EFFECT OF SUBCUTANEOUS ZERANOL IMPLANTS ON MEAT QUALITY OF AWASSI LAMBS AND CROSS-BRED KHALKHALI AND ABADEH GOAT KIDS

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ABSTRACT

This experiment was conducted to evaluate the effects zeranol implant on meat quality of Awassi lambs and cross-bred Khalkhali and Abadeh goat kids. Ten ram lambs (33.7 ± 0.5 kg and 4-month-old) and ten male goat kids (18.8 ± 0.7 kg and 4-month-old) were randomly assigned into two treatments (5 each treatment); C (control, 0 mg zeranol) and Z (24 mg zeranol implant) for each species. After 43 days experimental period. The animals were slaughtered, left for 24 hr post-mortem period and *Biceps femoris* muscle of each species were separated for measuring the proximal composition, TBARS values, cooking loss, drip loss and free fatty acids concentrations. Zeranol implants had significant (P<0.05) effect on dry matter, but did not have any effect on all other meat characteristics. However, dry mater was significantly higher for zeranol-implanted group as compared with control one. Moreover, significant effect of animal species was found for cooking loss, being highest in goat meat than ram lamb meat. Similarly, significant effect of animal species was noticed on TBARS values being highest in ram lamb meat. An obvious increase (P<0.005) in drip loss and TBARS values were observed for meat of both zeranol-implanted and non-implanted groups with storage period. It can be concluded that zeranol implant did not have any effect on meat quality for both ram lambs and male goat kids' meat.

Keywords: **drip loss, ram lambs, goat kids, zeranol, meat, TBARS, fatty acids , dry matter**.

مجلة العلوم الزراعية العراقية -2021 :52 (5):1084-1093 الدوسكي وآخرون

تاثير حقن الزيرانول في نوعية لحوم ذكور الحملان العواسية و جدايا الماعز المحلي المضرب الخلخالي × العبادي

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المستخلص

اجريت هذه الدراسة لدراسة تاثير حقن الزيرانول على نوعية اللحوم لدى الحملان العواسي وذكور جداء الماعز المحلي المضرب الخلخالي × العبادي. تم استعمال 10 من الحملان العواسية (33.7 ± 0.5 كغم وزن حي وعمر 4 شهر) و 10 من جدايا الماعز المحلي (18.8 ± 0.7 كغم وزن حي وعمر 4 شهور) قسمت عشوائيا الى مجموعتين متساويتين. تركت مجموعة السيطرة بدون حقن في حين حقنت حيوانات المجموعة المجموعة الثانية بهرمون الزيرانول (24 ملغم بشكل غرزة). وبعد مرور 43 يوم من المعاملة الهرمونية، تم ذبح الحيوانات وتبريد لحومها لمدة 24 ساعة ثم تم دراسة الصفات الآتية: التحليل الكيمياوي و اكسدة الدهن و نسبة الماء المفقودة بعد الطبخ و الماء المفقودة اثناء الخزن و احماض الدهنية الحرة . اظهرت النتائج ان حقن الزيرانول لم يكن له تاثير معنوي على معظم صفات اللحم بأستثناء زيادة المادة الجافة بنسبة 5 %. كما كانت اكسدة الدهن اعلى معنويا في لحوم حملان العواسية مقارنة بلحوم جدايا الماعز الاسود بنسبة 5 %. يمكن الاستنتاج ان حقن الزيرانول (24 ملغم لكل حيوان) لم يكن له تاثير على نوعية و صفات اللحم في كل من لحوم الحملان العواسية و جدايا الماعز الاسود.

كلمات مفتاحية: حملان, جدايا الماعز, الزيرانول, اللحوم

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INTRODUCTION

Sheep and goat production are rapidly developing among farmers in the broader Kurdish region in both commercial and small-scale farms (25). Both species are considered the most important farm livestock in Iraq and considered the main source of income for Iraqi farmers (2). Lamb and goat meat production and subsequent consumption have increased significantly due to an increased interest in this kind of meat (21). Recently, farmers have been seeking a better price for their animals to improve profitability. Improvements in profitability are pursued through shorter production periods and lower feed cost (24). Shorter production periods have been pursued through the use of growth promoters (31). There is a range of growth promoters available on the market; these vary from enzyme supplementation in feed, to the prophylactic use of antibiotics, probiotics, herbal metabolic stimulants, and the use anabolic steroids. The use of feed enzymes aid in the overall digestibility of feed which can yield an improved utilisation of nutrients (22). while on the other side of the scale anabolic steroids can be used to increase the growth rate and nutritional efficiency (13, 14). Anabolic steroids have been reported that the utilization of anabolic steroid implants offers the greatest rate of profitability identified with expanding efficiency outside of giving the most satisfactory feeding to beef cattle (26). Hence, application anabolic steroid implants increased about 10 to 21%, average daily gains and enhance feed: gain proportions in feedlot cattle by 6 to 14% (7). In addition, anabolic implanting tends to decrease fat deposition, resulting in declined marbling scores and reduced thickness of fat. It has been observed that zeranol implants is capable of modifying some of the fatty acid composition in meat (31), particularly polyunsaturated fatty acid content including n-3 and *n*-6 fatty acids, in both, intramuscular and subcutaneous fat tissues. An increasing polyunsaturated fatty acid is very important to human health (18) which have a great biological and cellular functions including producing of prostaglandins, improving of immune function, and organization of response to potential pain and inflammation (10). However, these are considered more susceptible to oxidative reactions which adversely influence the sensorial attributes of meat through the formation of lipid oxidation by-products such as hydroperoxides, malondialdehyde, 4-hydroxynonenal and volatile compounds (19). These lipid oxidation by-products are responsible for undesirable tastes, flavours, odours and discolouration of meat (23) and can cause a reduction in the nutritional value by the degradation of essential fatty acids (19). Several studies have been applying zeranol as an anabolic agent in feedlots that have shown increases in weight gain and enhances characteristic of carcass quality (18, 7, 2). However, to our knowledge, the effects of zeranol implants on the meat quality of sheep and goats in terms of changes in the physicochemical and chemical variables is not well documented. Thus, increasing meat yield by this mean without taking meat quality and consumer appeal in consideration is questionable. Meat quality is necessary to be evaluated. Therefore, the main purpose of this study was to address the use of zeranol implantations under common small-scale farming, pastured, practices, and the subsequent lamb and goat meat quality.

MATERIALS AND METHODS

Experimental animals and design

The experiment included two species of animals (Awassi lambs and cross-bred Khalkhali and Abadeh goat kids) and two treatments. Each species consisted of 10 male group, where males of Awassi lambs with 33.7 ± 0.5 kg of live weight and 4 months of age, while, males of local cross-bred Khalkhali and Abadeh goat kids with 18.8 ± 0.7 kg of live weight and 4 months of age. Each group of animals were randomly divided into two equal treatments. The treatments were: C (control, 0 mg zeranol) and Z (24 mg zeranol). The zeranol tap was implanted subcutaneously as two 12 mg implants, one behind each ear, making up the 24 mg for 42 days.]. All animals received similar diet while grazing on a lush local pasture. Water was available as *ad libitum* and refreshed daily. Following 43 days treatment period, the animals were slaughtered according to the procedures legislated by Kurdistan government and the average slaughter weights of animals were 23.15 ± 0.5

and 39.65 ± 0.5 kg. At 24 hours post-slaughter, the carcasses were dissected and the *Biceps femoris* (BF) muscles removed and vacuum packed and immediately frozen at -20 °C. The samples were transferred to the Meat Products Laboratory located at Animal Production Department, College of Agricultural Engineering Sciences for analysis.

Materials

Chemical composition of zeranol implants

Zeranol ((Ralgro® Implants for Beef Cattle, California, US), potassium hydroxide, Ethanol, Chloroform, Malonaldehyde bis (diethyl acetal), Hydrochloric acid, trichloroacetic acid, Acetic acid, Petroleum ether, Sodium hydroxide, Sulphuric acid, Boric acid (Scharlab S. L, Sentmenat, Spain) and 2-Thiobarbituric acid, phenolphthalein, Sodium thiosulfate, potassium iodide from Chem-Lab NV, Zedelgerm, Belgium)

Preparation of samples

This experiment was designed as factorial design consisted of a 2 x 2 x 3 with two species (lamb and goat), two treatments (zeranol treated and non-treated control), and three storage times (0, 4 and 8 days). For the preparation of meat samples, *Biceps femoris* muscle samples were thawed overnight at refrigeration temperatures (4-5°C). All meat samples were then stored in polyethylene bags and refrigerated at 4 °C for 0, 4, and 8 days, the meat samples were taken at each point of storage period and analysed as outlined below.

Proximate analysis

Biceps femoris muscle of each individual animals was minced by grinder (Heilbron powder grinder model watt HN-1019, Germany). Chemical composition of minced meat was measured according to the procedures described in AOAC (3). Dry matter of muscle samples was measured utilizing an oven drying method; meat samples were dried at 60°C in an oven for 72 h AOAC (3). Protein component was determined utilizing a Kjeldahl Analyzer and to calculate the protein content a conversion factor of 6.25 g of nitrogen/gram of protein was used AOAC (3). Total fat content was determined using Soxhlet extraction AOAC (3). Ash content was measured by burning dried meat in a 550°C muffle furnace for 3.5 hrs AOAC (3).

Thiobarbituric Acid Reactive Substances (TBARS) Determination

TBARS value was meaured in meat samples according to the procedure initially described by Buege and Aust (8). Roughly 0.5 g of ground meat was weighed and put in a 10 ml test tube to which 2.5 ml of TBA stock solution was added. Samples were then vortexed for 15 sec before being placed in a 95 °C water bath for 15 min, after which the tubes were rapidly cooled down and centrifuged (K Centrifuge PLC Series, Taiwan) at 2500 g for 10 min. The supernatant was transferred to a cuvette and the absorbance determined by spectrophotometer (Jenway, 6300 spectrophotometers, UK) at 532 nm against a blank. The TBARS in meat samples was determined and expressed as mg of malondialdehyde equivalents / kg meat using an appropriate malondialdehyde standard curve.

Drip Loss

Drip loss of meat was determined according to the method described by Honikel (17). Approximately 80 g of meat was weighed and placed individually into a netted bag and then suspended inside an airtight plastic container at 4 °C. After 24 h the meat samples were removed and dried using paper towel and reweighed. This represented the '0 day' results. The same procedure was used at days 4 and 8. Subsequently, the drip loss was calculated using the following formula: Drip loss (%) = [(Initial weight of raw meat (g)– final weight of meat (g))/ Initial weight of raw meat (g)] ×100.

Cooking Loss

Approximately 100 g of raw meat was weighed and wrapped with aluminium foil before being cooked in an oven at 160 °C until the internal temperature reached 71°C (approximately 10 minutes) as measured with a digital calibrated thermometer (ThermoPro TP025 thermometer, UK). After the cooking process, the meat was cooled to ambient temperature (23 °C). The meat samples were then blot-dried with paper towel and reweighed. Cooking loss was calculated using the following formula:

Cooking loss (%) = [(Initial weight of raw meat – weight of cooked meat)/ Initial weight of raw meat] ×100

Free fatty acid value

Free fatty acid (FFA) value was measured according to the procedure of Rukunudin et al. (26). Approximately 2.5 g of meat sample was mixed with 15 mL chloroform by means of a homogenizer for 1 min, after which the mixture was filtered using Whatman number-1 filter paper. Five drops of 1% ethanolic phenolphthalein as indicator were added to 10 mL of the filtrate and titrated with a 0.01 N ethanolic potassium hydroxide solution. The FFA value was determined using the following formula:

Free fatty acid (FFA %) = [((mL of titration × Normality of KOH × 28.2))/ (Initial weight of meat sample (g))] x100

Statistical Analysis

All data were analysed using Genstat (GenStat version 17, VSN International Ltd, UK). The data of TBARS value and drip loss of meat were analysed using factorial design of a 2 x 2 x 3 where the three factors were the animal species (lambs and goats), treatments (animal implanted with zeranol and control), and three storage periods (0, 4 and 8 days), with statistical general model as follow:

Yijkl= μ +Ai + Bj+ Ck+ ABC + eijk

Where: Yijkl is the observation value of the animals, μ is the overall mean, Ai is the effect of zeranol implantation, Bj is the effect of animal species, Ck is the effect of storage period, ABC is the interaction between zeranol implantation and animal species and eijk is the experimental error. The parameters (cooking loss, approximate analysis, free fatty acids) were analysed using two-way analysis of variance (ANOVA). The experiment was conducted in triplicate (n = 3). When the main factors and interactions was significant, Tukey's HSD test was used to identify the significant differences between means and the significance level of all data was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of proximate analysis of the meat from lamb and goat kids treated with zeranol and those without zeranol treatment are presented in Table (1). Non- significant differences were detected between species (lambs and goats) for dry matter, fat, protein and ash contents of the meat (Table 1). However, the percentages of all parameters, with exception of crude protein, were found to be higher in meat from lamb compared to the meat from goat. The mean values for the dry matter, fat, crude protein and ash components were 25.80 and 25.18%, 5.02 and 4.03%, 20.27 and 21.42%, and 1.11 and 1.10% for the goat and lamb meat samples respectively. These results were similar to values stated in the literature for goats and lamb's meat (27; 29). However, the current results were lesser than those reported by (6). Excluding data of dry matter, the proximate composition, did not significantly affected by the treatment and there were no significant interaction among factors (Table 1). These findings were consistent with those reported by Xiong et al (33) who pointed out that proximate chemical composition of *semimembranosus* muscle from steers implanted with zeranol did not differ from those unimplanted with zeranol. Furthermore, the fat content observed in the present study for meat of both species were similar to those reported by Vestergaard et al. (30), who found that fat content of meat from steers and heifers was not affected by subcutaneous injections of growth hormone like a pituitary-derived bGH (15-20 mg).

Effect of zeranol implant on lipid oxidation (TBARS value)

The lipid oxidation (TBARS value) of the *biceps femoris* muscle in goat kids and lambs either implanted with zeranol or not are noticed in Table (2). As compared with goat kids, Awassi lambs exhibited a significantly ($p=0.001$) higher level of TBARS values (0.99 and 0.67 mg MDA/kg meat) for lamb's and goat kids' meat respectively. This might be due to the fact that lamb meat had higher intramuscular fat than goat meat (4). TBARS is a secondary lipid oxidation product generated from the decomposition of hydroperoxides and considered a good indicator of oxidation status (19). An increase of TBARS in meat is indicative of advanced lipid oxidation (5). Elevated levels of TBARS are associated with off-odours and off-flavours which have a negative effect on sensory properties of meat (9), and as such decreases the shelf-life and nutritional values of meat (19).However, the effect of zeranol implants on TBARS value in muscle of both animal species was not significant ($p=0.144$).

Elevated values of TBARS were detected in meat of unimplanted animals compared to zeranol-implanted animals (Table 2). After the meat samples of both species were subjected to a storage period for 8 days, regardless of animal species and treatments, storage time had a significant (p = 0.002) impact on TBARS value. The TBARS value was 0.73 mg/kg meat at day 0, and decreased to 0.69 at day 4, while it markedly increased to 1.07 mg/kg meat at day 8 (Figure 1). The increasing in TBARS values on day 8 is a good indicator of occurring lipid oxidation which may be associated with decomposition of hydroperoxides and formation secondary lipid oxidation products such as malondialdehyde during storage period (5). similar phenomenon was detected in goat and lamb's meat (12).

Table 1. Proximate analysis of meat (% fresh weight basis) for Awassi lambs and goat kids (Mean ± SE).

variables	Species	Treatment		s.e.d	p value		
		Control	Zeranol		Species	Treatment	Inter S x T
Dry matter (%)	Goat	25.24	26.37	1.081	0.440	0.023	0.222
	Lamb	23.61	26.76				
Fat (%)	Goat	5.01	5.02	0.943	0.178	0.441	0.450
	Lamb	3.50	4.57				
Protein (%)	Goat	20.07	20.47	2.172	0.476	0.489	0.655
	Lamb	20.51	22.33				
Ash (%)	Goat	1.06	1.16	0.0658	0.849	0.084	0.979
	Lamb	1.06	1.15				

Table2. Effect of subcutaneous implants of zeranol on TBARS value (mg MDA/kg meat) of Awassi lambs and goat meat during storage period at 4 °C (Mean ± SE).

Main factors	TBARS value (mg MDA/kg meat)			SED	p value
Animal species (AS)	Goat	Lambs			
	0.67	0.99		0.086	0.001
Treatment (T)	Control	Zeranol			
	0.89	0.76		0.086	0.144
		Storage period (days)			
Storage period (SP)	0	4	8		
	0.73	0.69	1.07	0.106	0.002
Interaction AS x T	Treatment				
	Control	Zeranol			
Goat	0.74	0.59		0.122	0.795
Lamb	1.04	0.93			
Interaction AS x SP		Storage period (days)			
	0	4	8		
Goat	0.49	0.55	0.96	0.149	0.452
Lamb	0.96	0.83	1.18		
Interaction T x SP		Storage period (days)			
	0	4	8		
Control	0.77	0.77	1.14	0.150	0.940
Zeranol	0.68	0.61	1.00		

Effect of zeranol implant on drip loss /water holding capacity

The drip loss of the *biceps femoris* muscle in goat kids and lambs either implanted with zeranol or not are observed in Table (3). There was non- significant effect of animal species and treatment on drip loss proportion of meat. Moreover, drip loss was lower in animal implanted with zeranol compared to those unimplanted with mean values of 4.86% and 4.52% for control and zeranol treatment (Table 3). Drip loss is shown to be inversely proportional to water-holding capacity (15). Drip loss can be defined as water losing in meat during the storage period or following a cooking process (1), which is mainly dependent upon the capability of myofibrillar protein to retain and bind water (32). Hence, proteins of meat like fat, undergo–oxidation process by a free radical mechanism (15). These values were in contrary in terms of statistically aspects to those obtained by (11), who found that the water holding capacity of the two muscles *Longissimus dorsi and Biceps femoris* of lambs was affected significantly by the zeranol implants (P <0.05) which in both

muscles, a higher water holding capacity and less drip loss were observed in the muscles from implanted animals compared to the control group. Furthermore, regardless of animal species and treatments, storage time had a significant ($p < 0.001$) influence on drip loss (Table 3). Meanwhile, the drip loss of meat from both implanted and unimplanted group of animals significantly ($P<0.005$) increased with storage period. An elevating of drip loss in both group of meat during storage period is more likely returns to more oxidation of meat protein, which could decrease the capability of proteins to hold water. Khurshid (19) reported that meat exhibited the largest percentage of drip loss is considered an undesirable impact on meat quality. According to the results reported by Wang et al. (32), sarcoplasmic and myofibrillar protein solubility in meat decreased with an increase in time. Similar findings were reported by Maqsood, et al. (20), who found progressively an increase of drip loss in camel meat under refrigeration temperature with increasing storage time. Non-significant animal species x treatment x storage period interaction for drip loss ($p = 0.879$) were detected in meat (Figure2). However, drip-loss in all samples increased with increasing the storage periods.

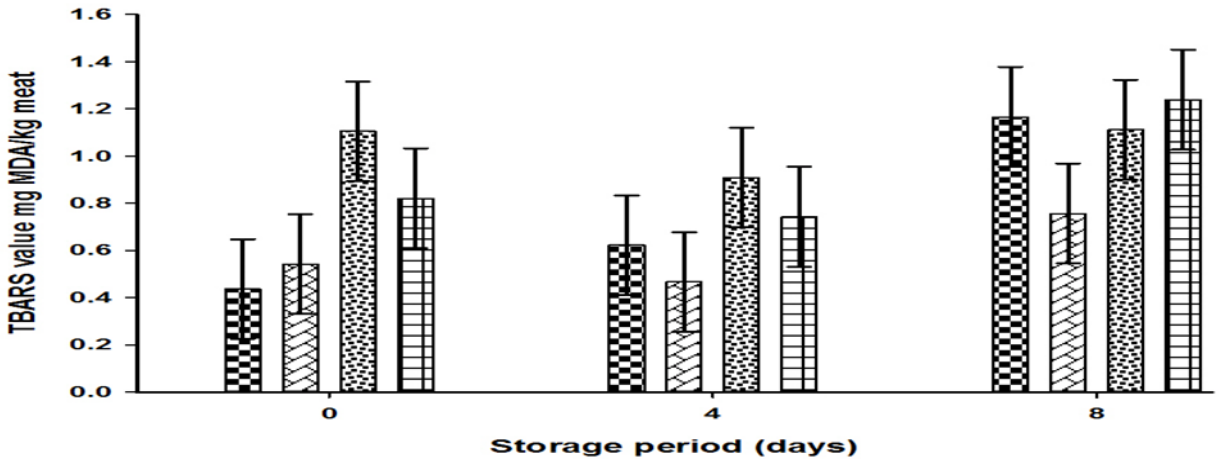

Figure 1. Interaction between subcutaneous implants of zeranol and storage period in Awassi lambs and goat meat., goat control (without zeranol) ( ), goat kids with zeranol ( ); Lambs control ( ); lambs treated with zeranol ( ). The data shown are the average and standard errors of differences of means

Table 3. Effect of subcutaneous implants of zeranol on drip loss (%) of Awassi lambs and goat meat during storage period at 4 °C (Mean ± SE).

Main factors	Drip loss (%)			SED	p value
	Goat	lambs			
Animal species (AS)	4.72	4.66		0.263	0.824
Treatment (T)	Control	Zeranol			
	4.86	4.52		0.263	0.206
	Storage period (days)				
Storage period (SP)	0	4	8		
	2.47	4.18	7.43	0.323	<0.001
Interaction AS x T	Treatment				
	Control	Zeranol			
Goat	4.87	4.58		0.373	0.853
Lamb	4.86	4.47			
Interaction AS x SP	Storage period (days)				
	0	4	8		
Goat	2.59	4.10	7.47	0.456	<0.001
Lamb	2.34	4.25	7.40		
Interaction T x SP	Storage period (days)				
	0	4	8		
Control	2.64	4.43	7.52	0.456	0.879
Zeranol	2.29	3.93	7.35		

Effect of zeranol implant on cooking loss

The drip loss of the *biceps femoris* muscle in goat kids and lambs either implanted with zeranol or not are showed in Table (3). Hence, regardless of zeranol implants cooking loss were significantly affected by animal species ($p<0.001$). Hence, highest proportion of cooking loss was detected in goat kids' meat (27.82%) compared to the Awassi lamb's meat (16.36%), respectively (Figure 3). Meat of unimplanted goat kids' group had highest percentage of cooking loss following by implanted goat meat, implanted Awassi lambs and unimplanted Awassi lamb's meat with mean values of 30.60, 25.04, 18.63 and 14.08 % respectively (Figure 3). Cooking loss is known total loss of water that occurred in meat during the cooking process and have been linked to the thermal process (1; 16), which denature and oxidize protein (32). Thus, reducing the ability of the meat proteins to retain water (1). Furthermore, the proportion of cooking loss of meat from both Awassi lambs and goat kids statistically did not affected ($p=0.289$) by zeranol implants. These results are consistent with those reported by Thompson et al. (28), who notifying that implanting heifers and steers with growth promoter Revalor-S. (28 mg oestradiol and 140 mg trenbolone acetate) resulted non-significantly effect on cooking loss, however, they found that implanted animals had slightly higher cooking loss than unimplanted group of animals. Similar findings were reported by Vestergaard et al. (30), who found that cooking loss of heifer's meat was not affected by subcutaneous injections of growth hormone like a pituitary-derived bGH (15-20 mg) during the breeding. Stability of lipid during the storage period. Figure (4) showed that the free fatty acids were not significantly affected by animal species ($p= 0.071$), while there was a trend of an increasing the amount of free fatty acid in Awassi lamb's meat compared to the goat kids' meat with mean values of 0.35 and 0.44 % for goat and Awassi lamb's meat respectively. These slightly elevated free fatty acids were detected in Awassi lambs could be due high fat content. Moreover, non-significant differences were found between implanted and unimplanted animals for both species with zeranol in respect of free fatty acids content in meat (Figure 4).

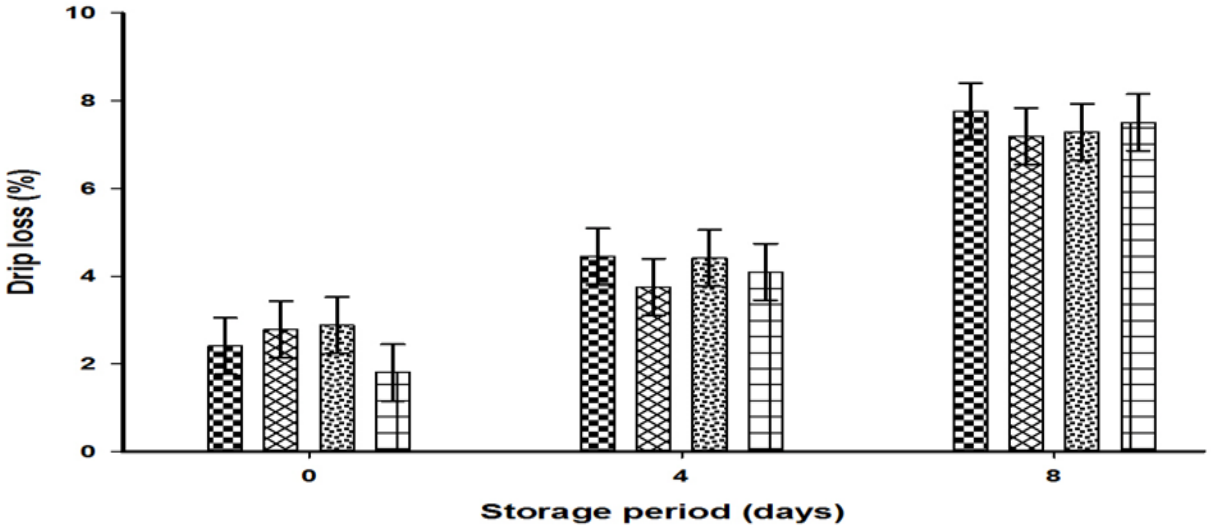

Figure 2. Interaction between subcutaneous implants of zeranol and storage period in Awassi lambs and goat meat. goat control (without zeranol) ( ), goat kids with zeranol ( ); Lambs control ( ); lambs treated with zeranol ( ). The data shown are the average and standard errors of differences of means

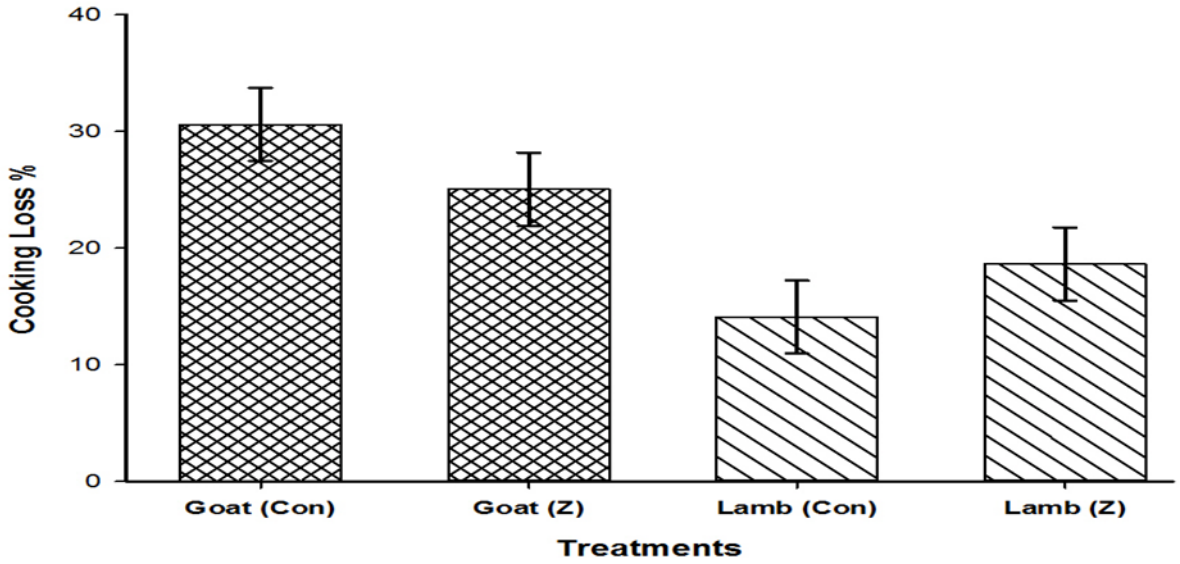

Figure 3. Effect of subcutaneous implants of zeranol on cooking loss (%) of Awassi lambs and goat meat (Mean ± SE).

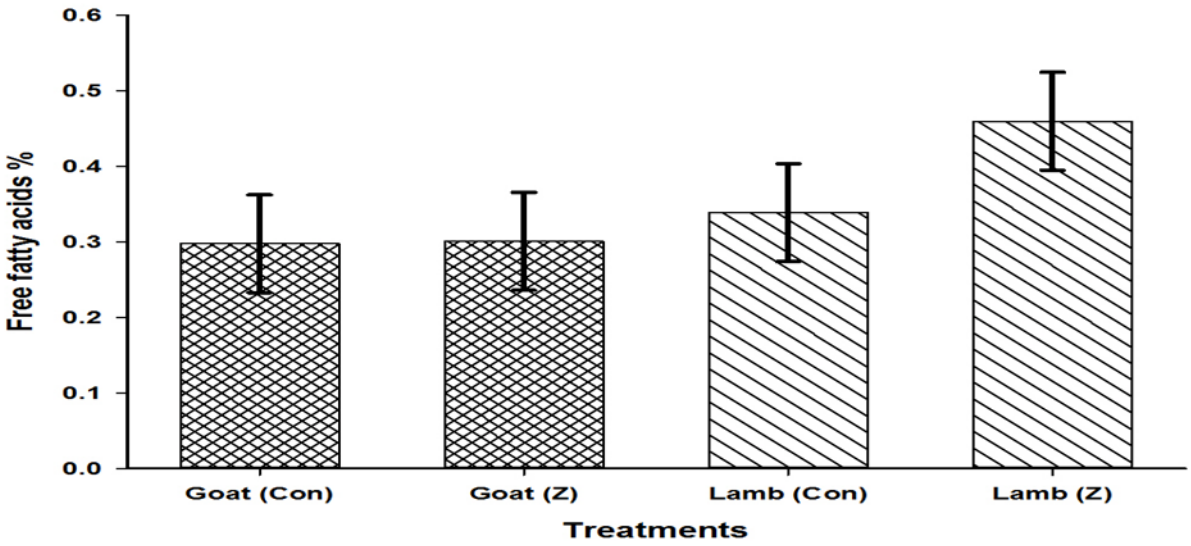

Figure 4. Effect of subcutaneous implants of zeranol on free fatty acids (%) of Awassi lambs and goat meat (Mean ± SE).

These data indicated that zeranol implants had no effect on all meat measurements of goat kids and Awassi lambs with exception of dry matter. Significantly an increase of drip loss and TBARS value of meat from both implanted and unimplanted animals were recorded during storage period. The amount of TBARS and cooking loss were found significantly higher in Awassi lambs than goat kids. Significant effect of animal species was detecting for cooking loss, which highest values observed in goat meat than lamb meat. Similarly, significant effect of animal species was observed on TBARS values which were highest in sheep meat.

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