MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (4): 634-642, 2009
ISSN: 1680-5593

Tanniniferous Oak (*Quercus hartwissiana*) Leaves Do Not Affect Plasma Levels of Leptin, IGF-I and LH in Lambs

[1]M. Cenesiz, [2]S. Yildiz, [1]M. Kaya, [2]F. Onder, [3]O. Ucar, [2]M. Uzun,
[4]M. Blackberry, [4]D. Blache, [2]I. Kaya, [2]Y. Unal, [5]A. Oncüer and [4]G.B. Martin
[1]Department of Physiology, Faculty of Veterinary Medicine,
Ondokuzmayis University, Kurupelit, Samsun, Turkiye
[2]Department of Physiology and Animal Nutrition, Faculty of Veterinary Medicine,
Kafkas University, Kars, Turkiye
[3]Department of Artificial Insemination, Faculty of Veterinary Medicine,
Ataturk University, Erzurum, Turkiye
[4]Department of Agriculture, University of Western Australia, Crawley, Perth, Australia

Abstract: Aim of the current study, was to evaluate the effects of diets containing different levels of tanniniferous oak (*Quercus hartwissiana*) leaves in the absence and presence of a tannin binding substance, polyethylene glycol, on plasma leptin, IGF-I and LH levels in ewe-lambs. Lambs (n = 42) were kept in individual metabolism cages and a total of 7 groups (n = 6 per group) were formed (Group I, control; Group II, 185 g leaf; Group III, 370 g leaf; Group IV, 185 g leaf plus 10 g PEG; Group V, 185 g leaf plus 20 g PEG; Group VI, 370 g leaf plus 20 g PEG; Group VII as 370 g leaf plus 40 g PEG). All groups were given 272 g concentrate and varying amounts of hay in a way that the amount of roughage was equal to 645 g. The diets were isonitrogenous and isoenergetical and the experiment continued for 60 days. Blood samples were collected fortnightly for the measurement of leptin and IFG-I. Additionally, on day 55 post-prandial rhythm of leptin was assessed with 30 min intervals for 8 h and LH response to naloxone was assessed for 2 h with 15 min intervals. For the determination of LH pulsatility, blood samples were collected on day 45 of the experiment with 15 min intervals for 6 h. IGF-I levels, fortnigtly leptin secretion, LH pulsatility and LH response to naloxone did not differ among the groups. Post-prandial leptin secretion appeared to be episodic but it was not affected by dietary treatments ($p>0.05$). Both leptin and IGF-I concentrations were positively correlated to LH pulse frequency ($R^2 = 0.235$, $p = 0.027$ and $R^2 = 0.248$, $p = 0.006$, respectively). In conclusion, the results suggest that tanniniferous Q. hartwissiana leaves do not have any effect on leptin, IGF-I and LH secretion and that leptin secretion appears to be in episodic manner and that leptin and IGF-I secretions appear to signal reproductive axis in ewe-lambs.

Key words: Oak leaves, ewe-lambs, sheep, tannins, LH, IGF-I, leptin

INTRODUCTION

Tannins are polyphenolic substances with various molecular weights and variable complexity (Makkar, 2003). They are found in many plants used as foods or feeds (Hagerman and Carlson, 1998) and their effect varies from beneficial to harmful (Makkar, 2003). Tannins are also present in oak leaves (Makkar and Singh, 1992a-c; Yildiz *et al*., 2002a, 2005) and Polyethylene Glycol (PEG) is used to deactivate tannins due to its strong affinity to tannins (Landau *et al*., 2000; Villalba *et al*., 2002). Number of literatures on the effect of tanniniferous plants is quite limited and most of the studies have focused on their effect on digestive system (Makkar, 2003). On the other hand, apart from digestive physiology, tanniniferous diets have been shown to be biological antioxidants (Hagerman and Carlson, 1998) and implicated to play roles in lipid metabolism (Barry *et al*., 1986; Yugarani *et al*., 1992, 1993; Wrisez and Lambert, 2001).

In ruminants, energy and related phenomena is the main determinant of reproductive activity especially that of LH pulse frequency (Chilliard *et al*., 1998; Yildiz *et al*., 2002b; 2003a, b). Leptin and IGF-I serve as the mediators

Corresponding Author: Metin Cenesiz, Department of Physiology, Faculty of Veterinary Medicine, Ondokuzmayis University, Kurupelit, Samsun, Turkiye

for nutrition-reproduction interactions in these animals (Blache *et al*., 2000; Yildiz *et al*., 2003a). Consumption of tanniniferous plants is widespread but to the best of our knowledge, there is no study investigating the effects of such plants on leptin/IGF-I and LH axis. Considering their effects on digestive physiology and lipid metabolism, it is plausible to hypothetise that consumption of tanniniferous oak leaves (*Quercus hartwissiana*) will affect leptin, IGF-I and LH secretion. To test this hypothesis, we offered fat-tailed Tuj lambs daily for 60 days either 185 or 370 g oak leaves in the absence or presence of PEG (5 or 10% of shade-dried leaf sample) and investigated long-term and post-prandial leptin secretion, pulsatile secretion of LH and plasma IGF-I levels.

MATERIALS AND METHODS

Animals and experimental design: Experimental design and digestive performance is given elsewhere (Yildiz *et al*., 2005). Briefly, 42 fat-tailed Tuj lambs were used for the current experiment and they were divided into 7 groups (n = 6 per group). The lambs were randomly allocated to 1 of 7 treatment groups as follows: control group (645 g hay with no leaf no PEG); 185 g leaf + no PEG group; 185 g leaf + 10 g PEG group; 185 g leaf + 20 g PEG group; 370 g leaf + no PEG group; 370 g leaf + 20 g PEG group; 370 g leaf + 40 g PEG group. Amount of PEG (0, 5, or 10%) was calculated in relation to amount of sun dried leaves. All groups were given 272 g concentrate and varying amounts of hay, such that the amount of leaf plus hay (hay/alfalfa mix, containing the same amount of CP as leaves) was equal to 645 g. Leaves were given first, followed by hay and the concentrate. PEG was mixed with concentrate. The lambs were housed in individual metabolism cages and the experiment lasted for 60 days following 17 days of adaptation period to the cages and dites (except leaves and PEG). Feed was offered twice daily at 08.00 and 16.00 h and animals had access to water at all times.

Blood samples for leptin and IGF-I was taken from jugular venepuncture on Days 0, 25, 45 and 60 of the experiment before morning feeding. Additionally, on day 55, blood samples were taken with 30 min intervals starting from 9.00 am until 5.00 pm and on this day animals were fed at 9.30 am and 4.00 pm. Naloxone was injected at 1.1 mg kg^{-1} dosis at 10.30 am (0 h) on Day 55 and blood samples were collected for 15 min intervals for 2 h. For the determination of LH pulsatility, serial blood sampling was carried out with 15 min intervals for 6 h on Day 45.

Body condition scoring: Body condition scoring was carried out on Days -17, 20 and 56 according to Russel *et al*. (1969) on the scale of 1-emaciated to 5-obese.

Leptin assay: Leptin concentrations were measured using a sensitive ovine radioimmunoassay developed by Blache *et al*. (2000). Briefly, antibodies against b/o-leptin were raised in a male emu *(Dromaius novaehollandiae)*. B/o-leptin was iodinated by the chloramineT method and labelled hormones were separated from free iodine on a Sephadex G25 column (Pharmacia, Sydney NSW, Australia). Peak fractions obtained were stored at 4°C. In the assay, 2 triplicates of standard (b/o-leptin) and 100 μL duplicates of unknown samples, 50 μL anti-b/oleptin (1:5000) and 50 μL normal emu serum (1:500) were added into glass tubes. After incubation overnight at 4°C, 50 μL ^{125}I-b/o-leptin (approximately, 10.000 c.p.m.) was added and the mixture was incubated for 48 h at 4°C. To precipitate the antibody-antigen complex, 100 μL sheep anti-emu immunoglobulin serum (diluted 1: 12) was added and tubes were incubated again for 48 h at 4°C. Before centrifugation at 2000 g for 30 min polyethylene glycol 6000 (Sigma, St Louis, MO, USA) was added to the tubes. Supernatants were decanted, pellets were allowed to dry overnight and radioactivity was counted. Limit of detection of the assay was 0.07 ng mL^{-1}. Samples taken on days 0, 25, 45 and 60 were analyzed in one assay and the intra-assay coefficients of variation at 0.80, 1.79 and 2.57 ng mL^{-1} levels were 6.9, 7.7 and 2.1%, respectively. Samples taken for post-prandial secretion of leptin were also analyzed in one assay and the intra-assay coefficients of variation at 0.71, 1.54 and 2.36 ng mL^{-1} levels were 9.0, 3.9 and 3.4%, respectively.

IGF-I assay: Plasma concentrations of IGF-I were measured in duplicate by chloramine-T method described by Gluckman *et al*. (1983). Interference by binding proteins was minimised by acid-ethanol cryoprecipitation method validated for ruminants by Breier *et al*. (1991). Briefly, recombinant hIGF-I (Amersham Australia, North Ryde, NSW) was used as a standard to give the range of 0.039-10 ng mL^{-1} concentration. Fraction of recombinant hIGF-I was used for iodination. Iodinated fractions were purified with a pre-albuminated Sephadex G 25 column and re-purified on a pre-albuminated 9×100 Sephadex G 100 column. Dilutions of rabbit antiserum against hIGF-I (AFP4892898, NIDDK, NIH, USA) and normal rabbit serum and donkey antirabbit IgG were 1:10000, 1:500 and 1:20, respectively. Minimum detection limit of the assay was 2 ng mL^{-1}. Intraassay coefficients of variation, measured at 9.2 and 50 ng mL^{-1} levels, were 9.6 and 3.2%, respectively.

LH assay: Plasma concentration of LH was measured using a sensitive competitive enzyme immunoassay method developed by Mutayoba *et al*. (1990) for bovine LH and modified by Yildiz *et al*. (2003b). Briefly,

D-Biotinyl-ϵ-aminocaproic acid N hydroxy-succimidine ester (Biotin-X-NHS; Sigma-Aldrich, Taufkirchen, Germany) was used for labeling oLH [NIDDK-oLH-I-4 (AFP8614B)]. Affinity purified goat IgG antirabbit IgG was attached to the solid phase and labelled and nonlabeled (sample) oLH were competed against the anti-oLH raised in rabbit [NIDDK-anti-oLH-1 (AFP192279)]. Dilutions of biotinyl LH and oLH antiserum were found to be 1:5,000 and 1:3,200,000, respectively. Standards used in the current study were between 0.39 and 50 ng oLH mL^{-1}. The minimum detection limit of the assay was 0.70 ng oLH mL^{-1}. Intra- and inter-assay coefficients of variation were calculated at 2 levels of quality control samples and as quadriplicates in 2 different locations of the plate. At 5.91 ng mL^{-1} level, the intra- and inter-assay coefficients of variation were 7.3 and 14.3% and for 12.01 ng mL^{-1} level they were 3.9 and 6.3%, respectively.

Statistical analyses: For the analyses of episodic secretion of LH and leptin, PCPULSAR program (Merriam and Wachter, 1982) was used. The G parameters (the number of standard deviation by which a peak must exceed the baseline in order to be accepted as pulse) used were 8, 6, 4, 3 and 2 for LH and 2.5, 2.1, 1.9, 1.5 and 1.2 for leptin for G_1-G_5, these being the requirements for pulses composed of one to 5 succesive samples that exceed the baseline, respectively.

For the repeated measurements throughout the experiment (BCS, leptin, IGF-I), a date (or time) by diet analysis was carried out using General Linearised Models within MINITAB statistical package (Minitab Inc., State College, PA). For post-prandial leptin secretions the same analysis was carried (time of the day by diet). At each time point, groups were also compared by using one-way ANOVA (Tukey's t-test). Results are given as mean and Standard Error of Mean (S.E.M).

RESULTS

Body condition score: BCS did not differ between the groups throughout the feeding period ($p>0.05$) (Table 1).

Leptin concentrations: Plasma leptin concentrations throughout the experiment is given in Table 2. Date of measurement affected plasma leptin concentrations significantly ($p<0.001$; 1.32±0.09, 1.49±0.09, 1.15±0.07 and 1.08±0.07 ng mL^{-1}, respectively on days 0, 25, 45 and 60) but there was no difference between the dietary groups in general ($p>0.05$). However, there was an interaction between dietary groups and date of measurement ($p<0.05$).

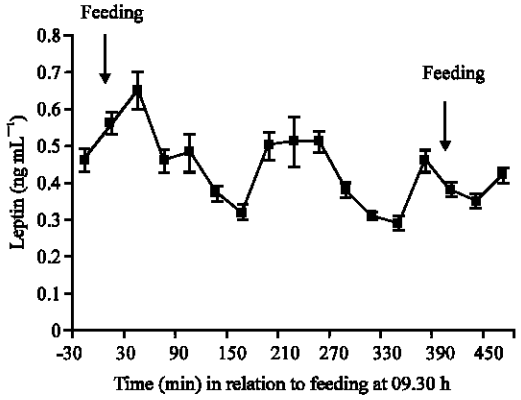

Fig. 1: Postprandial leptin secretion in fat-tailed tuj ewe-lambs on day 55 of the experiment

Table 1: Body Condition Scores (BCS) of ewe-lambs fed one of 7 diets

		185 g leaf (PEG%)			370 g leaf (PEG%)			
Days	Control	0	5	10	0	5	10	SEM
BCS (units)								
-17	2.45	2.47	2.43	2.44	2.42	2.44	2.41	0.05
20	2.46	2.29	2.25	1.93	2.41	2.05	2.26	0.07
56	2.65	2.60	2.37	2.65	2.41	2.51	2.45	0.07
BCS change (unit)								
-17	0.20	0.13	-0.06	0.21	-0.01	0.07	0.04	0.05

No significant differences were observed ($p>0.05$)

Table 2: Leptin concentrations (ng mL^{-1}) in ewe-lambs during the study period

		185 g leaf (PEG%)			370 g leaf (PEG%)			
Days	Control	0	5	10	0	5	10	SEM
0	1.12	1.81	1.29	1.05	0.67	1.18	1.66	0.09
25	1.77	1.21	1.22	1.59	1.44	1.74	1.46	0.09
45	1.04^{a}	1.03^{a}	1.16^{ab}	1.05^{a}	1.75^{b}	0.99^{a}	1.03^{a}	0.07
60	1.31	1.12	0.93	0.82	0.93	1.33	1.13	0.07

Within rows, means with different superscripts are significantly different ($p<0.05$)

Table 3: Postprandial leptin secretion (ng mL^{-1}) in ewe-lambs on Day 55 of the experiment

		185 g leaf (PEG%)			370 g leaf (PEG%)			
Groups	Control	0	5	10	0	5	10	SEM
Mean	0.41	0.44	0.41	0.46	0.45	0.46	0.42	0.01
Smoothed mean	0.39	0.43	0.36	0.39	0.39	0.40	0.38	0.01
Number of pulses per 8 h	0.67	0.17	1.67	1.33	1.33	1.50	1.00	0.15
Pulse amplitude	0.66	0.65	0.67	0.84	0.68	0.61	0.61	0.06

There was no statistical difference between the groups (p>0.05)

Table 4: Insulin-like Growth Factor-I (IGF-I) concentrations (ng mL^{-1}) in ewe-lambs fed one of seven diets

		185 g leaf (PEG%)			370 g leaf (PEG%)			
Days	Control	0	5	10	0	5	10	SEM
0	11.99	16.06	16.34	11.87	3.84	9.50	10.36	0.99
25	16.00	11.41	15.86	19.14	13.27	16.18	18.51	1.27
45	10.42	12.80	10.24	11.12	11.93	11.52	8.36	0.66
60	11.64^{a}	13.56^{ab}	11.46^{a}	11.58^{a}	9.72^{a}	21.10^{b}	16.87^{ab}	0.88

Different superscripts within a row differ significantly at p<0.05

Table 5: Luteinizing Hormone (LH) data in ewe-lambs fed one of five diets on Day 45

		185 g leaf (PEG%)		370 g leaf (PEG%)		
Groups	Control	0	5	0	5	SEM
Mean concentration (ng mL^{-1})	1.53	1.93	1.77	2.45	1.90	0.17
Smoothed mean (ng mL^{-1})	1.21	1.50	1.30	1.51	1.45	0.13
Pulse frequency (pulse/6 h)	2.00	2.33	3.17	3.17	2.50	0.30
Pulse amplitude (ng mL^{-1})	1.88	2.04	2.20	3.46	2.10	0.24

No significant differences were found between the groups (p>0.05)

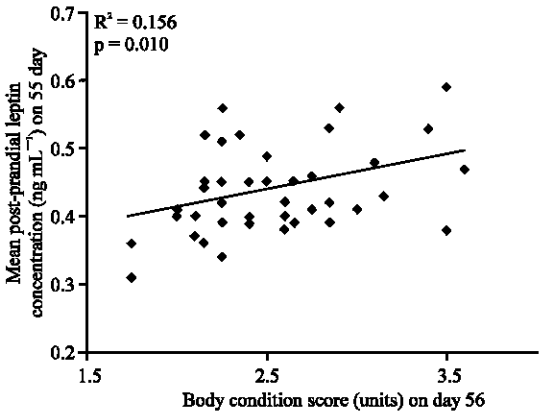

Fig. 2: The relationship between body condition score and mean post-prandial leptin concentration

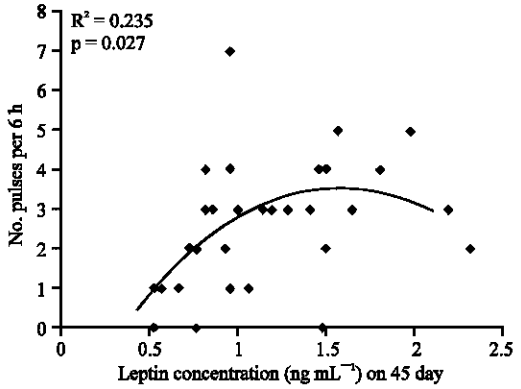

Fig. 3: The relationship between leptin concentration and number of LH pulses in ewe-lambs

Post-prandial leptin concentrations did not differ between the groups at any time of measurement (p>0.05). However, leptin levels significantly differed between times of measurements (p<0.001) with showing three elevations between 0-150th, 150-330th and 330-450 min (Fig. 1). Additionally, there was no difference between the groups in terms of number of pulses, mean and smoothed mean levels of leptin (p>0.05; Table 3). A positive significant relationship was found between mean post-prandial leptin concentration and body condition score (R^2 = 0.156, p = 0.01; Fig. 2).

IGF-I secretion: Diets did not affect IGF-I concentrations (p>0.05) but significant fluctuations were observed throughout the study period (p<0.001) with mean levels being 11.94±0.99, 15.83±1.27, 10.91±0.66, 13.70±0.88 and 20.02±1.10, respectively for Day 0, 25, 45 and 60 (Table 4).

Luteinizing hormone secretion: Mean and smoothed mean LH levels, number of LH pulses and pulse amplitude did not differ between the groups (Table 5). There was significant positive relationship between number of LH pulses and leptin concentration (R^2 = 0.235, p = 0.027; Fig. 3) and between number of LH pulses and IGF-I

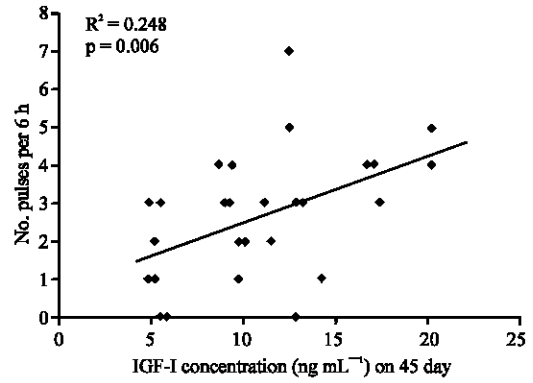

Fig. 4: Relationship between IGF-I concentration and number of LH pulses

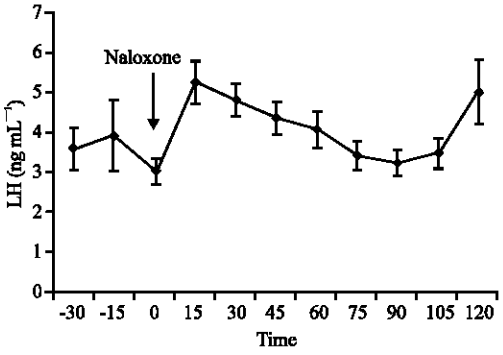

Fig. 5: Response to naloxone injection on day 55 of the experiment on LH release in ewe-lambs

concentration (R^2 = 0.248, p = 0.006; Fig. 4). Naloxane injection increased the LH release on day 55 of the experiment but this response was not affected by the experimental treatments (Fig. 5).

DISCUSSION

This is the first study reporting the effects of tanniniferous oak (*Q. hartwissiana*) leaf intake on leptin, IGF-I and LH pulsatility in sheep and the results suggests that the leaves used in the current study had no affect on these parameters. Furthermore, PEG appeared to be ineffective even though total tannin level was within the range at which PEG addition reported to be beneficial (Makkar, 2003). Several speculations might be done on the ineffectiveness of tannins. First, it might be that absorbable part of total tannins was lower in the current study (6.4% total tannins, 1.1% condensed tannins, 1.3% hydrolisable tannins, Yildiz *et al*., 2005). Astringency (level of tannins) is related to the level of voluntary intake of tanniniferous plants (Bate-Smith, 1973; Ben Salem *et al*., 1997). The levels used in the current study was determined prior to the experiment and they were found to be the maximum level that could voluntarily be consumed. Thus, it is possible to say that the amount of leaf offered was the levels that the lambs could tolarate.

On the other hand, level of hydrolisable tannins, the absorbable part, were similar with the levels observed for *Quercus* species (Inoue and Hagerman, 1988). Garg *et al*. (1992) reported intoxication (hepato and nephrotoxicity and death) of cattle consuming immature *Quercus incana* leaves and attributed these signs to hydrolisable tannins and simple phenols in *Q. incana* leaves. It is known that vegetatif stage (Makkar and Singh, 1993), species (Makkar and Singh, 1991b; Makkar *et al*., 1991a) and habitat (Goncalves-Alvim *et al*., 2004) affect tannin type and content. Thus, variable tannin content is likely to result in variable effects in the animal. Secondly, absorbable forms might probably have interacted with other substances in the digestive system resulting in reduced absorption. It has been reproted that salivary proteins form complexes with tannins (Makkar and Becker, 1998; Naurato *et al*., 1999). It has also, been shown that one of the green tea extracts, epigallocatechin gallate, a condensed tannin (Hagerman *et al*., 1997), has greater impact on growth hormone and other parameters if it is given intraperitoneally rather than orally (Kao *et al*., 2000). Thirdly, adaptations in the digestive system might have occurred against long-term tanniniferous feeding. It has been reported that microorganisms in the rumen possess adaptive mechanisims by time and become resistant to the effects of condsensed tannins (Brooker *et al*., 2000; McSweeney *et al*., 2001). Although, these microorganisms are not able to degrade condensed tannins (Makkar *et al*., 1995a,b; Brooker *et al*., 2000; McSweeney *et al*., 2001), they degrade hydrolisable tannins (Odenyo *et al*., 1999).

Thus, effects of absorbable tannins might have been reduced. Lastly, some forms of tannins or simple phenolics were probably absorbed but they did not cause toxicity or subtoxicity. Apart from some fractions (e.g., epigallocatechin gallate of green tea extract), the level of tannin absorption into bloodstream cannot be determined since they have variable and complex structure and there is a lack of commercially pure standards (Santos-Buelga and Scalbert, 2000; Makkar, 2003). Nevertheless, animals on the experiment showed no signs of toxicity and preliminary liver and kidney function tests (unpublished data) indicated no signs of subtoxicity suggesting that none of the components of oak leaves were harmful to the animal when compared to the control diet.

There are limited number of studies investigating the effects of tanniniferous feedstuffs on LH secretion

and their results are not equivocal. For example, Vera-Avila *et al.* (1997) observed increases in GnRH-induced LH secretion in a study carried out in male Angora goats grazing *Acacia berlandieri* and *Acacia rigidula* dominated pastures. However, in different studies, if phenolic amines present in *Acacia berlandieri* and *Acacia rigidual* were given parenterally, reproductive activity was impaired in female goats (Forbes *et al.*, 1994). Furthermore, Luque *et al.* (2000) observed increases in ovulation rate in ewes grazing on *Lotus corniculatus*. Interestingly, in the studies of Vera-Avila *et al.* (1997) and Luque *et al.* (2000) increased reproductive activity appeared to be due to superior nutritional value of the tanniniferous plants used. Because in their experiment tanniniferous plants appeared to by-pass valuable feed proteins and hence increase liveweight over the control group. In the current study, the diets were designed to be isoenergetical and isonitrogenous and concentrate/ roughage ratio was also similar among the groups. Thus, nutrient intake among the groups were similar. Additionally, tannins present in oak leaves did not affect body weight and body condition score suggesting that energy balance was similar accross the dietary groups (Yildiz *et al.*, 2005). In this experimental setting, tannins in oak leaves did not affect LH secretion. However, further studies are required to elucidate the effects of types of tannins or different tanniniferous plants, especially those rich in hydrolisable tannins, on IGF-I/leptin and LH axis.

The current study also shows that leptin secretion in fat-tailed sheep might be episodic as reported for other sheep breeds (Blache *et al.*, 2000; Daniel *et al.*, 2002). The pulses were characterised with about 2-fold increases in smoothed mean levels (Table 3). Blache *et al.* (2000) tried 5 or 20 min frequency of blood sampling for the determination of leptin episodes and they concluded that higher frequency did not improve the definition of the episodes. In the current study, our aim was to investigate whether a post-prandial trend is observed in leptin secretion and therefore we applied a 30 min sampling interval. Yet, this was sufficient to observe episodes. Throughout the sampling period 3 elevations were observed in leptin secretion: one after morning feeding, one before noon feeding and one in between (Fig. 1). The reason for this cluster is not known but interestingly a similar pattern, coinciding in terms of time of the day, was observed for the ewes that were in good body condition (Daniel *et al.*, 2002). In their expeirment, these ewes were fed *ad libitum* and hence together with data in the current study, the clusters might not be related to feeding regiments. It should also, need to be taken into account that, in our experiment, each lamb had 1 or 2 episodes throughout the 7.5 h sampling period (Table 3). Thus, some animals did not have all episodes appearing in the general picture in Fig. 1. Nevertheless, data in the current study and in that of Daniel *et al.* (2002) suggests, that further studies are necessary for elucidation of the cause and roles of these episodes.

The current study confirms, previous findings in sheep that body condition score is correlated with leptin secretion (Yildiz *et al.*, 2003a, b) and in turn, leptin informs hypothalamus about the sufficiency of energy stores and hence regulates pulsatility of LH secretion (Clarke and Henry, 1999; Foster and Nagatani, 1999; Blache *et al.*, 2000; Chilliard *et al.*, 2001; Yildiz *et al.*, 2003a, b). Additionally, IGF-I levels, sign of level of nutrition, were also positively correlated to LH pulse frequency as reported previously (Nugent *et al.*, 1993). Thus, it appears that rather than secondary plant compounds in *Q. hartwisiana* leaves, its feeding value is more important.

Oak trees or bushlands are widespread in many parts of the world (from Nepal to Greece and in America) and in these areas shortage in feedstuffs are common. Therefore, the current study suggests, that leaves of *Quercus hartwissiana* can be used to replace some of the roughage ration without any negative effects on leptin, IGF-I and LH.

CONCLUSION

It appears that tanninifreous oak leaves, when fed for two months, do not affect body condition score, plazma IGF-I concentration, long-term and post-prandial leptin levels and pulsatile secretion of LH and also that leptin is secreted in episodic manner as has been reported for other sheep breeds and that leptin and IGF-I appear to signal hypothalamus to regulate pulsatile secretion of LH in fat-tailed Tuj lambs.

ACKNOWLEDGEMENT

This study was supported by International Atomic Energy Agency (IAEA, Project No: TUR10272). We wish to thank NIDDK and AF Parlow for provison of LH reagents.

REFERENCES

Barry, T.N., T.F. Allsop and C. Redekopp, 1986. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep 5. Effects on the endocrine system and on adipose tissue metabolism. Br. J. Nutr., 56: 607-614. DOI: 10.1079/BJN19860141.

Bate-Smith, E.C., 1973. Haemanalysis of tannin: The concept of relative astringency. Phytochemistry, 12: 907-912. DOI: 10.1016/0031-9422(73)80701-0.

Ben Salem, H., A. Nefzaoui, L. Ben Salem and J.L. Tisserand, 1997. Effect of *Acacia cyanophylla* Lindl. foliage supply on intake and digestion by sheep fed lucerne hay-based diets. Anim. Feed Sci. Technol., 68: 101-113. DOI: 10.1016/S0377-8401(97)00015-1.

Blache, D., R.L. Tellam, L.M. Chagas, M.A. Blackberry, P.E. Vercoe and G.B. Martin, 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. J. Endocrinol., 165: 625-637. DOI: 10.1677/joe.0.1650625.

Breier, B.H., B.W. Gallaher and P.D. Glukman, 1991. Radioimminoassay for insulin-like growth factor-1: solution to some potential problems and pitfalls. J. Endocrinol., 128: 347-357. http://joe.endocrinology-journals.org/cgi/reprint/128/3/347.

Brooker, J.D., L. O'Donovan, I. Skene and G. Sellick, 2000. Mechanisms of Tannin Resistance and Detoxification in the Rumen. In: Brooker, J.D. (Ed.). Tannins in Livestock and Human Nutrition. ACIAR Proc., 92: 117-122. ISBN: 1 86320-276-5.

Chilliard, Y., F. Bocquier and M. Doreau, 1998. Digestive and metabolic adaptations of ruminants to undernutrition and consequences on reproduction. Reprod. Nutr. Dev., 38: 131-152. http://rnd.edpsciences.org/index.php?option=article&access=standard&Itemid=129&url=/articles/rnd/pdf/1998/02/RND_0926-5287_1998_38_2_ART0001.pdf.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Leroux, J. Djiane and F. Bocquier, 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland and regulation of plasma concentration. Domest. Anim. Endocrinol., 21: 271-295. DOI: 10.1016/S0739-7240(01)00124-2.

Clarke, I.J. and B.A. Henry, 1999. Leptin and reproduction. Rev. Reprod., 4: 48-55. DOI: 10.1530/ror.0.0040048.

Daniel, J.A., B.K. Whitlock, J.A. Baker, B. Steele, C.D. Morrison, D.H. Keisler and J.L. Sartin, 2002. Effect of body fat mass and nutritional status on 24 h leptin profiles in ewes. J. Anim. Sci., 80: 1083-1089. PMID: 12002315. http://jas.fass.org/cgi/reprint/80/4/1083.

Forbes, T.D.A., B.B. Carpenter, R.D. Randel and D.R. Tolleson, 1994. Effects of phenolic monoamines on release of luteinizing hormone stimulated by gonadotropin releasing hormone and on plasma adrenocorticotropic hormone, norepinephrine and cortisol concentration in wethers. J. Anim. Sci., 72: 464-469. http://jas.fass.org/cgi/reprint/72/2/464.

Foster, D.L. and S. Nagatani, 1999. Physiological perspectives on leptin as a regulator of reproduction: Role in timing puberty. Biol. Reprod., 60: 205-215. PMID: 9915983. http://www.biolreprod.org/cgi/reprint/60/2/205.

Garg, S.K., H.P. Makkar, K.B. Nagal, S.K. Sharma, D.R. Wadhwa and B. Singh, 1992. Oak (*Quercus incana*) leaf poisoning in cattle. Vet. Hum. Toxicol., 34: 161-164. PMID: 1509680.

Gluckman, P.D., J.J. Johnson-Barrett, J.H. Butler, B.W. Edgar and T.R. Gunn, 1983. Studies in insulin-like growth factor-I and-II by specific radioligand assys in umbilical cord blood. Clin. Endocrinol., 19: 405-413. DOI: 10.1111/j.1365-2265.1983.tb00014.x.

Goncalves-Alvim, S.J., R.G. Collevatti and G.W. Fernandes, 2004. Effects of genetic variability and habitat of *Qualea parviflora* (Vochysiaceae) on herbivory by free-feeding and gall-forming insects. Ann. Bot., 94: 259-268. DOI: 10.1093/aob/mch136.

Hagerman, A.E., Y. Zhao and S. Johnson, 1997. Methods for Determination of Condensed and Hydrolisable Tannins. In: Shahidi, F. (Ed.). Antinutrients and Phytochemicals in Food. Am. Chem. Soc. Washington, DC (USA), pp: 209-222. ISBN: 08-412-34981.

Hagerman, A.E. and D.M. Carlson, 1998. Biological responses to dietary tannins and other polyphenols. Recent. Res. Dev. Agric. Food Chem., 2: 689-704.

Inoue, K.H. and A.E. Hagerman, 1988. Determination of gallotannin with rhodanine. Anal. Biochem., 169: 363-369. DOI: 10.1016/0003-2697(88)90296-5.

Kao, Y.H., R.A. Hiipakka and S. Liao, 2000. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology, 141: 980-987. http://endo.endojournals.org/cgi/reprint/141/3/980.

Landau, S., N. Silanikove, Z. Nitsan, D. Barkai, H. Baram, F.D. Provenza and A. Perevolotsky, 2000. Short-term changes in eating patterns explain the effects of condensed tannins on feed intake in heifers. Applied Anim. Behav. Sci., 69: 199-213. DOI: 10.1016/S0168-1591(00)00125-8.

Luque, A., T.N. Barry, W.C. McNabb, P.D. Kemp and M.F. McDonald, 2000. The effect of grazing *Lotus corniculatus* during late summer and autumn on reproductive efficiency and wool production in ewes. Aust. J. Agric. Res., 51: 385-391. DOI: 10.1071/AR99078.

McSweeney, C.S., B. Palmer, D.M. McNeill and D.O. Krause, 2001. Microbial interactions with tannins: Nutritional consequences for ruminants. Anim. Feed Sci. Technol., 91: 83-93. DOI: 10.1016/S0377-8401(01)00232-2.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res., 49: 241-256. DOI: 10.1016/S0921-4488(03)00142-1.

Makkar, H.P.S. and K. Becker, 1998. Adaptation of cattle to tannins: Role of prolin-rich proteins in oak-fed cattle. Anim. Sci., 67: 277-281. http://www.bsas.org.uk/downloads/Vol67Part2_Oct_1998.pdf.

Makkar, H.P.S., M. Blümmel and K. Becker, 1995a. Formation of complexes between polyvinyl pyrrolidones and polyethylene glycols and tannins and their implication in gas production and true digestibility in *in vitro* techniques. Br. J. Nutr., 73: 897-913. DOI: 10.1079/BJN19950095.

Makkar, H.P.S., K. Becker, H.J. Abel and C. Szegletti, 1995b. Degradation of condensed tannins by rumen microbes exposed to Quebracho Tannins (QT) in Rumen Simulation Technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. J. Sci. Food Agric., 69: 495-500. DOI: 10.1002/jsfa.2740690414.

Makkar, H.P.S., R.K. Dawra and B. Singh, 1991a. Tannin levels in leaves of some oak species at different stages of maturity. J. Sci. Food Agric., 54: 513-519. DOI: 10.1002/jsfa.2740540403.

Makkar, H.P.S. and B. Singh, 1991b. Distribution of condensed tannins (proanthocyanidins) in various fibre fractions in young and mature leaves of some oak species. Anim. Feed Sci. Technol., 32: 253-260. DOI: 10.1016/0377-8401(91)90030-V.

Makkar, H.P.S. and B. Singh, 1992a. Effect of wood ash on tannin content of oak (*Quercus incana*) leaves. Bioresour. Technol., 41: 85-86. DOI: 10.1016/0960-8524(92)90103-5.

Makkar, H.P.S. and B. Singh, 1992b. Effect of steaming and autoclaving oak (*Quercus incana*) leaves on levels of tannins, fibre and lignin and in sacco dry matter digestibility. J. Sci. Food Agric., 59: 469-472. DOI: 10.1002/jsfa.2740590407.

Makkar, H.P.S. and B. Singh, 1992c. Detannification of oak (*Quercus incana*) leaves: Treatments and their optimization. Anim. Feed Sci. Technol., 36: 113-127. DOI: 10.1016/0377-8401(92)90091-J.

Makkar, H.P.S. and B. Singh, 1993. Effect of storage and urea addition on detannification and *in sacco* dry matter digestibility of mature oak (*Quercus incana*) leaves. Anim. Feed Sci. Technol., 41: 247-259. DOI: 10.1016/0377-8401(93)90017-E.

Merriam, G.R. and K.W. Wachter, 1982. Algorithms for the study of episodic hormone secretion. Am. J. Physiol., 243: E310-318. http://ajpendo.physiology.org/cgi/reprint/243/4/E310.

Mutayoba, B.M., H.H. Meyer, D. Schams and E. Schallenberger, 1990. Development of a sensitive enzyme immunoassay for LH determination in bovine plasma using the streptavidin-biotin technique. Acta Endocrinol. (Copenh), 122: 227-232. PMID: 2180243.

Naurato, N., P. Wong, Y. Lu, K. Wroblewski and A. Bennick, 1999. Interaction of tannin with human salivary histatins. J. Agric. Food Chem., 47: 2229-2234. DOI: 10.1021/jf981044i.

Nugent, R.A., T.G. Jenkins, A.J. Roberts and J. Klindt, 1993. Relationship of postpartum interval in mature beef cows with nutritional environment, biological type and serum IGF-1 concentrations. Anim. Prod., 56: 193-200.

Odenyo, A.A., C.S. McSweeney, B. Palmer, D. Negassa and P.O. Osuji, 1999. *In vitro* screening of rumen fluid samples from indigenous African ruminants provides evidence for rumen fluid with superior capacities to digest tannin-rich fodders. Aust. J. Agric. Res., 50: 1147-1157. DOI: 10.1071/AR98117.

Russel, A.J.F., J.M. Doney and R.G. Gunn, 1969. Subjective assessment of body fat in live sheep. J. Agric. Sci. Cambridge, 72: 451-454.

Santos-Buelga, C. and A. Scalbert, 2000. Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutrition and health (Review). J. Sci. Food Agric., 80: 1094-1117. DOI: 10.1002/(SICI)1097-0010(20000515)80:7<1094::AID-JSFA569>3.0.CO;2-1.

Vera-Avila, H.R., T.D. Forbes, J.G. Berardinelli and R.D. Randel, 1997. Effect of dietary phenolic amines on testicular function and luteinizing hormone secretion in male angora goats. J. Anim. Sci., 75: 1612-1620. http://jas.fass.org/cgi/reprint/75/6/1612.

Villalba, J.J., F.D. Provenza and R.E. Banner, 2002. Influence of macronutrients and polyethylene glycol on intake of a quebracho tannin diet by sheep and goats. J. Anim. Sci., 80: 3154-3164. http://jas.fass.org/cgi/reprint/80/12/3154.

Wrisez, F. and B. Lambert, 2001. Differential long-term effects of tannic acid on adenyl cyclase activity and lipolysis in rat adipocytes. Phytomedicine, 8: 292-297. DOI: 10.1078/0944-7113-00047.

Yildiz, S., D. Blache, F. Celebi, I. Kaya, M. Saatci, M. Cenesiz and B. Guven, 2003a. Effects of short-term high carbohydrate or fat intakes on leptin, growth hormone and luteinizing hormone secretions in prepubertal fat-tailed Tuj lambs. Reprod. Dom. Anim., 38: 182-186. DOI: 10.1046/j.1439-0531.2003.00414.x.

Yildiz, S., M. Saatci, M. Uzun and B. Guven, 2003b. Effects of ram introduction after the 2nd prostaglandin $F_{2\alpha}$ injection on day 11 on the LH surge characteristics in fat tailed ewes. Reprod. Dom. Anim., 38: 54-57. DOI: 10.1046/j.1439-0531.2003.00396.x.

Yildiz, S., M. Uzun, M. Cenesiz, M. Kaya and I. Kaya, 2002a. Effects of different levels of oak (*Quercus hartwissiana*) leaf consumption on GnRH-induced LH secretion in rams. Reprod. Dom. Anim., 37: 381-382. DOI: 10.1046/j.1439-0531.2002.00368.x.

Yildiz, S., M. Uzun, M. Cenesiz, O. Ucar, M. Kaya and F. Onder, 2002b. Effects of sexually activated rams or ewes on pulsatile LH secretion in anoestrous sheep. Acta Vet. Brno., 71: 297-302. http://vfu-www.vfu.cz/acta-vet/vol71/pdf/71_297.pdf.

Yildiz, S., I. Kaya, Y. Unal, D. Aksu Elmali, S. Kaya, M. Cenesiz, M. Kaya and A. Oncuer, 2005. Digestion and Body Weight Change in Tuj Lambs Receiving Oak (*Quercus hartwissiana*) Leaves with and Without PEG. In: Makkar, H.P.S. (Ed.). Predicting and Improving the Safety and Efficiency of Feeding Ruminants on Tanniniferous Tree Foliage, 122: 159-172. DOI: 10.1016/j.anifeedsci.2005.04.005.

Yugarani, T., B.K. Tan, M. Teh and N.P. Das, 1992. Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. Lipids, 27: 181-186. DOI: 10.1007/BF02536175.

Yugarani, T., B.K. Tan and N.P. Das, 1993. The effects of tannic acid on serum and liver lipids of RAIF and RICO rats fed on high fat diet. Comp. Biochem. Physiol. Comp. Physiol., 104: 339-343. DOI: 10.1016/0300-9629(93)90326-Y.