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The usefulness of leptin measurements and ultrasound fat thickness for assessment of body fat reserves of Awassi lambs

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ABSTRACT

The objective of this study was to investigate the usefulness of leptin measurements for predicting back fat thickness and the amount of carcase fat in lambs with varying body weights (BWs). Blood samples were taken from 20 male Awassi lambs at 20, 25, 30, 35, and 40 kg BW. Tail, omental, dissected, and total fat were measured at slaughter (40 kg BW). Ultrasound fat thickness (UFT) increased with BW ($p < .05$) whereas leptin concentration tended to increase ($p = .09$). Serum leptin concentration was correlated with BW for 25, 30, 35, and 40 kg BW ($r = 0.53$, 0.52, 0.51 and 0.61, respectively; $p < .05$) and with UFT for 30, 35, and 40 kg BW ($r = 0.50$, 0.51 and 0.59, respectively; $p < .05$); therefore it appears that leptin concentration is not a suitable predictor for BW and UFT in low BW lambs. In addition, similar correlations were found for leptin concentration and hot carcase, omental, dissected, and total fat weights ($r = 0.62$, 0.64, 0.66 and 0.76, respectively; $p < .05$) but not for tail fat ($p > .05$ for 40 kg BW). The introduction of UFT and BW as independent variables in addition to leptin in the multiple regression equations improved the predictions for carcase fat only (57.6%, $p < .05$; 57.8%, $p < .05$ and 60.7%, $p > .05$ for leptin, leptin + UFT and leptin + BW, respectively). Leptin was a single predictor for omentum and dissected fat (41.4%, $p < .05$ and 43.5%, $p < .05$ respectively) whereas no improvement was observed for tail fat ($p > .05$). The other variables (UFT and BW) were not a predictor ($p > .05$) except for total fat ($p > .05$). These results indicate that serum leptin concentration in association with BW and UFT could be used to estimate total fat (tail, omental and dissectible carcase fat) in male Awassi lambs.

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Awassi; leptin; fat tissues; carcase fat amount

Introduction

Managed of fatness is a high economic interest in farm animals for today's breeders. It is well accepted that there might be a relationship between consumption of animal fat and an increased risk of cancer and heart disease (Department of Health 1994). Therefore consumer preference has changed. Consumers are increasingly interested in healthy foods and usually prefer lean instead of fatty meats. This consumer choice has potentially caused a reduction in retail price for fat animals (Sanudo et al. 2000; Romdhani and Djemali 2006). Additionally, fat synthesis is energetically more expensive than muscle synthesis leading to more costly animal production and reduced farm profitability (Macit 2002; Romdhani and Djemali 2006). High adipose tissue deposition in farm animals negatively affects not only meat quality but also the whole body metabolism, production efficiency and reproduction (Mácajová et al. 2004). As a result, a priority target in the sheep industry is the reduction of fat in order to improve the efficiency and profitability of commercial lamb production (Beermann et al. 1995).

Predicting the carcase composition of live animals, the optimum age of slaughter can be identified and real-time ultrasound technology has a practical value for producers in predicting an animal's readiness for slaughter or for a selection of sheep with superior carcase traits (Stanford et al. 2001; Romdhani and Djemali 2006; Leeds et al. 2007). Ultrasound is the most common method for estimation of carcase in live animals today (Orman et al. 2008, 2010). On the other hand, ultrasound has some problems and limitations for

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utility in sheep such as, the equipment cost, a low accuracy of measurement because of its small size and the mobility of subcutaneous fat layer, the lack of variation in subcutaneous tissue, a necessity to restrain animals, experiences of the operator and the presence of wool which is an added complicating factor (Stanford et al. 1998; Teixeira et al. 2006).

The practical use of hormones for carcase composition has been discussed over several years and leptin is one potential hormone in this respect. Ultrasound fat thickness (UFT) measurements were similar to the accuracy for the estimation of carcase fat by serum leptin concentrations at slaughter time in lambs (Altmann et al. 2005). On the other hand, the relationship between serum leptin concentrations in early life and carcase characteristics are poorly found out in lambs (Altmann et al. 2005, 2006).

The objectives of this study were (1) to compare UFT and serum leptin levels in live male Awassi lambs at different body weights (BWs) and (2) to estimate the relationship between carcase fat, UFT and serum leptin levels in male Awassi lambs.

Table 1. Nutrient compositions of starter concentrate and alfalfa hay on dry matter basis.

Item	Starter concentrate[a,b]	Alfalfa hay
Dry matter, %	92.95	88.29
CP, %	21.24	17.79
Ether extract, %	4.44	2.51
NDF, %	27.98	44.56
ADF, %	14.15	35.12
ADL, %	4.31	8.10
NFC[c], %	38.94	25.18
Ash, %	7.40	9.96

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.
[a]Saf Feed Industry, Eskişehir, Turkey.
[b]Contained the main ingredients: ground corn grain, ground barley grain, wheat bran, soybean oil, soybean meal, corn gluten meal, sunflower meal, molasses, mineral and vitamin mix, limestone, salt.
[c]NFC (Nonfiber carbohydrate) = 100 − (NDF% + CP% + Ether extract% + Ash%).

Materials and methods

Lambs were handled according to the EU directive number 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes and all procedures were approved by The Ethical Committee of Uludag University (2008-7/1).

Animals

A total of 20 single born Awassi male lambs were selected randomly from a sheep flock of the Research and Applied Center at The Faculty of Veterinary Medicine, Uludag University that is placed within the north west Turkey, 40° north latitude, 29° east longitude and at an altitude of 120 m above sea level. Lambs were born in first half of February. They were reared with their dams and weaned in 60 days. Dams were multiparous. There were lambs of 2–4 years old and live weights were similar (average was 50 kg). All animals were kept under the same management conditions and they were fed ad libitum (16% crude protein and 2.78 Mcal/kg metabolisable energy) according to the NRC (2007) recommendations. Feeding was based on dry alfalfa and commercial lamb concentrate feed (Table 1). They were watered free and kept during the whole study in a semi open sheep yard and weighed at first in 20 kg and slaughtered when they had a slaughter weight of 40 kg BW. Descriptive data are given in Table 2.

Ultrasound equipment

The ultrasound measurements were performed using a portable real-time ultrasound (Dynamic Imaging – MCV Concept model) which is a version of B-mode, producing images almost instantaneously using a 7.5 MHz and 6 cm linear transducer. The resolution of the scanner callipers was 0.01 cm. Scanner maximum penetration depth was 8 cm with a 7.5 MHz probe.

Table 2. Serum leptin levels and UFT at different BW and descriptive data.

			Descriptive data					
					Fat			
BW, kg	UFT, mm Mean ± SE	Leptin, ng/ml Mean ± SE	Exact BW, kg Mean ± SE	Exact age, days Mean ± SE	Omental, g Mean ± SE	Half carcass dissected, g Mean ± SE	Tail, kg Mean ± SE	Total, kg Mean ± SE
20	2.20 ± 0.05[a]	2.09 ± 0.16*	20.31 ± 0.44	70.00 ± 3.78				
25	2.73 ± 0.14[bc]	2.12 ± 0.13	25.29 ± 0.40	79.21 ± 2.86				
30	2.96 ± 0.14[cd]	2.29 ± 0.12	30.41 ± 0.30	104.00 ± 3.98				
35	3.41 ± 0.17[de]	2.33 ± 0.14	34.85 ± 0.30	117.04 ± 2.98				
40	3.71 ± 0.11[e]	2.67 ± 0.20*	39.72 ± 0.20	141.50 ± 5.57	360.00 ± 63.53	810.56 ± 94.20	1.94 ± 0.18	3.04 ± 0.26

BW: body weight; UFT: ultrasound fat thickness.
[a,e]Different superscripts indicates statistical significance ($p<.05$).
*Tendency.

Ultrasound measurements

Primarily, wool was removed from the measurement areas by a clipper. For ultrasound scanning, lambs were individually restrained to minimal movements and to ensure they were in a normal standing position. Acoustic gel was used as a coupling medium to allow better contact between the probe and the skin of the animal. Lambs were scanned just before slaughter. Real-time ultrasound was used to measure just the subcutaneous fat thickness (FT), over the longissimus dorsi muscle between the 12th and 13th ribs. The transducer was placed perpendicular to the backbone between the 12th and 13th ribs lateral to the vertebral column following physical palpation and preparation. All measurements were taken on the right side and 4 cm on the vertebral column of the lambs. After capturing the scan image, the thickness of back fat (UFT) at the same point was measured using the electronic callipers of the scanner.

Slaughter procedure and carcase measurements

All lambs were slaughtered by exsanguination at a commercial slaughterhouse using a standard slaughtering procedure. The fore and the rear limbs (feet) were then separated at the radiocarpal and tarsometatarsal articulations respectively and the pelt, head and all internal organs were removed. After slaughter, omental and tail fat were weighed. After being stored at +4 °C for 24 h in a conventional chill cooler, carcases were weighed without separating the tail and carcase yields were determined according to these weights. Then, carcases were split down the vertebral column with a band saw into two halves (right and left). The right half carcase was dissected as fat, muscle and bone. The right half of each carcase was ribbed at the 12th and 13th ribs (on a slice cut through the side parallel to the back bone and passing through the probe sites) at the same anatomical point where measurements were taken on the live animal using ultrasonics (Orman et al. 2008). Cross-sectional fat tissue was imaged over a transparent film. The chemical carcase composition was not analysed.

Blood sampling and leptin assay

Blood samples were taken from each lamb at 20, 25, 30, 35, and 40 kg BW. Exact BWs and blood sampling age were given as mean ± standard error in Table 2. Blood samples were stored at −80 °C until they were analysed. Serum leptin levels were analysed with a radioimmunoassay (RIA) that has been validated for use in several ungulate species, including ovine samples (Multispecies leptin RIA kit; Linco Research, St. Charles, MO, USA). The intra-assay coefficient of variation was 6.3%, the inter-assay coefficient of variation was 13.9%, and the limit of detection was 0.3 ng/ml.

Statistical analysis

Data were analysed by the GLM procedure, Pearson correlations and regressions used the SPSS (version 23.0, SPSS Inc., St. Louis, IL, USA) statistical programme. GLM procedure was used in comparison of UFT and leptin levels by time. Pearson correlation coefficients were determined among leptin and UFT, BW, fat tissues (tail and omentum), fat amount (dissected and total) and hot carcase weight and correlation coefficients were interpreted according to Evans and Over (1996). Regression analysis was used to determine the equations to predict carcase fat tissues and carcase fat amount. Tail, omentum and carcase fat amounts were estimated by multiple regression equations using leptin, UFT and BW. All regression analyses were performed to determine the independent variables that were predicting the fat amounts best. Stepwise regression equations were evaluated with the R^2 and the residual SD (RSD).

Results and discussion

Serum leptin levels and ultrasound fat measurements

Table 2 shows leptin levels and UFT at different BW in male lambs. Leptin concentration is getting higher by BW, but this increase did not show a statistical significance. However, there was a tendency between leptin levels of 20 and 40 kg BWs. Similar results were reported by Altmann et al. (2006) who reported that serum leptin did not change significantly in lambs (East Frisian and Blackheaded Mutton × East Frisian crosses) of 20 and 30 kg BW but the authors found that it increased at 35 kg and until 40 kg. In the present study, leptin levels did not reach to 3 ng/ml even in 40 kg lambs. On the other hand, serum leptin levels were at least 3 ng/ml for all BWs lambs in previous study (Altmann et al. 2006). Additionally, in the present study, leptin level increase was approximately 30% (no data info) until 40 kg but Altmann et al. (2006) reported that leptin increase was more than 30% between 30–35 kg BW, also Radwańska and Kosior-Korzecka (2016) reported that leptin level significantly increased after fifth month age, probably

that leptin level would be increasing significantly after 40 kg in Awassi male lambs. But consumers prefer lamb carcases of 40 kg live weight lamb in Turkey (TURKSTAT 2017). It can be concluded that age and live weight progress could be based on fat tissue increases. It is natural that fat tissue accumulation can show a difference in terms of age and BW in different breeds. Additionally, it is probable that blood leptin level could show a statistical difference after live weight.

Ultrasound fat thickness was significantly increased by BW ($p < .05$). Various results have been reported for UFT by several researchers. According to Orman et al. (2008, 2010) UFT was increased with the increase of live weight of Awassi lambs. Also, a similar result was reported by Altmann et al. (2005) for different breeds (East Frisian and Blackheaded Mutton × East Frisian crosses). On the other hand, Kuźnicka et al. (2017) reported that fat thickness over the eye loin did not change with live weight in merino male lambs. It stood out that back fat thickness was faster in increase than serum leptin levels by BW in Awassi male lambs. As mentioned previously, various results originated from a breed difference.

Relationship between leptin and fat tissues amount

Simple correlation coefficients obtained in leptin and some variables such as BW, UFT, hot carcase, omental, tail, dissected and total fat amounts for different BWs Awassi lambs are given in Figure 1.

Correlation coefficient between serum leptin concentration and BW was found 0.23 ($p < .05$) for Awassi male lambs and correlation coefficient was ($R^2 = 0.67$) strong. A higher result was reported by Wang et al. (2013) ($r = 0.44$, $p < .05$) between serum leptin concentration and BW in humans. Catunda et al. (2013) reported that leptin and BW correlation coefficients changed between 0.08 ($p > .05$) and 0.32 ($p < .05$) in different ages of pregnant and non pregnant ewes. On the other hand, the correlation coefficient between leptin and BW was not significant in beef cattle as stated by Geary et al. (2003). Naturally, various results can be obtained if species, gender and BW are different.

Also, a positive significant correlation coefficient was found between serum leptin level and UFT ($r = 0.42$; $p < .05$) in the Awassi male lambs and the correlation coefficient was ($R^2 = 0.88$) strong. A similar correlation coefficient ($r = 0.38$, $p < .05$) was reported by Swelum et al. (2017) for 39 kg BW male lambs. Daniel et al. (2002) reported that serum leptin concentrations were highly correlated with UFT ($r = 0.73$ and 0.89, $p < .05$ in fed and fasted Blackface ewes respectively), but in that study, ewes were heavier than the present study and had weights of 70.9–111.8 kg. On the other hand, the correlation coefficient between the serum concentration of leptin and backfat thickness was low ($r = 0.31$, $p < .05$) in castrated adult male Merino sheep as stated by Blache et al. (2000). Different results point out that the correlation coefficient the between serum leptin level and UFT is more related with BW and gender than breed.

Results showed that there was a positive significant correlation ($r = 0.62$, $p < .05$) between serum leptin concentration and hot carcase weight in 40 kg BW lambs. Altmann et al. (2005) reported the value for the same parameter to be 0.17 and 0.15 ($p > .05$)

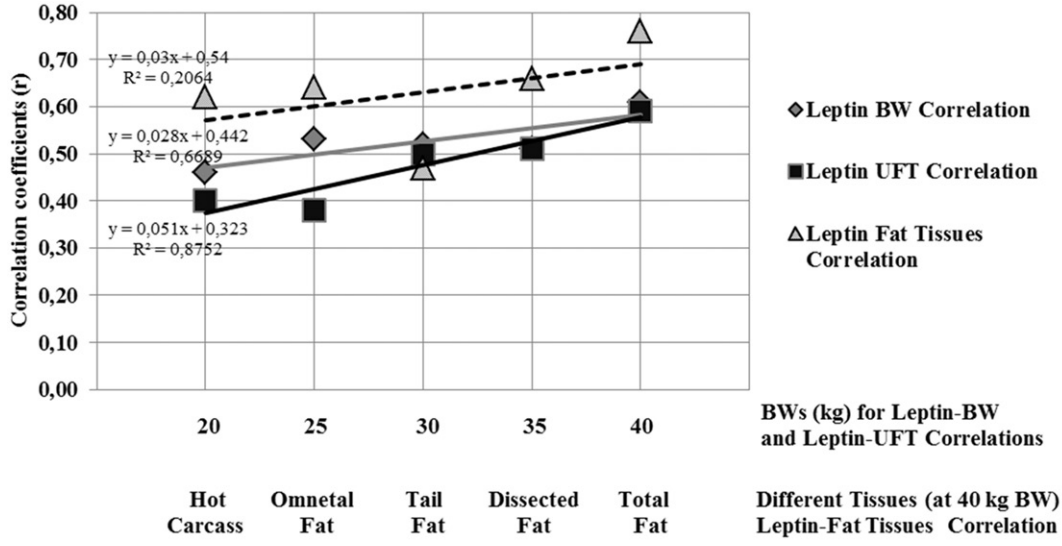


Figure 1. Correlation coefficients (*r*) between leptin levels and body weight (BW), ultrasound fat thickness (UFT) and fat tissues at different BW.

in 35 and 45 kg BW Merino and Blackheaded mutton breeds (male lambs) respectively, whereas same researchers reported the value for the same parameter to be 0.45 ($p < .05$) in whole lambs. On the other hand, Geary et al. (2003) reported the value for the same parameter to be 0.14 and not significant in beef cattle. Because of inadequate and inconsistent results on the correlations between serum leptin concentration and hot carcase weight, it is difficult to make a conclusion.

A similar positive and significant correlation coefficient ($r = 0.64$, $p < .05$) was found between serum leptin level and omental fat in Awassi male lambs and a similar correlation coefficient ($r = 0.60$, $p < .05$) was reported by Altmann et al. (2006) for 40 kg BW East Frisian and Blackheaded Mutton x East Frisian crosses lambs. A similar correlation coefficient ($r = 0.66$, $p < .05$) was reported by Swelum et al. (2017) between leptin level and internal fat in male lambs. These findings show that similar correlation coefficients can be found between serum leptin level and omental or internal fat if BW and gender are the same in different breeds.

Our data indicate that leptin was not affected by tail fat. The correlation coefficient between serum leptin level and tail fat was not significant ($p > .05$) for 40 kg BW Awassi male lambs. It is known that fat tail is a natural piece of some sheep in which energy reserves are stored and it helps sheep adaptable and able to survive the tough challenges of extreme climatic conditions. Therefore, fat tail cannot be considered as a simple body fat and it cannot be reflected in serum leptin level. Additionally, regional variations in fat deposition can affect serum leptin level (Altmann and von Borell 2007). In other words, every fat tissue cannot affect the serum leptin level.

An other correlation was detected between leptin and dissected carcase fat ($r = 0.66$, $p < .05$). The results were anticipated because previous studies indicated similar results for different species and breeds in this trait. For instance, Higashiyama et al. (2003) reported serum leptin level was positively related to carcase fatness ($r = 0.69$, $p < .05$) in Japanese Black steers and Altmann et al. (2006) reported a lower correlation coefficient ($r = 0.41$, $p < .05$) for male lambs at 40 kg BW. Also, Kuźnicka et al. (2017) reported an other lower correlation coefficient ($r = 0.41$, $p < .05$) but it was for low weight (20, 25 and 30 kg) BWs male merino lambs. These results mean that fat accumulation for different tissues can occur at different BWs in different breed and species.

The highest correlation coefficient was detected between serum leptin level and total fat ($r = 0.76$, $p < .05$). Different results have been reported in this trait. For instance, Ehrhardt et al. (2000) reported the value for the same parameter to be 0.91 ($p < .05$) in Holstein bull cows, whereas Yamada et al. (2003) reported the values changing between negative (not significant) and 0.81 ($p < .05$) in different BW Japanese Black × Holstein cross breed steers. Also, in male lambs, the various correlation coefficients were reported by Altmann et al. (2005, 2006) as 0.51, 0.49 and 0.48 ($p < .05$) for 35, 40 and 45 kg BW. A similar correlation coefficient was reported by Delavaud et al. (2000) as 0.68 ($p < .05$) for Lacaune ewes. Similar results from different researchers supported the idea that serum leptin level reflected more total fat than the other fat tissues.

Estimation of fat tissues and fat amount from leptin

Multiple regression equations with leptin, BW and UFT for predicting carcase fat tissues weights in Table 3 (for male lambs in 40 kg BW) were developed by multiple linear regression using leptin, BW and UFT as an independent variable and tail, omentum, dissected fat and total fat as dependent variables. Leptin was taken into account as the first and dominant variable in the models.

The results of tail fat data recorded in the current study showed that the tail fat variation could not be explained by using leptin alone (22%, $p > .05$) and an inclusion of BW or UFT measurements to the multiple regression equation did not improve the variation in male Awassi lambs. There is no research yet in lambs predicting the tail fat using leptin, BW and UFT, therefore, an objective comparison was not possible. According to these findings, it can be concluded that leptin, BW and UFT were not the best predictors for tail fat in male Awassi lambs.

We observed that 41.4% ($p < .05$) of the omental fat variation was explained using leptin alone and an addition of BW and UFT measurement to the multiple regression equation did not improve the multiple regression equation for male Awassi lambs. Higashiyama et al. (2003) reported that, the leptin mRNA expression levels were more related with subcutaneous adipose tissue but not with omental adipose tissue in different breed cattle. Also, Montague et al. (1998) reported similar results in humans. On the other hand, Kumar et al. (1998) reported that the expression of leptin mRNA in omental, perirenal and subcutaneous fat tissue was similar in lambs. The level of leptin gene expression may reflect considerable differences among adipose tissues and the reason is not

Table 3. Multiple regression equations for leptin, ultrasound fat thickness and BW for predicting different fat tissues and carcase fat content of male Awassi lambs at 39.72 ± 0.20 kg BW.

		Dependent variables															
		Tail				Omentum				Dissected fat				Total fat			
Equation No	Independent variables	Coefficients	R^2	RSD	p	Coefficients	R^2	RSD	p	Coefficients	R^2	RSD	p	Coefficients	R^2	RSD	p
1	Leptin	0.31	22.0	0.53	.17	0.15	41.4	0.16	.04	0.21	43.5	0.23	.05	0.73	57.6	0.56	.01
2	BW	0.31	15.6	0.52	.29	0.17	30.2	0.19	.13	0.18	17.5	0.27	.26	0.66	27	0.78	.15
3	UFT	0.27	5.0	0.58	.53	0.19	20.4	0.19	.19	0.29	22.8	0.26	.19	0.92	28.9	0.72	.11
4	Leptin	0.46	25.6	0.55	.35	0.17	41.6	0.17	.15	0.22	43.6	0.25	.18	0.78	57.8	0.60	.05
	UFT	−0.35				−0.03				−0.02				−0.12			
5	Leptin	0.38	40.8	0.47	.21	0.13	47.8	0.18	.14	0.21	43.5	0.25	.18	0.72	60.7	0.62	.06
	BW	0.01				0.07				0.01				0.08			
6	BW	0.20	30.9	0.51	.33	0.13	43.0	0.19	.18	0.12	30.4	0.27	.34	0.46	47.0	0.72	.15
	UFT	0.48				0.18				0.23				0.88			
7	Leptin	0.35	40.9	0.51	.41	0.10	48.4	0.19	.31	0.22	43.6	0.27	.37	0.68	60.8	0.68	.17
	BW	0.01				0.08				0.01				0.10			
	UFT	0.06				0.05				−0.02				0.10			

BW: body weight; UFT: ultrasound fat thickness; RSD: residual standard deviation.

known yet why the leptin expressions are various in different fat depots (Altmann and von Borell 2007). In the light of these findings it can be concluded that leptin is not a strong predictor but it offers an opinion and on the other hand, BW and UFT were not the predictors for omental fat in male Awassi lambs.

In the present study, it was found that carcase dissected fat variation could be explained by using leptin alone (43.5%, $p < .05$) but an addition of BW and UFT measurement to the multiple regression equation did not improve that for male Awassi lambs. Different were reported by previous researchers for different species. For instance, Altmann et al. (2005, 2006) reported that carcase fat variations were explained by using leptin alone between 23% ($p < .05$) and 34% ($p < .05$) for different sheep breeds. On the other hand, Higashiyama et al. (2003) reported that carcase fatness was explained by leptin 68% ($p < .05$) and 39% ($p > .05$) for different cattle breeds. It can be concluded that leptin is not a strong predictor like for omental fat but it offers an opinion and on the other hand, BW and UFT were not the predictors for dissected fat in male Awassi lambs.

Leptin was once again, the first variable admitted in the prediction model for total fat, accounting for 57.6% ($p < .05$) and when UFT was included in the model which consisted of leptin and UFT the explained variation was higher (57.8%, $p < .05$) and the best prediction was calculated when BW was included in the model which consisted of leptin and BW (60.7%, $p > .05$) for male Awassi lambs. Similarly, Altmann et al. (2005) reported leptin was the best predictor for carcase fat and they reported that when the empty BW was included in the model which consisted of leptin and the empty BW, the explained variation was higher and the highest prediction was calculated when UFT was included in the model which consisted of leptin and UFT in different BW sheep breeds.

Conclusions

This study has shown that there is a modest and significant relationship in plasma leptin level and different fat tissues and fat amount (Omental, Dissected and Total Fat) can be predicted moderately by leptin in 40 kg male Awassi lambs. Nevertheless, plasma leptin level is not a reliable predictor yet and cannot be recommended as an early predictor of carcase fat amount in comparison to ultrasound measurement of fat thickness. When leptin and UFT were combined UFT did not provide a contribution. It is likely that plasma leptin level would be a more reliable predictor of fat in heavier lambs, therefore, there is a need

for further investigations about it especially on higher BWs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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