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Effects of Food Restriction on Oxidative Stress Indices in Ghezel Ewes

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Abstract: We conducted this study to determine the effects of low energy diets (i.e., food restriction) on the oxidative stress indices of ewes in late pregnancy. About 21 clinically intact Ghezel ewes with an average weight of 50±3 kg used for the study. They subjected for oesterus synchronization by using CIDR. Data were analyzed using split-plot design in time. The major factor of T in two levels (T_1: food restriction; T_2: control) and the 2nd factor P in four levels (P_0: control or 4th week of pregnancy; P_1: overnight feed deprivation treatment in 5th week; P_2: 1st food restriction treatment in 14th week; P_3: 2nd food restriction treatment in 19th week (8th week to probable date of gestation)). Serum concentration of glucose, BHB: β-hydroxybutyrate; NEFA: Non Esterified Fatty Acids; MDA: Malondialdehyde and activities of SOD: Superoxide Dismutase and GSH-Px: Glutathione Peroxidase in red blood cells and TAS value measured in those periods. The findings showed that there is a statistically significant difference between BHB and NEFA ($p<0.05$) and TAS: Total Antioxidant Status of different treatments ($p<0.01$). In addition, NEFA, BHB, TAS, MDA, SOD and GSH-Px had statistically significant difference in periods ($p<0.05$). Only BHB and TAS were statistically significant difference in T*P, respectively ($p<0.01$ and $p<0.05$). We concluded that in comparison with food restriction, pregnancy period was the major agent for oxidative stress in late pregnancy of Ghezel ewes. We also concluded that food restriction had not a significant effect on oxidative stress indices. However, period of gestation especially in late pregnancy imposed lots of stress on anti-oxidative system of ewes.

Key words: Food restriction, ghezel ewe, pregnancy, oxidative stress, glucose, serum concentration

INTRODUCTION

Underfeeding is a frequent situation for small ruminants in different countries. In temperate areas, it is mostly of moderate extent and sometimes due to an economic consideration when food is scarce and/or expensive (concentrate supply, preserved forages) animals underfed (Doreau *et al*., 2003). Forage is the major and cheaper ingredient of sheep diet. So, the usual method of feeding in sheep rearing systems in Iran especially when concentration ingredients are expensive is naturally grazing on pasture. Practically, it is impossible to meet metabolizable energy requirements of 3.6 Mcal day^{-1} DMI for maintenance and pregnancy in a given sheep herd. National Research Centre recommends (NRC, 1985) energy requirements of pregnant ewes should be increase by 20%. Though, pasture grazing result in a food restriction state and negative energy and nitrogen balance especially in late pregnancy period. Sheep have a seasonally oesterus cycle and winter is the lambing season in the province. Naturally, the quality of food decreases in winter. Then a sheep with a negative energy and protein balance should graze on a poor pasture (or eat low quality hay) and tolerate different levels of food restriction. Insufficient energy intake thought to give rise to a fallin the mitochondrial proton leak which positively related to the Reactive Oxygen Species (ROS) (Brookes, 1998; Papa and Skulachev, 1997; Tanaka and Ando, 1990). In animals, leaky mitochondrial electron transport is the major source of oxidative stress with perhaps 2-5% of the electron flow generating superoxide and ultimately hydrogen peroxide (Grisham, 1992). On the other hand, long-term energy deficiency causes mobilization in the bodies fat deposits and an increase in ketone bodies, especially acetoacetate and β-Hydroxybutyrate (BHB), relative to energy metabolism (Ives *et al*., 2000; Sahlu *et al*., 1999).

There is a close correlation between energy expenditure and the available oxygen. The decrease in cellular oxygen consumption causes a dramatic loss in the amount of oxygen molecules produced in the mitochondria and a decrease in the amount of ROS (Jamme *et al*., 1995; Ramsey *et al*., 2000). Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of free radicals (Miller *et al*., 1993; Castillo *et al*., 2001) which are

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produced continuously during metabolism and may increase as a result of pathological and other circumstances (Roth, 1997). However when free radical generation exceeds the body's antioxidant production capacity, oxidative stress develops (Castillo *et al*., 2005). In fact, oxidative stress is commonly defined as an imbalance between oxidants and reductants (antioxidants) at the cellular or individual level (Lykkesfeldt and Svendsen, 2007). In the last few years, the detection of free radical damage and the body's defenses against it have become increasingly important in clinical medicine as a complementary tool in the evaluation of metabolic status (Castillo *et al*., 2005).

Ruminant embryos do not need high levels of oxidative metabolism and a large amount of oxygen in the early phases of pregnancy. Later, however, especially in the final months, they do have high levels of both oxidative and glycogenic metabolism (Boland *et al*., 2001; Frettly *et al*., 1995). When the pregnant animal is subjected to some oxidative stress, antioxidant activities increase in the placenta. This increase indicates that the effects of free oxygen radicals are limited in the embryonic tissue and this is known as the placental block (Georgeson *et al*., 2002).

Oztabak *et al*. (2005) showed that the activity of some oxidative indices increase in ewe late pregnancy. In this study, we induced higher level of food restriction by a pattern close to what happening commonly in sheep rearing systems of the country.

The question is does food restriction in pregnant Ghezel ewes (especially in the last 8 weeks of pregnancy) makes a large amount of challenge for antioxidant system and this leads in oxidative stress. Oxidative stress in farm animal diseases has primarily been studied in pigs, cattle (only in two disease: pneumonia and mammilitis) and horses (Lykkesfeldt and Svendsen, 2007) but there are limited study in sheep.

MATERIALS AND METHODS

Study date and experimental animals: The study was carried out in a dry and cold climate of Tabriz (East Azerbaijan province of Iran). Winter is the reproduction season in this region (around January). The oesterus synchronization was in March that was out of reproduction season. Ewes had not an access to pasture and we used manual nutrition system. The study was carried out using 21 healthy multiparous non-pregnant. About 21 Iranian Ghezel ewes with an average weight of 50±3 kg were used for the study. Supplementary drugs such as AD_3E (A, D_3 and E vitamins) and CMP (Calcium-magnesium-phosphor) injected for 3 days for all of animals. Albendazole was used as a wide spectrum anti parasite (10 mg kg^{-1} BW). Prior to flushing period all ewes were fed a ration of 1.9 Mcal ME kg^{-1} of Dry Matter Intake (DMI) and 8.8% Crude Protein (CP) (60% hay+40% straw) and the overall DMI was 1.4 kg day^{-1} per head in the morning (08:00) and evening (16:00) (NRC, 1985). All ewes provided drinking water *ad libitum*. An adaptation period for this phase was 2 weeks.

Table 1: The content of diets fed ewes in different periods of study

Diet contents	Flushing (%)	up to 13th week (%)	T_2 (%)	T_1(%) (P_2)	T_1(%) (P_3)
Wheat straw	42.8	40	42.8	66.6	-
Straw	22.2	60	22.2	23.4	100
Corn grain	14.3	-	14.3	-	-
Barley grain	14.4	-	14.4	-	-
Wheat bran	3.5	-	3.5	-	-
Soybean meal	1.8	-	1.8	-	-
Vitamin-mineral premix [1]	0.4	-	0.4	-	-
Lime stone	0.2	-	0.2	-	-
DCP [2]	0.2	-	0.2	-	-
With salt	0.2	-	0.2	-	-
Dry matter (kg)	1.5	1.2	1.5	1.5	1.5

[1]Vitamin-mineral premix provides (per kg of concentrate): 11,000 mg Cu, 10 mg co, 100 mg I, 350 mg Fe, 800 mg Mn, 600 mg Zn, 8 mg Se and 2,000,000 IU vitamin A, 200,000 IU vitamin D3, 2,200 mg vitamin E and 15,000 mg antioxidant. [2]Die calcium phosphate

Flushing and oesterus synchronization: In order to boost ovulation, conception and embryo implantation rates, the ewes flushed for 5 weeks. They fed a ration of 2.4 Mcal ME kg^{-1} of Dry Matter (DM) and 11% Crude Protein (CP) (1.5 kg day^{-1} per head) in 3 weeks before and 2 weeks after mating (NRC, 1985). The ration was contained of 35% of concentrate and 65% of forage (Table 1).

Total 1 week after a flushing diet an EAZI-BREED CIDR (containing 0.3 g progesterone) placed in vagina. According to recommendation of the commercial company in 14th day, CIDRs expelled and 500 IU PMSG (Intervet, Poland) injected intra-muscularly. About 2 rams introduced to herd for natural conception. After a 4 weeks period, 14% ewes of close body condition score chosen and were used up to the end of study.

Experimental diets: After flushing period, all the ewes fed a ration of 2.12 Mcal ME kg^{-1} of Dry Matter (DM) and 8.9% Crude Protein (CP) (1400 g day^{-1} per head) in the first 13 weeks of gestation. On day 95 after mating, the ewes examined by ultrasound for pregnancy and 14 pregnant were selected. At the beginning of 14th week of pregnancy the elected ewes were randomly assigned into T_1 (4 ewes) and T_2 groups (10 ewes). The T_1 group received a normal diet until their parturition but the T_2 group fed a restricted ration in 2 phases of P_1 (1st food restriction period) and P_2 (2nd food restriction

period). About 10 randomly selected ewes assigned to each group then placed in individual pens and held under the same environmental conditions. All animals fed twice a day (6:00 a.m. and 03:00 p.m.) shown in Table 1. Drinking water and salt and vitamin-mineral lick provided *ad libitum*. All the rations was formulated according to NRC (1985).The feeds given to each group were analyzed for CP and DM (Table 2) Chemical analyses of the ration performed as AOAC (1990) method.

Sample collection: Animals were sampled in 4 periods (P_0-P_3) as schematic Fig. 1: P_0-control (4th week of pregnancy); P_1-overnight feed deprivation treatment (in 5th week); P_2-1st food restriction treatment (in 14th week); P_3-2nd food restriction treatment (in 19th week). Blood samples obtained by jugular venipuncture in tube without anticoagulant. Tubes for serum collection allowed clotting at room temperature for 2 h. Then centrifuged at 2000 g for 20 min. The supernatant serum was frozen at -70°C until analysis.

Analytical procedures: All biochemical parameters measured on UV/VIS spectrophotometer (Alycon 300). Serum BHB and NEFA were measured using standardized kits supplied by Randox Laboratories Ltd. Serum glucose was measured using commercial kit (Pars Azmoon, Karaj, Iran).

TAS measurement: TAS was estimated using Randox Total Anti Oxidant Status test in serum (Randox Laboratories Ltd., United Kingdom, Cat. No. NX 2332, Lot. No.: 023195). ABTS®(2,2′-Azino-di-[3-ethylbenzthiazoline ulphate]) was incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS®. This has a relatively stable blue-green colour which is measured at 600 nm. Antioxidants in added sample cause suppression of this colour production to a degree which is proportional to their concentration.

Table 2: Chemical analyses of diets in different periods of study

Analyes of diet	Flushing	Control	Up to 13th week pregnancy	1st food restriction	2nd food restriction
DM (%)	89.10	88.70	89000	88.20	91.10
Mcal ME kg^{-1}	2.40	2.40	1.800	2.00	1.74
CP (%)	11.60	11.20	8.200	11.00	4.80
Ca (%)	0.77	0.83	0.744	0.99	0.18
P (%)	0.30	0.27	0.110	0.18	0.05

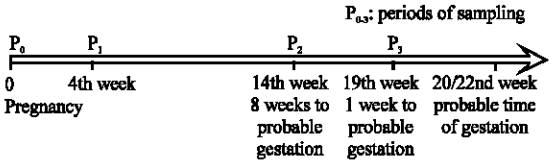

Fig. 1: Schematic view of sampling periods

SOD measurement: SOD activity in erythrocytes was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2- (4-iodophenyl)-3- (4-nitrojenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction (Ransod, Randox Laboratories Ltd. United Kingdom, Cat. No. SD 125, Lot. No. 0019J). Results obtained SOD U mL and then converted to SOD Units g^{-1} Haemoglobin.

GSH-Px measurement: GSH-Px activity in red blood cells was measured using the method described by Paglia and Valentine (1967). GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately is converted to reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured (Ransel, Randox Laboratories Ltd., United Gingdom, Cat. No: RS 504, Lot. No.:1764J). Results obtained GSH-PX U/L and then converted to GSH-Px Units g^{-1} Haemoglobin.

MDA measurement: Oxidized lipids are indirectly determined as aldehydes such as MDA using either simple colorimetric methods including Thiobarbituric acid Reactivity (TBARS assay) or more selective HPLC methods. MDA and related methods are indirect as the aldehyde is a degradation product of the oxidized lipid (Close and Hagerman, 2006) (Fig. 2). We used MDA method. MDA reacts with thiobarbituric acid then extracts with butanol. The optic density of the aqueous extract is measured spectrophotometrically at wavelengths 532 nm and compare with standard curve (Bilici *et al*., 2001; Yagi, 1994).

Statistical analysis: We used split-plot design in time. The major factor (T) in 2 levels (T_2: control and T_1: food restriction treatment) and the second factor (P) in 4 levels (P_0: control or 4th week of pregnancy; P_1: overnight feed deprivation treatment in 5th week; P_2: 1st food restriction treatment in 14th week; P_3: 2nd food restriction treatment

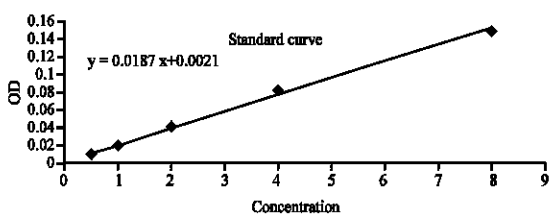

Fig. 2: Standard curve of MDA measurement

in 19th week (8th week to probable date of gestation). Data were analysed by Analysis of Variance (ANOVA) using the General linear model procedure of the Statistical Analysis System (SAS Institute Inc.). Duncan's multiple-range test used to separate and compare the means. All results expressed as the mean±SD. A value $p<0.05$ considered statistically significant. Spearman correlation tests used to evaluate the relationship among serum parameters.

RESULTS AND DISCUSSION

Analyzed data of average oxidative stress indices and serum concentration of glucose, BHB and NEFA shown in Table 3-6. Data are reported as mean±SD. Analysis of variance of hierarchical factorial design for traits (Table 3) showed that there is a statistically significant difference between BHB and NEFA ($p<0.05$) and TAS of different treatments ($p<0.01$). In addition, NEFA, BHB, TAS, MDA, SOD and GSH-Px had statistically significant difference in periods ($p<0.05$). Only BHB and TAS were statistically significant difference in T*P, respectively ($p<0.01$ and $p<0.05$). In this study, we decreased the total energy of animal's diet 16.5 and 27.5% from recommended level (NRC, 1985) in the last 8 and 3 weeks of pregnancy. However, only one ewe aborted and ceased her pregnancy in the last week of gestation. This can be mean as a relative resistance of Ghezel sheep to food restriction or could be a true result of flushing at the beginning of pregnancy. Robinson *et al.* (2002) stated that if nutrition before mating was good and there was a sufficient fat reserve, resistant of ewes to food restriction is more than those ewes with insufficient fat reserves.

Undoubtedly, the most sensitive period for nutritional modification of placental growth with an associated effect on lamb birth weight is between 50 and 90 days of gestation, the period of rapid proliferating growth of the placenta (Robinson *et al.*, 2002). As we exerted a food restriction from 14th week of gestation so may be this is the reason why restricted diet could not affect conceptus growth and did not cease the gestation. The overall mean concentration of serum glucose in

Table 3: Analysis of variance of hierarchical factorial design for measured traits

		Mean[2]						
Source of variation	df	Glucose (mg dL^{-1})	BHB (mmol L^{-1})	NEFA (mmol L^{-1})	TAS (mmol L^{-1})	MDA (nmol mL^{-1})	SOD (U g^{-1} Hb)	GSH-Px (U g^{-1} Hb)
Treatment T)	1	150.42^{NS}	0.4289^{*}	0.4889^{*}	0.1954^{**}	0.3604^{NS}	96705.29^{NS}	2582.98^{NS}
Error I (R/T)	24	43.21	0.0578	0.0363	0.0106	0.2020	1169271.25	4792.99
Period (P)	3	58.66^{NS}	0.0704^{*}	0.2934^{*}	0.3790^{**}	3.6200^{**}	3149685.25^{**}	26765.20^{**}
P*T	3	34.84^{NS}	0.1250^{**}	0.0955^{NS}	0.0747^{*}	0.1148^{NS}	12007.65^{NS}	688.06^{ns}
Error II	26	53.51	0.0188	0.0572	0.0101	0.2046	291974.56	728.56
	C.V	16.70	32.4200	32.2100	8.8300	16.9800	16.73	11.07

BHB: β-hydroxybutyrate; NEFA: Non Esterified Fatty Acids; TAS: Total Antioxidant Status; MDA: Malondialdehyde. NS: Non Significant; *$p<0.05$; **$p<0.01$

Table 4: Comparison of mean values for traits of treatments by Duncan's method

Treatments	Glucose (mg dL^{-1})	BHB (mmol L^{-1})	NEFA (mmol L^{-1})	TAS (mmol L^{-1})	MDA (nmol mL^{-1})	SOD (U g^{-1} Hb)	GSH-Px (U g^{-1} Hb)
T_1	40.50 ± 6.67^{a}	0.56 ± 0.25^{a}	0.9 ± 0.54^{a}	1.046 ± 0.21^{b}	2.92 ± 0.59^{a}	3183.5 ± 807.2^{a}	257.92 ± 68.18^{a}
T_2	46.88 ± 5.42^{a}	0.30 ± 0.09^{b}	0.6 ± 0.25^{b}	1.223 ± 0.21^{a}	2.42 ± 0.82^{a}	3271.9 ± 727.5^{a}	230.34 ± 50.32^{a}

T_1: Restricted energy; T_2: Control. Different superscripts within lines indicate significant differences ($p<0.05$)

Table 5: Comparison of mean values for traits of periods by Duncan's method

Periods	Glucose (mg dL^{-1})	BHB (mmol L^{-1})	NEFA (mmol L^{-1})	TAS (mmol L^{-1})	MDA (nmol mL^{-1})	SOD (U g^{-1} Hb)	GSH-Px (U g^{-1} Hb)
P_0	51.75 ± 4.84^{a}	0.27 ± 0.11^{b}	0.51 ± 0.15^{b}	1.240 ± 0.03^{b}	1.63 ± 0.76^{c}	3176.1 ± 561.5^{a}	206.64 ± 41.98^{b}
P_1	44.22 ± 6.62^{a}	0.31 ± 0.07^{b}	0.54 ± 0.26^{b}	1.400 ± 0.25^{a}	2.40 ± 0.48^{b}	2414.7 ± 591.9^{b}	173.95 ± 40.75^{c}
P_2	42.61 ± 7.19^{a}	0.53 ± 0.29^{a}	0.82 ± 0.43^{a}	0.970 ± 0.04^{c}	2.49 ± 0.28^{b}	3519.5 ± 804.1^{a}	271.53 ± 37.90^{a}
P_3	41.00 ± 5.73^{a}	0.50 ± 0.20^{a}	0.97 ± 0.65^{a}	0.963 ± 0.06^{c}	3.56 ± 0.51^{a}	3776.8 ± 424.3^{a}	302.04 ± 31.99^{a}

P_{0-3} means different period of pregnancy.Different superscripts within lines indicate significant differences ($p<0.05$)

Table 6: Comparisons of an interaction mean values for traits by Duncan's method

T*P	Glucose (mg dL^{-1})	BHB (mmol L^{-1})	NEFA (mmol L^{-1})	TAS (mmol L^{-1})	MDA (nmol mL^{-1})	SOD (U g^{-1} Hb)	GSH-Px (U g^{-1} Hb)
T_1P_1	43.8 ± 7.00^{a}	0.30 ± 0.08^{b}	0.447 ± 0.25^{a}	1.23 ± 0.20^{b}	2.66 ± 0.41^{a}	2455.86 ± 706.31^{a}	184.99 ± 46.17^{a}
T_1P_2	41.1 ± 7.21^{a}	0.73 ± 0.32^{a}	0.918 ± 0.37^{a}	0.945 ± 0.04^{c}	2.57 ± 0.27^{a}	3284.23 ± 734.96^{a}	284.65 ± 37.27^{a}
T_1P_3	42.7 ± 6.17^{a}	0.64 ± 0.21^{a}	0.838 ± 0.77^{a}	0.962 ± 0.06^{c}	3.61 ± 0.39^{a}	3673.41 ± 448.76^{a}	319.88 ± 24.07^{a}
T_2P_0	49.0 ± 4.84^{a}	0.27 ± 0.11^{b}	0.229 ± 0.15^{a}	1.24 ± 0.03^{b}	2.09 ± 0.76^{a}	2886.70 ± 561.48^{a}	210.44 ± 41.98^{a}
T_2P_1	46.0 ± 6.16^{a}	0.32 ± 0.02^{b}	0.263 ± 0.28^{a}	1.613 ± 0.25^{a}	2.13 ± 0.48^{a}	2446.33 ± 147.05^{a}	174.26 ± 26.28^{a}
T_2P_2	47.8 ± 5.07^{a}	0.29 ± 0.04^{b}	0.292 ± 0.11^{a}	0.985 ± 0.04^{c}	2.44 ± 0.31^{a}	3576.93 ± 1048.59^{a}	255.86 ± 35.61^{a}
T_2P_3	42.0 ± 5.29^{a}	0.31 ± 0.07^{b}	0.782 ± 0.22^{a}	1.055 ± 0.03^{c}	3.50 ± 0.82^{a}	3888.45 ± 363.13^{a}	284.59 ± 38.89^{a}

T*P means treatments * periods. Different superscripts within lines indicate significant differences ($p<0.05$)

sheep is 50-80 mg dL^{-1} (Kaneko *et al*., 2008). Mean value of serum glucose in control group throughout this study was 46.88±5.42 mg dL^{-1} and a little lower than those mentioned range (Table 4).

The average serum concentration of glucose in all periods and treatments in comparison to the control group of the same period and treatment (Table 4-6) had not statistically significant difference at the level of 5 %. Then food restriction were tolerated by animals and could not decrease serum concentration of glucose. Ruminants appear to be an animal well adapted to a carbohydrate economy based on the endogenous synthesis of glucose from non-carbohy drate sources, i.e., gluconeogenesis (Kaneko *et al*., 2008).

In ruminants, little glucose is absorbed from the gut, so the overwhelming bulk of glucose is synthesized (Lindsay, 1959; Otchere *et al*., 1974). Most (approximately 90%) of this synthesis occurs in the liver with the remainder occurring in the kidney (Bergman, 1982). The chief substrates are propionate and amino acids, with the former being most important in animals on a high-grain diet. Other precursors are branched chain Volatile Fatty Acids (VFAs) and lactate absorbed from the rumen and glycerol released during lipolysis (Bergman, 1975). In the other hand, fetal glucose demands increase with increasing body size (Kaneko *et al*., 2008) and it can increase susceptibility to pregnancy toxemia. A fetus of sheep in late pregnancy utilizes about one-third to one-half of the daily glucose turnover of 100 g (Kaneko *et al*., 2008). However, the mean value of serum glucose did not decreased in the study.

Therefore, we can conclude that during food-restricted period of pregnancy, gluconeogenesis powerfully supplies lamb and maternal requirements to glucose. Ideal body condition score at the start of gestation and a period of exerted food restriction may be other causes of statistically non-significant change of serum concentration of glucose.

The finding is in agreement with Schlumbohm and Harmeyer (2004) findings and they believe that due to a metabolic adaptation, efficiency of hepatic gluconeogenesis from glucose precursors increases during pregnancy. Mean value of serum BHB in control group (T_2P_0) at the beginning of pregnancy was 0.27 mmol L^{-1} and had a statistically significant difference with T_1P_3 (0.64 mmol L^{-1}) and T_1P_2 (0.73 mmol L^{-1}) groups ($p<0.05$). Generally, researchers believe that BHB concentration is a golden marker for diagnosis of pregnancy toxemia and/or ketosis in ewes and cattle (Kaneko *et al*., 2008) but there are different ideas about cut-off point of BHB. Smith introduce levels >1 mmol L^{-1} as a cut-off point. According to this criteria, mean value of all treatments in the study were lower than this point and could not be classified as pregnancy toxemia (albeit serum BHB concentration were a few >1 mmol L^{-1} in one case in P_2 and one case in P_3). Kaneko *et al*. (2008) proposed 0.55±0.04 mmol L^{-1} as a reference value of serum BHB concentration.

Considering the latter criteria food restriction resulted in a mild to moderate Ketosis in T_1P_2 and T_1P_3 groups. These mentioned changes were statistically significant ($p<0.05$) in comparison to the corresponding control group. It means that food restriction clearly increased serum BHB and caused ketosis. This rise in serum BHB is a compensatory mechanism and a reflectionary response to carbohydrate deficiency and inhibition of Kreb's cycle (Reece, 2004). In fact following β-oxidation of ketones, some acetyl-CoA is combusted in the citric acid cycle. However, during fasting, gluconeogenesis is quite active in the liver and much of the mitochondrial oxaloacetate is used for that purpose and is unavailable for citrate formation with acetyl-CoA; consequently, large quantities of acetyl-CoA are shunted into ketogenesis (Kaneko *et al*., 2008) and hyperketonemia occurs. Acetone, acetoacetate and BHB are three main product of ketogenesis, the latter is more important in ruminants (Murray *et al*., 2003; Dhanotiya, 2004). A negative feedback of hyperketonemia on glucose production renders the pregnant or lactating ruminant into a vicious circle (Schlumbohm and Harmeyer, 2004). As Ramin *et al*. (2005) showed no correlation observed between BHBA and glucose concentrations. There was not statistically significant correlation between other parameters (MDA, TAS, SOD, GSH-Px) too.

Mean value of NEFA in P_2 and P_3 periods were lower than P_0 and P_1 that was statistically significant ($p<0.05$). The rise of NEFA concentration simultaneously with BHB increment was another reason for importance of lipolysis during food restriction.

Food restriction had not a statistically significant effect on activities of SOD and GSH-Px of red blood cells and serum level of MDA. However as Table 4 shows TAS levels were decreased (1.046±0.21 in T_1 groups vs. 1.223±0.21 in T_2 groups) this means that food restriction challenged antioxidants system to solve the problem. Caloric restriction in animals extends life span at least in part by minimizing age-related accumulation of oxidative damage. Table 5 clearly proves by the time that pregnancy develops all indices of oxidative stress have a statistically significant change ($p<0.05$).

Activity of SOD and GSH-Px in red blood cells and serum concentration of MDA (an index of lipid peroxidation) increased (simultaneously with BHB and NEFA) in P_3 that means an oxidative stress had occurred.

The increased rate of NEFA oxidation because of negative energy balance-generates a large amount of Reactive Oxygen Species (ROS) resulting in raised lipoperoxidative processes and changes in the prooxidative/ antioxidative status (Mudron *et al*., 1999). This supported by results of Bionaz *et al*. (2007) showed elevated plasma levels of NEFA and ROS around partus. Research in dry period of cows by Turk *et al*. (2008) showed that the higher level of lipoperoxidative products (i.e., the MDA concentration) suggests an exceeding degree of lipid peroxidation caused by ROS in late pregnancy. The results are match with these findings.

Changes of SOD, GSH-Px and MDA was statistically significant shown in Table 4 then the effects of food restriction on oxidative stress indices was statistically non significant and surprisingly food restriction did not amplified the effects of pregnancy period. Therefore, previous-mentioned significant changes were purely the effect of period of pregnancy on oxidative stress indices. Oztabak *et al*. (2005) demonstrated that catalase and GSH-Px activities increased on day 148 of pregnancy. They claimed this rise of oxidative stress markers might explained by production of hydrogen peroxide that has been enhanced by the mobilization of fatty acids from the body deposits during pregnancy.

Special pattern of TAS changes clearly matched with SOD, GSH-Px and MDA. An interaction (T*P) mean values for TAS were statistically significant ($p<0.05$). In Table 6, TAS had the lowest value in T_1P_3 and T_1P_2 ($p<0.05$).

It means that food restriction and pregnancy period lowered TAS and caused an oxidative stress. However, the control groups of these two (T_2P_3 and T_2P_2) had not a statistically significant difference. Then, we concluded pregnancy period was the major agent for a reduction in TAS value in comparison with food restriction.

CONCLUSION

The results suggest that in comparison with food restriction, pregnancy period was the major agent for oxidative stress in late pregnancy of Ghezel ewes. We founded that food restriction had not a significant effect on oxidative stress indices. However, period of gestation especially in late pregnancy imposed lots of stress on anti-oxidative system of ewes.

May be a native and custom opinion believe in a causative responsibility of underfeeding for a oxidative stress in late pregnancy period but considering the results further studies can make clear a true reason of oxidative stress in late pregnant ewes.

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