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Some Reproductive and Metabolic Responses to Food Restriction and Re-Feeding in Egyptian Native Goats

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Abstract: This study aimed to evaluate the effect of diet restriction and re-feeding on some reproductive and metabolic aspects. Ten native multiparous non-pregnant goats were equally divided according to their body weight into light (<15 kg) and heavy (≥15 kg) groups. Goats were subjected to 50% diet restriction for 35 days followed by re-feeding for another 35 days. Estrus was synchronized using 60 mg medroxyprogesterone acetate intra-vaginal sponges for 10 days starting on day 4-post re-feeding. Blood samples were collected twice a week with each endorectal ultrasound examination and body weight was recorded every week. Progesterone, insulin like growth factor-1, leptin and nitric oxide concentrations as well as total proteins, albumin, urea, total cholesterol and triglycerides levels were analyzed. In both groups, goats lost their weight during diet restriction and regained it with re-feeding. Ultrasonography revealed that feed restriction persisted corpora lutae in 80% of goats in both groups. One goat in each group (20%) showed signs of estrus and ovulated near the end of the diet restriction period. During the period of re-feeding, all animals in the heavy group and 80% of the light one responded to synchronization and ovulated. During the first estrus after synchronization, heavy group goats showed estrus earlier than the light one. Two out of five (40%) goats had double ovulation and two out of three goats with single ovulation were conceived after mating. All the ovulated light group goats had a single ovulation and one of them conceived after mating. During the 2nd estrus after sponge removal, all of the three remnant goats of the heavy group had double ovulation and all conceived after mating, but in the light group, three out of the four had a single ovulation, one did not ovulate and only one conceived after mating.

Key words: Food restriction · Reproduction · Metabolites · Hormones · Goats

INTRODUCTION

The adaptation of animals to different levels of nutrition is a powerful tool that breeders in large areas of the world have been exploiting with evident advantages [1, 2]. Inducing an animal to store reserves (mainly as fat) in periods when fodder availability is high and mobilize those reserves to cope with production needs when affordable feeds are scarce, is a common and economic strategy [3]. Reproductive performance is commonly correlated with body weight changes. Severe body weight loss is usually accompanied by anestrus [4] suggesting that there is a critical body weight to maintain the estrous cycle in female farm animals.

Fasting effects on the hypothalamic-pituitary-ovarian axis may be modulated by metabolic mediators, including glucose, insulin, growth hormone (GH), insulin like growth factor-1(IGF-I) and insulin like growth factor binding proteins (IGFBP) [5]. So, indicators, such as blood metabolites, could also be very useful in predicting and avoiding metabolic shortages before any serious or even irreparable damage is caused [6]. However, the effect of limited feed resources that can decrease reproductive efficiency to an extent depends on the degree [7] and reproductive status [8] at the time of feed restriction. Ovulation rate was decreased in protein-restricted [8] and fasted ewes [9]; an effect which is pronounced when ewes were fasted during the luteal phase of the estrous cycle [9]. Moreover, restriction of dietary energy to 0.4 maintenance for 13 to 15 days suppressed ovarian follicular development and resulted in anovulation in 60% of beef heifers [10] and decreased secretion of estradiol and LH in milk-fed ovariectomized-prepubertal lambs [11,12].

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Leptin is a hormone which is secreted in a pulsatile pattern in sheep [13]. It appears to modulate feeding behaviour and energy expenditure and therefore plays an important role in body weight regulation, metabolism and reproductive functions [14] and it plays a specific role in mediating the response of reproductive hormones to the nutritional status [15]. Chronic and acute changes in nutrition affect systemic leptin concentrations in sheep [13] and cattle [16]. Leptin stimulates gonadal function and increases uterine weight [17]. Furthermore, leptin receptors have been found in ovaries [18] and circulating leptin are responsive to short term nutrient flux and is associated with changes with insulin, IGF-1 and LH pulsality in prepubertal heifers [19].

Nitric oxide (NO) is a short-lived gaseous radical, which is generated enzymatically by nitric oxide synthase (NOS). Three different isoforms of NOS are known. Two forms are constitutively expressed: the neuronal NOS (called nNOS or NOS1) and endothelial NOS (eNOS or NOS3) [20]. NO stimulates GnRH secretion by activation of the ovary and is hypothesized to play a role in steroidogenesis [21]. It is implicated in the control of gonadotrophin secretion at both hypothalamic and hypophyseal levels, LH surge mechanism, sexual behaviour, estradiol synthesis, follicle survival and ovulation [22].

The objective of this study was to investigate the effect of diet restriction then re-feeding on body weight changes, ovarian activity and some serum metabolic aspects in cyclic native Egyptian goats.

MATERIALS AND METHODS

Animals and treatments: Ten Egyptian native non-pregnant adult goats were divided into two equal groups according to their body weight after their purchase. Light group, included does of body weight <15 kg and heavy group included goats of body weight =15 kg. Ration consisted of commercial pelleted concentrate mixture and wheat straw was offered to all animals twice per day. Water was provided *ad libitum*. Initially, both groups received 0.75 kg/day of pellets for 15 days to cover 100% of their maintenance nutritional requirement and metabolic energy (ME) equal 2.04 Kcal/head/day [23]. Diet was restricted for 35 days to 50% of the previous diet (about 0.4 kg/head/day, where the ME equal 1.02 Kcal/head/day). Re-feeding for another 35 days by giving each animal 0.75 kg of pelleted ration in addition to 0.275 kg barely where the ME of both equal 2.94 Kcal/head/day. Animals were weighed weekly. Starting from day 4 post re-feeding, estrus was synchronized using vaginal sponges impregnated with 60 mg medroxyprogesterone acetate (Veramix, Upjohn) for 10 days. Estrus was detected by a fertile buck that was introduced at the day of sponge removal.

Ultrasound examination and blood sampling: An endorectal linear array 6-8MHz transducer (Scanner 240, PieMedical, the Netherlands), modified to be handled externally was used during this work. Ultrasonographic examination was performed twice a week. Does were examined on lateral recumbence. Ovaries were located and follicles >2mm were counted and their diameters were measured. Corpora lutea were also counted and their diameters were measured [24]. Follicles were classified into small (= 0.3cm), medium (>0.3 to <0.5 cm) and large (=0.5cm). Blood samples were collected via jagular venipuncture with each ultrasonographic examination. Blood serum was separated and stored at -20°C until assayed.

Hormones and metabolic assays: Serum total protein[25], urea[26], cholesterol[27], triglycerides[28] and glucose[29] were measured by spectrophotometer using chemical kits from (Stanbio laboratory). Progesterone was assayed using ELISA commercial kit for quantitative determination of progesterone in serum with inter and intra-run precision coefficients of variation of 2.9 and 4.8 %, respectively and a sensitivity of 0.05ng/ml [30]. Insulin like growth factor-1 (IGF-1), (BioSource Europe S. A. Belgium) and Mutli-Species Leptin RIA Kit (Linco Research) were estimated by radioimmunoassay (RIA), [31,32]. The limit of sensitivity, intra- and inter-assay coefficients of variation were 3.4ng/ml, 1.9% and 4.1% and 1.0ng/ml, 2.8% and 8%,respectively. Nitric oxide (NO) was also assayed using ELISA [33].

Statistical analysis: Data were pooled according to the food handling into acclimation, food restriction and re-feeding periods. Data were analyzed using SPSS data analysis computer software [34] and the effect of feed restriction and re-feeding using split analysis of variance [ANOVA] was performed for each group of goats and the statistical significance between light and heavy goats was compared using Student's *t*-test; $P < 0.05$ was considered significant. Data are presented as Mean ±SEM

RESULTS

1. Body weight: Light and heavy goats insignificantly lost weight during restriction and started to gain weight at

Table 1: Effect of food restriction and re-feeding on body weight (Mean ±SEM)

	Body weight / kg	
Treatment	Light group	Heavy group
At the end of acclimation period	12.34±0.74	19.05±1.24
At the end of food restriction period	11.84±0.72	17.20±0.20
At the end of re-feeding period	13.21±1.03	21.06±1.29

Table 2: Effect of food restriction and re-feeding on follicular development and ovulation

	Light group			Heavy group		
periods	0-15	16-50	51-85	0-15	16-50	51-85
SF	4.00±0.63	4.33±0.44^{a}	3.21±0.34	4.00±1.23	2.11±0.46^{b}	2.83±0.28
MF	1.40±0.25	1.11±0.20^{a}	2.04±0.27	1.50±0.50	3.11±0.51^{b}	2.17±0.24
LF	0.00±0.00^{A}	0.13±0.07^{A}	0.35±0.10^{B}	0.25±0.15^{A}	0.18±0.13^{A}	0.74±0.19^{B}
TF	5.25±0.48	5.56±0.32	5.54±0.30	5.75±0.75	5.39±0.26	5.59±0.29
DF/cm	0.40±0.03	0.41±0.02	0.46±0.03	0.46±0.06	0.45±0.01	0.49±0.02
Ov.R	1.20±0.20	0.80±0.09^{a}	1.00±0.12	1.40±0.41	1.20±0.11^{b}	1.40±10

0-15 = acclimation period 16-50 = restriction period 51-85 = re-feeding period. SF = small folliclesnumber, MF = medium follicles number, LF = large follicles number, TF = total follicles number, DF = dominant follicle diameter, Ov.R= ovulation rate. Means ± SEM with different superscript within period (a,b,c) and within group (A,B,C) are significantly at P<0.05.

re-feeding. The level of weight loss and regain was more obvious in heavy in comparison to light goats (Table 1).

2. Ovarian activities during the food restriction period: During the food restriction period (Table 2), the number of small follicles in the light group significantly increased (4.33±0.44 Vs 2.11±0.46, P<0.003) as compared to the heavy one. On the other hand, the medium follicles in the light group significantly decreased in number than that in the heavy one (1.11±0.20 Vs 3.11±0.51, P<0.001). In both groups, there was a neglected growth of large follicles. The total number of follicles was not significantly affected. Food restriction decreased the diameter of the dominant follicle in light group compared to heavy one. Ultrasonographic examination revealed that food restriction led to persistence of the corpora lutae in 80% of goats in both groups (Table 3). One goat in each group (20%) showed signs of estrus and ovulated near the end of the diet restriction period. Progesterone levels, ultrasound scanning and estrous observation confirmed the prolonged life span of the corpora lutea in most of the goats during diet restriction.

3. Effect of re-feeding on ovulation and reproductive performance: During the period of re-feeding, all heavy goats responded to synchronization and ovulated. In the first estrus after synchronization, the heavy group showed estrus earlier than the light one. Two out of the five (40%) had double ovulation and the rest had single ovulation. Two out of those with single ovulation conceived after mating. Ovulated goats in the light group (4/5) had single ovulation and one of them conceived after mating.

In the second estrus after sponge removal, the rest three goats of the heavy group had double ovulation and

Table 3: Effect of food restriction and re-feeding on ovulation and reproductive performance

Treatment periods	Light group (n=5)	Heavy group (n=5)
During food restriction		
Estrus activities and ovulation	20% (1/5)	20% (1/5)
Persistent corpora lutea	80% (4/5)	80% (4/5)
1st Estrus after synchronization		
Response to synchronization	80% (4/5)	100% (5/5)
Time to estrus	>72 hrs	<72hrs
Double ovulation	00% (0/4)	40% (2/5)
Single ovulation	100% (4/4)	60% (3/5)
Conception after mating	25% (1/4)	40% (2/5)
2nd Estrus after synchronization		
Double ovulation	00%	100% (3/3)
Single ovulation	66.6% (2/3)*	00%
Conception after mating	50% (1/2)	100% (3/3)

*One (33.3%) did not showed estrus or ovulate until the end of the experiment

Table 4: Effect of food restriction and re-feeding on some metabolic factors (Mean+SEM)

Groups	Light group			Heavy group		
periods	0 - 15	16- 50	51- 85	0 -15	16- 50	51- 85
Progesterone (ng/mL)	1.4±0.35	1.39±0.18	1.29±0.16	1.40±0.44	1.37±0.16	1.02±0.13
IGF-1 (ng/mL)	269.7±54^{A}	394±67^{Ba}	368±35^{B}	223.4±40	241±15^{b}	255±44
Leptin (ng/mL)	0.71±0.62	0.98±0.20	0.65±0.10	1.11±0.42	0.95±0.13	1.05±0.30
Nitric oxide (µmol)	15.7±8.9	12.5±1.1	16.5±2.5	17.8±5.1	16.2±1.1	17.8±1.8
Total cholesterol (mg/dL)	84.9±3.4	65.2±6.1	76.0±5.6	79.7±3.8	60.7±5.6	77.9±7.2
Triglycerides (mg/dL)	78.7±3.1	55.4±10.9	82.8±7.9	65.6±4.8^{A}	55.4±1.1^{A}	81.1±5.9^{B}
Total proteins (mg/dL)	8.4±0.27	7.6±0.40	9.1±0.5	9.59±0.41^{B}	8.2±0.3^{A}	10.0±0.4^{B}
Urea (mg/dL)	16.2±1.95	15.9±0.7	14.1±1.6	15.8±2.1	13.7±0.7	13.1±2.0
Glucose (mg/dL)	101.6±12	85.6±6.0	91.0±5.0	83.6±15	83.6±4.0	92.3±3.0

0-15 = acclimation period 16-50 = restriction period 51-85 = re-feeding period. Means ± SEM with different superscript within period (a,b,c) and within group (A,B,C) are significantly at P<0.05.

all conceived after mating. In the light group, two out of the three does had single ovulation and only one conceived after mating.

4. Blood serum metabolites and hormones: During the acclimation period, there are no significant differences between both light and heavy groups in concentrations of progesterone, IGF-1, Leptin and NO, fats (total cholesterol and triglycerides), proteins (total proteins and urea) and glucose.

Levels of progesterone were high during restriction compared with re-feeding in both groups of goat with no observed significant variation in progesterone between both groups.

During food restriction, the IGF-1 showed an increased level, in both groups which reached a significant (P<0.05) level in the light group only, however, the level declined in light goats only after re-feeding period. During restriction, a significant increase in IGF-1 levels (P<0.05) was observed in the light compared to heavy goats (394±67 Vs 241±15 ng/mL). Leptin levels were insignificantly affected by restriction in heavy goats but its levels declined insignificantly in light goats during re-feeding period compared to restriction one. A decrease in circulating levels of NO levels in light and heavy goats during restriction period followed by a pronounced but non significant increase during the re-feeding intervals in both goat groups was evident. In heavy goats, (Table 4) a significant decline in values of triglycerides and total proteins and a non significant decline in total cholesterol, urea and glucose and also their levels declined during restriction in light goats.

A highly significant negative correlation was found between leptin and IGF-1 levels (r= -1.0, P<0.0001) in light goats but was not significant in heavy one (r= -0.97) and also a significant positive high correlation between body weight and leptin levels was recorded in light goats only (r=0.53, p<0.05). The correlation between body weight and progesterone was positive and significant in each group of goats but was higher in heavy compared to light goats(r=0.63, P<0.0001, 0.44, <0.05). NO levels correlated significantly with body weight in heavy goats only (r=0.43, P<0.003) and negatively correlated with levels of IGF-1(r=-0.66, P<0.05).

DISCUSSION

In domestic animals, body weight is generally used to evaluate energy status. Similar to the present findings, goats fed 30% of diet [35], mares fed restricted energy diets [36] and lactating sows fed restricted diet [37] lost weight compared to the control. Moreover, ewes kept losing weight at slow and steady rate, demonstrating the adaptation to under-nutrition after an initial period of a rapid decrease in live weight [3].

Regarding the follicle population, the increased diameter of the dominant follicles at re-feeding is in agreement with those recorded in heifers whereas re-alimentation of nutritionally induced anovulation in resulted in a gradual increase in maximum diameter and persistenc of dominant follicles. [38,39]. Undernutrition in beef heifers temporarily reduced the number of medium-sized ovarian follicles[40] which is in agreement with that recorded in this study in light goats. Similarly, during an estrous cycle, commencing approximately 5 weeks after di*et al*location, the maximum diameter and persistenc of the maximum diameter attained by the dominant follicle (DF) was smaller in heifers fed 0.7 [41] and 0.4 maintenance (Mn) diets [10], the DF decreased in the restricted heifers though they continued to ovulate [41].

Therefore, 75% of heifers fed 0.4 Mn became anovulatoy after 13-15 days of feed restriction. They referred ovulation not to difference in growth rate but to the increase in the number of days of growing which may explain the delayed response of light goats in this study. Moreover, all goats subjected to 30% diet restriction entered ovarian quiescence and the interval to the onset of ovarian quiescence depended on the body weight of the animal at the start of food restriction [35]. Undernutrition in beef heifers increased the length of the estrous cycle and reduced the proportion of heifers with normal fertilized ova [40]. The previous observations explain the ovulation recorded in this study in one animal in each group at the end of restriction period and the prolonged life span of their corpora lutea. Conversely, no significant difference in the length of luteal phase of goat subjected to 30% diet restriction was observed [35].

Similarly to the current findings, ovulation was recorded in one fasted Ardi goat during the second estrous cycle but seven out of eight goats did not show any sign of estrus [42]. Although, no ovulation was recorded after CIDR-G removal in any of the dietary-restricted goats whose body weight decreased to around 18 kg [43]. As well as, in two heifers fed 0.4 Mn, the dominant follicle (DF) failed to ovulate and one of these heifers failed to exhibit estrus [10].

The improved ovulation rate during re-feeding period in the present results corresponds with that recorded in control and fasted ewes determined 13 d after re-alimentation and the administration of prostaglandine [44].

Contrary to our results, a decline in progesterone levels during the mid luteal phase was recorded in Shiba goat subjected to 30% food restriction [35]. Moreover, significantly lower progesterone concentrations was recorded in fasting compared to fed Ardi goat during the second estrous cycle [42]. Undernutrition reduced plasma levels of progesterone within 5 days in beef heifers subjected to 15% food restriction and weights of corpora lutes formed in undernourished heifers were about 70% of control values[40]. On the other side, increased progesterone was noted in response to feed restriction in sheep [45, 46] because feed restriction may have altered the metabolic clearance of progesterone [47].

Decreasing triglycerides concentrations during the period of under-nutrition is similar to those observed by [48, 49] and were justified by the decline on the substrate availability from the gastro-intestinal tract. Lipid metabolism of goats is regulated by light even in constant temperature and feeding conditions [50]. The recorded decline in total proteins in our results is similar to that recorded in 30 % nutritionally restricted sheep [51]. The low urea values coincide with the onset of the intake reduction periods resulted probably from a decrease in nitrogen intake illustrating the direct effect of nitrogen intake on urea serum concentrations [51, 52].Moreover, serum urea concentrations fluctuated in almost all groups [3]. Although, under-nutrition led to a slight reduction of glucose concentrations prior to the initiation of anoestrus in food-restricted beef cows [4] and the decline of blood glucose concentration after fasting in light goats was greater than that in heavy goats [53] but there were no significant differences in the glucose concentration between control and restricted goat [53] which coincides with our findings. The absence of a significant change in glucose suggests that the central nervous system was not deprived of this primary energy source [43].

Conversely to the present results, plasma IGF-I was reduced by fasting in heifers [54] and sheep [55]. After 48 h of fasting, serum concentrations of IGF-I decreased in fasted ewes and remained lower than in control animals until 72 h following realimentation [44]. During weight gain, by contrast, IGF-I increased significantly with every unit change in body condition up to BCS of 4 and plateaued thereafter [56]. It has also been reported that a 25% restriction of feed intake did not affect plasma IGF-I concentrations in gilts [57].

In agreement with the present results, leptin concentrations were similar in the fasted and fed Ardi goats [42]. No diurnal or meal-related fluctuations in plasma leptin in rams, attributing the apparent species difference to differences in nutritional physiology [13]. However, the dynamics of the leptin response to fasting in the ruminant and the factors that modulate leptin secretion in the short-term remain unclear.

Since the circulating levels of NO follow the pattern of uterine angiogensis and uterine blood flow [5] and the increased expression of iNOS protein during the late proestrous phase which is also accompanied by the preovulatory increase in serum estradiol and LH levels [59, 60].Moreover,the detected eNOS protein during the periovulatory phase [61] and the cyclic changes in expression of NOS subtypes in ovary which lends support to the role of NO in directly affecting the ovarian function. Increase in NOS activity in ovary corresponding to the time of the preovulatory surge suggests that NO may be assisting the process of ovulation via its stimulatory effect on prostaglandin production [62]. All this may explain the increase in circulating levels of NO in the current work during the refeeding period in both light

and heavy goats and confirms the response of goats to synchronization and the preovulatory follicle growth.

It is concluded that short term diet restriction could be practically tried in animals of good body condition at the time of feed shortage but it must be followed by re-alimentation to compensate the loss in body reserves since short term diet restriction in this study had no serious effect on body weight, follicle population, ovulation, ovulation rate and metabolic aspect in animals of higher body weight. Body weight is a detrimental factor that could severely affect reproduction.

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