linoleic) acid with conjugated double bonds. The most extensively investigated CLA isomer is *cis-9,trans-11* octadecadienoic acid, which is thought to be biologically active (Chin *et al*., 1992, Bessa *et al*.,2000). Zinc is a micro-mineral involved in various processes of animal metabolism. Since it was originally demonstrated that zinc is necessary for healthy growth rats (Underwood and Somers, 1977), the role of zinc in the animal organism began to gain special attention. Zinc participates actively in protein synthesis and carbohydrate metabolism. The discovery that the enzyme carbonic anhydrase contains 0.33% of zinc in its molecule (Underwood and Somers, 1977) is considered the first acceptable explanation of the mechanism of action of this element. After that, many other enzymes have been identified as containing zinc: alcohol dehydrogenase, carboxipeptidase and DNA-polymerase, this latest being fundamental in cell division process. This mineral stabilizes the quaternary structure of enzymes and large quantities of zinc were found to provide stability to the structures of RNA, DNA and ribosomes (Mc Dowell, 1992). Zn superoxide dismutase (ZnSOD) is a dietary factor, which contributes to the antioxidant defence system (Morrissey *et al*. 1998). Some studies suggest that Zn content in diets of dairy cows can modify the level of blood lipids, as lower levels of Zn in the diet increase the lipid oxidation rate in the liver (Roussel *et al.,*1993). In most studies reviewed no adverse effects occurred when dietary Zn concentration was below 600 PPM. The requirement should be 46 PPM. Many factors influence Zn toxicity and dietary Pb , Cu deficiency. Marginal Se intake exacerbates it, while soybean protein appears to protect against excess Zn compared with casein. Details of the toxic effects of Zn have been reviewed .Selenium is implicated in antioxidant functions, and Se-cysteine is essential in the active centres of Se-enzymes that carry out redox reactions, glutathione peroxidase (GPx), thyroid hormone deiodinase families and thioredoxinreductase (Tapiero *et al*., 2003). In several studies using rats , it was found that the content of polyunsaturated fatty acids (PUFA), especially in serum cholesterol esters and phospholipids were positively correlated with Se concentration in the diet (Crespo *et al.,* 1995). Czauderna *et al*. (2004) suggested that the interaction between Se and the CLA isomers mixture protected CLA from peroxidation damage in muscles and increased the level of CLA in the muscles of rats. Supplementing the cows with selenium significantly decreased the level of cholesterol in blood plasma, including HDL and LDL lipoproteins (Brzóska and Brzóska, 2004). Reklewska *et al.* (2002) indicated that supplementing the diet with minimum doses of linseed and mineral mixture (Mg, Fe, Cu, Co, Mn, Zn, Se, Cr, Ca) significantly increases CLA isomers and decreases cholesterol content in cows milk. Vitamin E is primarily active as an antioxidant protecting polyunsaturated fatty acids in *in vivo* and *post-mortem* animal tissues and muscle nutrients from free-radical attack (Morrissey *et al.,* 1994). The aim of the present experiment was to check the feasibility of decreasing the cholesterol level of blood and milk and increasing the CLA level of ewes milk by applying intramuscular *pre-* and *post-partum* injections of Zn, Se and vitamin E .

MATERIALS AND METHODS

This study was carried out at the Experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena, Egypt where there is a prevailing tropical climate. The experiment was conducted on 30 Ossimi ewes divided randomly into two groups: control (G1) and experimental (G2), each of 15 animals. On thirty day before lambing, and then on day one, week three and week six after lambing all 15 ewes from group G2 received intramuscular injections with 5 ml 0.1% Na_2SeO_4 (2.09 mg Se), 10 ml 10% $ZnSO_4$ (227 mg Zn) and 250 mg vitamin E (α- tocopherol).Both groups were kept with their lambs under the same management conditions eight weeks post lambing. Feeding was based on: maize silage, alfalfa hay, concentrate mixture (Soybean meal 8%, Yellow maize 36.5%, sunflower meal 16%, Barley meal 20%, Wheat bran 16%, Calcium carbonate 2%, Sodium chloride 1% and Vitamin-mineral mixture 0.5%). The daily dry matter (DM) intake per ewe during pregnancy was 0.51 kg from alfalfa hay, 0.67 kg from maize silage and 0.86 kg from concentrate feed mixture while during lactation period were 0.68, 0.67 and 1.04 kg/head /day respectively. The declared content of Zn and Se in the mineral premix was 6000 PPM and 12 PPM, respectively. Milk samples were taken at week 1 and week7 post lambing, following the injection of 1ml oxytocin per ewe to stimulate milk let-down. Proximate analysis of feeds was performed using standard methods A.O.A.C. (2005) The level of metabolizable energy of feed was calculated on the basis of the results of proximate chemical analyses using the equation recommended by (MAFF, 1975). The concentrations of SFA, MUFA, Polyunsaturated Fatty Acids (PUFA) and (CLA) were measured in milk. To determine fatty acids milk samples were freeze-dried and extracted with chloroformmethanol-water mixture (4:2:1.v/v). Hydrolization and derivatization reaction was carried out according to Czauderna *at al.* (2001). The derivatized samples were filtered through 0.2μm membrane filter (Whatman) and the filtrates injected on two chromatographic column Spheri-5 RP-18, 5 μm, 220 × 4.6 mm (Perkin Elmer , USA). The CLA isomer mixture standard (the *cis-9,trans-11* and *trans-10,cis-12*) of CLA and other fatty acid standards were provided by SIGMA (USA).The blood was withdrawn six weeks after lambing. To determine total HDL (highdensity lipoprotein), cholesterol and Triacylglycerol (TAG) in blood plasma determined using Enzyme-linked tests 12 ppm . To determinate cholesterol in milk, samples were saponified and extracted with hexane according to Fletouris *et al.* (1998). Total cholesterol in blood serum was determined colorimetrically according to Searcy and Berquist (1960) . Zn content in feeds was determined by atomic absorption spectrometry. Samples (0.5 g) were mineralized in a mixture of 5 ml HNO_3 and 1 ml H_2O_2 in hermetic high-pressure vessels by heating in microwave oven. Total Se content was determined by flame (air-acetylene) atomic absorption spectrometry using hydrogen generation system. Selenium hydride was generated with $NaBH_4$. Hallow cathode lamp (196.0 nm) with deuterium background correction was used.

Statistical analysis:

Data were statistically analyzed using SAS (2001), Duncan Multiple range Test was used to compare the differences among means (Duncan, 1955). The effect of Zinc, Selenium and vitamin E injections were considered to analyze milk traits and chemical composition of blood Ossimi ewe. The following model used for milk traits and chemical composition of blood Ossimi ewe was as follows:

Yik = U + B i + eik where:

Yijk = The trait of study

U = The overall mean;

Bi =The fixed effect of ith Zinc, Selenium and vitamin E injections;

Eijk = random error

RESULTS AND DISCUSSION

The results of proximate analyses of feeds and their nutritive value , as well as Zn and Se contents of DM are shown in Table 1. Daily Se intake per ewe from feeds and mineral premix during pregnancy and lactation period amounted 0.39-0.44 mg while Zn from 115-123 mg. However, National Research Council (NRC, 1992) suggested that Se requirements for sheep vary from 0.1 to 0.2 PPM of feed DM. Mc Dowell (1992) suggested that dietary may have some beneficial effects when it's above those generally accepted levels.

Injecting Se, Zn and vitamin E intramuscular before and after lambing of ewes was effective in increasing the contents of these nutrients in milk (Gabryszuk *et al.,* 2005). However, when the Se level of blood plasma was within the normal range, injecting additional Se and vitamin E has adverse effects, decreasing fertility and lamb body live weight at birth (Gabryszuk, 1994).

In comparison to the control (G1), four intramuscular injections of Se (each of 2.09 mg), Zn (each of 227 mg) and vitamin E (250 mg) applied in group (G2) led to a significant decrease in the cholesterol content of milk and total cholesterol of blood plasma with simultaneously higher levels of HDL fraction (Table 2). Simultaneously the concentration of Triacylglycerol (TAG) of blood plasma in group (G2) was lower than in control ewes (Table 2)

Results of proximate analysis of feeds and metabolizable energy (MJ/Kg DM)are shown in Table1.

The Selenium (Se) content of crops below 0.1 ppm is the level considered adequate for preventing deficiency in sheep (NRC, 1983).

The most commonly noticed lesion in sheep resulting from an inadequate supply of Selenium is degeneration of the cardic and skeletal musculature (white muscle disease), but unthriftiness, early embryonic death, and periodontal disease are also signs of a possible Selenium deficiency (Underwood, 1981).

Supplementation with 0.1 mg Se/ Kg DM (as sodium selenite) in the diet of ewes during gestation, through weaning consistently provided essentially complete protection against white muscle disease in their lambs (Schubert *et al.,* 1961).

In the present study daily selenium intake per ewe from diet during pregnancy and lactation period were 0.27 mg/ Kg DM and 0.30 mg/ kg DM in addition to premix containing 12 PPM selenium, while the Zinc was 55.56 mg/Kg DM and 63.43 mg/Kg DM during pregnancy and lactation period respectively and from premix concentration 6000 PPM.

Although (Davies *et al.,* 2006), showed that the maximum tolerable level of inorganic Se for sheep is much higher than 2 mg/kg DM as suggested earlier, feeding up to 12 mg sodium selenite / 1kg DM of feed, to ewes under the stress status of production (i.e gestation and lactation) for 72 weeks did not produce any clinical or pathogenic signs of Se intoxication .

Zinc supplementation of 40 PPM to the diet of Pregnant and lactating ewes has yet to be determined the few studies that have been conducted indicated that the lactating ewe is clearly susceptible to zinc deficiency, but whether zinc is necessary for normal parturition in sheep as it is in rats is still unclear (Moir, 1983).

The suggested minimum requirements are 20 mg Zn/ Kg DM for growth and 33 mg Zn / Kg DM for maintenance of normal reproductive function in males and for pregnancy and lactation in females. Diets high in calcium (1.2 to 1.8 percent calcium) have been reported to adversely affect Zinc utilization (Mills and Dalgarno,1967) .

NRC (1992) suggested that the requirements of sheep for Zn vary from 20 to 33 mg / Kg DM of feed.

Although Mc Dowell (1992) showed that ruminants overt Zn toxic sis first appears when levels around 1000 PPM are in corporated into a natural – ingredient diet .

Injecting Se, Zn and vitamin E intera muscular before and after lambing were effective in increasing the contents of these nutrients in milk (Gabryszuk *et al.*, 2005). However, when the Se level of blood plasma was within the normal range, injecting both Se and V. E decreased fertility and lamb body live weigh at birth (Gabryszuk, 1994).

In comparison to the control (G^1), three intra muscular injections of Se (each 2.09 mg), Zn (each of 227 mg) and vitamin E (250 mg) applied in (G^2) led to significant decrease in the cholesterol content of milk 10.7 mml/100 ml in (G^2) vs 14.24 mml/100ml in (G^1) . The same trend was observed . Simultaneously the concentration of TAG of blood plasma in (G2) was lower than (G1) .

On the contrary high density lipproteinsn (HDL) recorded higher levels for (G2) than

(G1) the figures were 0.98 +0.13 vs 0.85+ 0.14 mmol/L (Table 2). These results agree with the findings of Brz oska and Brz oska (2004) and Reklewska *et al*., (2002).

(Table 3) exhibits the concentration of SFA%, MUFA%, PUFA % and CLA% in milk at week 1 and week 7.

Saturated fatty Acids (SFA) showed higher % at week 1 and week 7 in (G1) compared with week 7 in (G1) , the figures were 71.3 % and 74.4 % vs 70.4 % and 72.2%, respectively, while the enhancement was 2.95 %in the decline of SFA.

Monounsaturated fatty Acids (MUFA%) 22.2% and 20.2% compound for (G1) compared with 22.4% and 21.5% respectively, for week 1 and week 7, therefore the enhancement in MUSFA for week 1 and week 7 were 0.9% & 6.44 %, respectively. While Polyunsaturated Fatty Acids (PUFA) were raised from 6.4 % to 7.2 % in week 1 and from 5.4 % to 6.3% in week 7. Therefore the enhancement of (PUFA) in week 1 and week 7 were 12.51% and 16.62 % respectively.

This simultaneously showed in Conjugated Linolic Acids Isomers(CLA), which enhanced by 42.59 % in (G2) when compared with (G1) in week 1, while in week 7 the enhancement was 53.49% in (G2) when compared with (G1), (Table 3).

These findings agree with those found by Reklewska *et al*. (2002), who reported significant increases in CLA and decreases in cholesterol.

Falkowska *et al.* (2000) showed that supplementation of a diet with Se and vitamin E significantly increased HDL fraction in the blood of cows from 0.440 to 0.552 mmol/l. Brzóska and Brzóska (2004) observed that when the level of dietary Se was increased from 0.04 to 0.48 mg/kg DM, the cholesterol content of blood plasma in cows declined from 228.6 to 183.9 mg/dl, with simultaneous decreases in HDL and LDL. The level of Zn ranging from 39 to 59 mg/kg DM of the diet of dairy cows did not affect cholesterol or other metabolite levels in blood plasma or milk.

(Brz óska and Kowalczyk, 2002). The long-chain n-3 fatty acids are known to be a factor in decreasing the LDL content of the liver, which at a declining tendency in blood plasma, could promote the increase of HDL blood plasma content (Brzóska and Kowalczyk, 2002).

While concentrations of CLA in milk was higher in (G2) (0.77 vs 0.54) in (G1) mmol/L (Table 3), Reklewska *et al.* (2002) reported significant increases in CLA and decreases in the cholesterol content of cows' milk, fed a diet with minimum doses of linseed and a mineral bioplex mixture (Mg, Fe, Cu, Co, Mn, Zn, Se, Cr, Ca). In an earlier study (Gabryszuk *et al*. 2007) which demonstrated that Se, Zn and vitamin E administered orally to growing ram-lambs induced a decrease in cholesterol content of blood and meat, and led to increased CLA isomer levels in meat and liver. Emanuelson and Bertilsson (1995) reported a tendency to increase oxidation of milk fat when linseed was added to a cows' diet. Spontaneous oxidation of milk fat may occur at a low concentration of milk antioxidants. Since increased dietary concentration of antioxidants may lead to improved oxidative stability of milk fat (Niki *et al.,* 1989) it seemed worth trying to produce milk of increased content of antioxidants as well as of some functional fatty acids. We suggest that the interactions between Se, Zn and vitamin E are factors which can modulate desaturase and chain elongase activity as well as inhibit fatty acid β- oxidation in mammary glands. The genetically determined ability of the subcellular membrane to assimilate and store peroxidation antagonists such as vitamin E, GSH-Px, catalase and superoxide dismutase. Crespo *et al*. (1995) reported that the concentration of PUFA was positively correlated with the level of Se in diets of rats. The *cis-9,trans- 11* CLA found in ruminants milk and meat is an intermediate in the biohydrogenation of linoleic acid (*cis-9,cis-12* C18:2) to stearic acid (C18:0) – Bessa *et al*. (2000) The major part of conjugated linoleic acids (*cis-9,trans-11* C18:2) is synthesized in the ruminant tissues and particularly in the mammary glands by the desaturation of trans-vaccenic acid (*trans-11* C18:1) resulting from the action of the stearoyl-CoA desaturaze (SCD). The introduction of *cis*-double bond is catalysed by the set of microsomal electron-transport proteins composed sequentially of NADH cytochrome b5 reductase, cytochrome b5, and the terminal SCD. Stearoyl-CoA desaturase is the rate-limiting component in this reaction. Its activity is regulated by different factors such as diet, hormones, temperature, metals, peroxisomal proliferators, vitamin A, and developmental processes (Ntambi, 1999; Miyazaki and Ntambi, 2003). More than 80% of *cis-9,trans-11* CLA in milk is produced endogenously by Δ9-desaturase from *trans*-11 C18:1 in the mammary gland. Cows on the same diet have different milk fat *cis-9,trans-11* CLA concentrations that may be partially explained by differences in Δ9-desaturase activity between cows . Increasing the activity of Δ9-desaturase in the mammary gland may offer greater potential for enhancing the *cis-9,trans-11* CLA content of milk fat (Lock and Garnsworth 2002). We presume that supplementation of Se, Zn and vitamin E can regulate activity of SCD. We also suggest that dietary Se, Zn and vitamin E decreased β-oxidation of the CLA isomers in mammary gland. It can be hypothesized that the Se (as Na2SeO4), Zn and vitamin E as strong antioxidants, have a protective effect against per oxidation damage in CLA isomers metabolism

This study demonstrated that Se, Zn and vitamin E administered intramuscularly to pregnant and lactating ewes induced a decrease in cholesterol content of blood and milk, and led to increased the CLA isomers level in milk.

Based on the above observation, it is suggested that the intramuscular administration of Se, Zn and vitamin E improved the lipid profile of ovine milk which should be evaluated for consumer uptake/acceptance .

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Table 1. Approximate Chemical analysis of feeds (g/kg/DM)

Component	concentrate mixture	alfalfa hay	Maize silage
Dry matter	882	895	336
In dry matter :-			
Crude protein	168	195	88
crude fibre	163	267	261
ether extract	29	12	24
crude ash	75	122	98
N-free extractives	565	404	529
Neutral detergent Fibre	302	634	460
Acid detergent fibre	137	365	241
ADL	43	42	22
Metabolizable Energy(MJ/kg)	12.6	10.2	11.8
Se (mg/kg DM)	0.16	0.10	0.12
Zn (mg/kg DM)	29.4	15.1	33.7

Table 2. Means±SE of cholesterol content of milk, and total cholesterol, HDL fraction and TAG contents of blood plasma of Ossimi ewes

Item	G[1]	G[2]
Week7 Milk		
cholesterol (mg/100 g)	14.23±2.89	10.6±1.96
Week 6 Blood		
total cholesterol (mmol/l)	3.79± 0.60	3.02± 0.51
HDL (mmol/l)	0.85± 0.14	0.98± 0.13
TAG (mmol/l)	0.54± 0.09	0.44± 0.05

HDL = Highdensity lipoprotein TAG = triacylglycerol

Table 3. Means±SE of Fatty acid composition (% of total fatty acids) of Ossimi ewes milk

Item	Week 1 Milk		Week 7 Milk	
	G1	G2	G1	G2
SFA (%)	71.3 ±10.2	70.4 ±17.4	74.4 ±14.3	72.2 ±22.8
MUFA (%)	22.2± 16.6	22.4± 21.1	20.2 ±16.6	21.5± 22.8
PUFA (%)	6.4± 19.3	7.2 ±28.8	5.4 ±19.9	6.3 ±7.3
CLA (%)	0.54b± 19.9b	0.77a± 28.8a	0.43b± 24.3b	0.66a± 26.6a

[a] and [b] means in the same row followed by different letters are significantly different ($p<0.05$).
PUFA=Polyunsaturated Fatty Acids . CLA= Conjugated Linolic Acid
MUFA=Monounsaturated fatty acids. SFA= Saturated fatty acids.

دراسة تأثير الحقن بعنصرى الزنك والسلينيوم وفيتامين هـ على تركيز الأحماض الدهنية والكلسترول فى حليب نعاج الاوسيمى

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أجريت هذه الدراسة بمزرعة الإنتاج الحيواني - كلية الزراعة - جامعة جنوب الوادي بقنا من شهر يونيو إلى أكتوبر ٢٠١٠ لدراسة تأثير الحقن قبل وبعد الولادة بعنصرى الزنك والسلينيوم وفيتامين **هـ** على تركيز الأحماض الدهنية والكلسترول فى دم ولبن النعاج ألآوسيمى واستخدم في هذه الدراسة ثلاثون من النعاج الاوسيمى الحوامل . وزعت الحيوانات عشوائيا تبعا لأوزانها وأعمارها في مجموعتين متساويتين في كل مجموعة (خمسة عشرة نعجة)، الأولى مقارنة حيث غذيت النعاج فيها على العليقة الأساسية فقط أما الثانية غذيت النعاج فيهاعلى العليقة الأساسية وحقنت فى العضل قبل الولادة بشهر وعقب الولادة مباشرة وعند واحد وعشرون يوما وكذلك عند اثنين واربعون يوما بعد الولادة بعنصرى الزنك والسلينيوم وفيتامين **هـ** بسبة (١٠مل بتركيز ١٠ % من $ZnSO_4$ +٥ مل بتركيز ٠,١ % من $Na2SeO_4$ + ٢٥٠ملجرام من فيتامين هـ) وتم تقدير الكلسترول باللبن فى الاسبوع السابع بعد الولادة والاسبوع السادس فى الدم وكذلك تم تحليل نسبة الأحماض الدهنية المشبعة وغير المشبعة فى دهن اللبن المنتج بالاسبوع الاول والسابع وفى بلازما الدم بالاسبوع السادس وخلصت النتائج الى وجود زيادة معنوية فى نسبة الأحماض الدهنية الغير مشبعة ونقص معنوى فى الأحماض الدهنية المشبعة فى دهن لبن النعاج المعاملة مقارنة بمجموعة الكنترول عند مستوى معنوية ٥% وكذلك نقص فى الكلسترول ببلازما دم النعاج المعاملة مقارنة بمجموعة الكنترول عند مستوى معنوية ٥% وهذه النتيجة تكون مفيدة من الناحية الصحية .