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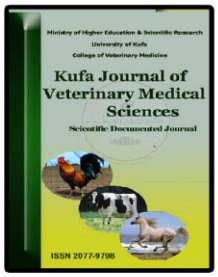

Role of seasonal changes on serum ghrelin and lipid profile in Turkish Awassi Rams

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Abstract

The present study was designed to investigate the role of sesonality changes on some physiological aspect of Ghrelin , lipid profile and their relation to body weight.Ten adult Turkish Awassi rams (1.2-1.3 years in age) and bodies weights ranged (45-58kg) were used in this study , which lasted from the beginning of January 2016 to the end of October 2016. Body weights of each individual ram were taken for each ten days. Blood samples were collected every ten days along the experiment at fasting and feeding states . and then blood samples was collected one hour after feeding for the same ten adult rams(considered feeding state). Ghrelin , cholesterol, triglycerids ,HDL-C,LDL-C,VLDL-C concentrations were estimated . The environmental temperature was measured by using special thermometer every day along experimental peroid. The results of this study revealed a significant increase in ghrelin hormone,cholesterol, triglyceride,HDL-C,and VLDL-C concentrations ,specially at Winter and Spring seasons in Turkish awassi rams serum at fasting state as compared to feeding .Additionally , the concentration of LDL-C shows a significant decrease during feeding state. The correlation coefficient between studies parameters indicated that ghrelin has a positive significant correlation with Cholesterol, Triglyceride, HDL-C and VLDL-C level in rams serum. On the other hand , the environmental temperature seems to has a highly negative significant($p \leq 0.01$) correlation with serum levels of cholesterol, Triglyceride ,HDL-c,LDL-c and VLDL-c So we concluded the positive relationship between the ghrelin hormone and parameters related to lipid profiles and nagativelly relationship between environmental temperature and lipid profile under the effect of seasonality in the Turkish awassi rams.

Key word: Ghrelin, cholesterol, seasonality, Turkish awassi rams

(دور التغيرات الموسميه في مستوى هرمون الكرلين ومعايير الدهون في الكباش العواسية التركية في العراق).

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الخلاصه

استهدفت هذه الدراسة معرفة دور التغيرات الموسميه على بعض الجوانب الفسلجيه لهرمون الكريلين المتعلقه بايض الدهون في الاغنام العواسي التركيه.ثم استخدم عشرة كباش بالغه بعمر (1.2-1.3 سنه) وبوزن يتراوح مابين(45-58 كغم

) بدات التجربه من بدايه شهر كانون الثاني 2016 واستمرت لغايه نهايه شهر تشرين الاول 2016 .اجريت عمليه سحب الدم كل عشرة ايام وعلى طول فترة التجربه في مرحلتي التصويم و الاكل . عزل المصل لاجراء الفحوصات الهرمونيه والتي تشمل (قياس مستويات كل من الكرلين و قياس تركيز الكولسترول والكليسيريدات الثلاثيه وتراكيز البروتينات المرتبطه بالكولسترول بانواعها الثلاثه(HDL-C,LDL-C,VLDL-C). تم قياس اوزان الكباش العواسي كل عشرة ايام وعلى طول فترة التجربه ولكل كبش على حدا . خضعت النتائج للتحليل الاحصائي المسمى (TWO WAY ANOVA) فحص دنكن وباستخدام متوسط القراءات ± الخطا القياسي. اظهرت النتائج تفوقا معنويا في كل من مستوى هرمون الكرلين في مصل الدم للكباش العواسيه خلال فترة الصيام مقارنه بفترة الغذاء. اضافه لما تقدم, لوحظ زياده معنويه في تراكيز كل من الكولسترول والكليسيريدات الثلاثيه , HDL-C,VLDL-C في مصل الكباش ضمن فترة التصويم مقارنه ب فترة التغذيه, اما عن تركيز LDL-C فقد اوضحت النتائج وجود زياده معنويه خلال مرحله التغذيه مقارنه بمرحله التصويم. لوحظ ان اعلى معدلات HDL-Cو LDL-C, VLDL-C كانت خلال فصل الشتاء والربيع مقارنه ببقيه الفصول خلال السنه.

ان مقياس الترابط مابين المعايير المدروسه في هذة الدراسه قد اوضحت وجود ترابط معنوي ايجابي مابين هرمون الكرلين وتراكيز مستويات كل من الكولسترول و HDL-C, VLDL-C المقاسه في مصل الكباش العواسيه . من جانب اخر لوحظ علاقه عكسيه معنويه ($p\leq0.01$) مابين درجات الحرارة و وزن الجسم من جانب ومعايير الدهون ن جانب اخر نستنتج من النتائج الظاهرة لهذه الدراسه العلاقه الايجابيه التي تربط مابين هرمون الكرلين وتراكيز كل من الكولسترول والكليسيريدات الثلاثيه والبروتينات العاليه الكثافه والواطئه جدا المرتبطه بالكولسترول وعلاقه سلبيه تربط درجات الحرارة واوزان الجسم والمعايير المذكورة انفا في مصل دم الكباش العواسي تحت تاثير عامل الموسميه في سلاله الكباش العواسيه التركيه المستحدمه في هذه الدراسه.

الكلمات المفتاحيه: هرمون الكرلين. الكولسترول. الموسميه. الكباش العواسيه التركيه

Introduction.

Ghrelin is octanoylated peptide containing a 28 amino acid which is chiefly produced by the stomach, is the natural ligand of the type 1a growth hormone secretagogue receptor (GHS-R1a) as reported by (1).The gastric ghrelin hormone originates from 117 amino acid,and its precursor preproghrelin encoded by the gene GHRL found on chromosome 3 (3p25-26) as mentioned by (2). It is well known that ghrelin has been found in several Peripheral tissues, such as the gastrointestinal tract, adrenal gland, thyroid, breast, ovary, placenta, fallopian tube, testis, prostate, liver, gallbladder, fat tissue, human lymphocytes, spleen, kidney, lung, skeletal muscle, myocardium, vein, and skin (3). The brain, containing the neurones which producing ghrelin specially in the pituitary, hypothalamic arcuate nucleus, and in a group of neurones adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei (4). Ghrelin is a participant in regulating the complex process at which both energy has to be adjusted. Energy input by adjusting hunger signals and energy output by adjusting the proportion of energy going to ATP production, glycogen storage, fat storage and short term heat loss. The net result of these processes is reflected in body weight under continuous monitoring and adjustment based on metabolic signals and needs. Ghrelin and synthetic ghrelin mimetics (growth hormone secretoguge) led to increase appetite and body weight and fat mass by triggering receptors in the arcuate nucleus that include the orexigenic (NPY) and (AgRP) neuron (5,6,7). The chronic administration of ghrelin either systemically or in the intra cerebro-ventricular is produced hyperphagia, weight gain, and increased adiposity .Meanwhile, the central administration of ghrelin increases adipose deposition without relating to food intake in pair-fed animals (8). Environmental change is seen as a remarkable danger to survival of numerous species, and the economic sustainability of pastoral system in various parts of the world, especially in developing countries (9). The ambient temperature is the main factor affecting animal's production, also there are other effectors like humidity, solar, radiation and wind. The temperature was increased over the last century are 0.74 C° and 0.80 C° (10). It was expected that the temperature will be rise up

to 3 C° more in this century. Thus, livestock can be under heat stress during certain periods of the year (11). It was informed that the level of ambient temperature affects the production traits in ruminants such as growth rate, milk production, milk composition and reproduction in both male and female. On the other hand, the hormonal fluctuations that occur in response to heat stress can also play a vital role in reduced production (12). Increasing temperature results in reduce activity of thyroid gland and the production of the gonadotropins that lead to inadequate estrogen and progesterone production. Thus, a reduction in thyroid activity decreases feed consumption and energy supply which inversely affects production potential and product quality of livestock . Recently (14) had found an interaction among ghrelin, photoperiod and metabolic status that influenced growth hormone (GH) and prolactin hormone (PRL) in ewes. Therefore, from the available literature and till this moment we couldn't find any study about ghrelin hormone in Iraq concerning ruminants neither nor its relation with seasonal variation and their effect on production. Moreover, knowledge of overlap between the temperature and the concentration of Ghrelin hormone with other related hormones in different the environmental conditions in Iraq on this type of Awassi sheep which is the most common breed of sheep in the Middle East countries and the only native breed in Jordan.

3- Materials and Methods :.

This study was conducted in Ruminant Researches Station at Abu–Ghraib/ Department of Animal Resource Researches/ Office of Agricultural Researches/ Ministry of Agriculture. It was carried out from 7th January 2016 up to 31st October 2016, to estimate of the total serum ghrelin level, explore the relationship between ghrelin hormone and some parameters related to lipid metabolism .Ten Turkish Awassi rams were of 1.2-1.3 years age and 45-58 Kg body weight were used in this study . the environmental temperature was recorded two times daily by special thermometer. Rams was weighted individualy every each ten days ,and were starved every 10 days' intervals throughout the study for 24 hours from the concentrate diet and roughage,(while the drinking water was available for the animals constantly). Blood samples were taken from all animals from the jugular vein before and one hour after feeding . Then serum was isolated by centrifugation at (3000 rpm) for 20 minutes. Sera were obtained to estimate the ghrelin hormone , cholesterol, triacylglycerol, Low Density Lipoprotein-Cholesterol (LDL-C), High Density Lipoprotein-Cholesterol (HDL-C), Very Low Density Lipoprotein-Cholesterol,(VLDL-C) . All data were subjected to two way analysis of variance test and presented as mean ±SE Duncans test was used to expresss the correlation between variables.

1- **Deteremination of Ghrelin hormone assay(pg/ml).**

The coated well immunoenzymatic assay for the quantitative measurement of GHRL utilizes a multiclonal anti-GHRL antibodyand an GHRL-HRP conjugate. The assay sample and buffer are incubated together with GHRL-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the GHRL concentration since GHRL from samples and GHRL-HRP conjugate compete for the anti-GHRL antibody binding site. Since the number of sites is limited, as more sites are

occupied by GHRL from the sample, fewer sites are left to bind GHRL-HRP conjugate. Standards of known GHRL concentrations are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density) to the concentration of GHRL. The GHRL concentration in each sample is interpolated from this standard curve.

2 -Dertermination of Serum cholesterol , triglyceride, HDL-C and LDL-C concentration(mg/dl) :

The determination was made by using Kit test provided from Biolabo SA, Company France (14).

3- Dertermination of Serum VLDL-Cholesterol concentration(mg/dl) .

The assessed of serum VLDL-C concentration (mg/dl) was made by dividing serum triglyceride concentration on 5 (15).

VLDL-C conc. =triglyceride Conc */5*

. **Results :**

1 : Serum Ghrelin concentration (pg/ml):

The effect of season on serum ghrelin concentration in Turkish awassi rams during fasting and feeding status is illustrated in table(4-1).Although ,ghrelin concentration shows at non-significant($p \geq 0.05$) increase during fasting compared with feeding state during all seasons, this level is highly significant ($p \leq 0.01$)during Spring months at fasting state ,in comparison with feeding state.

Table (1): Effect of seasonality changes and nutritional status on serum Ghrelin concentration(pg/ml) in Turkish awassi rams

Season	Nutritional Status		Level of sig.
	Fasting	Feeding	
Winter	3.06 ± 0.57 A a	2.14 ± 0.28 A a	NS
Spring	2.81 ± 0.23 A a	2.12 ± 0.07 B a	**
Summer	2.32 ± 0.17 A a	2.03 ± 0.06 A a	NS
Autumn	2.25 ± 0.08 A a	2.02 ± 0.11 A a	NS
Level of sig.	NS	NS	----

Values represent mean±SE, n=10

* ($P \leq 0.05$), ** ($P \leq 0.01$)

NS=Non- significant.

Different capital letters denote significance difference between stats of feeding

Different small letter denote significance difference between seasons of year

2- Serum Cholesterol concentration(mg/dl)

The effect of season and nutritional status on serum cholesterol concentration are demonstrated in table (2). Rams serum showed a highly significant($p \leq 0.01$) increase concentration in serum

cholesterol at fasting state compared with feeding state during Winter, Spring and Summer seasons .On the other hand , this concentration has the highly significant ($p \leq 0.01$) decrease during Autumn and Summer months in comparison to other seasons at both fasting and feeding states.

Table (2): Effect of seasonality changes and nutritional status on serum cholesterol concentration(mg/dl) in Turkish awassi rams.

Season	nutritional status		Level of sig.
	Fasting	Feeding	
Winter	48.40 ± 1.20 A a	37.00 ± 1.55 B a	**
Spring	46.33 ± 0.95 A a	38.53 ± 1.52 B a	**
Summer	43.26 ± 1.25 A a	27.40 ± 1.03 B b	**
Autumn	30.90 ± 1.35 A b	31.50 ± 1.99 A b	NS
Level of sig.	**	**	----

Values represent mean±SE, n=10
* ($P \leq 0.05$), ** ($P \leq 0.01$)
NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

3-Serum Triglyceride concentration(mg/dl)

Table (3) illustrates the mean values of triglycerides concentration in serum of turkish awassi rams during different seasons at fasting and feeding states. This table reveals a highly significant ($p \leq 0.01$) increase in triglyceride concentration in rams serum at the fasting state in comparison to feeding state during all seasons of the year .At the same time,this concentration is significantly($p \leq 0.01$) lower in serum of Turkish awassi rams at the feeding state during Summer and Autumn as compared with Spring and Winter.

Table (3): Effect of seasonality changes and nutritional status on serum triglyceride concentration(mg/dl) in Turkish awassi rams

Season	nutritional status		Level of sig.
	Fasting	Feeding	
Winter	32.90 ± 1.21 A a	27.00 ± 0.93 B a	**
Spring	31.40 ± 1.20 A a	26.26 ± 1.53 B a	**
Summer	29.13 ± 1.31 A a	20.53 ± 1.55 B b	**
Autumn	31.40 ± 1.43 A a	21.20 ± 1.31 B b	**
Level of sig.	NS	**	----

Values represent mean±SE, n=10
** (P≤0.01)
NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

4-Serum High Density Lipoprotein-Cholesterol HDL-c concentration (mg/dl):

The concentration of HDL-c according to different seasons at fasting and feeding states is represented in table(4-). There is a highly significant (p≤0.01) increase in HDL-c concentration in rams serum at the fasting state as compared with the feeding state during the four seasons of the year. On comparing this level during different seasons,this table reveals highly significant(p≤0.01) increase in HDL-c concentration during Winter and Spring months at both fasting and feeding states in comparison to its level during Autumn and Summer months.

Table(4) : Effect of seasonality changes and Nutritional status on serum high density lipoprotein-Cholesterol (HDL-c) concentration(mg/dl) in Turkish awassi rams.

Season	Nutritional status		Level of sig.
	Fasting	Feeding	
Winter	92.50 ± 8.11 A a	62.60 ± 5.06 B a	**
Spring	80.06 ± 4.18 A a	63.20 ± 3.37 B a	**
Summer	51.93 ± 4.58 A b	39.86 ± 3.49 B b	*
Autumn	35.60 ± 2.48 A c	23.70 ± 1.51 B c	**
Level of sig.	**	**	----

Values represent mean±SE, n=10
** (P≤0.01)
NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

5- Serum Low Density Lipoprotein-Cholesterol (LDL-c) concentration(mg/dl).

The serum of rams at the feeding state shows a significant (p≤0.01) increase in LDL-C concentration during Winter and Spring and Summer seasons as compared with those levels at the fasting state. On the other hand ,LDL-c concentration is significantly (p≤0.01) increase in rams serum during spring in comparison to other seasons of the year at both fasting and feeding states. Table(5).

Table 5-: Effect of seasonality changes and nutritional status on serum Low density lipoprotein-Cholesterol (LDL-c) concentration(mg/dl) in Turkish awassi rams.

Season	Nutritional status		Level of sig.
	Fasting	Feeding	
Winter	34.40 ± 2.14 B b	46.55 ± 4.54 A b	*
Spring	46.33 ± 5.39 B a	59.40 ± 3.80 A a	**

Summer	28.26 ± 2.26 B b	42.47 ± 3.03 A b	**
Autumn	25.10 ± 2.14 A b	29.60 ± 2.70 A c	NS
Level of sig.	**	**	----

Values represent mean±SE, n=10
* (P≤0.05), ** (P≤0.01)
NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

6-:Serum Very Low Density lipoprotein-Cholesterol(VLDL-c)concentration(mg/dl):

Table (6) demonstrate the effect of season and state of feeding on serum VLDL-c concentration .the results showed a highly significant (p≤0.01) increase in serum VLDL-c concentration at the fasting state as compared to the feeding state during the fall seasons of the year. At the meantime ,the values of serum VLDL-c concentration shows a highly significant (p≤0.01) increase during Winter and Spring as compared to Summer and Autumn at both fasting and feeding states.

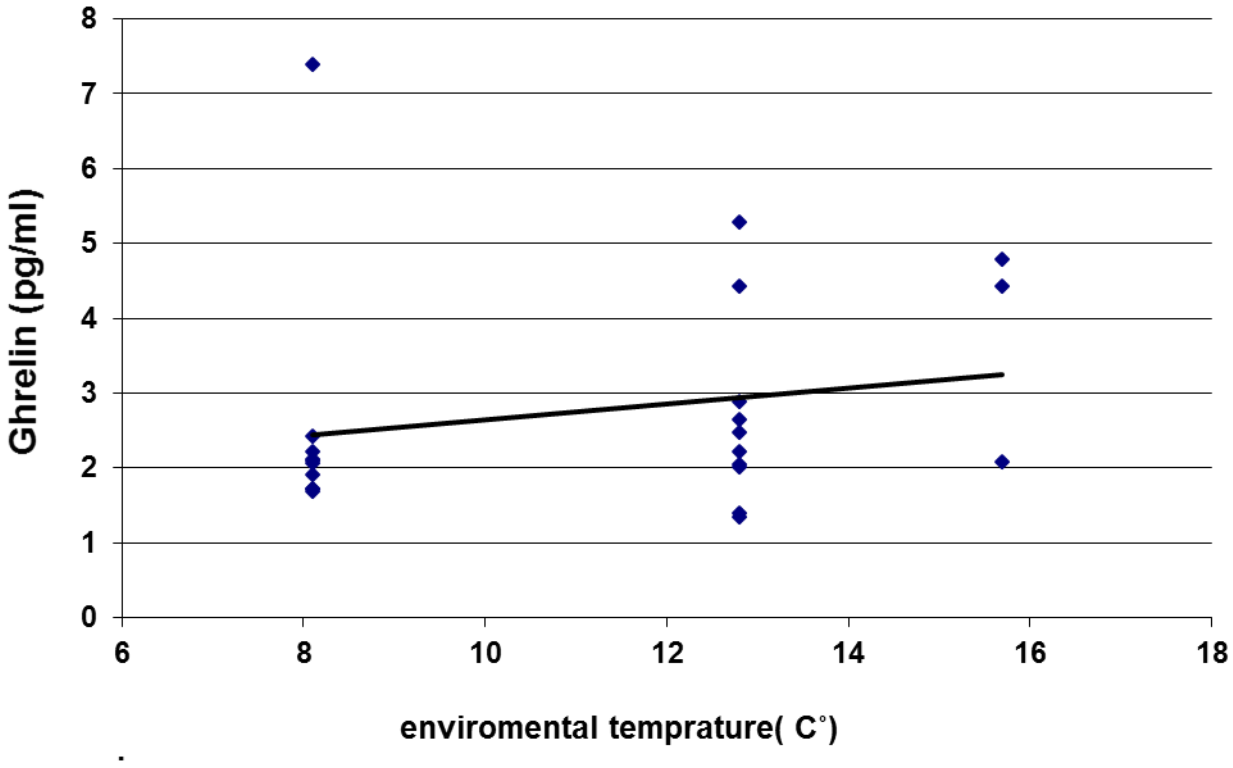
Table (6): Effect of seasonality changes and nutritional staus on serum Very Low density lipoprotein-Cholesterol(VLDL-c) concentration(mg/dl) in Turkish awassi rams.

Season	Nutritional status		Level of sig.
	Fasting	Feeding	
Winter	6.58 ± 0.19 A a	5.40 ± 0.19 B a	**
Spring	6.65 ± 0.17 A a	5.47 ± 0.32 B a	**
Summer	6.02 ± 0.19 A ab	4.31 ± 0.26 B b	**
Autumn	5.60 ± 0.23 A b	4.04 ± 0.21 B b	**
Level of sig.	**	**	----

Values represent mean±SE, n=10
** (P≤0.01)
NS=Non- significant.
Different capital letters denote significance difference between stats of feeding
Different small letter denote significance difference between seasons of year.

7: Correlation coefficient between Ghrelin hormone and environmental temperature .

The relationship between the ghrelin hormone and the environmental temperature was revealed in figure(1).The results showed that ,a negative significant (p≤-0.05) the increasing of the environmental temperature was associated with decreasing in ghrelin hormone concentration in this study.



Figure(**1**):relationship between ghrelin and environmental temperature.

8: Correlation coefficient between environmental temperature and body weight.

The correlation between the environmental temperature and body weight was clarified in figure(2). There is a positive significant ($p \leq 0.01$) increasing in ram's body weights which was associated with elevation in environmental temperature.

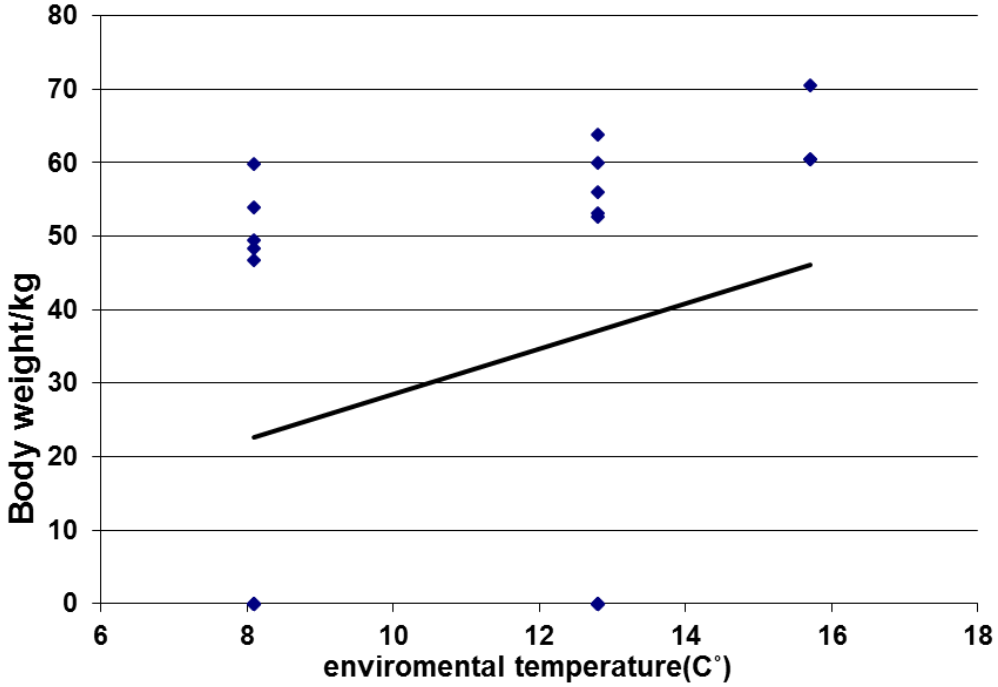


Figure 2. Relationship between Body weight & Temperature.

Discussion

The results of this study demonsetrated that, there is a significant increase in (bad) cholesterol concentration during feeding state with a significant increasing in each of (total cholesterol ,triglyceride, HDL-C ,and VLDL-C) in the fasting state as compared to feeding state. On comparing , serum level of these nutrients during different seasons , Winter and Spring months represent the higher concentrations Moreover, serum ghrelin concentration in the studied animals showed a positive correlation with the above mentioned nutrients i.e. increased during fasting state . Serum ghrelin increases steadily during long term of fasting in rats(15) and returns to normal after re-feeding which may be due to the effect of ghrelin hormone on lipid metabolism. The peripheral administration of ghrelin has been implicated on the regulation of lipid metabolism, with effects on liver, skeletal muscle and adipose tissue In the liver, ghrelin increases lipogenic genes expression and triglyceride content (16). Furthermore, in the gastrocnemius muscle, ghrelin increases mitochondrial oxidative enzyme activities and reduces triglyceride content. Ghrelin selectively increases peroxisome proliferator activated receptor to reduce muscle fat content in skeletal muscle. It was shown also that , ghrelin acts on stimulation of the lipogenesis processs in adipocyte by the insulin-induced glucose uptake It antagonizes lipolysis induced by isoproterenol and stimulates the proliferation and differentiation of pre adipocytes (17). It was demonstrated that ghrelin increases white adipose tissue volume by either stimulating adipogenesis or inhibiting lipolysis and lipid efflux from adipocytes. Acyl-ghrelin-induced lipid accumulation is not limited to white adipose tissues. In mouse liver, acyl-ghrelin significantly elevated the number of lipid droplets, total lipid area and triacylglycerol content (18) as well as lipogenesis related genes. In contrast, ghrelin demonstrated no effect on hepatic lipogenesis in GHSR1a null mice (19). Climate change is the most serious long-term challenge faced by small ruminants' owners worldwide. Heat stress results in decreased growth, reproduction, production, milk quantity and quality, as well as natural immunity, making animals more vulnerable to diseases, and even death(20).Heat stress(HS), is the most concerning issue nowadays in the ever-changing climatic (21).high and low environmental temperature, stress, or administration of insulin reciprocally affect plasma levels of ghrelin and leptin(21). The prolactin and ghrelin effects are season dependent and influenced by the nutritional status of the ewes.our current study confrming that ghrelin concentration in serum is negatively correlated with environmental temperature i.e higher level of ghrelin during Winter and Spring more than Summer and Autumn. This could be explained in relation to the great variation of Iraqi climate between very high temperature (Summer) and very low temperature (Winter). The influence of ghrelin is only known peripherally produced and centrally which act as peptide hormone that ,stimulating feed intake.

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