Vol 16 No 2, p 201-207

RELATIONSHIP OF LEPTIN HORMONE WITH INSULIN AND GLUCOSE IN ARABIAN CAMEL (*Camelus dromedarius)*

AL–Suhaimi Ebtesam*, Abu-se'da Khalid and Akbar Amal
*Physiology-Biology Department, Science College, KFU, Dammam, Kingdom of Saudi Arabia

ABSTRACT

The aim of current study was to know relationship of leptin hormone with insulin and glucose in Arabian camel (*Camelus dromedarius)*. Two hundreds and forty male and female camels were used and divided into 3 groups according age (below 1 year, 1–3 years and above 3 years) for both sex and season (summer and winter). Blood samples were collected and plasma was separated. Leptin and insulin hormones were determined immunoenzymatically on ELISA and Elecsys systems, respectively. Glucose was estimated enzymatically by Reflotron system.

Leptin levels were affected by season variation, i.e. its level increases in winter than summer in female and vice versa in males. There was positive correlation between leptin and age in males and negative one in females. A synchronised increase in level of leptin and insulin was noticed with age advancement in males and decrease of those levels in females. Season variations had effects on glucose and insulin in camel plasma. There was an increase of both in male at winter than summer, while glucose and insulin levels increased in female at summer than winter. The results revealed that there was negative relation between glucose and insulin levels with age progression.

Key words: Arabian camels, glucose, insulin, leptin

Leptin is a protein hormone like cytokine produced from white fatty cells (Sayed *et al,* 2003 and Bartha *et al,* 2005). It has been reported to be secreted is humans and animals (Sinha *et al,* 1996 and Blache *et al,* 2000). Its receptors are found in placenta and foetal tissue (Muhlhausler *et al,* 2003), skeletal muscles (Wang *et al,* 1998), brain, CNS and pituitary (Yonekura *et al,* 2003), kidney medulla (Yanagihara *et al,* 2000), hotheaded digestive organs (Yonekura *et al,* 2002) and in most regions of dairy cattle (Chelikani *et al*, 2003 b), mammary gland (Yonekura *et al,* 2006). Leptin levels have been reported to be increased in human and ruminants after feeding and decreased due to food deficiency or prolonged starvation in ruminants (Morrison *et al,* 2001; Thomas *et al,* 2001). Plasma leptin level increases in acute infection (Bornstein *et al,* 1998) and during gestation (Lage *et al,* 1999). Leptin was also affected by seasonal variation in different environmental ruminants (Chilliard *et al,* 2005 b). It reached minimum limit in winter in ovariectomised cows (Garcia *et al*, 2002), decreased in autumn when compared with spring in cow calves (Reist *et al*, 2003), also increased in warm than cold (Asakuma *et al,* 2003), Delavaud *et al* (2004) reported that camel leptinemia return to humped weight (fatty cells mass), but insensitive to large variations in nutrition levels, it increased gradually during 3 weeks of water deprivation (Chilliard *et al,* 2005a).

Insulin is responsible for glucose utilisation by liver, muscles and fatty tissues. Earlier workers have reported variation in the insulin levels due to dehydration (Charnot, 1967 and Yagil, 1985).

Glucose is an important carbohydrate substance for energy production in animals. In ruminants, glucose is produced from gluconeogenesis in liver, external layer of kidney and from intestine absorption. It is controlled by pancreatic enzyme, insulin and glucagon, cortisol, thyroxin and growth hormones. Camel's glucose levels are affected by many factors, i.e. nutrition, water deprivation, physiological state like pregnancy, lactation, age (MacSween and Whaley, 1992; Shier *et al,* 1999). In camel, levels of glucose varies widely (Al – Rehaimi *et al,* 1989). Yagil and Berlyne (1977a) and Nazifi *et al* (1998) reported that glucose rise in camels was more than other ruminants. Yagil and Berlyne (1977b) studied effects of dehydration on female camels aged (5-12 year) which led to increase in blood glucose. Mirgani and Bakhit (1987) found an increase in blood glucose in camel after treatment with molasses

SEND REPRINT REQUEST TO AL–SUHAIMI EBTESAM email: Dr_suhaimi2008@windowslive.com

and following two hours of nutrition. Soliman *et al* (2002) found a glucose level decrease after 24 hours of starvation while Al–Katheeri *et al* (2004) recorded low glucose levels in plasma of camels treated with dexamethasone. Knight *et al* (1994) noticed gradual increase in serum glucose during exercise of training race camel. The aim of current study was to know relationship of leptin hormone with insulin and glucose in Arabian camel (*Camelus dromedarius)*.

Materials and Methods

Blood samples were collected from 240 male and female camels which were divided into 3 sub groups according to age.

I- < 1 year.

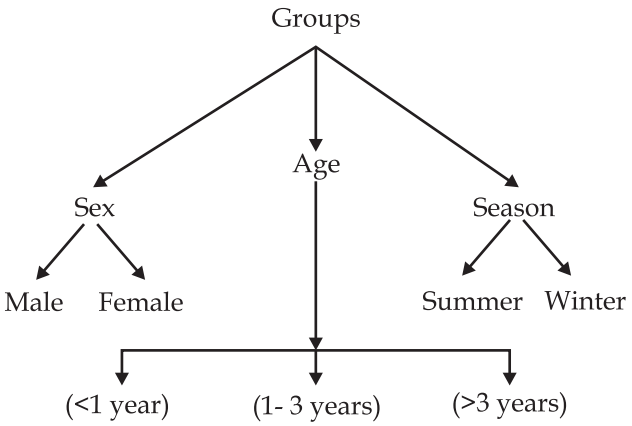
II- 1 – 3 years.

II- > 3 year.

Blood samples were collected from jugular vein in glass bottles containing EDTA and plasma was separated by centrifugation (Essamadi *et al,* 2002) and stored at - 20°C.

Leptin was estimated by The RayBio®Mouse Leptin ELISA (Enzyme – Linked Immunosorbent Assay), RayBiotech, Inc., Cat# : ELM-LEPTIN-001 (Zhou, 1997 and Way, 2001).

Insulin was estimated by the electrochemiluminescence immunoassay "ECLIA" (Roche Elecsys 1010/2010 and MODULAR ANALYTICS E170 - Elecsys module - immunoassay analysers), 12017547 122 (Bablok, 1988; Tietz, 1995; Chevenne *et al,* 1998; Clark, 1999; Sapin *et al,* 2001; Spain, 2003 and Owen and Robert, 2004).



Glucose was determined by–Quant (hexokinase) of Roche Diagnostics, Germany, test strips, REF 10744948 (Greiling and Gressner, 1995 and Thomas, 2000)* on Reflotron system.

Statistical analysis

Mean, SE, and F values were calculated using ANOVA test. Post Hoc test (Least significant differences) was done to compare the means with each other. Pearson correlation test and SPSS statistics programme were used for above analysis.

Results and Discussion

Table 1 showed that leptin levels in male and female camels ranged 0. 29 ± 0.00 – 8.12 ± 0.06 ng / ml, which was close to leptin levels of (8.9 ± 0.5 ng / ml), (7.3 ± 0.5 ng / ml) in calves this value (Ehrhardt *et al,* 2000), (0.16 – 1.03 ± 0.13 ng / ml) lambs (Nagatani *et al,* 2000; Soliman *et al,* 2002) and horse (3.81 ± 0.45 ng / ml) at rest (Piccione *et al,* 2005). The current study showed the effects of season on leptin levels in camel. There was an increase in summer than winter in males, whereas increase in winter than summer in female, it ranged 0.60 ± 0.01 – 8.12 ± 0.06 ng / ml, 0.29 ± 0.00 – 5.50 ± 0.09 ng / ml, 0.60 ± 0.02 – 5.20 ± 0.09 ng / ml and 0.60 ± 0.01 – 6.20 ± 0.08 ng/ ml in females respectively. Previous studies found that plasma leptin levels in males decreases with cold (Trayhun *et al,* 1998).

In this study there was synchronisation of an increase in leptin and insulin with decrease in glucose level in male camels with advancement of age while there was decrease in leptin and insulin with an increase in glucose was age advanced in female camel. These results agree with Kulcsar *et al* (2005) who reported that age affected leptin levels.

These results pointed that there was a high significant positive relationship between leptin and insulin whereas a high significant negative correlation with glucose, as shown in table 4. References were

Table 1. The effect of studied factors (age, sex and season) on leptin levels (ng/ml) in camel's plasma.

Season	Winter		Summer	
Sex Age (Years)	Female	Male	Female	Male
< 1	3.8 ± 0.17	2.31 ± 0.94	2.46 ± 0.72	2.15 ± 0.56
1 - 3	*4.73 ± 0.55	1.41 ± 0.48	1.22 ± 0.05	2.14 ± 0.44
> 3	*1.13 ± 0.19	*2.77 ± 0.86	1.59 ± 0.56	2.4 ± 0.62

F = 2.041* = The mean difference is significant at 0.05

Table 2. The effect of studied factors (age, sex and season) on glucose levels (mg/dl) in camel's plasma.

Season	Winter		Summer	
Sex Age (Years)	Female	Male	Female	Male
< 1	131.00±4.06	125.23 ± 16.13	139.50 ± 4.28	2.15 ± 0.56
1 - 3	*129.85 ± 2.81	160.81 ± 9.188	159.00 ± 9.79	149.43 ± 8.24
> 3	*166.21 ± 15.00	*166.55 ± 44.58	158.80 ± 10.20	148.83 ± 9.43

F = 2.769 * = The mean difference is significant at 0.05

Table 3. The effect of studied factors (age, sex and season) on insulin levels (mIU/ml) in camel's plasma.

Season	Winter		Summer	
Sex Age (Years)	Female	Male	Female	Male
< 1	57.30 ± 4.81	20.00 ± 0.01	14.50 ± 2.24	22.95 ± 2.47
1 - 3	*44.62 ± 6.88	*19.90 ± 1.43	*16.00 ± 1.63	**20.72 ± 0.56
> 3	**23.20 ± 1.67	*20.00 ± 0.01	23.07 ± 1.33	*32.50 ± 7.50

F = 11.066 * * = The mean difference is significant at 0.01
* = The mean difference is significant at 0.05

not found for camels about these relations. On other hand, previous researchers did not agree with current results. They found that there was negative correlation regulation between leptin and insulin release (Kieffer and Habener, 2000; Schwartz *et al*, 2000).

The difference in leptin levels may be attributed to sensitivity of the technique used in this study. Radio immunoassay could determine leptin levels as low as 0.5 ng / ml and could give difference of plasma leptin which was induced by nutrition, fats and sex (Ehrhardt *et al,* 2000). The high levels of leptin in current study may be explained by other hormonal factors such as glucocorticoids (Havel, 2004), growth hormone and prolactin (Flint *et al,* 2003; Zieba *et al,* 2003), progesterone and cortisol (Kulcsar *et al,* 2005).

Glucose levels showed wide range in camels. The values in table 2 ranged 92.00 ± 33.47 – 280.00 ± 0.03 mg/dl, previous study recorded 76.2 ± 4.4 mg/dl (Siam *et al,* 1993), 121 ± 17.3 mg/dl (Agrawal *et al,* 2005). In camel, glucose level are higher than other ruminant and animals (Duehlmeier *et al,* 2007) .

Insulin levels in current study (Table 3) ranged from 11.00 ± 0.01 to 75.60 ± 0.08 mIU / ml. There are few studies for camel insulin showing variation (Yagil, 1985 and Siam *et al,* 1993) recorded 5.8 ± 1.4 µ / l.

Table 2 and 3 shows that glucose and insulin levels increase in males during winter than summer, while in females camel those levels increase in summer than winter. This result agree with Yagil and Berlyne (1977a). The high level of glucose in camel plasma could be explained by Abdulaziz (1999) who reported that large quantity of water could be saved by camels in summer season as at this time the blood contains traces of solid substance which leads to a increase in glucose concentrations.

Insulin level in males as shown in table 3 ranged 17.08 ± 2.92 – 50.00 ± 0.03 mIU / ml and in summer 11.00 ± 0.01 – 75.60 ± 0.08 mIU / ml in winter. In females it ranged 14.50 ± 2.24 – 72.00 ± 0.06 mIU / ml in summer and 20.00 ± 0.01 – 67.00 ± 0.04 mIU / ml in winter. This study showed that there is an effect of age on glucose and insulin levels in both male and female camels. It was noted that there was a high significant negative correlation between glucose and insulin as age increase showed in correlation. The rise of glucose level in camel returned to low insulin level which has role in glucose consumption by cells, also glucose level increase as a result of water deprivation and it followed by decrease in insulin. Khatim *et al* (1985) and Yagil (1985) showed that thirstiness of camel is like diabetic person while the low insulin level in camel is not abnormalities in secretory organ but resulted in water shortage and ratio of insulin releasing tissue to total tissue of Islet is affected by water control. It is possible that basal insulin level decreased to 30% during 10 days of water deprivation, whereas, glucose infusion leads

Table 4. Pearson correlation test of studied parameters in camel's plasma.

	Leptin	Insulin	Glucose
Leptin	-	-	-
Insulin	.455**	-	-
Glucose	-0.255**	-0.205**	-

** Correlation is highly significant at the 0.01 level (1 – tailed)

to increased insulin levels in camels. which in return lead to increase in glucose consumption by tissues (Yagil and Berlyne, 1977b).

The ability of camel to a climate with dry weather lead to save high glucose levels reach to 1300 mg% in blood with loss of water in urine. By this way, camel can retain water in plasma which lead to other factors for resistance of water deprivation (Yagil, 1985). It could be said that insulin secretory organ decrease response to loss of water, the released insulin depend on water availability (Jehad, 1995).

There is a poor insulin response and low sensitivity to insulin in camel tissues (Elmahdi *et al*, 1997; Kaske *et al*, 2001). In current study, there was a synchronisation between insulin and leptin. Morrison *et al* (2001) showed that insulin has cooperation ability with leptin for appetite reduction in ewe.

On other hand, the ability of camels to keep high glucose level in blood reflects active gluconeogenesis (Al–Ali *et al*, 1988). Also, variations in ambient temperature has effects on physiological process through direct effect on metabolism, which vary according to animal species or seasons (Faye *et al*, 1995).

High glucose level may be attributed to thyroid activity. Ganong (1995) found that an increase in basal metabolic rate is due to high thyroxin level. Abdel–Fattah *et al* (1999) explained that high level of glucose in camel blood return to higher glucagon level in camels than human and other ruminants. Nutrition play an important role in insulin levels (Morrison *et al*, 2001).

Bartha *et al* (2005) reported that energy metabolism in ruminants under control of hormonal factors (thyroid hormones, leptin). The low and high levels of leptin were induced by catabolism and anabolism, respectively. Leptin levels dominance in plasma is required for function maintenance.

Camels leptin needs further studies in details to evaluate physiological and other functions of it to use in economic and applied fields.

Acknowledgement

We thank to king Abdulaziz city for science and technology for their supporting.

References

Abdel–Fattah M, Amer H, Ghoneim MA, Warda M and Megahed Y (1999). Response of one – humped camel (*Camelus dromedarius*) to intravenous glucagon injection and to infusion of glucose and volatile fatty acids, and the kinetics of glucagon disappearance from the blood. Zen. Veterinary A. 46(8):473-81.

Adam CL, Archer ZA and Miller DW (2003). Leptin actions on the reproductive neuroendocrine in sheep. Reproduction in Domestic Animals Supplement 61:283-97.

Agrawal RP, Beniwal R, Kochar DK, Tuteje FC, Ghorui SK, Sahani M and Sharma S (2005), Letter to the editor. Diabetes Research Clinical Practice 68:176-7.

Al–Ali AK, Husayni HA and Power DM (1988). Purification and comparison of phosphoenolpyruvate carboxy kinase from the liver and kidney of the arabian camel (*Camelus dromedarius)*. Comparative Biochemical Physiology 89:335-8.

Al–Attas OS (1989). Comparative stadies on the major features of insulin receptors in mammalian and non-mammalian liver membranes. Comparative Biochemical Physiology 93(1):125-33.

Al–Katheeri NA, Wasfi IA, Lambert M and Saeed A (2004). Pharmacokinetics and pharmacodynamics of dexamethasone intravenous administration in camels: effect of dose. Veterinary Research Communications 28(6):525-42.

Al–Rehaimi AA, Al–Ali AK, Mutairy AR and Dissanayake AS (1989). A comparative study of enzyme profile of camel (*Camelus dromedarius*) hump and sheep (*Ovis aries)* tail tissues. Comparative Biochemistry Physiology 93(4):857-8.

Andrews JF (1998). Leptin : energy regulation and beyond to a hormone with pan – physiological function. Proc. Nutr. Soc. 57:409-11.

Asakuma S, Morishita H, Sugino T, Kurose Y, Kobayashi S and Terashina Y (2003). Circulating leptin response to feeding and exogenous infusion of insulin in sheep prexposed to thermoneutral and cold environments. Comp. Biochem. Physiol. AMOL In Tegr. Physiological 134(2):329-35.

Bablok W (1988). A general regression procedure for method transformation. Journal of Clinical Chemistry 26:783-90.

Barakat MZ and Abdel–Fattah M (1971). Seasonal and sexual variations of certain constituents of normal camel blood. Zentr. Vet. Reihe. A. 18:174-8.

Bartha T, Sayed AA and Rudas P (2005). Expression of leptin and its receptors in various tissues of ruminants. Domestic Animal Endocrinology 29(1):193-202.

Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE and Martin GB (2000). Level of nutrition affects leptin concentrations in plasma, and cerebrospinal fluid in sheep. Journal of Endocrinology 165:625-37.

Boden G, Chen G, Mozzoli M and Rayan I (1996). Effect of fasting on serum leptin in normal human subjects. The Journal of Clinical Endocrinology and Metabolism 81:3419-23.

Bornstein SR, Licinio J, Tauchnitz R, Engelman R, Negrao AB, Gold P and Chrousos GP (1998). Lipopolysaccharide – induced changes in monoamines in specific areas of the brain : blockade by interleukin – 1 receptor antagonists. Journal of Endocrinology 164:361-9 .

Casanueva FF and Dieguez C (1999). Neuroendocrine regulation and actions of leptin. Front. Neuro Endocrinol. 20:317-63.

Charnot Y (1967). Regulation endocrinenne du metabolism de leau chez le dromedaire. Bull. Sco. Sci. Nat. Phys. Marco. 47:1-7.

Chelikani PK, Glimn DR and Kennelly JJ (2003). Short communication : Tissue distribution of leptin and leptin receptor mRNA in the bovine. Journal of Dairy Science 86(7):2369-72.

Chevenne D, Letailleur A, Trivin F and Porquet D (1998). Effect of haemolysis on the concentration of insulin in serum determined by RIA and IRMA. Clinical Chemistry 44(2):354-6.

Chilliard Y, Ben–Goumi M, Delavaud C, Faulconnier Y and Faye B (2005 a). Body lipids and adaptation of camel to food and water shortage : new data on adipocyte size and plasma leptin. In: Faye, B, Esenor, P. (Eds), Desertification combat, food Sayety : the added value of camel producers, NATO Science Seriers : Life and Behavioural Science IOS Press. 362:135-45.

Chilliard Y, Delavaud C and Bonnet M (2005b). Leptin expression in ruminants : nutritional and physiological regulations in relation with energy metabolism. Domestic Animal Endocrinology 29(1):3-22.

Clark PM (1999). Assays for insulin, proinsulin (s) and c – peptide. Annal Clinical Biochemistry 18(6):313-26.

Delavaud C, Bengoumi M, Faye B, Tabarani A, Faulconnier Y, Sghiri A and *et al* (2004). Plasma leptin measurement in the dromadery camel and its relationships to adiposity and feeding level. Biotechnol. Agron. Soc. Environ. 8:45.

Dubuce GR, Phinney SD, Stem JS and Havel PJ (1998). Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. Metababolism 47:429-34.

Duehlmeier R, Sammet K, Widdel A, Von Engelhardt W, Wernery U, Kinne J and Sallmann HP (2007). Disrtibution patterns of the glucose transponters GLUT4 and GLUT1 in skeletal muscles of rats (*Rattus norvegicus)*, pigs (*Sus scrofa)*, cows(*Bos taurus)*, adult goats, goat kids (*Capra hircus)* and camels (*Camelus dromedarius)*. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 146(2):274-82.

Ehrhardt RA, Slepetis RM, Siegal–Willott J, Van Amburgh ME, Bell AW and Boisclair YR (2000). Development of a specific radioimmunoassay to measure physiologyical changes of circulating leptin in cattle, and sheep. Journal of Molecular Endocrinology 166(3):519-28.

Elmahdi B, Sallmann HP, Fuhrmann H, Vonengelhadt W and Kaske M (1997). Comparative aspects of glucose tolerance in camels, sheep, and pories. Comp. Biochem. Physiol. Aphysiol. 118(1):147-51.

Essamadi AK, Bengoumi M, Zaoui D, Faye B, Bellenchi GC, Musci G and Calabrese L (2002). Purification and partial characterization of camel (*Camelus dromedarius)* ceruloplasmin. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 131(3):509-17.

Fain JN, Bahouth SW, Breakthroughs and Views (2000). Regulation of leptin release by mammalian adipose tissue. Biochemistry Biophysics Research Communication 274:571-5.

Faye B, Ratovonanahary M, Chacornac JP and Soubre P (1995). Metabolic profiles and risks of diseases in camels in temperature conditions. Comp. Biochem. Physiol. A. Physiol. 112(1):67-73.

Ferreira DG, Claudino F, Carvalho H, Agricola R, Alpoim MJ and Robalo SJ (2005). Seasonal reproduction in the mare: Possible role of plasma leptin body weight and immune status. Domestic Animal Endocrinology 29(1)203-13.

Flint DJ, Binart N, Kopchick J and Kelly P (2003). Effects of growth hormone and prolactin on adipose tissue development and function. Pituitary 6(2):97-102.

Ganong W F (1995). Review of Medical Physiology. 17th Ed., Appleton and Lange California pp.

Garcia MR, Amstalden M, Williams SW, Stanko RL, Morrison CD, Keisler DH and *et al* (2002). Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. Journal of Animal Sciences 80:2158-67.

Greiling H and Gressner AM (Hrsg.) (1995). Lehrbuch Der Klnischen Chemie und Pathobiochemie. Stuttgart / New York. Schattauer. Verlag.

Havel PJ (2004). Update on adipocyte hormones : regulation of energy balance and carbohydrate / lipid metabolism. Diabetes S. 53(11):143-51.

Huszenicza G, Janosi S, Gaspardy A and Kulcsar M (2004). Endocrine aspects in pathogenesis of mastitis in postpartum dairy cows. Animal Reproduction Science 82-83:389-389.

Jehad (1995) is arabic

جهاد - السيد أحمد (1995) : الإبل العربية " إنتاج و تراث " . المطبعة الثقافية ، جامعة الملك فيصل المكتبة المركزية - الأحساء .

Kaske M, Elmahdi B, Von–Engelhardt W and Sallmann HP (2001). Insulin responsiveness of sheep, ponies, miniature pigs and camels : results of hyperinsulinaemic clamps using porcine insulin. Journal of Comparative Physiology B. 171(7):549-56.

Kassab SMD, Abdul–Ghaffar T, Nagalla DSMD, Sachdeva UMD and Nayar UMD (2004). Interactions between leptin, neuropeptide – Y and insulin with chronic diurnal fasting during Ramadan. Annals Saudi Medicine 24(5):345-9.

Khatim MS, Gumaa KA, Petersson B, Lundqvist G, Grimelius L and Hellerstrom C (1985). The structure and hormone content of the endocrine pancreas of the one – humped camel (*Camelus dromedarius*). Anat. Anz. 159(1–5):181-6.

Kieffer TJ and Habener JF (2000). The adipoinsular axis : effects of leptin on pancreatic beta cells. American Journal of Physiology - Endocrinology and Metabolism 278:1-14.

Knight PK, Rose RJ, Evans D, Cluer D, Henckel P and Saltin, B. (1994). Metabolic responses to maximal intensity exercise in the racing camel. Acta Physiologica Scandinavica Supplement 671:61-77.

Kulcsar M, Janosi SZ, Lehtolainen T, Delavaud C, Chilliard Y and Pyorala S (2004). Mastitis – related endocrine

alterations in postpartum dairy cows. Acta Veterinaria Hungarica Submitted for publication.

Kulcsar M, Janosi SZ, Lehtolainen T, Katai L, Delavaud C, Balogh O, Chilliard Y, Pyorala S, Rudas P and Huszenicza GY (2005). Feeding – unrelated factors in fluencing the plasma leptin level in ruminants. Domestic Animal Endocrinology 29(1):214-26 .

Lage M, Garcia MR, Tome MA, Cordido F, Valle IF, Considine RV, Caro JF, Dieguez C and Casanueva FF (1999). Serum leptin levels in women throughout pregnancy, and the postpatum period, and in women suffering spontaneous abortion. Clinical Endocrinology Oxford 50:211-6.

MacSween RNM and Whaley K (1992). Muirs Text Book of Pathology. 13th Ed., ELBS. (Eductional Low – Priced Books Scheme). Funded by the British Government pp.

Mirgani T and Bakhit SMA (1987). Effect of intraluminal administration of molasses on blood glucose concentration and rumen VFA concentration in camels, sheep and goats. In : Camel Research Project University of Khartoum 107-19.

Morrison CD, Daniel JA, Holmberg BJ, Djiane J, Raver N, Gerller A and Keisler DH (2001). Central infusion of leptin in to well – fed, fed, and undernourished ewe lambs : effects on feed intake and serum concentrations of growth hormone, and luteinising hormone. Journal of Endocrinology 168:317-24.

Muhlhausler BS, Roberts CT, Yuen BS, Marrocco E, Budge H, Symond ME and *et al* (2003). Determinants of foetal leptin synthesis, fat mass, and circulating leptin concentrations in well – nourished ewes in late pregnancy. Endocrinology 144:4947-54.

Nagatani S, Zeng Y, Keisler DH, Foster DL and Jaffe CA (2000). Leptin regulates pulsatile luteinising hormone and growth hormone secretion in the sheep. Endocrinology 141(11):3965-75.

Nazifi S, Rezakhani A and Gheisari HR (1998). Physical, biochemical and cytologic properties of blood and synovial fluid in clinically normal adult camel (*Camelus dromedarius*). Zentralbl. Veterinary Medicine A. 45(3):155-60 .

Owen, WE and Roberts WL (2004). Letter to the editor : cross – reactivity of 3 recombinant insulin analogs with 5 commercial insulin immunoassays. Clinical Chemistry 50(1):257-9.

Piccione G, Grasso F, Costa A, Fazio F and Caola G (2005). Influence of short – term exereise on serum leptin concentration in the horse. Veterinarski Arhiv 75(1):15-22.

Qayyum MA, Fatani JA, Shaad FU and Mohajir AM (1987). A histochemical study on the innervation of the pancreas of the one – humped camel (*Camelus dromedarius*). Journal of Anatomy 151:117-23.

Reist M, Erdin D, VonEuw D, Tschuemperlin K, Leuenberger H, Delavaud C and *et al* (2003). Concentrate feeding strategy in lactating dairy cows : metabolic and endocrine changes with emphasis on leptin. Journal of Dairy Science 86:1690-706.

Sapin R, Legaludec V, Gasser F, Pinget M and Grucker D (2001). Elecsys insulin assay : free insulin determination and the absence of cross – reactivity with insulin lispro. Clinical Chemistry 47:602-5.

Sapin R (2003). Review; insulin assays : previously known and new analytical features. Clinical Chemistry 49:113-21.

Sayed AA, Elmorsy, SE, Rudas P and Bartha T (2003). Partial cloning and localisation of leptin and leptin receptor in the mammary gland of the egyptian water buffalo. Domestic Animal Endocrinology 25(3):303-14.

Schwartz MW, Woods SC, Potte DJ, Seeley RJ and Baskin DG (2000). Central nervous system control of food intake. Nature 404:661-71.

Shier D, Butler J and Leulis R (1999). Hole's Human Anatomy and Physiology. 8th Ed., WCB / Mc. Graw – Hill.

Siam AA, Mona MA, Wafaa, EM and Ismail A (1993). Plasma levels of glucose and insulin in camels during dehydration. Journal of Veterinary Scince 9(3):93-6.

Sinha MK, Sturis J, Ohannesian JP, Magosin S, Stephens TW, Heiman ML, Polonsky KS and Caro JF (1996). Ultradian oscillations of leptin section in humans. Biochemistry. Biophysics Research Communication 228:733-8.

Skidmor JA, Billah M and Allen WR (2002). Investigation of factors affecting pregnancy rate after embryo transferrin the dromedary camel. Reprod. Fertil. Dev. 14:(1-2):109-16.

Soliman M, Abdelhady S, Fattouh I, Ishioka K, Kitamura H, Kimura K and Saito M (2001). No alteration in serum leptin levels during acute endotoxaemia in sheep. Journal of Veterinary Medical Scince 63(10):1143-5.

Soliman M, Ishioka K, Kimura K and Saito M (2002). Plasma leptin responses to lipopolysaccharide and tumour necrosis factor alphain cows. Japanese. Journal of Veterinary Research 50(2–3):107-14.

Thomas L (Hrsg) (2000). Labor und Diagnose. Frankfurt : TH Books Vertagsgesellschaft. 5 Erw. Auflage.

Thomas L, Wallace JM, Aitken RP, Mercer JG, Trayhurn P and Hoggard N (2001). Circulating leptin during ovine pregnancy in relation to maternal nutrition, body composition, and pregnancy outcome. Journal of Endocrinology 169:465-76.

Tietz NW (1995). Clinical Guide to Laboratory Tests. 3rd Ed., Philadelphia. Pa. WB Saunders Co. 594 and 366-7.

Tokuda T, Kimura D and Fujihara T (2000). Changes in plasma leptin level in growing lambs fed with timothy hay and concentrate, In : Proceedings of the 9 th Congress of the Asian – Australasian Association of Animal Production. University of New South Wales, Sydney, Australian Asian – Australian Science 13:145.

Trayhum P, Duncan JS, Hoggard N and Rayner DV (1998). Regulation of leptin production : a dominant role for the sympathetic nervous system. In. Proceedings of the Nutrition Society 57:413-19.

Wang J, Liu R, Hawkins M, Barzilail N and Rossetti L (1998). A nutrient – sensing pathway regulates leptin gene expression in muscle and fat. Nature 393:684-8.

Way JM (2001). Adipose tissue resisting expression is severely suppressed in obesity and stimulated by peroxisome

proliferators – activated receptor gamma agonists. Journal of Biology Chem. 276(28):25651-3.

Wernery U, Haydn EJ and Kinne J (1998). Amprolium – induced cerebro-cortical necrosis (CCN) in dromedary racing camel. Zeutralbl. Veterinarmed. B. 45(6):335-43.

Yagil R and Berlyne GM (1977a). Glucose loading and dehydration in the camel. J. Appl. Physiol. 42(5):690-3.

Yagil R and Berlyne GM (1977b). Renal handling of creatinine in various stages of hydration in the camel. Comp. Biochem. Physiol. A. 56:15-8.

Yagil R (1985). The Desert Camel : Comparative Physiological Adaptation Krager, Basel. Switzerland pp.

Yanagihara N, Utsunomiya K, Cheath TB, Hirano H, Kajiwara K, Hara K, Nakamura E, Toyohira Y, Uezono Y, Ueno S and Izumi F (2000). Characterisation and functional role of leptin receptor in bovine adrenal medullary cells. Biochem. Pharmac. 59:1141-5.

Yonekura S, Kitade K, Furukawa G, Takahashi K, Katsumata N and Katoh K, *et al* (2002). Effects of aging and weaning on mRNA expression of leptin and CCK receptors in the cal frumen and abomasum. Domestic Animal Endocrinology 22:25-35.

Yonekura S, Senoo T, Kobayash Y, YoneZawa T, Katoh K and Obara Y (2003). Effects of acetate and butyrate on the expression of leptin and short – form leptin receptor in bovine and rat anterior pituitary cells. General and Comparative Endocrinology 133:165-72.

Yonekura S, Sakamoto K, Komatsu T, Hagino A, Katoh K and Obara Y (2006). Growth hormone and lactogenic hormones can reduce the leptin mRNA expression in bovine mammary epithelial cells. Domestic Animal Endocrinology 31(1):88-96.

Zhao XX, Li XL and Chen BX (2001). Isolation of ovulation – inducing factors in the seminal plasma bactrian camel (*Camelus bactrianus)* by DEAE – cellulose chromatography. Reprod. Domestic Animal Endocrinology 36(3-4):177-81.

Zhou YT (1997). Induction by leptin of uncoupling protein – 2 and enzymes of fatty acid oxidation. Proceedings of The National Academy of Science (USA) 49:6386-90.

Zieba DA, Amstalden M, Morton S, Gallina JL, Edwards JF, Harms PG and Williams GL (2003). Effects of leptin on basal and GHRH – stimulated GH secretion from the bovine adenohypophysis are dependent up on nutritional status. J. Endocrinol. 178(1):83-9.