



RESEARCH ARTICLE

Leptin Levels and Lipids Profile Determination in Different Sheep Breeds

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ABSTRACT

Leptin hormone is produced by fat cells and is believed to coordinate the control of body weight. This study was conducted to determine the levels of leptin, triglyceride, cholesterol, VLDL, LDL, HDL and glucose in serum apart from the body weight of animals, and to find out the correlations between them. Four breeds of sheep (Karakul; semi-fat tailed breed, Morkaraman and Norduz; fat tailed breeds, Tahirova; thin tailed breed) were used as research material under the same feeding conditions. Fifteen sheep of each group -for a total of 60- were selected as research material. ELISA tests were used to determine serum leptin levels; auto analyzer was used for the estimation of triglyceride, cholesterol, VLDL, LDL, HDL and glucose levels. Body weights were measured by a weighing scale. Leptin levels, along with triglyceride, cholesterol, VLDL, LDL, HDL levels and body weight, were higher in Tahirova sheep breed, and the difference was found statistically significant ($P < 0.05$). There was no statistically significant difference between the HDL levels of sheep breeds. Glucose levels were high in Karakul breed and statistically significant difference was determined between Karakul, Norduz and Tahirova breeds ($P < 0.05$). In conclusion, thin tailed sheep breeds were found to have a high leptin level, which shows that leptin levels are independent of the tail fat ratios. Leptin has been studied in detail in human medicine, but researches on animals were scarce. Apparently, further studies are needed to elucidate the factors of production of leptin in animals.

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INTRODUCTION

Leptin is a small peptide (16 kDa) (Coelho *et al.*, 2013; Tourkantonis *et al.*, 2013), with the structure of a single-chained polypeptide and synthesized by adiposities that signals available energy reserves to the brain and thereby influences development, growth, metabolism and reproduction (Denver *et al.*, 2011). Leptin is produced primarily in fat cells and also in the placenta, where it is regulated by estradiol and the stomach, where it is released into the intestine and then absorbed. Body mass index and body fat are strongly correlated with leptin production under conditions of regular food intake, leptin concentrations reflect the proportion of adipose tissue (Gambino *et al.*, 2010; Haleem *et al.*, 2014).

The location and size of the adiposities in the body are effective in the production of leptin (Chillard *et al.*, 2001). Big fat cells contain higher amount of leptin than small fat cells. Despite this, the largest remaining part of the mechanism has not yet been recognized.

Leptin gene and leptin release areas differ between storage cells of ruminants (Gregorio *et al.*, 2014; Qureshi *et al.*, 2015). Plasma leptin concentration correlated with total and relative visceral fat mass in lambs. It is reported that the plasma level of leptin and mRNA expression in sheep have a positive correlation with the body fat mass (Blasio *et al.*, 2010).

Increasing the body fat mass, food intake, glucocorticoids and insulin levels also increases the amount of ob gene mRNA levels and the plasma leptin levels. Insulin, glucose, glucocorticoids, and TNF- α appear to stimulate leptin secretion, whereas sympathetic and adrenergic stimulation, growth hormone, thyroid hormones, androgens, and melatonin appear to reduce leptin levels (Benatti *et al.*, 2011). A positive relationship was observed between circulating leptin levels and intramuscular fat content in cattle (Tokuda *et al.*, 2000).

A few studies report correlation between the expression of the leptin gene in food intake (Delavaud *et*

al., 2000) and the localization of storages of fat (Kim *et al.*, 2000; Chilliard *et al.*, 2001). In addition, there are also studies showing the effect of the nutritional, physiological and endocrine factors (Chilliard *et al.*, 2005) and addition of zinc to the ration (Avcı *et al.*, 2013) on the leptin level in ruminants. However, no research has been found in the literature to study the leptin levels of different breeds of sheep raised in the province of Van in Turkey.

This study is conducted on four different sheep breeds that are raised in the province of Van in Turkey: Karakul (semi-fat tailed breed), Morkaraman (fat tailed breed), Norduz (fat tailed breed) and Tahirova (thin tailed breed). The study aims to find body weights of animals and serum levels of leptin, triglyceride, cholesterol, VLDL, LDL, HDL and glucose in blood samples taken from the different tail types (fat tail-thin tale). The final step is to determine the correlation between body weights, tail types and these levels.

MATERIALS AND METHODS

Experimental group: The research material consisted of 4 different sheep breeds (Karakul, Morkaraman, Norduz, Tahirova) under the same care and nutrition conditions, with age of 2-3 years, all non-pregnant and healthy, and all are raised in Yuzuncu Yil University Research and Practice Farm in Van, Turkey. Fifteen from each sheep breed -for a total of 60- were studied. The sheep having the highest body weight were selected from each breed. No additional nutrient was given to any animal raised in the farm during July; and they were moved to pasture only in the mornings and afternoons.

Blood Samples: Initially the sheep of morning session were weighed and blood samples were taken from the jugular vein into 5 ml non-anticoagulant tubes. The tubes were centrifuged for 10 minutes at 4°C at 1240 x g (3000 RPM) and the blood serums were separated. Leptin, triglyceride, cholesterol, VLDL, LDL, HDL and glucose levels in these sera were examined.

Analysis: Measurement of leptin: Cusabio brand sheep leptin ELISA kit was used (Catalog number: CSB-EL 012870 SH), the examination was carried out on Stat Fax 2100 ELISA Reader.

Triglyceride, cholesterol, VLDL, LDL, HDL and glucose levels were measured by colorimetric test, using Roche Cobas brand kit stigmatized Modular PP auto-analyzer.

Body weights were determined by weighing all the sheep in the morning at 7:00 before moving to grazing.

Statistical analysis: One-way variance analysis was performed to compare the group means for the emphasized features. Afterwards, Duncan's test was applied. Pearson correlation coefficients were calculated in groups individually in order, to clarify the relationship between these variables. The statistical significance was considered to be 5% and SPSS statistical software program was used for calculations.

RESULTS

The average serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the sheep breeds of Karakul, Morkaraman, Norduz and Tahirova are presented in Table 1.

Serum leptin, triglyceride, cholesterol, VLDL, LDL levels and body weights were found to be at the highest level and statistically significant in breed of sheep Tahirova (P<0.05). The high level of glucose observed in the Karakul breed and the calculated difference between Norduz and Tahirova breeds was found significant (P<0.05).

As a result of the correlation analysis performed in Karakul sheep breed, values of positive correlation were observed between: leptin and cholesterol, HDL, body weight; triglyceride and cholesterol, VLDL; cholesterol and VLDL, HDL and body weight. On the other hand, negative correlation was observed between LDL and glucose (Table 2).

As a result of correlation analysis performed in the sheep breed Morkaraman, significant positive correlation was found between: leptin and body weight; triglyceride and cholesterol, VLDL, LDL, glucose; cholesterol and VLDL, LDL, glucose; VLDL and LDL; LDL and glucose; HDL and body weight. Negative correlation was found between triglyceride and HDL; VLDL and HDL (Table 3).

In sheep breed Norduz, a positive correlation was observed between: leptin and LDL, body weight; triglyceride and cholesterol, VLDL; cholesterol and VLDL. Also, a negative correlation was found between triglyceride and HDL; cholesterol and HDL; VLDL and HDL (Table 4).

As a result of correlation analysis performed in the sheep breed Tahirova, significant positive correlation was found between leptin and triglyceride, cholesterol, VLDL, LDL, body weight; triglyceride and cholesterol, VLDL, LDL, body weight; cholesterol and VLDL, LDL, body weight; VLDL and LDL, body weight; LDL and body weight. Furthermore, a negative correlation was observed between HDL and leptin, triglyceride, VLDL, LDL, body weight (Table 5).

Table 1: The mean serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the sheep breeds of Karakul, Morkaraman, Norduz and Tahirova

Parameters	Karakul n=15	Morkaraman n=15	Norduz n=15	Tahirova n=15
Leptin (ng/ml)	12.08±0.82 ^b	6.50±3.35 ^c	5.04±2.61 ^c	16.68±6.78 ^a
Triglyceride (mg/dl)	36.10±7.02 ^b	56.86±31.79 ^b	43.13±24.33 ^b	179.08±113.08 ^a
Cholesterol (mg/dl)	70.40±7.84 ^c	120.73±48.75 ^b	83.53±16.40 ^c	187.20±44.30 ^a
VLDL (mg/dl)	7.00±1.46 ^b	13.93±10.05 ^b	7.87±3.35 ^b	35.80±22.66 ^a
LDL (mg/dl)	17.60±4.01 ^b	25.87±6.22 ^b	18.16±3.84 ^b	107.00±30.96 ^a
HDL (mg/dl)	45.80±6.91	49.20±9.58	49.71±7.12	44.40±11.36
Glucose (mg/dl)	57.80±11.53 ^a	50.40±10.35 ^{ab}	46.00±9.05 ^b	46.53±11.46 ^b
Body Weight (kg)	37.28±1.12 ^c	47.90±3.32 ^b	46.37±2.12 ^b	55.14±2.46 ^a

Values (mean±SE) bearing different letters in a row differ significantly (P<0.05).

Table 2: Correlation of serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the Karakul sheep

Karakul	Leptin	Triglyceride	Cholesterol	VLDL	LDL	HDL	Glucose	Body Weight
Leptin	1							
Triglyceride	0.37	1						
Cholesterol	0.80*	0.74*	1					
VLDL	0.38	1.00*	0.74*	1				
LDL	0.46	0.24	0.28	0.25	1			
HDL	0.55*	0.48	0.81*	0.48	-0.31	1		
Glucose	0.02	-0.29	0.10	-0.30	-0.78*	0.63*	1	
Body Weight	0.85*	-0.05	0.60*	-0.04	0.31	0.51*	0.32	1

*P<0.05.

Table 3: Correlation of serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the Morkaraman sheep

Morkaraman	Leptin	Triglyceride	Cholesterol	VLDL	LDL	HDL	Glucose	Body Weight
Leptin	1							
Triglyceride	-0.27	1						
Cholesterol	-0.20	0.94*	1					
VLDL	-0.18	0.97*	0.94*	1				
LDL	-0.15	0.92*	0.97*	0.90*	1			
HDL	0.41	-0.51*	-0.42	-0.61*	-0.30	1		
Glucose	-0.23	0.61*	0.64*	0.44	0.72*	0.26	1	
Body Weight	0.90*	-0.24	-0.13	-0.22	-0.06	0.53*	0.02	1

*P<0.05.

Table 4: Correlation of serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the Norduz sheep

Norduz	Leptin	Triglyceride	Cholesterol	VLDL	LDL	HDL	Glucose	Body Weight
Leptin	1							
Triglyceride	-0.12	1						
Cholesterol	-0.17	0.93*	1					
VLDL	-0.03	0.98*	0.92*	1				
LDL	0.74*	-0.13	-0.01	-0.02	1			
HDL	0.14	-0.73*	-0.53*	-0.63*	0.28	1		
Glucose	0.27	-0.23	0.02	-0.24	0.39	0.44	1	
Body Weight	0.84*	-0.05	-0.13	-0.04	0.43	-0.06	0.23	1

*P<0.05.

Table 5: Correlation of serum leptin, triglyceride, cholesterol, VLDL, LDL, HDL, glucose levels and body weight of the Tahirova sheep

Tahirova	Leptin	Triglyceride	Cholesterol	VLDL	LDL	HDL	Glucose	Body Weight
Leptin	1							
Triglyceride	0.70*	1						
Cholesterol	0.70*	0.87*	1					
VLDL	0.70*	1.00*	0.87*	1				
LDL	0.70*	0.77*	0.96*	0.77*	1			
HDL	-0.59*	-0.69*	-0.46	-0.69*	-0.51*	1		
Glucose	0.34	-0.26	0.11	-0.27	0.22	0.36	1	
Body Weight	0.94*	0.86*	0.87*	0.86*	0.84*	-0.61*	0.17	1

*P<0.05.

DISCUSSION

Circulating leptin level in serum is accepted as a good indicator of changes in the storage of body fat in ruminants, contrary to the mono-gastric species (Delavaud *et al.*, 2000). Studies on ruminants show that leptin could affect the physiological functions of the animals mentioned. It is reported that nutrition, physiological and endocrine factors affect leptin levels in ruminants (Chilliard *et al.*, 2005).

Fat tail sheep breeds store fat in their tails as opposed to European thin tailed sheep breeds. Some biochemical means within the breeding programs may have modified the storage of lipids in the tail. However, the molecular mechanisms leading to fat storage in different parts of the body between the thin tailed and fat tailed sheep breeds are not yet clear.

The essential factor in the production of leptin in ruminants and other species is adipose tissue and peripheral leptin concentration increases in case of risen in adipose tissue (Wegner *et al.*, 2001). Therefore, the plasma leptin is a good factor indicating body weight in ruminants (Delavaud *et al.*, 2000). In early studies

conducted on ruminants, not only anatomical variety in the body of ruminants and but also the difference between adipose tissue indicate the intensity of the expression of the leptin gene (Kim *et al.*, 2000).

In this study, the statistical significance was detected at a level of P<0.05 between Tahirova -which has the highest leptin level- and Karakul, and Morkaraman and Norduz breeds. There are studies that show changes in leptin levels between fat and non-fat tail sheep breeds are insignificant (Eryavuz *et al.*, 2007; Avcı *et al.*, 2013). In this study, serum leptin levels were found higher in thin tailed breed and lower in fat tailed breeds

Leptin is a hormone that regulates energy balance and body weight controls, provides information to the brain about the storages of body fat (Lemor *et al.*, 2010). Increased leptin mRNA was observed in a very low level of visceral fat in the subcutaneous adipose tissue in sheep. High leptin mRNA was found in the subcutaneous adipose tissue in goats (Chilliard *et al.*, 2001). A high mRNA leptin would be synthesized and most of these results may be due to high leptin levels in the thin tails. Therefore, in this study, the leptin level was found higher in thin tailed sheep breeds. In addition to these results,

although the level of leptin is proportional to the adipose tissue in the body, it is found not to totally be dependent on it. The most important indicator of this condition is the reduced level of leptin in the absence nutrition; as serious depletion occurs in adipose tissue in the case of hunger and higher leptin levels appear when fat storages are not full of nutrition (Frederich *et al.*, 1995). Therefore, more research is needed to explain the effective and regulatory factors in the production of leptin.

Basal level of leptin plays a key role as it forms more than half of triglyceride in serum. It is closely related to changes in leptin levels, regardless of variations in cholesterol, triglyceride, VLDL, blood glucose and body weight. In addition, this information shows that leptin is closely related to triglyceride, more than glucose (Gültürk *et al.*, 2005). Additionally, 31% rise of triglyceride level was observed when exogenous leptin was applied to animals. At the same time, it leads to decrease in index of triglyceride in the liver, skeletal muscle and pancreatic langerhans islet cells of rats (Cohen *et al.*, 1998). In a study conducted in rats, it has been reported that leptin increases the impact of lipoprotein enzyme lipase, and increases the level of triglyceride (Sarmiento *et al.*, 1997).

Analyzing the lipids profile; it was found that serum triglyceride, cholesterol, VLDL and LDL levels were statistically higher in Tahirova sheep breed compared to other breeds, and the difference was found to be significant at $P < 0.05$. No statistical difference could be found among the breeds of sheep in the level of HDL. In the analyzes of correlation, it was found that there is a positive correlation between leptin and cholesterol ($P < 0.5$) and HDL ($P < 0.05$) in the Karakul breed; positive correlation between leptin and LDL ($P < 0.05$) in Norduz breed; positive correlation between leptin and triglyceride, cholesterol, VLDL and LDL ($P < 0.05$) and negative correlation with HDL ($P < 0.05$) in Tahirova breed. The high level of serum triglyceride, cholesterol, VLDL and LDL in Tahirova breed over other breeds may result from the high levels of leptin in serum. Indeed, it is a fact that leptin hormone stimulates lipolysis; and therefore an increase may have occurred in the creation parameters of the lipids profile.

The most important factor in the regulation of body weight is leptin. The total mass of adipose tissue by body mass index is positively related to serum concentrations of leptin (Daniel *et al.*, 2002). In a study Thomas *et al.* (2002), it was reported that there is a correlation between body weight, increased body weight per day and the concentration of leptin. Leon *et al.* (2004), reported that there is a positive relationship between body weight, weight gain and the leptin levels in heifers.

In this study, body weight in Tahirova sheep was considered statistically significant at $P < 0.05$ compared to other breeds. In addition, a positive correlation was observed between leptin levels and body weights of the Karakul, Morkaraman, Norduz and Tahirova sheep breeds ($P < 0.05$). Although animals with the same care and nutrition conditions have the highest body weight among all breeds of sheep analyzed were selected for the study, there were statistically significant differences in the body weight in classified basis of breed. It is known that insulin and leptin plays an important role in the distribution of nutrients and regulation of their use (Chilliard *et al.*, 2005).

Rumen fermentation in polygastric animals plays a very important role in the ruminant digestive system, and provides the passage of glucose into volatile fatty acids in the liver. It has been reported that there is a positive correlation between glucose and fleece weight in sheep (Mert *et al.*, 2003). Ruminants generally absorb the little part of the glucose directly from the digestive system; however, they use various substrates such as lactate, glycerol and amino acid via gluconeogenesis (Demigne *et al.*, 1991).

It was reported that factors such as physiological situations, reproduction status, nutrition, age, climate and breed affect the concentration of glucose in ruminants (Nachtomi *et al.*, 1991).

In one of several studies investigating the relationship between glucose and leptin (Sivitz *et al.*, 1997), it is reported that leptin administration subcutaneously, increases the level of plasma glucose in rats and in another study it is reported that intravenous administration of leptin is not effective on the concentration of glucose in sheep (Henry *et al.*, 1999). Tokuda *et al.* (2000) reported that the concentration of glucose decreased only a long time after leptin administration and it is not effective when it is administrated for a short period. Block *et al.* (2003) expressed that the concentration of leptin has a tendency to show positive correlation with the concentration of glucose in cattle.

In this study, high level of serum glucose was observed in Karakul breed and the statistically significant was found at $P < 0.05$ between this breed and Norduz and Tahirova sheep. Correlation could not be found between leptin and glucose in all breeds tested. As Nachtomi *et al.* (1991) reported, a variety of factors in ruminants can affect the level of glucose.

Conclusions: Serum leptin, triglyceride, cholesterol, VLDL, LDL, glucose levels and body weights were observed in Karakul, Morkaraman, Norduz and Tahirova sheep breeds whose tail types are different. Although statistically significant was detected between serum leptin, triglyceride, cholesterol, VLDL, LDL, glucose levels and body weights in these four breeds of sheep; no difference was observed in the serum levels of HDL. Serum leptin, triglyceride, cholesterol, VLDL, LDL, glucose levels and body weights of Tahirova sheep were high ($P < 0.05$) compared to Karakul, Morkaraman and Norduz sheep. No statistical difference was found in the amounts of serum HDL. The high level of glucose was observed in Karakul sheep and significance was detected ($P < 0.05$) between Karakul, Norduz and Tahirova breeds. As a result, high level of leptin in thin tailed sheep shows that production of leptin is not related to the ratio of tail fat. Detailed studies have been conducted on humans; however, further research is needed to explain efficient regulatory and mechanically factors in the production of leptin in animals, as there is a lack of study on the effectiveness of leptin in them.

Authors' contributions: HM designed the experiments. AC and BC collected and analyzed the samples. All authors interpreted the data, critically revised the manuscript and approved the final version and agreed to publication.

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