

**EFFECT OF ENERGY OR PROTEIN RESTRICTION ON SOME  
PHYSIOLOGICAL RESPONSES OF SHEEP: 1. PUBERTY AND  
ESTROUS PHENOMENA**

M.A.A. El-Barody<sup>1</sup>, A.K.I. Abd El-Moty<sup>1</sup>, T. Klopfenstein<sup>2</sup>, J. Kinder<sup>2</sup>,  
F.M.R. El-Feel<sup>1</sup> and S.T.M. Fahmy<sup>1</sup>

1) Department of Animal Production, Faculty of Agriculture,  
University of Minia, Minia, Egypt 2) Department of Animal Production,  
University of Nebraska, Lincoln, U.S.A.

**SUMMARY**

Eighty-five young crossbred ewe lambs, aged three and half months and weighing  $22.11 \pm 0.83$  kg, were used in this study. The lambs were divided randomly into five groups each of seventeen and were fed ad lib. as follows: Group 1 was fed the control diet (TDN= 75.0%, CP= 15.00%), group 2 was fed on low energy (LE) diet (TDN =60.0%, CP = 13.57%), group 3 was fed on low protein (LP) diet (TDN= 65.0%, CP = 11.39%), group 4 was fed on LE diet for nine weeks then switched to control diet (LE/C) and the last group was fed on LP diet for nine weeks then switched to control diet (LP/C). The experiment was terminated after 140 days of feeding. Animals were weighted weekly, and blood samples were collected weekly from each ewe for progesterone measurement and assessment of puberty. The average daily gain during the experimental period was higher ( $P < 0.002$ ) in lambs fed the control diets in comparison with those fed on LE, LP or LE/C diets. Lambs fed the control diet reached puberty 16 days earlier ( $P < 0.05$ ) than those fed on the LE diet, while there was no significant difference in age at puberty between lambs fed on the control and those fed on LP diet.

Progesterone concentrations during estrous were higher in ewes fed LE rations compared to those fed control ( $P < 0.004$ ) or LP ( $P < 0.05$ ) diets. Dietary changes from the LE diet to control diet led to a decrease ( $P < 0.007$ ) in progesterone concentration compared to ewes fed LE diet throughout the experiment. There was no significant difference in serum concentrations of progesterone in ewes switched

from LP to control diet compared to those fed LP diet throughout the experiment.

**Keywords:** Energy or protein restriction, sheep, puberty, estrous phenomena

#### INTRODUCTION

The nutritional level and quality of feeds play a great role in controlling the endocrine system that regulates reproductive functions. Onset of puberty and estrous in ewe lambs were affected by energy or protein restriction (Foster and Olster, 1985). Adequate nutrition and good body conditions lead to increased ovulation rate of ewes (Thomas *et al.* 1987). Nutritional restriction and decreased body weight affect the length of anestrus in ewes (Hall *et al.*, 1986). Progesterone concentrations in peripheral blood have been reported to decrease (Beal *et al.*, 1978); Imakawa *et al.*, 1983), increase (McCann and Hansel, 1986) or remain constant (Hamadeh *et al.*, 1989; Schlich *et al.*, 1990) after dietary restriction in ovine and bovine females. Several investigators have demonstrated that puberty of ewe lambs was delayed by undernutrition (Fitzgerald *et al.*, 1982; Foster *et al.*, 1984; Foster and Olster, 1985). The present study was conducted to investigate the effect of energy or protein restriction on onset of puberty and estrous in ewe lambs as monitored by serum progesterone concentrations.

#### MATERIAL AND METHODS

Eighty-five young crossbred (1/2 Finn, 1/4 Dorset and 1/4 rambouillet) ewe lambs belonging to the experimental farm of the Animal Science Department, University of Nebraska, U.S.A., were used in this experiment. The experiment was initiated as ewes aged three and half months and weighing  $22.11 \pm 0.83$  kg. The animals were stratified by weight and randomly divided into five groups each of 17. They were fed *ad lib.* as follows: group (1) was fed on control diet (C), group (2) was fed low energy diet (LE), group (3) was fed low protein diet (LP), group (4) was fed LE diet for nine weeks then switched to control diet (LE/C) until the end of the experiment, and the last group was fed LP diet for nine weeks, then switched to control diet (LP/C) until the end of experiment. The experiment was terminated after 140 days of feeding. Body weight of lambs was measured weekly. Diet composition, dry matter (DM%), total digestible nutrients (TDN%) and crude protein (CP) of diets fed are presented in Table 1. Ewes were group fed in indoor lots that had adequate bunk space for all ewes to consume feed at the same time. Weekly blood

samples were collected from each ewe before feeding by Jugular vein puncture. The blood samples were placed on ice upon collection and stored at 4°C for 24 h., then centrifuged at 3000 x g for 20 minutes. Serum samples were separated and stored at -20°C until use for progesterone assay. Serum concentration of progesterone was measured by the radioimmunoassay (Walf et al., 1989). Serum concentration of progesterone was used to estimate the day of puberty (first ovulation) as described (Fitzgerald et al., 1982; Day et al., 1984).

Analysis of variance (SAS, 1985) was used to evaluate the effect of dietary treatments on live weight gain, body weight, age at puberty and serum progesterone concentrations during estrous activity. Least significant rang was applied to detect the significant variance among treatment means.

Table 1. Diet composition, dry matter (DM%), total digestible nutrients (TDN) and crud protein (CP) of control, low energy and low protein diets

Items	Control (C)	Low energy (LE)	Low protein (LP)
Alfalfa pellets	46.60	-	-
(Rolled) corn	49.14	-	-
Soyhulls	-	55.29	33.23
Corn cobs	-	30.02	56.61
Feather meal	0.30	2.40	-
Blood meal	0.30	2.10	-
Urea	0.20	1.00	1.00
Dicalcium	0.13	0.85	0.82
Salt	0.26	0.27	0.27
Trace minerals	0.05	0.05	0.05
Selenium premix	0.02	0.02	0.02
Molasses	3.00	8.00	8.00
Dry matter (DM%)	90.36	88.94	88.79
TDN %	75.00	60.00	65.00
Crude protein (CP%)	15.00	13.57	11.39

## RESULTS

Average daily gain during 140 days of the experimental period was greater ( $P < 0.002$ ) for lambs fed the control diet ( $186 \pm 6.0$  g/day) than those fed on LE, LP and LE/C diets ( $107 \pm 6.0$ ,  $104 \pm 6.0$  and  $162 \pm 6.4$  g/day, respectively). There was no significant difference in daily

gain between lambs fed LE and LP nor between LE/C and LP/C diets. Final body weight of lambs fed on the control diet was heavier ( $P < 0.01$ ) than those fed on LE and LP, but there was no significant difference between LE/C and LP/C diets. Animals fed the control diet also had heavier ( $P < 0.01$ ) body weight at puberty than those fed LE and LP diets. Dietary switchover of low energy or low protein treatment to the control diet led to increased live weight gain and body weight at puberty (Table 2). Animals fed the control diet reached puberty 16 days earlier ( $P < 0.05$ ) than those fed on LE, but there was no significant difference in age at puberty between the control and LP fed groups (Table 2). Switchover of LE or LP to the control diet resulted in no significant change in age at puberty compared to those fed LE or LP throughout the experiment.

Table 2. Effect of feeding regimen live body weight, body weight gain and age at puberty .

Items	Control	LE	LP	LE/C	LP/C	±SE	P<
Number of lambs	17	17	17	17	17	-	-
Initial age (day)	105	107	104	106	108	1.3	-
Initial body weight (kg)	22	22	22	23	22	1.9	-
Final body weight (kg)	48a	37b	37b	45c	47ac	2.7	(1.01)
Daily weight gain (g)	186a	107b	104b	162c	177ad	6.0	(0.002)
Age at puberty (day)	169a	186b	178ab	181cb	179a	5.2	(0.05)
Body weight at puberty (kg)	35a	31b	32b	33ac	34ac	1.0	(0.01)

Control = Group of lambs fed the control diet.

LE = Group of lambs fed the low energy diet.

LP = Group of lambs fed the low protein diet.

LE/C = Group of lambs fed the low energy diet followed control diet.

LE/P = Group of lambs fed the low protien diet followed control diet

a, b, c = Means within the same row with different superscript letters differ significantly.

The progesterone profile (Fig. 1,2 and 3) of all animal groups fed either control or restricted diets showed that none of animals had achieved puberty at the beginning of the experiment (progesterone concentration in serum was less than 1 ng/ml). The occurrence of first behavioral estrous (puberty) generally followed normal ovulation and corpus luteum development, which was associated with increasing progesterone concentrations in serum during the estrous cycles. Averages of serum progesterone concentrations during estrous activity after puberty were  $1.0 \pm 0.12$ ,  $1.5 \pm 0.12$ ,  $1.1 \pm 0.12$ ,  $1.0 \pm 0.12$  and  $1.1 \pm 0.12$  ng/ml for control, LE, LP, LE/C and LP/C, respectively, (Table 3). Animals fed LE diet showed higher values of progesterone

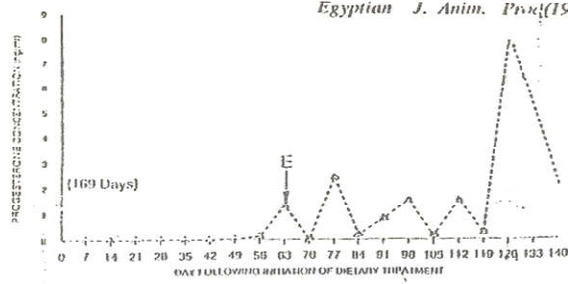


Fig 1 Serum progesterone concentrations before and after puberty in female lambs that fed control diet (E indicates puberty or first estrus; number between parentheses represent age of ewe lambs)

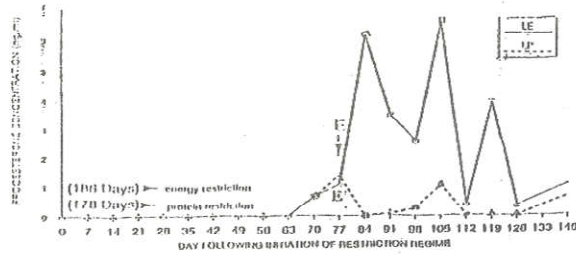


Fig 2 Serum progesterone concentrations before and after puberty in female lambs that fed restricted energy and protein diets (E indicates puberty or first estrus; numbers between parentheses represent age of ewe lambs).

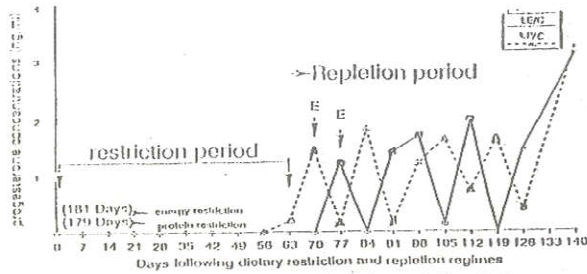


Fig 3 Serum progesterone concentrations before and after puberty in female lambs that fed restricted diets followed by control diet (E indicates puberty or first estrus; numbers between parentheses represent age of ewe lambs).

concentrations compared to those fed control ( $P < 0.004$ ) or LP ( $P < 0.05$ ) diets. Dietary changes (low energy/control or low protein/control treatments) caused a decrease in progesterone concentration ( $P < 0.007$ ) compared to those fed LE diet throughout the experiment. On the contrary no significant difference was noted in serum progesterone concentrations in ewes switched to control diet (LE/C or LP/C) compared to those fed low protein diet throughout the experiment.

Table 3. Number of ewes reaching puberty during the experimental period and mean ( $\pm$ SE) of progesterone concentrations during the estrous cycle for different dietary treatments.

Treatments	Number reaching Puberty (n) N*/n	Mean of progesterone concentrations (ng/ml)
Control group	17/17	1.0 $\pm$ 0.12 a
Low energy group	17/17	1.5 $\pm$ 0.12 a
Low protein group	17/17	1.1 $\pm$ 0.12 a
Low energy/control	17/17	1.0 $\pm$ 0.12 a
Low protein/control	17/17	1.1 $\pm$ 0.12 a

\*N : Total number of animals per each treatment.

a, b : Mean within column with different letters superscript differ significantly ( $P < 0.007$ ).

#### DISCUSSION

Final body weight, average daily gain and body weight at puberty were significantly decreased as a result of energy or protein restriction. Greater average daily gain of ewes fed the control diet encouraged earlier puberty than ewes fed on the low energy or protein regime. During sexual development, inadequate level of dietary energy of ovine and bovine females resulted in a delay in onset of puberty (Foster and Olster, 1985.; Day *et al.*, 1986; Kurz *et al.*, 1989). In the present study energy restriction of lambs delayed puberty 16 days compared with those fed adequate diet (control). Two explanation can be advanced in this respect : firstly, the low level of energy intake may have suppressed GnRH secretion and the production of the high frequency LH that are required for preovulatory for follicular development (Foster *et al.*, 1984). Secondly, the delay in puberty might be attributed to an inadequate response of the pituitary to LHRH and therefore a decrease in the secretion of LH. Similar observations were reported in heifers by Day *et al.* (1986). In fact

the physiological signal linking the first ovulation, normal corpus luteum function and progesterone secretion to nutrition and growth rate appears to be directly related to pulsatile LH secretion (Foster et al., 1984). In the present study low energy intake may reduce the ability of slowly growing females to generate this hormone at the required level.

The restriction of protein intake did not result in a significant delay of puberty in lambs. This result is interesting and is in agreement with Blaxter (1957) in his review on the effect of defective nutrition on reproduction in farm animal. The restriction in protein intake in the present study may not have been severe enough to affect significantly age at puberty. This explanation is supported by the study of Widdowson (1977). The present study indicated that realimentation regime had no effect on age at puberty compared to those fed on the restriction regime throughout the experimental period. These results are in contrast with previous conclusion by Foster and Olster (1985). They reported that ad-libitum feeding of lambs after a restriction regime, resulted in rapid catch-up growth and onset of reproductive cycles, while continuous feed restriction of another group of lambs led to a decrease in their body weight and associated anovulation. The difference between the two results may be due to the difference in the initial age of animals (10 weeks vs. 12 weeks in the present study). The difference in the nature of the restriction regime (feed amount restriction vs. nutrient constituent restriction in the present study) and the age of animals at the beginning of realimentation (28 weeks vs. 21 weeks in the present study) may account for the different findings.

The general pattern of serum progesterone concentrations throughout the estrous activity for ewes was similar to that observed in the previous studies in sheep (Fitzgerald et al., 1982; Oyedipe et al., 1986). Increased serum progesterone concentration during estrous activity in ewes fed low energy diets is contradictory, see results by Cumming et al. (1971) and Lamond et al. (1972). The low serum progesterone level in females fed low protein diet compared to those fed low energy diet in the present study (1.1 vs 1.5 ng/ml) are consistent with the observation of Giannina and Leathem (1974). They reported that animals fed either protein-free or restricted diet showed a dramatic decrease in progesterone concentration. The decrease in serum progesterone concentrations in ewes fed the low protein diet is interesting. Whether, it represents a reduction in corpus luteum function or an alteration in the rate of hepatic clearance of the steroid is not known. Realimentation of ewe lambs fed low protein diet had no effect on serum progesterone concentration. Although, this result is in agreement with the previous studies by Hudgens and Hallford (1983) and Hamadeh et al. (1989). It may be due

to the short interval between realimentation and blood sampling. Whether a longer period of realimentation would alter the finding is not known. More work is needed to elucidate the effect of inadequate dietary protein on ovarian functions and the temporal changes in circulating progesterone concentration following realimentation.

## REFERENCES

- Beal, W.E., R.E. Short, R.B. Stagmiller, R.A. Bellows, C.C. Kaltenbach and T.G. Dunn 1978. Influence of dietary energy intake in bovine pituitary and luteal function *J. Anim. Sci.* 46:181.
- Blaxter, K.L., 1957. The effects of defective nutrition during pregnancy in farm livestock. *Nut. Soc. Proc.* 16:52.
- Cumming, I.A., B.J. Mole, M.A. Obst, De. B. Blockey, C. G. Winfield, R.W. Blaxter and J.R. Goging 1971. Increase in plasma progesterone caused by undernutrition during early pregnancy in the ewe. *J. Reprod. Fert.* 24:146.
- Day, M.L., K. Imakawa, D.D. Zalesky, R.J. Kittok and J.E. Kinder, 1986. Effects of restriction of dietary energy intake during the prepubertal period secretion of luteinizing hormone and responsiveness of the pituitary luteinizing hormone-releasing hormone in heifers. *J. Anim. Sci.* 62:1641.
- Day, M.L., K. Imakawa, D.M. GarciaWinder, D.D. Zalesky, B.D. Schanbacher, R.J. Kittok and J.E. Kinder, 1984. Endocrine mechanisms of puberty in heifers Estradiol-negative feedback regulation of luteinizing hormone secretion and first ovulation. *Biol. Reprod.* 31. 332.
- Fitzgerald, J.F., R. Michel and W.R. and Buttler 1982. Growth and sexual maturation in ewes. Dietary and seasonal effects modulating luteinizing hormone secretion and first ovulation. *Biol. Reprod.* 27:864.
- Foster, D.L. and D.H. Olster, 1985. Effect of restricted nutrition on Puberty in the lamb. Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. *Endocrin.* 116:375.
- Foster, D.L., S.M. Yellon and D.H. Olster, 1984. Endocrine physiology of puberty in female sheep. Tenth. Inter. Cong. on Anim. Reprod. Artif. Insem, Urbana, Illinois, USA, 7:16.
- Giannina, T. and J.H. Leatham, 1974. Serum progesterone levels in pregnant rats fed a protein-free diet. *Proc. of the Soc. Exp. Biol. Med.* 146:957.
- Hall, D.J., N.M. Fogarty and A.R. Gilmour, 1986. Seasonality of ovulation and estrus and the ram effect in poll Dorset ewes. *Theriogenology* 25:455.



- Hamadeh, S.K., C.V. Hulet, T.T. Ross and D.M. Hallford, 1989. Ovarian cyclicity and serum progesterone and lutenizing hormone in fine wool ewes supplemented with alfalfa or pinto beans. *Theriogenology*, 32:149.
- Hudgens, R.E. and D.M. Hallford, 1983. Effects of long-term consumption of sewage solids on reproductive performance and serum progesterone and estradiol-17 $\beta$  in mature fine wool ewes. *Theriogenology*, 19:249.
- Imakawa, K., R.J. Kittok and J.E. Kinder, 1983. The influence of energy intake on progesterone concentrations in beef heifers. *J. Anim. Sci.* 56:454.
- Kurz, S.C., R.M. Dyer, M.D. Wright, Y. Hu and M.L. Day, 1989. Alteration of LH secretion by dietary energy in prepubertal heifers. *Biol. Reprod.* 40:83.
- Lamond, D.R., R.G. Gaddy and S.W. Kennedy, 1972. Influences of season and nutrition on luteal plasma progesterone in Rambouillet ewes. *J. Anim. Sci.* 34:626.
- McCann, J.P. and W. Hansel, 1986. Relationships between insulin and glucose metabolism and pituitary function in fasted heifers. *Biol. Reprod.* 34:630.
- Oyedips, E.O., N. Pathiraja; L.E. Edqvist and V. Buvanendran, 1986. Onset of puberty and estrous cycle phenomena in Yankase ewes as monitored by plasma progesterone concentrations. *Anim. Reprod.* 12 : 195.
- SAS (1985): SAS User's Guide Statistic. SAS inst., Inc., Cary, NC.
- Schrich, F.N., J.C. Spitzer; T.C. Jenkins; D.M. Henricks and T.G. Althen, 1990. Effect of dietary energy restriction on metabolic and endocrine responses during the estrous cycle of suckled beef cows. *J. Anim. Sci.* 68 : 3313.
- Thomas, D.L, P.J. Thomford; J.G. Crickman; A.R. Cobb and P.J. Dziuki, 1987. Effects of plane nutrition and phenobarbital during the pre-mating period on reproduction in ewe fed differentially during the summer and mated in the fall. *J. Anim. Sci.* 64:1144.
- Wolfe, M.W.; T.T. Stumpf; M.S. Robertson; P.L. Wolfe; R.J. Kittok and J.E. Kinder, 1989. Estradiol influences on pattern of gonadotropin secretion in estradiol feedback in age matched females. *Biol. Reprod.* 41:626.
- Widdowson, E.M., 1977. Undernutrition and retarded growth before and after birth. *Nutr. Met.* 21:76.

## تأثير تحديد الطاقة او البروتين على الاستجابات الفسيولوجية للاغنام ١- البلوغ ومظاهر الشيع

محمد عبد الفتاح احمد البارودي ، عبد المعطي خيرى ابراهيم ، تبرى كلوبفن شتين\*، جيم كيندر\*،  
فوزى محمود رحيم الفيل ، وسمير توفيق محمد فهمى  
قسم الانتاج الحيوانى - كلية الزراعة - جامعة المنيا

استخدم فى هذه الدراسة عدد ٨٥ حمل عمر ٣,٥ شهر بمتوسط وزن  $22,11 \pm 83$  وقد قسمت الحملان عشوائياً الى خمس مجموعات كل مجموعة بها ١٧ حمل وتم تغذيتها كالاتى : المجموعة الاولى غذيت على عليقة قياسية (المركبات الكلية المهضومة = ٧٥,٠٠٪ والبروتين الخام = ١٥,٠٠٪) والمجموعة الثانية غذيت على عليقة منخفضة فى الطاقة (المركبات الكلية المهضومة = ٦٠,٠٠٪ والبروتين الخام = ١٣,٥٧٪) المجموعة الثالثة غذيت على عليقة منخفضة فى البروتين (المركبات الكلية المهضومة = ٦٥,٠٠٪ والبروتين الخام = ١١,٣٩٪) المجموعة الرابعة غذيت على عليقة منخفضة فى الطاقة لمدة ٩ اسابيع ثم على عليقة قياسية لنهاية التجربة المجموعة الاخيرة غذيت على عليقة منخفضة فى البروتين لمدة ٩ اسابيع ثم عليقة قياسية حتى نهاية التجربة استمرت فترة التجربة ١٤٠ يوم وقد تم وزن الحيوانات اسبوعياً وتم تجميع عينات من الدم اسبوعياً من كل حيوان وذلك لقياس تركيز هرمون البروجسترون وكذلك لتحديد البلوغ . كان متوسط الزيادة اليومية فى الوزن مرتفعاً باحتمال ( $P > 0.002$ ) للحملان التى غذيت على عليقة قياسية عند مقارنتها بالحملان التى غذيت على عليقة منخفضة فى الطاقة او البروتين أو التى غذيت على عليقة منخفضة فى الطاقة ثم العليقة القياسية. الحملان التى غذيت على عليقة قياسية وصلت الى البلوغ مبكراً ( $P < 0.05$ ) بمقدار ١٦ يوم عند مقارنتها بالحملان التى غذيت على عليقة منخفضة فى الطاقة . بينما لم يكن هناك فروق معنوية فى عمر البلوغ بين الحملان التى غذيت على قياسية والتي غذيت على عليقة منخفضة فى البروتين.

كان تركيز هرمون البروجسترون مرتفعاً معنوياً فى الحيوانات التى غذيت على عليقة منخفضة فى الطاقة عند مقارنتها بالحيوانات التى غذيت على عليقة قياسية ( $P < 0.004$ ) ومنخفضة فى البروتين ( $P < 0.5$ ). الانتقال من التغذية على العليقة المنخفضة فى الطاقة الى العليقة القياسية ادى الى نقص فى تركيز هرمون البروجسترون عند المقارنة بالحيوانات التى غذيت على عليقة منخفضة فى الطاقة طوال مدة التجربة ولم يوجد فرق معنوى فى تركيز الهرمون عند التحول من العليقة المنخفضة فى البروتين الى العليقة القياسية عند مقارنتها بالحيوانات التى غذيت على عليقة منخفضة فى البروتين طوال التجربة.

\* قسم الانتاج الحيوانى - جامعة نيبرا سكا - لنكولن - نيبراسكا - الولايات المتحدة الامريكية.