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Correlation Effects of Nano Selenium and Conjugated Linoleic Acid on the Performance, Lipid Metabolism and Immune System of Male Moghani Lambs

Research Article

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ABSTRACT

This study was carried out to evaluate the effects of nano selenium (nano-Se) and conjugated linoleic acid (CLA) on performance, biometric and blood parameters and selenoprotein W1 (SEPW1) and lipid gene expression in male Moghani lambs. Thirty male Moghani lambs, 3-months-old and weighing 30 ± 0.25 kg, were used in completely randomized design in a 2 × 3 factorial arrangement with dietary supplemental the CLA (0 and 15 g/kg DM) and nano-Se (0, 1 and 2 mg/kg DM). The lambs were slaughtered on day 90 (end of experiment). Feeding a mixture of CLA and nano-Se tended to reduce the body weight of lambs comparing to the control group. The experimental diets had no impact on biometric parameters. Some blood parameters like high density lipoprotein (HDL), low density lipoprotein (LDL), thyroxine (T4), triiodothyronine (T3) and glutathione peroxidase (GPx) and glutathione peroxidase depended on dietary nano-Se supplementation ($P<0.05$); but glucose, triglycerides (TG), very low density lipoprotein (VLDL), total protein (TP) and cholesterol had no considerable effects. The results of qPCR analysis showed that nano-Se supplemental at highest level (2 mg/kg DM) increased gene expression of GPX1 and SEPW1 in liver ($P<0.05$). Dietary inclusion of CLA enhanced the peroxisome proliferator-activated receptor gamma expression and decreased stearoyl COA desaturase 1 genes in tail ($P<0.01$). In conclusion, nano-Se and CLA differently increased the gene expression in liver and tail and had good impacts on some blood parameters; suggesting that nano-Se and CLA have not synergism interaction in the above parameters.

KEY WORDS conjugated linoleic acid, lambs, Moghani, nano selenium, performance.

INTRODUCTION

Minerals are essential nutrients for all the species due to their role in preserving the health, growth, safety and reproduction (Zhan *et al.* 2014). Selenium, a trace mineral, has positive effects in livestock. National research council (NRC, 2007) have recommended 0.1 to 0.2 mg of selenium per kilogram of dry matter (DM) for growing lambs but later editions (NRC, 2007) recommended 0.22 to 0.44 mg/kg DM. Selenium also applied in the antioxidant system by participating in the structure of selenoproteins (Ponce *et al.* 2018). Selenoenzymes are the product of selenoproteins produced by cellular metabolism and peroxide radicals (Kieliszek *et al.* 2017). Glutathione peroxidase 1 (GPX1) contains the amino acid selenocysteine, which is used for specific function in body. The function of this enzyme is to reduce the intracellular hydrogen peroxide (Lubos *et al.* 2011). Selenoproteine W1 (SEPW1), another selenoproteins, which is like GPX1 and it plays main role in protecting cells from oxidative stress in the cellular defense system (Wang *et al.* 2010). It has been reported a positive correlation between SEPW1 expression and selenium concentration in spleen of rat and sheep, and vice versa (Li *et al.* 2011; Yu *et al.* 2011). Selenium supplemental could en-

hance the expression corresponding to selenium-related genes in muscle and lipid in adipose tissue (Pinto *et al.* 2012).

Peroxisome proliferator-activated receptor gamma (PPARγ) produces bands with non-esterified fatty acids and long-chain unsaturated fatty acid and converts fat signals into transcriptional changes; affecting the metabolic processes such as synthesis, storage, transfer and oxidation of fats (Tan *et al.* 2008; Malapaka *et al.* 2012). It has been found that the profile of dietary fatty acids, especially unsaturated fatty acids, regulates PPARγ gene expression, it affects the differentiation of the adipocyte cells and the pathway of energy-related genes (Sato *et al.* 2004). Stearoyl COA desaturase 1 (SCD1) enzyme regulates liver lipogenesis and lipid oxidation (Mainieri *et al.* 2006). It was reported the different amounts for synthesis and contexture of saturated fatty acid to unsaturated in heifers and beef cattle is associated with the key enzyme responsible for this differences is SCD1 (Barton *et al.* 2011).

Conjugated linoleic acid (CLA) is known as a position of geometric for linoleic acid with conjugated double bonds. It has positive impacts on animal models, such as reducing fat in milk and body (Harvatine *et al*. 2014).

However, investigation have indicated that dietary inclusion of selenium increases polyunsaturated fatty acids particularly CLA in bull meat (Netto *et al.* 2014), meat and liver of sheep (Gabryszuk *et al.* 2007), cow's milk (Ran *et al.* 2010) and goat (Pechova *et al.* 2008). Payne *et al.* (2005), in a research on dietary CLA and selenium supplementation in rats, have seen less reduction in weight gain of rats fed CLA + selenium-supplemented diet than CLA alone.

Moghani breed is fat-tailed sheep which is raised in Ardabil province located in the north-west of Iran. Regarding positive impacts of CLA in reducing fat and beneficial impacts of selenium on liver and fat metabolism, it hypothesized that dietary inclusion of nano-selenium (nano-Se) and CLA may have synergism interaction effect on performance and key genes expression associated with liver enzymes and lipid metabolism.

Therefore, this research was performed to study the impacts of nano-Se and CLA on performance, biometric and blood parameters and expression of GPX1 and SEPW1 genes in the liver and PPARγ and SCD1 genes in the tail of male Moghani lambs.

MATERIALS AND METHODS

Animals and experimental procedure

Thirty male Moghani lambs, 3-months-old and body weight of 30 ± 0.25 kg, were assigned in a completely randomized design with a 2 × 3 factorial arrangement with dietary supplemental of CLA (0 and 15 g/kg DM) and nano-Se (0, 1 and 2 mg/kg DM). Animals were classified into 6 groups with 5 animals and experimental treatments were including 1) control; without nano-Se and CLA, 2) 1 mg nano-Se/kg DM, 3) 2 mg nano-Se/kg DM, 4) 15 g CLA/kg DM, 5) 15 g CLA + 1 mg nano-Se/kg DM, 6) 15 g CLA + 2 mg nano-Se/kg DM.

The selenium (liquid) and CLA (powder) utilized in current research were purchased from the Pishgaman Nano Mavade Iranian and Golbar Navid Bahar company, respectively. All lambs were kept in individual pens (1 m×1.85 m) with wood shavings as bedding. The room temperature was controlled at 18 ˚C with 60 to 70% humidity. The lighting was scheduled from 7 am to 9 am. Lambs were fed the experimental diets (Table 1) twice (8 am and 7 pm) a day and had free access to water.

Slaughter and sampling

The lambs' growth performance was measured monthly. On day 90, the animals were slaughtered at the slaughter house of Ardabil. The protocol for animal research was approved by the Ethics Committee of the University of Mohaghegh Ardabili (Ethics committee reference number: IR.ARUMS.REC.1395.46). Weights of head, feets, wool and skin, liver, heart, testicular, kidney, lungs, hot and cold carcass, neck, tail, ribs and thigh were calculated. Liver and tail tissue specimens were obtained and frozen directly in liquid nitrogen and kept at -80 ˚C to subsequent analysis. Two-hundred mg of liver and tail tissues was immediately collected in RNA-later Stabilization Reagent (Thermo Scientific, USA) and processed for storage at -80 ˚C, following the instructions of producer.

RNA isolation and cDNA synthesis

Total RNA was separated from liver and tail samples by the High Pure RNA Tissue Kit (Sinaclon, Cat. No: RN7713C/EX6101, USA) as recommended by instructions of producer.

ND-1000 Nano Drop (NanoDrop Technologies, USA) spectrophotometer was used to assess qualitative and quantitative the separated RNA. Only specimens with RNA above 100 ng and absorbance ratios of A260/280 and A260/230 of about 1.9 were utilized for more analyses. The RNA was reverse transcribed into cDNA with the Transcription First Strand cDNA Synthesis Kit (Thermo Scientific, USA). Denaturing 1.0 µg of RNA was performed at temperature of 95 ˚C for 5 min in the existence of 50 µM Oligo (dT). The reverse-transcription mixture (20 µL in total volume) included of RNA (13 µL), reverse transcriptase buffer (4 µL), 10 mM Dntp (2 µL), protector RNase Inhibitor (40 U/µL) (0.5 µL), and reverse transcriptase (20 U/µL) (0.5 µL).

Table 1 Ingredients and chemical composition of the diet (DM basis)

Ingredients (%)	Control diet	Tested diets				
Alfalfa	30	30	30	30	30	30
Soybean meal	1	1	1	1	1	1
Barley	48	48	48	48	48	48
Wheat bran	17	15.5	15.5	17	17	17
Di-calcium phosphate	1	1	1	1	1	1
Soda	1	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin and mineral complement without selenium	1.5	1.5	1.5	1.5	1.5	1.5
Conjugated linoleic acid	0.0	0.0	0.0	1.5	1.5	1.5
Chemical composition[1]						
Dry matter (%)	90.12	90.01	90.01	90.12	90.12	90.12
Crude protein (%)	14.29	14.05	14.05	14.29	14.29	14.29
Neutral detergent fiber (%)	31.96	31.26	31.26	31.96	31.96	31.96
Acid detergent fiber (%)	17.05	16.83	16.83	17.05	17.05	17.05
Ether extract (%)	2.1	3.1	3.1	2.1	2.1	2.1
Calcium (%)	0.98	0.98	0.98	0.98	0.98	0.98
Phosphorus (%)	0.57	0.57	0.57	0.57	0.57	0.57
Selenium (mg/kg dry matter)	0.0	1	2	0.0	1	2
Metabolizable energy (Mcal/kg DM)	2.20	2.20	2.20	2.28	2.28	2.28

[1] The basal diet containing 0.3 mg per kilogram selenium in base of dry matter (premix composition per kg).

The incubation of resulted solution was then performed in temperature of 50 ˚C for 60 min, next at 70 ˚C for 10 min, and lastly kept at -20 ˚C.

Primer design

Designing primers of gene expression was performed using Primer 3Plus (http://www.bioinformatics.nl/) based on GenBank Ovis Aries sequences and amplicon sequence complied with boundaries of exon-exon.

The abundances of relative mRNA of genes were tested. Chosen targets were two selenoprotein genes (GPX1 and SEPW1) and two genes related with metabolism of lipid (PPARγ and SCD1).

In order to evaluate the expression of studied genes, the following primers were used. The sequence of the Glutathione peroxidase 1 (GPX1) forward primer was (CCTGGTCGTACTCGGCTTC) and GPX1 reverse was (CCTTCTCGCCATTCACCTC). The sequence of the selnoproteine W1 (SEPW1) forward primer was (CTATGGCGCTTGAGGCTACA) and SEPW1 reverse was (TGGAGTGAACCAGCTTTCCC).

The sequence for the peroxisome proliferator-activated receptor gamma (PPARγ) forward primer was (ATGGCTTCATAACCCGTGAG) and PPARγ reverse was (AATCCCCTCCTGCATTTTCT). The sequence for the stearoyl COA desaturase (SCD1) forward primer was (GTGCCGTGGTATCTATGGGG) and SCD1 reverse was (GGGGTTGATGGTCTTGTCGT) and the sequence for the

Beta-actin (ACTB) as the housekeeping gene forward primer was (CTCTTCCAGCCTTCCTTCCT) and ACTB reverse was (GCAGAAAGAGATCACTGCCC).

The PCR fragment length for *GPX1*, *SEPW1*, *PPARγ*, *SCD1* and *ACTB* genes were 154, 152, 206, 150 and 178 bp, respectively.

Quantitative real-time PCR assays (qPCR)

The PCR was performed in a total of 10 µL PCR mixture containing SYBR Green PCR Master Mix. Each reaction was run in triplicate and the average was utilized to compute the relative value of the target gene. Housekeeping gene ACTB, stability was examined by geNorm software version 3.4., which calculates the measure of gene expression constancy corresponding to a putative reference gene on the basis of the average pairwise variation between all investigated reference gene.

The relative gene expression was normalized against a factor which was on the basis of the geometric average of the expression level corresponding to the housekeeping gene, based on the recommendation of Vandesompele *et al.* (2002). For calculating the relative genes expression, the ΔCt and ΔΔCt formula was used the (Livak and Schmittgen, 2001).

Blood parameters

On day 90 of experiment, before slaughter blood samples (10 mL) were collected from each lamb and centrifuged at 2500 rpm for 15 min and kept at -20 ˚C until subsequent analyses. The blood specimens were tested for high density lipoprotein (HDL), low density lipoprotein (LDL), thyroxine (T4), triiodothyronine (T3), glutathione peroxidase (GPx), triglycerides (TG), total protein (TP) glucose, triglycerides (TG), very low density lipoprotein (VLDL),

total protein (TP) and cholesterol by using Pars Azmoon commercial kits (Karaj, Iran).

Statistical analysis

Effects of CLA and nano-Se and their interactions were evaluated by analysis of variance by the command PROC GLM of SAS (2003) software based on the model:

$$Yi= \mu + Ti + Sj + TSij + eijk$$

Where:
Yi: dependent variable.
μ: overall average.
Ti: effect of CLA.
Sj: impact of selenium.
eijk: residual error.

The effects of treatment based on the diet were determined with averages calculated by command LSMEANS and Duncan test was utilized for determination of the considerable differences amongst the treatment groups at significant level of $P < 0.05$.

RESULTS AND DISCUSSION

Our findings in Table 2 showed that addition of selenium and CLA in the amounts mentioned had no considerable impact on mean daily gain, final weight, feed intake and feed conversion ratio ($P<0.05$). Parallel to current results, Vignola *et al*. (2009) did not observe the significant difference in weight gain, feed intake and feed conversion ratio in animals receiving the diverse levels of selenium. Other investigations have indicated that dietary inclusion of 1 and 2 ppm of nano-selenium in the diet did not impact on growth rate and feed conversion ratio (Kumar *et al.* 2008). It has been found that dietary inclusion of selenium increased gaining weight and in taking feed in goat (Shi *et al.* 2011).

The conflicts in earlier investigations may be described by amount and kind of basal diet and diverse kinds of selenium in the diet. Other investigations have indicated that dietary inclusion corresponding to selenium and CLA had not important impact on growth efficiency (Vignola *et al.* 2009; Kumar *et al*. 2008). Payne *et al.* (2005) reported that dietary inclusion of CLA in the existence of selenium in the rat's diet, caused to reduction of the weight gain comparing with those only treated with CLA. It can be stated that selenium and CLA have not important influence on performance which is able to be attributed concentrations of selenium and CLA which could be insufficient for affecting on performance.

Effects of treatments on biometric and abattoir parameters are presented in Table 3. As results show the divers amounts of nano selenium and CLA had no important influence on these parameters. In support of these results, other investigations did not report significant effects of CLA and selenium on such parameters (Lambertini *et al.* 2005; Vignola *et al.* 2009; Schiavon *et al.* 2010). We did not see a positive correlation between carcass characteristics and growth performance.

The influence of treatments on blood metabolites is presented in Table 4.

Results indicated that nano selenium in singly form had significant effects on HDL, LDL, T3, T4 and GPx ($P< 0.05$), but it had not important influence on glucose, VLDL, TG and cholesterol.

It has been found that mice with selenium deficiency showed increased plasma cholesterol and the usage of appropriate amounts can cause a reduction in LDL and rise in HDL (Qu *et al*. 2000).

Serum cholesterol is transformed in the HDL fraction and is considered as index for lipoprotein concentrations (Herdt and Smith, 1996). The liver has low capacity for synthesizing cholesterol in ruminants; cholesterol is synthesized in adipose tissue and small intestine (Liepa *et al.* 1978; Chen *et al.* 1995).

Partly similar to our results, other investigations have reported that dietary inclusion of selenium caused to decrease T4 hormone and increase T3 hormone (Wichtel *et al.* 1996). Dalir-Naghadeh and Rezaei (2008) reported that concentrations of serum and T3 and ratio of T3 to T4 in muscle disease in lambs was less than healthy lambs. Nano-selenium supplementation to the diet increased GPx ($P<0.0001$). The activity of enzyme is taken into account as an important indicator of selenium status in animals (Yang *et al*. 1989). Kumar *et al.* (2008) indicated that addition of 0.15 ppm selenium in basal diet containing 0.19 ppm selenium increased glutathione peroxidase function in erythrocytes. The function of GPxs is to decrease hydrogen peroxides and lipid hydroperoxides.

Since selenium is a glutathione peroxidase component and a direct linear relationship is between blood selenium status and function of this enzyme this result is logical (Yang *et al*. 1989). Furthermore, the data corresponding to GPX1 expression confirmed our findings for serum. It can be concluded that selenium participates in GPX1 structure and prevented lipid peroxidation and thus improves blood parameters.

This study tried to include the highest amounts of selenium on Moghani male lambs (2 mg/kg DM) in nano form. The results of qPCR analysis showed that nano-Se differently improved the genes expression.

Table 2 The effects of treatments on the performance (n=5)[1]

Item	Month	CLA: 0			CLA: 15			Nano-Se	CLA	Nano-Se × CLA	SEM
		Se: 0	1	2	Se: 0	1	2				
	1	1541	1560	1504	1527	1451	1497	NS	NS	NS	47.62
FI (g/day)	2	1839	1875	1842	1811	1830	1830	NS	NS	NS	40.99
	3	1879	1894	1811	1851	1866	1886	NS	NS	NS	47.40
	1	37.39	36.11	36.02	36.45	34.54	36.69	NS	NS	NS	0.73
LW (kg)	2	42.81	41.93	42.07	41.08	40.24	42.12	NS	NS	NS	0.80
	3	52.12	49.23	50.65	49.33	48.84	51.36	NS	NS	NS	1.15
ADG (g/d)		232	200	216	201	196	223	NS	NS	NS	8.15
FCR		7.55	8.81	7.95	8.60	8.92	7.79	NS	NS	NS	0.09

[1] Conjugated linoleic acid (CLA) levels (0 and 15 g kg dry matter), nano-selenium levels (0, 1 and 2 mg per kilogram of dry matter).
FI: feed intake (grams per day); LW: live weight (kg); ADG: average daily gain (g) and FCR: feed conversion ratio.
SEM: standard error of the means.
NS: non significant.

Table 3 The effects of treatments on biometric parameters and carcass characteristics (n=5)[1]

Biometry (cm)	CLA: 0			CLA: 15			Nano-Se	CLA	Nano-Se × CLA	SEM
	Se: 0	1	2	Se: 0	1	2				
Body length	73.81	72.01	70.45	70.62	70.15	68.73	NS	NS	NS	0.81
Height of withers	66.72	67.59	68.27	67.16	67.12	66.57	NS	NS	NS	0.91
Height of buttock	68.67	68.23	67.97	67.99	67.87	68.83	NS	NS	NS	0.62
Hip length	11.8	11.98	12.16	11.44	11.6	11.39	NS	NS	NS	0.28
Abdominal circumference	95.01	89.48	92.88	89.57	89.50	89.94	NS	NS	NS	1.25
Chest circumference	95.01	89.48	92.88	89.57	89.50	89.94	NS	NS	NS	1.08
Testicles circumference	25.13	24.32	24.24	20.34	20.81	24.73	NS	NS	NS	1.26
Abattoir (kg)										
Head	2.44	2.56	2.41	2.44	2.48	2.35	NS	NS	NS	0.06
feet	0.96	1.01	1	0.93	0.97	0.93	NS	NS	NS	0.02
Wool and skin	6.49	6.28	6.52	5.81	6.19	5.95	NS	NS	NS	0.37
Liver	0.88	0.91	0.85	0.86	0.89	0.77	NS	NS	NS	0.04
Heart	0.25	0.21	0.21	0.23	0.19	0.21	NS	NS	NS	0.01
Testis	0.36	0.37	0.34	0.32	0.32	0.39	NS	NS	NS	0.03
Kidney	0.20	0.20	0.18	0.21	0.19	0.18	NS	NS	NS	0.01
Lung	0.55	0.58	0.52	0.53	0.57	0.52	NS	NS	NS	0.03
Hot carcass	23.28	24.98	24.26	24.12	24.48	23.63	NS	NS	NS	0.59
Cold carcass	22.65	14.38	23.76	23.47	23.85	22.99	NS	NS	NS	0.63
Neck	1.66	1.72	1.24	1.57	1.78	1.58	NS	NS	NS	0.06
Tail	4.49	5.35	5.5	4.96	4.3	4.56	NS	NS	NS	0.36
Ribs	6.9	7.18	6.77	6.88	7.37	6.63	NS	NS	NS	0.31
Thigh	6.09	6.42	6.29	6.33	6.69	6.26	NS	NS	NS	0.21

[1] Conjugated linoleic acid (CLA) levels (0 and 15 g kg dry matter), nano-selenium levels (0, 1 and 2 mg per kilogram of dry matter).
SEM: standard error of the means.
NS: non significant.

Nano-Se increased the expression of GPX1 and SEPW 1 in liver (P<0.05) but it had not important influence on PPARγ and SCD1 (P<0.05) (Figure 1). Conversely, CLA did not have important influence on the expression of GPX1 and SEPW 1 in liver (P<0.05) but its impacts on PPARγ and SCD1 was significant (P<0.01).

Many studies have reported dietary inclusion of selenium increased the expression of GPX1 and SEPW1 (Sunde, 2017; Yu *et al.* 2011), which is in accordance with our findings. On the other hand, other investigations have reported that the types and amounts of selenium did not improve the levels of selenoproteins enzymes and some genes including GPX1 and SEPW1 (Elsom *et al.* 2006; Zhou *et al.* 2009).

Conversely, Liu *et al.* (2012) stated that selenium supplemental at high levels (3.0 mg Se/kg) reduced SEPW1 mRNA amount in the pigs liver comparing to other levels (0.3 mg Se/kg diet). The enhanced in GPX1 can show to increase the health in animals, because it inhibits oxidation. Antioxidants including GPX1 and SEPW1 are synthesized within the cells and play role as primary defense system against free radicals (Sunde, 2017). The GPX1 and SEPW can reduce free radical production.

Table 4 The effects of treatments on blood metabolites (n=5)

Treatments	CLA: 0			CLA: 15			Nano-Se	CLA	Nano-Se × CLA	SEM
	Se: 0	1	2	Se: 0	1	2				
Glucose (mg/dL)	78.6	77.92	77.71	78.48	77.02	76.88	NS	NS	NS	1.86
HDL (mg/dL)	30.37^{b}	36.73^{a}	37.33^{a}	33.08^{b}	42.52^{a}	42.67^{a}	0.022	NS	NS	3.11
LDL (mg/dL)	32.27^{a}	25.62^{b}	25.53^{b}	30.88^{a}	25.07^{a}	23.92^{b}	0.006	NS	NS	2.14
VLDL (mg/dL)	9.46	9.80	9.98	8.95	10.62	10.73	NS	NS	NS	0.58
TG (mg/dL)	40.80	38.35	37.69	39.75	36.29	38.91	NS	NS	NS	1.79
T3 (nmol/L)	1.23^{b}	1.60^{a}	1.59^{a}	1.23^{b}	1.59^{a}	1.60^{a}	0.0001	NS	NS	0.03
T4 (nmol/L)	83.84^{a}	73.14^{b}	71.91^{b}	84.63^{a}	74.38^{b}	73.11^{b}	0.001	NS	NS	1.01
TP	5.06	5.20	5.18	5.15	5.42	5.53	NS	NS	NS	0.58
Cholesterol (mg/dL)	70.40	64.08	64.06	64.90	64.58	63.46	NS	NS	NS	2.11
GPx (kat/Lμ)	239.24^{b}	421.81^{a}	447.39^{a}	264.29^{b}	458.67^{a}	467.11^{a}	0.0001	NS	NS	31.60

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; TG: triglycerides; T3: triiodothyronine; T4: thyroxine; TP: total protein and GPx: glutathione peroxidase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

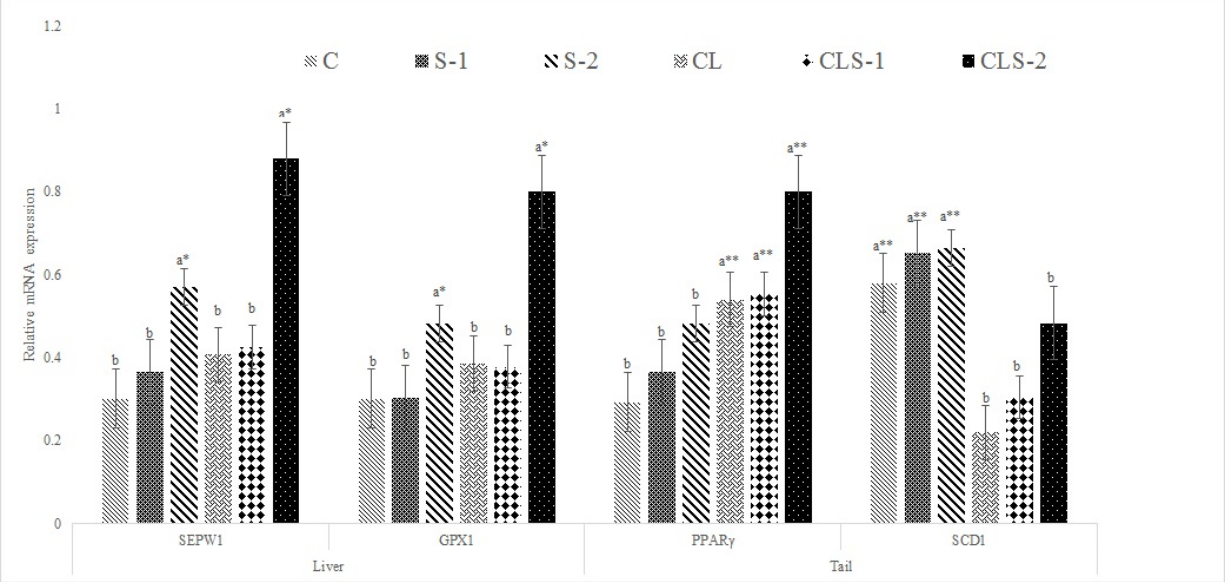

Figure 1 The effects of treatments on the expression of the desired genes
C: Control; S-1: Se 1 mg/kg DM; S-2: Se 2 1 mg/kg DM; CL: CLA (conjugated linoleic acid); CLS-1: 15 mg/kg DM CLA + 1 mg/kg DM Se and CLS-2: 15 mg/kg DM CLA + 2 mg/kg DM Se
GPX1: glutathione peroxidase 1; SEPW1: selenoprotein W1; PPARγ: peroxisome proliferator-activated receptor gamma and SCD1: stearoyl COA desaturase 1
Values are shown as mean ± SEM (n=5)
Columns with dissimilar letters indicate significant difference
* (P≤0.05) and ** (P<0.01)
NS: non significant

As has been previously reported, extensive oxidation increases the synthesis of unstable compounds known as free radicals (Levander O.A. and Beck, 1997), which would damage the biological components in the body and cause peroxidation of lipid, protein carboxylation and DNA strand breakages, ultimately causing various clinical consequences (Stone *et al.* 2010). Previous studies have been reported that some antioxidants such as vitamins and selenium are a secondary defense system (Ghaderzadeh *et al.* 2016).

Our results confirm the selenium role in these metabolic mechanism; suggesting that selenium is not only a key antioxidant but also a gene expression regulator.

CLA increased the expression corresponding to PPARγ and decrease the SCD1 genes. PPAR is active group of protein nuclear receptors that interfere with regulating the genes expression which affect energy metabolism, differentiation of cell, and death of cell, and plays main role in cellular differentiation, development and lipid, carbohydrates and proteins metabolism of the animals.

PPARγ is the key regulator corresponding the fat cells differentiation and production, and has a key role in controlling the overall metabolism of the body and is mainly expressed in fat tissues (Spiegelman, 1998).

CLA is considered as agonist component for PPARγ. PPARγ isoforms are part of the regulatory impacts of dietary fatty acids on gene expression and interferes in the fat storage and is observed to be great in adipose tissue. According to the outcomes of current research, CLA enhanced expression of PPARγ gene in fat tissue (Heikkinen *et al.* 2007). It can be stated a direct relationship presence of CLA in the diet and the amount of PPARγ gene expression, which will enhance the meat quality and change the profile of the adipose tissue. Other gene is SCD1 which plays main role in adding a double bond in the Delta 9 position in an extensive range of fatty acids that, leading to produce CLA in ruminants' meat and milk (Corl *et al.* 2001; Ntambi *et al.* 2002). SCD1 was the one of the first candidate genes to attract researchers to change the ratio of saturated to unsaturated fatty acids and increase the level of conjugated linoleic acids in milk (Medrano *et al.* 2007). The function of this SCD1 reduces with the existence of unsaturated fatty acids in the diet and increases with their absence because of the existence of CLA in the diet ($P<0.001$). It means that altering the diet can impact the function of genes that consequently changes the quality of animal products. Based on our findings, there was no synergism interaction effect was observed between selenium and CLA, which can be explained by presence of selenium in the diet, may cause the unsaturated fatty acids oxidation and affect the level of genes expression (Lawler *et al.* 2004). Furthermore, high amounts of selenium in the diet can damage the liver and consequently affect the metabolism corresponding to fatty acids and selenium (Galan-Cjllet *et al.* 2015).

Wang *et al.* (2014) reported that high levels of selenium could disrupt liver function and, consequently, impair the metabolism corresponding to fats, which concluded that high amounts of selenium may rise oxidative stress.

CONCLUSION

The outcomes of current research presented that nano-Se and CLA differently had improtant impacts on the expression of GPX1, SEPW1 and PPARβ1 and SCD1 genes and also some blood parameters. It means that nutrition can have significant role in changing the expression of gene which influences the amount of the products derived from ruminants through nutrition. Though, in current research, the synergism interaction between selenium and conjugated linoleic acid was not found and further research is needed.

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