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COMPARISON BETWEEN SOME METABOLIC HORMONES AND BIOCHEMICAL PARAMETERS BEFORE, DURING AND AFTER PUBERTY IN KARADI EWE LAMBS UNDER NATURAL CONDITIONS IN KURDISTAN REGION OF IRAQ

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Abstract

The aim of this paper is to determine the concentration of blood metabolic hormones (leptin, insulin and estradiol) levels in growing Karadi Ewe lambs. The research was carried out at bamerne Village, Duhok governorate, Kurdistan region of Iraq. Twenty four Ewe lambs randomly selected as per age and body weight were divided into four groups, Group I (>3 to 5 months)(20±0.4 or 16±1.5 Kg), Group II (>5 to 7 months)(26.4±1.2 or 26±4.4 Kg), Group III (>7 to 9 months) (34± 2.023 Kg) and Group IV (>9 to 11 months)(40.562± 2.202 Kg), with six animals in each group .Concentrations of insulin, leptin and estradiol were determined in the blood serum of ewe lambs during (pre pubertal puberty and post pubertal ewes) were determined by ELISA using ovine kits. The concentration of insulin decreased with increasing age, the level of estradiol elevated at the third groups it means the pubertal age in Karadi ewe, non- significant elevation of leptin concentration with increasing age. The serum biochemical parameters measured in the study included lipid profile (cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides), Total protein profile (TP) serum albumin, serum globulin, carbohydrate profile. Total protein increased, but by a small percentage, compared to the first group, the lipid and carbohydrate profile gradually decreased with increasing age. This decreasing in the percentage of insulin due to feeding on herbs and the weather conditions of the region. The aim of the current study is to estimate the baseline information of physiological concentration and relation of metabolic hormones leptin, insulin and estrogen levels before, during and after puberty in Karadi Ewe lambs under natural conditions of Iraqi Kurdistan region.

Keywords: Leptin; Insulin; Estradiol; Metabolic Hormones; Total Protein; Lipid Profile; Karadi Ewe; Puberty.

1. INTRODUCTION

The Karadi breed which comprises 18-20% of the Iraqi sheep population is native to the north-eastern mountains, villages, and undulating dry farming plains of the Kurdistan region of Iraq. The Karadi lambs are white in color with a black open face and pendulous ears. The black color often extends to the shoulders and other parts of the body (Alkass et al., 2013). Previously, it was noted that age at puberty of Karadi ewe lambs was 286.2 day (Alkass et al., 2022) Growth and development are the continuous and dynamic processes that require integration of numerous factors such as plane of nutrition, efficiency of metabolism, hormonal levels, immune responses, physiological status and maintenance of homeostasis. The majority of metabolic hormonal levels in young animals differ from normal values of adults and they change as per age of the animals (Ježek et al., 2006). The level of certain hormones can be used

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as physiological indicators of growth, the metabolism of energy in farm animals is controlled by important hormonal factors like leptin and insulin (Veena et al., 2018). In lambs, the correlation between blood leptin and insulin concentrations increases with the age. (Veena et al., 2018). There is a close association between general body growth and development of the reproductive organs (Pálsson & Vergés, 1952). Metabolic hormones such as insulin play an important role in animal metabolism. Insulin is a 5.8-kDa protein synthesized in the pancreatic β -cells and secreted in response to evaluation of plasma glucose level (Magistrelli et al., 2008). Insulin has an important role in glucose transport and modulates peripheral satiety signals and directly targets the central nervous system to inhibit food intake (Gale et al., 2004). Also, insulin has an important role in lipid metabolism, stimulating lipogenesis and inhibiting lipolysis (Ban-Tokuda et al., 2008). Leptin is an "adipokine" peptide hormone produced by fat cells, particularly in white adipose tissue (Ehrhardt et al., 2003). There are many roles of leptin in the animal organism. Production of leptin is modulated by insulin, glucocorticoids and sexual steroids and effects on the central nervous system inhibiting hypothalamic neuropeptide production, mainly neuropeptide Y, which stimulate appetite (Stephens et al., 1995). The initiation of puberty in sheep depends upon a complex neuroendocrine interplay. Many blood-borne substances have been proposed as metabolic signals, such as glucose (Nagatani et al., 1996), amino acids (Recabarren et al., 1996), free fatty acids (Rutter et al., 1983), insulin (Hileman et al., 1993), IGF-I (Hiney et al., 1991), and recently leptin (Mantzoros et al., 1997). Leptin receptors have also been identified in both granulosa and theca cells of the human, bovine and porcine (Agarwal et al., 1999) ovarian follicles .The effects of leptin on GnRH release and to cause an upregulation of LH pulses are mediated through inter neuronal pathways involving NPY, proopiomelanocortin (POMC) and Kisspeptin (Barash et al., 1996) Furthermore, leptin regulates reproductive function by altering the sensitivity of the pituitary gland to GnRH.

Recently, we described that deletion of both LepR and insulin receptor from POMC cells causes insulin resistance, hyperandrogenism and ovarian abnormalities with late onset of subfertility (Hill et al., 2010). Studies of the relevant role of the kisspeptin- GPR54 system in reproductive physiology and the co-expression of the Kiss1 and LepR genes in hypothalamic neurons have postulated the permissive role of leptin in the onset of puberty, relayed by Kiss1 neurons (Pinilla et al., 2012; Terasawa et al., 2012). With regard to regulation by reproductive hormones, rising estradiol levels are correlated with rising serum leptin in human and rodent females and estrogen treatment stimulates serum leptin (Fungfuang Nakada et al., 2013; Fungfuang Terada et al., 2013). Studies on the metabolic hormones especially leptin hormone attributes have not been well documented in Karadi sheep and specific physiological references are needed for appropriate interpretation of hormonal serum biochemical results. The present work is the first of its kind in Karadi sheep and could serve as a baseline reference of biochemical values for Karadi sheep in Kurdistan region of Iraq and the other countries having similar climate and nutritional conditions. The aim of the present study is to estimate the baseline information of physiological concentration and relation of metabolic hormones leptin, insulin and estrogen levels before, during and after puberty in Karadi Ewe lambs under natural conditions of Iraqi Kurdistan region.

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2. MATERIALS AND METHODS

Ethics statement

The Animal Ethics Committee of the Department of Animal Production at the University of Duhok in Iraq has approved the current study's protocol and procedures under authority number AEC19112021.

Animal and nutrition

The present study was conducted to determine the levels of leptin, insulin, estradiol hormones and (glucose, lipid, protein) profiles during the different stages of growth (pre-during-after) puberty in Karadi ewe lambs. Sheep maintained at Akre breeding farm, Shermin village, Duhok governorate, Iraq. Twenty-four Ewe lambs randomly selected as per age and body weight were divided into four groups, Group I (>3 to 5 months) (20±0.4 or 16±1.5 Kg), Group II (>5 to 7 months) (26.4±1.2 or 26±4.4 Kg), Group III (>7 to 9 months) (34± 2.023 Kg) and Group IV (>9 to 11 months) (40.562± 2.202 Kg), with six animals in each group. The animals were maintained under grazing in the green pastures in the mountains.

Collection of the samples

The blood samples collected from jugular vein in to the clot activator coated vacutainers were allowed to clot for 30 minutes at room temperature to obtain the serum. The samples were collected at fortnightly intervals for two months in each group, i.e., 15th, 30th, 45th and 60th day. Before each blood sampling, the animals were individually weighed on a digital scale to record the body weight in kilograms.

Hormonal assays

The serum leptin, insulin and estradiol levels were measured by radioimmunoassay (RIA) using a commercially available kits (Multispecies leptin RIA kit, Linco Research, St. Charles, MO, USA). The procedures were performed according to the vendor's protocol. Protein concentration was determined using the Bradford assay.

Biochemical analysis

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglyceride levels were measured by an automated clinical chemistry analyzer (Architect ci8200; Abbott GmbH Co KG, Wiesbaden, Germany) using commercially available assay kits (Abbott GmbH Co KG). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the formula: LDL-C (in milligrams per deciliter) = total cholesterol – (HDL – C + triglyceride/5).

Statistical analysis

The gathered data submitted to SPSS program (Statistical package for social sciences, Ver 26, 2019), in order to analyze it statistically. Descriptive statistics and analysis of variance (One-way ANOVA-repeated measures) was applied to testing the effect of time on the studied parameters (insulin, estrogen, leptin, protein profile, carbohydrate profile and lipid profile).

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However, the means differences were separated using Bonferroni test, that specify for repeated measure ANOVA.

3. RESULTS

3.1. The Effect of age on Insulin (Iu/ml) Plasma Concentration

The Effect of age on insulin (Iu/mL) Plasma Concentration. Insulin levels tended to decrease gradually during 1st, 2nd, 3rd and 4th groups. Baseline values of insulin (7.18Iu/mL) in 1st group decreased to (0.200Iu/mL) in the 4th groups ($P < 0.01$). Respectively (Figure 1; Table1).

Table 1: The effect of age on insulin plasma level (Iu/mL) of Karadi ewe

Time	Mean	± Std. Error	Sig. (p)
1	7.188 a	1.545	** (0.004)
2	3.490 ab	.777	
3	.923 bc	.482	
4	.200 c	.000	
Overall mean	2.950	.504	

Means having the same letters are didn't differed significantly.

***= Significant (p<0.01); *= Significant (p<0.05); NS= non-significant (p>0.05)*

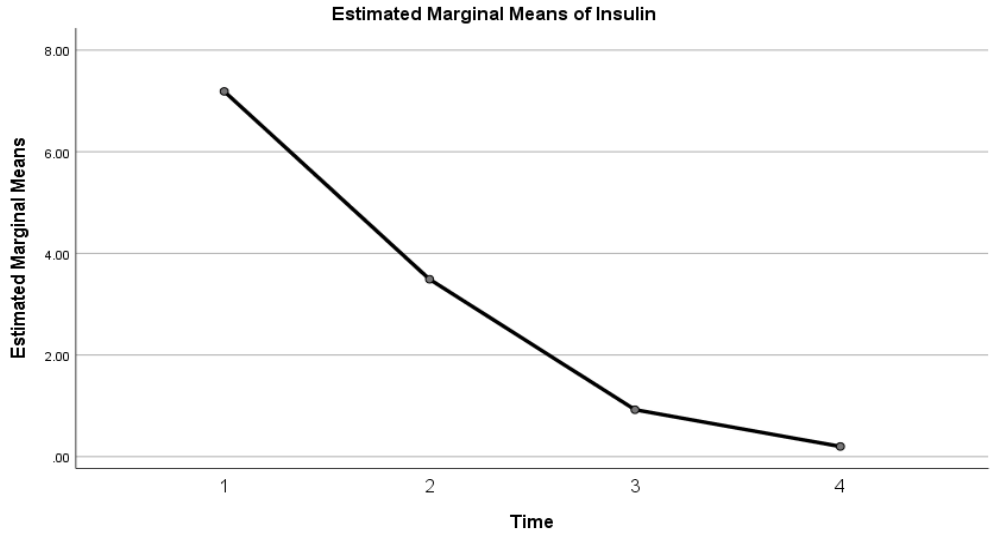

Figure 1: level of Insulin in plasma as affected by age in Karadi ewe lambs

3.2. The Effect of age on estradiol (Pg/ml) Plasma Concentration

2:2. Table and Figure (2) shows the summary of estradiol pulsatile secretion characteristics in normal growing of female lamb's 4th different groups of age. In the 1st group, 2nd and 4th groups mean plasma estradiol concentrations amplitude decreased ($p>0.05$), in the other side Mean estradiol concentrations amplitude significant increase in ($p<0.05$).

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Table 2: Characteristics of the pulsatile estradiol (pg/mL) hormone secretion in 4th groups of Karadi ewe

Time	Mean	± Std. Error	Sig. (p)
1	43.867 b	12.715	* (0.03)
2	25.132 b	1.440	
3	56.683 a	5.160	
4	33.568 b	1.012	
Overall mean	39.813	3.547	

Means having the same letters are didn't differed significantly.

***= Significant (p<0.01); *= Significant (p<0.05); NS= non-significant (p>0.05)*

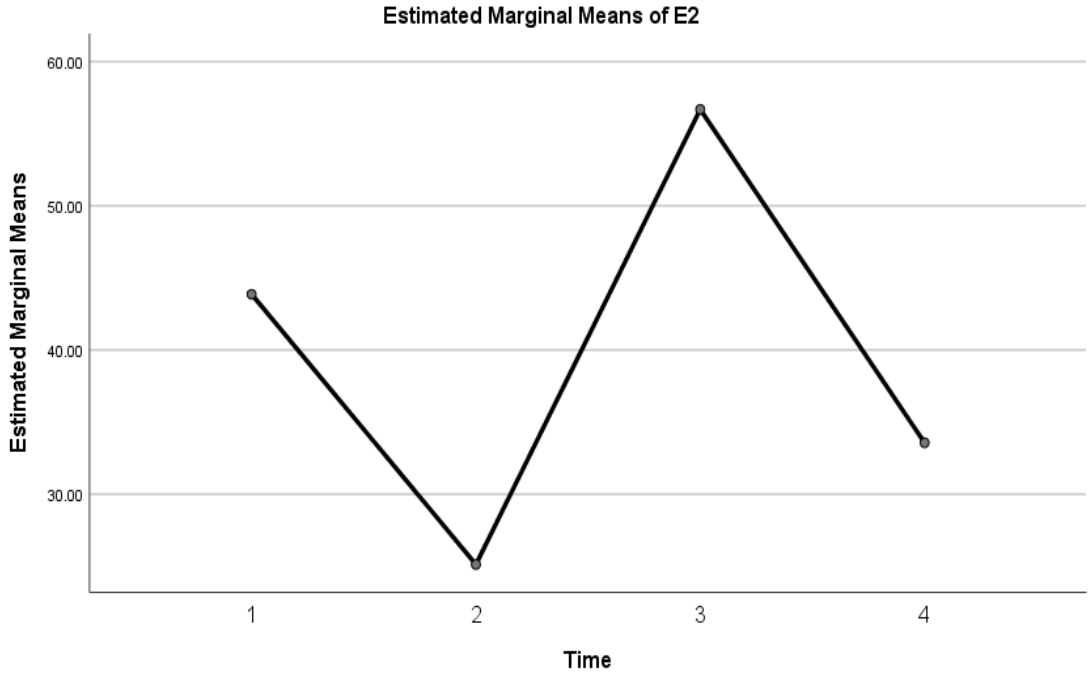

Figure 2: Plasma estradiol concentrations profiles in individual female sheep. Blood samples were taken from 24 ewe lambs in 4th groups compare the initiation of puberty

3.3. The Effect of age on leptin (Pg/ml) Plasma Concentration

The circulating concentration of leptin was lower although there were no significant differences (p>0.05) between the four groups. Moreover, it was found that the circulating concentration of leptin increased (p>0.05) ewes during measurements third group and reaching its highest level in the fourth group. (Table 3) (Figure 3)

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Table 3: The effect of age on Leptin (pg/mL) of Karadi ewe. The same Letters indicate no-significant differences between the groups, and the results

Time	Mean	± Std. Error	Sig. (p)
1	720.197 a	116.798	NS (0.69)
2	678.170 a	70.127	
3	812.228 a	192.240	
4	1019.885 a	311.652	
Overall mean	807.620	55.976	

Means having the same letters are didn't differed significantly.

***= Significant (p<0.01); *= Significant (p<0.05); NS= non-significant (p>0.05)*

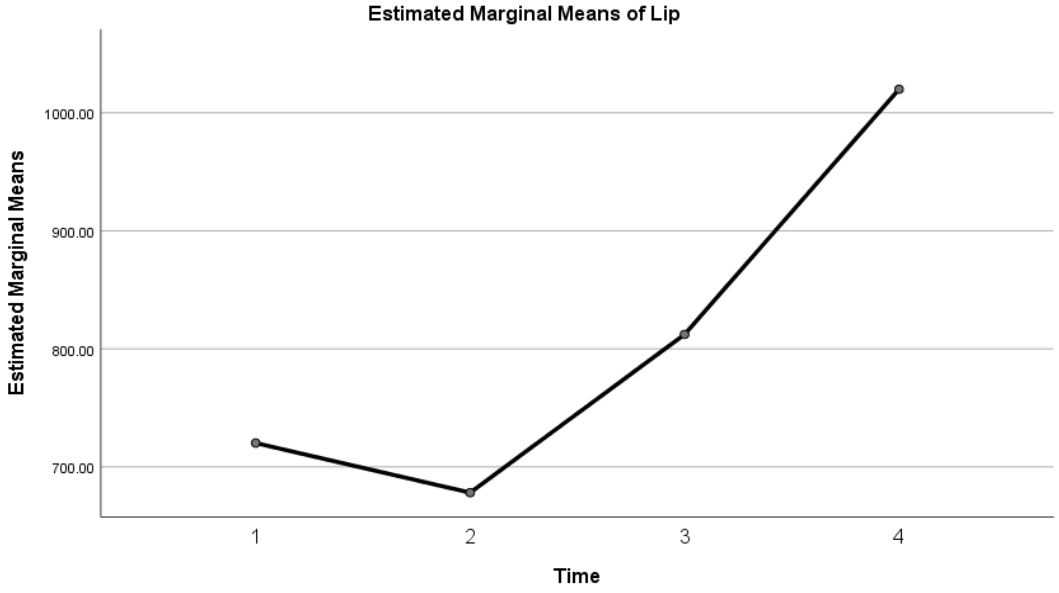

Figure 3: The effect of age on the plasma leptin concentration before and after puberty. Shows the non- statistically significant differences between before, during and after puberty

3.4. Serum biochemical parameters

The mean values (±SE) of serum biochemical parameters lipid profile involve (cholesterol, HDL, LDL, VLDL, triglyceride) protein profile (total protein, albumin and globulin) and carbohydrate profile (Table 4: Figure 4) there are high significant differences ($p<0.01$) between the profile of carbohydrate between the 1st group and the other 2nd, 3rd and 4th group. When comparing the lipid profile, it was found that there is a high significant difference ($p<0.01$) between the 1st group and the rest of the groups, as well as there is a high significant difference ($p<0.01$) between the 2nd group and the rest of the groups, but there was no significant difference between the 3rd and 4th groups, when observing the protein profile, it was found that there was a significant difference ($p<0.05$) between the 1st group and the rest of the groups,

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respectively. It was noted that there were no significant differences between the 2nd, 3rd and 4th groups. (Table: 5) lipid profile the significant decrease ($p<0.01$) of all its concentrations (cholesterol, HDL, LDL, VLDL, triglyceride) with age.

Table 4: Biochemical profile parameters as affected by age

Time		N	Mean	± Std. Error	Sig. (p)
Protein Profile (g/dl)	1	16	12.162 b	.335	* 0.03
	2	16	13.031 a	.163	
	3	16	13.032 a	.208	
	4	16	13.099 a	.169	
	Overall mean	64	12.831	.112	
Carbohydrate Profile (mg/dl)	1	16	209.56 a	16.705	** 0.001
	2	16	122.63 b	7.939	
	3	16	104.56 b	3.195	
	4	16	103.81 b	1.898	
	Overall mean	64	135.141	4.245	
Lipid Profile (mg/dl)	1	16	192.09 a	17.785	** 0.001
	2	16	102.31 b	7.647	
	3	16	71.90 c	4.960	
	4	16	75.28 c	2.372	
	Overall mean	64	110.394	4.594	

Means having the same letters are didn't differed significantly.

***= Significant ($p<0.01$); *= Significant ($p<0.05$); NS= non-significant ($p>0.05$)*

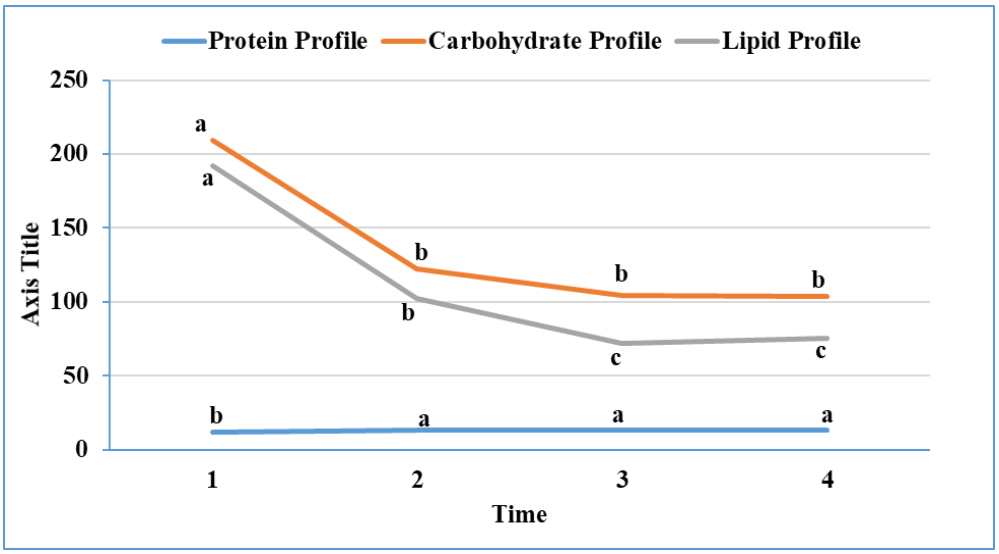

Figure 4: Curve of (protein, carbohydrate, lipid) profile affected by age in Karadi ewe

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Table 5: Lipid (mg/dl) profile affected by age

		N	Mean	± Std. Error	Sig. (p)
CHO	1	16	132.50 a	15.101	** 0.001
	2	16	61.63 b	4.046	
	3	16	49.94 b	2.486	
	4	16	53.75 b	2.069	
	Overall mean	64	74.45	5.769	
HDL	1	16	73.81 a	8.318	** 0.001
	2	16	40.13 b	2.444	
	3	16	37.69 b	1.884	
	4	16	38.38 b	1.110	
	Overall mean	64	47.50	2.904	
TG	1	16	42.56 a	3.934	** 0.001
	2	16	29.06 b	3.021	
	3	16	15.69 c	2.528	
	4	16	15.38 c	1.207	
	Overall mean	64	25.67	1.981	
LDL	1	16	67.200 a	7.6226	** 0.001
	2	16	27.313 b	2.1512	
	3	16	15.388 b	1.4572	
	4	16	18.450 b	1.5089	
	Overall mean	64	32.088	3.2899	** 0.001
VLDL	1	16	8.513 a	.7868	
	2	16	5.812 b	.6043	** 0.001
	3	16	3.138 c	.5055	
	4	16	3.075 c	.2414	
	Overall mean	64	5.134	.3963	

Means having the same letters are didn't differed significantly.

***= Significant (p<0.01); *= Significant (p<0.05); NS= non-significant (p>0.05)*

4. DISCUSSION

Unfortunately, very limited work has been carried out on the reproductive aspects in female Karadi sheep which live in Iraqi Kurdistan Mountains. Our findings noted was in agreement with the results of (Alkass et al., 2022) who indicated that the age at puberty of Karadi ewe lambs was 286.2 day and it appears that glucose was positively correlated with weight at puberty (P<0.05) and insulin was positively correlated with body weight (P<0.05), but negatively with age at puberty. The result was in agreement with the results of my research (Gatford et al., 2004) he shows that glucose homeostasis, insulin secretion, insulin sensitivity, and insulin action decrease with maturation, from before weaning to early adult life in the sheep. The results in my research is due to the impairment of glucose tolerance with aging from the young animal across puberty to the young adult is common to the sheep and the rat (Bracho-

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Romero & Reaven, 1977) and (Gatford et al., 2004). But these researchers did not agree with (Ehrhardt et al., 2003) in lambs, (Ban-Tokuda et al., 2008) in lambs and (Antunović, Zvonko et al., 2010), (Veena et al., 2018). When they confirmed that insulin levels increased as the age advanced indicating anabolic status during different stages of growth that finally could opined that the increased insulin level in fattening lambs may be related to an increase in body weight and body fat accumulation. The explanation for this difference in results is due to when Glucose concentration drop in the older ewes can be in connection to the weakened regeneration of glucose in the liver (Church, 1993). According to (Magistrelli et al., 2008) insulin can be absorbed from the milk and enters the system. Intense circulation, which increases its plasma level in lactation kids. This news points to a possible role for milk-borne insulin in regulating the functions of the endocrine glands and the gastrointestinal tract children before weaning. This explains the increased concentration of insulin and glucose in the first and second groups of my experiment.

The results of the present study were in accordance with the findings of (Delavaud et al., 2000; Veena et al., 2018)in sheep, (Chilliard et al., 2001) in ruminants, (Bispham et al., 2002) in sheep, (Čebulj-Kadunc & Cestnik, 2005) in Jezersko-Solcava lambs and (Antunović, Zvonko et al., 2010) in sheep. They have reported that the plasma leptin levels are closely correlated with body condition score and nutritional status of the animal and increase in leptin concentration in blood plasma was correlated to the progress in the fattening of the lambs as the age of the animal advanced. The increase in concentration of serum leptin after weaning during fattening period could have been due to the increase live weight and consequently increase adipose cells volume (Tokuda & Yano, 2001).

These findings may be associated with increased in size of adipocyte where leptin is secreted and fat proportion The rise in serum leptin was blunted during hypoglycemia compared to hyperglycemia in humans (Schmitz et al., 1997). Wellhoener et al. (2000) suggested that glucose metabolism rather than insulin is a main determinant of leptin secretion in humans (Wellhoener et al., 2000). Therefore, circulating glucose may have a stimulatory effect on leptin secretion in newborn lambs. Recent work has focused on interactions between leptin and insulin, proposing that insulin acts in an adipogenic/leptogenic fashion. But the results were not similar with (Kasap et al., 2018) leptin concentration was high in the prepartum and baseline periods, while it was at the lowest level in week 3, week 4, and week 12 of lactation. Fluctuating decline in leptin concentration continued until week 12 compared to the baseline might be related to changes in adipose tissue density in response to parturition in sheep There is a significant increase in the level of serum estradiol in the third group, and this indicates that Karadi ewe sheep reach puberty at this age, and these results were not identical with (Veena et al., 2018), (Gonzalez-Padilla et al., 1975) in heifer, (Huffman et al., 1987).

This difference is due to the factors such as inherent characters of different breeds, body weight at birth, nutrition and photoperiod and geological location of the farm. But my results were identical (Ehtesham & Vakili, 2015) and (Menatian et al., 2016). Estrogen may also have an indirect effect via reducing the insulin-like growth factor-1 feedback inhibition resulting in increased growth hormone secretion (Tilahun et al., 2016). Generally; leptin synthesis is down

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regulated by fasting (Chelikani et al., 2004), thyroid hormones, IL-6, growth hormone (GH), androgens and cAMP at cellular level. However, cold exposure (Asakuma et al., 2003), estrogens, prolactin, transforming necrosis factor-α-glucocorticoids and insulin (Faulconnier et al., 2003) rise the rate of leptin synthesis in white adipose tissue (Frühbeck et al., 2001) and (Reist et al., 2003). On the other hand the results of my research are agreement with (Delavaud et al., 2000). The researcher (Davis et al., 2003) results were identical to the results obtained in my estimation of protein, especially in the first group that owns young animals. Dietary amino acids are utilized efficiently for protein deposition in the neonate because the protein synthetic response to the ingestion of nutrients is elevated, particularly in skeletal muscle. High ribosome abundance and enhanced sensitivity of protein synthesis to both insulin and amino acids enable the elevated rate of protein synthesis in skeletal muscle of the neonate. The enhanced activation of the factors that regulate the binding of mRNA and met-tRNA to the40S ribosomal subunit contribute to the postprandial rise in protein synthesis in neonatal skeletal muscle. And our results were not in agreement with (Kaneko et al., 1997). An elevated feeding induced activation of the insulin signaling pathway leading to translation initiation in skeletal muscle of the neonate likely plays a crucial role in initiating these processes that result in the high rate of protein deposition in skeletal muscle during early postnatal life.

Decrease of total protein over the pregnancy and lactation could be explained by a rapid extraction of immunoglobulin from the plasma during the last few months of pregnancy when a colostrum is being formed in the mammary gland as well as the increased needs for proteins for the fetus development (Castillo et al., 1999). This explains the reason for the decrease in protein in young animals and elevation with increasing age, as well as the reason for the gradual decrease in insulin with age. The postprandial elevation in skeletal muscle protein synthesis during the early postnatal period is mediated, in part by insulin (Davis et al., 2001; Wray-Cahen et al., 1997). Each of (CHO, HDL, TG, LDL, VLDL) or lipid propyl descends gradually from young ages until puberty and after puberty if the ewe is not pregnant, and these results were identical to each of (Nazifi et al., 2002) and (Antunović, Z. et al., 2011).

The high level of glucose in the first group of our research, or in young animals, then its concentration decreased until puberty and after puberty, as the animal needs energy during pregnancy, especially the last period, due to the need for gain weight, as well as the stress of parturition. Therefore, my results were identical to the research that says that young animals have high glucose because of the energy it gets from the mother during pregnancy and the lactation period (Castillo et al., 1999; McNeill et al., 1998) and (Ramos et al., 1994). Glucose concentration drop in the older ewes can be in connection to the weakened regeneration of glucose in the liver (Church, 1993). It was concluded that the puberty in Karadi ewe lambs influenced by leptin, insulin and estradiol concentration in the growing Karadi ewe lamb and the findings could be used as basic metabolic indicators of puberty and metabolic hormone accelerate puberty in Karadi ewe lambs in Iraqi Kurdistan region.

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5. CONCLUSION

Leptin is classified as a metabolism modifier, through tissue-specific mechanisms, the sensitivity to insulin and hence glucose uptake by the cells, in order to direct nutrients towards organs or tissues that are metabolically more active. Moreover; the decrease in leptin plasma concentration puberty may also be correlated with the intensive lipolysis and fat mobilization. However, the lack of fat in the feeding of Karadi ewe lamb they have reached puberty in a standard time. We concluded that the leptin has non-significant increase but its level was increase and this will lead to reach puberty under the poor environmental nutrition and hard weather it lead to make test of leptin gene of Karadi ewe and used it to increase fertility because having strong effect on metabolism and steroid genesis.

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Data Availability Statement

All data in the manuscript are available from the corresponding author upon request.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1) Agarwal SK, Vogel K, Weitsman SR, Magoffin DA. Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. J Clin Endocrinol Metab. 1999; 84(3):1072–6.

2) Alkass J, Mustafa K, Baker I. Mesopotamia Journal of Agriculture Performance of Karadi Sheep in Kurdistan Region/Iraq: A Review. Vol. 50, Mesopotamia Journal of Agriculture. 2022 Dec.

3) Alkass J, Pedaey A, Abdulkareem T. Effect of different dietary protein levels and age on testicular development and ultastructure in Karadi ram lambs. International journal of agriculture and food research. 2013; 2:14–24.

4) Antunović Z., Novoselec J, Šperanda M, Vegara M, Pavić V, Mioč B, Djidara M. Changes in biochemical and hematological parameters and metabolic hormones in Tsigai ewes blood in the first third of lactation. Archives Animal Breeding. 2011; 54(5):535–45.

5) Antunović Zvonko, Novoselec J, Sauerwein H, Vegara M, Šperanda M. Blood Metabolic Hormones And Leptin In Growing Lambs. Poljoprivreda. 2010; 16(2):29–34.

6) Asakuma N, Hirashima H, Imai H, Fukui T, Toki M. Crystallization and Reduction of Sol-Gel-Derived Zinc Oxide Films by Irradiation with Ultraviolet Lamp. Journal of Sol-Gel Science and Technology. 2003; 26(1):181–4.

7) Ban-Tokuda T, Delavaud C, Chilliard Y, Fujihara T. Plasma leptin, feed intake and body fat accumulation in fattening castrated male and female lambs. Animal Science Journal. 2008; 79(1):58–67.

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/PZ23W

8) Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. Endocrinology. 1996; 137(7):3144–7.

9) Bispham J, Budge H, Mostyn A, Dandrea J, Clarke L, Keisler DH, Symonds ME, Stephenson T. Ambient temperature, maternal dexamethasone, and postnatal ontogeny of leptin in the neonatal lamb. Pediatr Res. 2002; 52(1):85–90.

10) Bracho-Romero E, Reaven GM. Effect of age and weight on plasma glucose and insulin responses in the rat. J Am Geriatr Soc. 1977; 25(7):299–302.

11) Castillo C, Hernandez J, Lopez-Alonso M, Miranda M, Benedito JL. Effect of physiological stage and nutritional management on some serum metabolite concentrations in Assaf ovine breed. Archives Animal Breeding. 1999; 42(4):377–86.

12) Čebulj-Kadunc N, Cestnik V. Circulating leptin concentrations in Lipizzan horses and Jezersko-solchava sheep. Slov Vet Res. 2005; 42:11–4.

13) Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Life Sci. 2004; 61(2):192–208.

14) Chilliard Y, Bonnet M, Delavaud C, Faulconnier Y, Leroux C, Djiane J, Bocquier F. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest Anim Endocrinol. 2001; 21(4):271–95.

15) Church DC. The Ruminant Animal: Digestive Physiology and Nutrition. Waveland Press; 1993. 564 p.

16) Davis TA, Fiorotto ML, Beckett PR, Burrin DG, Reeds PJ, Wray-Cahen D, Nguyen HV. Differential effects of insulin on peripheral and visceral tissue protein synthesis in neonatal pigs. Am J Physiol Endocrinol Metab. 2001; 280(5):E770-779.

17) Davis TA, Suryawan A, Bush JA, O'Connor PMJ, Thivierge MC. Interaction of amino acids and insulin in the regulation of protein metabolism in growing animals. Can J Anim Sci. 2003; 83(3):357–64.

18) Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A, Kann G. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J Endocrinol. 2000; 165(2):519–26.

19) Ehrhardt RA, Greenwood PL, Bell AW, Boisclair YR. Plasma leptin is regulated predominantly by nutrition in preruminant lambs. J Nutr. 2003; 133(12):4196–201.

20) Ehtesham S, Vakili A. The Effect of Spent Mushroom Substrate on Blood Metabolites and Weight Gain in Kurdish Male Lambs. Entomology and Applied Science Letters. 2015; 1:29–33.

21) Faulconnier Y, Delavaud C, Chilliard Y. Insulin and (or) dexamethasone effects on leptin production and metabolic activities of ovine adipose tissue explants. Reprod Nutr Dev. 2003; 43(3):237–50.

22) Frühbeck G, Gómez-Ambrosi J, Muruzábal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol Endocrinol Metab. 2001; 280(6):E827-847.

23) Fungfuang W, Nakada T, Nakao N, Terada M, Yokosuka M, Gizurarson S, Hau J, Moon C, Saito TR. Serum leptin concentrations, leptin mRNA expression, and food intake during the estrous cycle in rats. Lab Anim Res. 2013; 29(1):1–6.

24) Fungfuang W, Terada M, Komatsu N, Moon C, Saito TR. Effects of estrogen on food intake, serum leptin levels and leptin mRNA expression in adipose tissue of female rats. Lab Anim Res. 2013; 29(3):168–73.

25) Gale SM, Castracane VD, Mantzoros CS. Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. J Nutr. 2004; 134(2):295–8.

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/PZ23W

26) Gatford KL, De Blasio MJ, Thavaneswaran P, Robinson JS, McMillen IC, Owens JA. Postnatal ontogeny of glucose homeostasis and insulin action in sheep. Am J Physiol Endocrinol Metab. 2004; 286(6):E1050-1059.

27) Gonzalez-Padilla E, Ruiz R, LeFever D, Denham A, Wiltbank JN. Puberty in beef heifers. III. Induction of fertile estrus. J Anim Sci. 1975; 40(6):1110–8.

28) Hileman SM, Schillo KK, Hall JB. Effects of Acute, Intracerebroventricular Administration of Insulin on Serum Concentrations of Luteinizing Hormone, Insulin, and Glucose in Ovariectomized Lambs during Restricted and Ad Libitum Feed Intake1. Biology of Reproduction. 1993; 48(1):117–24.

29) Hill JW, Elias CF, Fukuda M, Williams KW, Berglund ED, Holland WL, Cho Y-R, Chuang J-C, Xu Y, Choi M, Lauzon D, Lee CE, Coppari R, Richardson JA, Zigman JM, Chua S, Scherer PE, Lowell BB, Brüning JC, Elmquist JK. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell Metab. 2010; 11(4):286–97.

30) Hiney JK, Ojeda SR, Dees WL. Insulin-like growth factor I: a possible metabolic signal involved in the regulation of female puberty. Neuroendocrinology. 1991; 54(4):420–3.

31) Huffman LJ, Inskeep EK, Goodman RL. Changes in episodic luteinizing hormone secretion leading to puberty in the lamb. Biol Reprod. 1987; 37(4):755–61.

32) ježek jožica, Klopčič M, Klinkon M. Influence of age on biochemical parameters in calves. 2006; 50:211–4.

33) Kaneko J, Harvey J, Bruss M. Clinical Biochemistry of Domestic Animals. Academy Press, San Diego, California, USA. 1997; 22.

34) Kasap S, Temizel EM, Akgul G, Senturk S. Practical Field Applications for Reducing Infectious Diseases of 0-6 Months Calves and Their Results. Harran Univ Vet Fak Derg. 2018; 7(1):102–7.

35) Magistrelli D, Dimel GP, Rosi F. Leptin, insulin and ghrelin levels in goat milk and in plasma of suckling kids. Small Ruminant Research. 2008; 79(1):38–41.

36) Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab. 1997; 82(4):1066–70.

37) McNeill DM, Murphy PM, Lindsay DR. Blood lactose v. milk lactose as a monitor of lactogenesis and colostrum production in Merino ewes. Australian Journal of Agricultural Research. 1998; 49(4):581–7.

38) Menatian S, Mirzaei Alamouti HR, Fatahnia F, Masoumi R. Effect of Pre-Pubertal Plan of Nutrition on Reproductive Performance, Hormone Concentrations and Milk Production in Kurdish Female Lambs. Iranian Journal of Applied Animal Science. 2016; 6(3):613–20.

39) Nagatani S, Bucholtz DC, Murahashi K, Estacio MA, Tsukamura H, Foster DL, Maeda KI. Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. Endocrinology. 1996; 137(4):1166–70.

40) Nazifi S, Gheisari HR, Shaker F. Serum lipids and lipoproteins and their correlations with thyroid hormones in clinically healthy goats. Veterinarski arhiv. 2002; 72(5):249–57.

41) Pálsson H, Vergés JB. Effects of the plane of nutrition on growth and the development of carcass quality in lambs Part I. The effects of High and Low planes of nutrition at different ages. J Agric Sci. 1952; 42(1–2):1–92.

42) Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev. 2012; 92(3):1235–316.

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/PZ23W

43) Ramos JJ, Verde MT, Marca MC, Fernández A. Clinical chemical values and variations in Rasa Aragonesa ewes and lambs. Small Ruminant Research. 1994; 13(2):133–9.

44) Recabarren SE, Escobar H, Lobos A, Recabarren MP, Parilo J. Luteinizing hormone pulse frequency is increased by arginine infusion in prepubertal sheep. Exp Clin Endocrinol Diabetes. 1996; 104(1):72–7.

45) Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Delavaud C, Chilliard Y, Hammon HM, Kuenzi N, Blum JW. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin. J Dairy Sci. 2003; 86(5):1690–706.

46) Rutter LM, Randel RD, Schelling GT, Forrest DW. Effect of Abomasal Infusion of Propionate on the GnRH-Induced Luteinizing Hormone Release in Prepuberal Heifers3. Journal of Animal Science. 1983; 56(5):1167–73.

47) Schmitz OJ, Beckerman AP, O'Brien KM. Behaviorally Mediated Trophic Cascades: Effects of Predation Risk on Food Web Interactions. Ecology. 1997; 78(5):1388–99.

48) Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature. 1995; 377(6549):530–2.

49) Terasawa E, Kurian JR, Keen KL, Shiel NA, Colman RJ, Capuano SV. Body weight impact on puberty: effects of high-calorie diet on puberty onset in female rhesus monkeys. Endocrinology. 2012; 153(4):1696–705.

50) Tilahun D, Hanlon C, Fekadu A, Tekola B, Baheretibeb Y, Hoekstra RA. Stigma, explanatory models and unmet needs of caregivers of children with developmental disorders in a low-income African country: a cross-sectional facility-based survey. BMC Health Serv Res. 2016; 16:152.

51) Tokuda T, Yano H. Blood leptin concentrations in Japanese Black cattle. Animal Science. 2001; 72:309–13.

52) Veena MP, Swamy M, Yathiraj S, Ramesh PT, Rao S, Shridhar D, Kalmath GP, Shwetha H s. Leptin, Insulin and Estradiol Profile in Bannur Female Sheep. International Journal of Current Microbiology and Applied Sciences. 2018; 7:507–11.

53) Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W, Born J, Fehm HL, Peters A. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. J Clin Endocrinol Metab. 2000; 85(3):1267–71.

54) Wray-Cahen D, Beckett PR, Nguyen HV, Davis TA. Insulin-stimulated amino acid utilization during glucose and amino acid clamps decreases with development. Am J Physiol. 1997; 273(2 Pt 1):E305-314.