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EFFECT OF ENERGY OR PROTEIN RESTRICTION ON SOME PHYSIOLOGICAL RESPONSES OF SHEEP 2. BODY WEIGHT AND PLASMA; GLUCOSE, UREA AND GROWTH HORMONE CONCENTRATIONS

M.A.A.El-Barody[1], F.M.R. El-Feel[1], T.Klopfenstein[2], J.Kinder[2], A.K.I.Abd El-Moty[1] and S.T.M. Fahmy[1]

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

Seventeen crossbred wethers, aged seven months were fed ad.lib. on control diet for 6 weeks (adjustment period). Thereafter, animals were assigned randomly to feed either a control diet, n = 5, or a energy restricted diet (ER), n = 6, or protein restricted diet (PR), n = 6. This scheme lasted for one week (short restriction period). The same feeding regime was extended for another 5 weeks (long restriction period). Wethers fed either ER or PR diet were shifted to feed the control diet for one week (realimentation period). Serial blood samples were collected at the end of each period from each animal for growth hormone, glucose and urea assay. Body weight and dry matter intake of animals were measured weekly.

Short and long restriction periods resulted in a significant (P<0.01) decrease in body weight and dry matter intake compared with ad.lib. feeding regime (control group). Mean plasma glucose concentration of wethers fed control diet was lower (P<0.01) than that fed ER diet, but it was similar to that fed PR diet. On the contrary, mean plasma urea concentration of wethers fed control diet was higher (P<0.01) than those fed PR diet, but was lower (P<0.01) than those fed ER diet. Feeding protein or energy restricted diets resulted in a significant (P<0.01) more plasma GH concentrations compared to normal feeding. Restriction of protein resulted in a significant (P<0.01) increase in mean plasma GH concentration compared to ER. Realimentation for one week failed to show significant in plasma glucose and urea concentrations. Realimentation of wethers previously fed PR diet resulted in a significant (P<0.01) decrease in mean plasma GH concentration, while realimentation of those perviously fed ER diet did not change the plasma GH concentration.

Keywords: Energy or protein restriction, sheep, plasma; glucose, urea and growth hormone

INTRODUCTION

The nutritional level and quality of feeds play a great role in controlling the animal performance. Energy or protein restriction for different periods lead to significant effects on body weight gain, dry matter and body composition of lambs (Drouillard, 1989). Changes in concentrations of plasma urea and blood glucose reflect possible metabolic alterations associated with energy or protein restriction. McNiven (1984) reported that sheep maintained on negative energy or protein nutritional balance, as a result of feed restriction, maintained blood glucose level by reducing tissue utilization or stimulating glycogenolysis, and maintaining a higher rate of glucose recycling than did sheep given food at or above maintenance. Plasma urea concentration was related inversely to energy intake, while it was related positively to protein intake as a result of restriction regime (Roberson et al. 1991). Growth hormone (GH) is considered the most important hormone affecting the animal's performance. Undernutrition increased GH concentration in most species, including sheep (Foster et al. 1989; Thomas et al. 1991). While, feeding regime has been shown to cause a reduction in plasma GH concentration of sheep (Driver and Forbes, 1981).

The present experiments were carried out to study the influence of energy or protein restriction for short and long periods on changes of body weight and plasma; glucose, urea, growth hormone concentrations.

MATERIAL AND METHODS

Seventeen crossbred wethers of seven months old and 30±0.5 kg body weight, belonging to the experimental farm of Animal Science Department, University of Nebraska, USA were used in this experiment. The wethers were placed in individual pens. Temperature was maintained at 20 °c with constant flourecent lighting and ventilation throughout the experimental period. Through the first 6 weeks of the trials, the wethers were fed ad.lib. on the control diet (adjustment period). At the end of 6 weeks, blood samples were collected from each wethers. One day before starting blood collection, wethers were fitted with indwelling jugular catheter. Blood samples were collected in 5cc heparinized syringes, collection was initiated 2 h after administration of diet then 15 min intervals throughout 6h period. Samples were placed into plastic tubes on ice. Plasma was harvested immediately from these samples after centrifugation at 6500 xg for 15 min and stored at - 20 C° until they were assayed for growth hormone according to the method of Klindt et al., (1985). Other blood samples were collected, using tubes containing sodium flouride, at hourly intervals for 6 h within each period and were placed immediately on ice. Plasma was harvested from these samples after centrifugation and were stored at -20 C° until they were assayed for glucose and urea concentrations. At the beginning of the seventh week, wethers were stratified by weight and randomly alloted to one of these treatments control (con., N = 5), energy restriction diet (ER, N=6) or protein restriction diet (PR, N= 6). Wethers assigned to control treatment received the same diet that was fed during the adjustment period. Diets restricted in energy or protein were formulated to be first limited in either net energy (NE) or metabolizable protein (MP). Metabolizable protein of food stuffs were estimated using calculation described by Burroughs et al., (1974), while energy values of food stuffs were based on NRC (1985) estimates. Diet composition, dry matter, metabolizable protein and net energy of the diets are presented in Table 1. This feeding design was continued for five weeks after which the restricted animals were switched back to the control diet. Blood samples collection scheme was reperformed at the end week from feeding the restricted diet, at the end of five weeks on restricted diets and at the end of one week repletion. Feed samples were taken twice weekly for determination of dry matter (DM). Total weekly DM intake was calculated. The wethers were weighed at weekly intervals during the experimental period.

Table 1. Diet composition and dry matter(DM), net energy(NE) and metabolizable protein (MP) of control (cont.), low energy (ER) and low protein (PR) diets.

Ingredient	Cont.	ER	PR
	% on	dry matter	basis
Alfalfa pellets	45.800		
Corn	45.800		
Ensiled corn cobs		89.590	83.430
Urea			2.026
Alfiet			13.507
Decalcium phosphate		0.680	0.680
Blood meal	2.140	3.300	
Corn gluten meal	2.920	6.080	
Molasses	3.000		
Salt	0.300	0.300	0.300
Sheep trace minerals	0.030	0.030	0.030
Selenium premix	0.015	0.015	0.015
Vitamin premix	0.010	0.010	0.010
DM %	88.270	54.520	52.500
NE, Mcal/Kg	1.060	0.570	1.150
MP, g/kg DM	84.450	82.000	41.000

- Control diet was formulated to maintain body weight of animals at maintenance requirements. Metabolizable protein was calculated using Burroughs system (A.S. Leaflet R190, 1974).
- The maintenance requirements of 30 kg body weight lambs are 0.718 Mcal/day and 112 g/day for net energy and metabolizable protein, respectively (NRC,1985).

Plasma urea was measured according to Marsh et al., (1965) technique, while plasma glucose was determined by a colorimetric assay using the method concentration, mean baseline, pluse frequency and pulse amplitude of GH secretion were identified by a modified version of the pulsar algorithmic method described by Merriam and Watcher (1985). Mean concentration of GH for each bleeding period was calculated by averaging the 25 samples in the 6h blood collection period. Frequency of GH pulses was calculated as the number of pulses detected in each blood collection period. Amplitude of GH pulses was the average height of all pulses detected in each period.

Data were analysed as a randomized complete block design. Analysis of variance procedure (SAS, 1985) was employed to evaluate the effects of feed restriction and realimentation on body weight changes, dry matter intake, plasma glucose, plasma urea, plasma growth hormone concentrations and pulsatile characteristics of GH. Linear contrasts were used to compare least square means for all variables previously indicated.

RESULTS AND DISCUSSION

1- Body weight:

Results in Table 2 revealed that after one week of restriction (short-term restriction), it can be observed that restriction of energy or protein resulted in a body weight loss, while wethers on control feeding achieved a weight gain. Differences in body weight between energy and protein restricted groups were not significant (Table, 2), but differences in body weight among control and restricted treatments were significant ($P<0.01$). The 7 days period of restriction in the present study were not sufficiently long to induce differences in body weight loss between ER and PR groups.

After five weeks of restriction (long-term restriction) differences in body weight between ER and PR animals and between restricted and control feeding groups were significant ($P<0.01$). Body weight loss of wethers on PR diet was greater ($P<0.01$) than those on ER diet. The greater body weight loss of wethers on PR may be due to the losses of protein, fat and water from the body, while ER with sufficient dietary protein may lead to losses of fat and water only. This explanation is in accordance with that of Drouillard (1989). He found that lambs restricted in protein intake lost protein. fat and water, while the protein mass in the energy restricted water, while the protein mass in the energy restricted lambs was unchanged during 42 days restriction, but losses of fat and water was noted. Another study by Fattet et al., (1984) indicated that adipose tissue in energy-restricted lambs was mobilized to provide energy for accretion of lean tissue when sufficient dietary protein was available.

Table 2. Body weight changes (kg), dry matter intake (g/d), plasma urea (g/dl) and plasma glucose (mg/dl) of wethers fed energy or metabolizable protein restricted diets.

	Body weight* kg	Dry matter intake (g/d)	Plasma glucose (mg/dl)	Plasma urea (mg/dl)
Period 1				
	44.27±1.93	1365±141	44.80±0.61	24.54±1.1
	45.51±1.76	1581±129	44.58±0.55	24.73±1.0
	45.36±1.76	1290±129	47.88±0.55	24.85±1.0
Period 2				
Con	46.99±1.93a	2001±141a	46.00±0.61b	24.90±1.1b
ER	42.49±1.76b	496±129b	75.20±0.55a	29.00±1.0a
PR	42.03±1.76b	243±129b	48.40±0.55b	4.90±1.0c
Period 3				
Con	56.25±1.93a	1896±141a	47.40±0.61b	25.30±1.1b
ER	39.84±1.76b	664±129b	78.60±0.55a	29.20±1.0a
PR	31.53±1.76b	567±129b	49.00±0.55b	5.10±1.0c
Period 4				
Con	62.69±1.93a	2364±141a	48.70±0.61b	22.90±1.1b
ER	48.91±1.76b	1543±129b	78.20±0.55a	30.80±1.0a
PR	43.77±1.76b	1670±129b	48.70±0.55b	4.10±1.0c

Con= control, ER=Energy restricted, PR=Protien restricted. Period 1= 6 weeks unrestrictedd regimen, Period 2= 1 week restricted regimen, Period 3= 5 weeks restricted regimen, Period 4= 1 week realimentation.
a,b,c: Means within columns (in the same period) having different superscripts are significantly different ($P<0.01$).
* Initial body weight of wethers = 30 kg.
Body weight: Measurements was averaged at the end of each period

During a one week realimentation period, differences in body weight between control and restricted groups (ER and PR) were significant (P<0.01). However the body weight of all animals in this period was clearly improved.

2- Dry matter intake (DMI):

Energy or protein restriction caused significant (P<0.01) reductions in DMI during short or long-term restriction. The depressions in DMI values in short-term restriction period were 75 and 88%, while the depression values after long-term restriction were 65 and 70% for ER and PR groups, respectively. Differences in DMI between energy or protein restricted groups were not significant in both short and long-term restriction periods. Also the difference during the realimentation period between ER and PR groups was not significant. These results are in agreement with those previously reported by Drouillard (1989) of the numerous studies conducted., it has been shown that feed intake may be increased (Abd Alla et al, 1988) or decreased (Foot and Tullough, 1977; Murray and Slezacek, 1980) as a result of previous feed restriction. Factors that determine compensatory feed intake have not been clearly identified, but it may be related to change in gastrointestinal capacity and/or altered satiety mechanisms.

3- Plasma glucose and urea concentrations:

During short and long-term restriction, the mean of plasma glucose concentrations of wethers fed energy-restricted diet was significantly higher (P<0.01) than that of control, while protein restricted diet did not induce significant alteration in plasma glucose concentration in comparison with the control diet (Table, 2). Differences in plasma glucose concentrations between ER and PR fed wethers were significant (P<0.01). Increased plasma glucose of wethers fed ER diet compared to wethers fed control diet are in agreement with the results of McNiven (1984). He showed that sheep maintained on negative energy balance maintained the blood glucose level by reducing tissue utilization or stimulating glycogenolysis for the utilization of first tissues or organs and levelling a higher rate of recycling glucose than did sheep given food at or above maintenance. The relatively similar plasma glucose concentration in wethers fed PR ration compared to wethers fed control diet are in consistence with the results of Waghorn (1987). He indicated that low intake of dietary protein did not cause a decline in plasma glucose concentration. After one week of realimentation (period 4) the plasma glucose concentration showed the same picture observed during the restriction period (period 3).

Short or long-term restriction had a significant effect on plasma urea concentration. Mean concentration of plasma urea of wethers fed unrestricted diet (control) was higher ($P<0.01$) than that of those fed PR diet, but it was lower ($p<0.01$) than in case of those fed ER diet (Table, 2). Differences in plasma urea concentrations between ER and PR wethers were significant ($P<0.01$). On the other hand concentration of plasma urea was positively related to protein intake in short and long-term restriction regime. These results showed that plasma urea values were inversely related to energy intake. Similar observation have been reported in heifers fed two levels of metabolizable energy by Roberson et al., (1991). Also, these findings are in consistence with results of Bull, (1978). This result is quite acceptable as urea is one of the end products of protein metabolism.

After one week of realimentation, the trend of plasma urea concentration did not return to the normal (control) level. Biddle et al., (1975) indicated that plasma urea declined in growing animals during protein depletion, and increased up to a peak after 3 weeks of repletion. This may explain that one week realimentation to the control diet was insufficient to refill protein storages and rebalance protein metabolism pathways. In the present study, it is shown that changes in plasma concentrations of urea and glucose reflect possible metabolic alterations associated with ER and PR at different periods of restriction. The significant ($P<0.01$) increase of plasma glucose and urea during both short and long-term ER are logically due to gluconeogenesis, i.e. the formation of glucose from protein as response to dietary ER stress (Kotby, 1967).

4- Growth hormone (GH):

Nutritional status and food intake markedly influence GH secretion, and chronic undernutrition was reported to result in increased GH concentrations in sheep (Foster et al. 1989), Thomas et al. 1990). In the present study energy or protein restriction was accompanied with increase in mean concentration, mean baseline level and mean pulse amplitude of GH (ng/ml) in plasma. These results can be attributed to the major role of GH in controlling and regulating anabolic processes, that take place at various rates depending upon the productive potential of the animal and the availability of required absorbed nutrients. Increasing plasma GH concentrations may help to increase concentrations of energy governing anabolic reactions leading to more efficient utilization of absorbed feed nutrients, specially under feed restriction circumstances. Plasma GH concentrations were significantly higher in sheep given low protein diets than in those given high protein diets(Waghorn, 1987). A recent study by Thomas et al., (1991) showed that restricted feeding of ewes resulted in a significant increase in mean plasma GH concentrations and mean GH pulse amplitude.

In this experiment mean concentration, mean baseline and mean pulse amplitude of GH were significantly higher when PR diet was fed in comparison with ER diets. This result is in agreement with Waghorn (1987) and Chilliard (1988) findings. The closer relationship between GH and protein compared to GH and lipid or energy is probably due to the need for closer control of GH in protein sparing (low dietary protein level) or protein accretion (high dietary protein level) than in energy or lipid mobilization (Chilliard, 1988). This explanation is confirmed on the light of GH concentration profile during realimentation period. This period seems to be short (one week), but obvious change in GH concentration values were obtained. Realimentation of wethers previously restricted in protein resulted in a significant ($P<0.01$) decrease in mean concentration , mean pulse amplitude of GH, while realimentation of wethers previously restricted in energy showed insignificant changes in GH parameters (Table 3).

Table 3. Mean plasma growth hormone (GH) concentrations and pulsatile characteristics in wethers fed energy and metabolizable protein restricted diets.

	Mean GH (ng/ml)	Mean baseline (ng/ml)	Pulse amplitude (ng/ml)	Pulse frequency (pulse/6h)
Period 1				
	2.170±1.4	1.74±0.78	2.580±2.15	1.60±0.44
	2.520±1.3	2.26±0.71	2.480±2.78	1.67±0.57
	2.190±1.3	1.98±0.71	2.340±2.41	1.50±1.50
Period 2				
Con	2.304±1.3c	1.43±0.78c	4.430±2.41c	2.00±0.50
ER	4.715±1.2b	3.24±0.71b	6.930±1.97b	2.50±0.40
PR	8.672±1.2a	5.84±0.71a	8.320±1.97a	2.70±0.40
Period 3				
Con	1.442±1.3c	0.95±0.78c	3.430±2.41c	2.50±0.50
ER	3.187±1.2b	2.21±0.71b	4.550±1.97b	2.30±0.40
PR	10.550±1.2a	5.53±0.71a	14.770±1.97a	3.30±0.40
Period 4				
Con	1.486±1.3b	1.26±0.78b	2.085±2.41c	1.80±0.50
ER	3.267±1.2a	1.99±0.71a	6.962±1.92b	1.80±0.40
PR	4.188±1.2a	2.98±0.71a	10.230±1.92a	3.10±0.40

Con=Control, ER=Energy restricted, PR=Protein restricted. Period 1= 6 weeks unrestricted regimen.
Period 2= 1 week restricted regimen.
Period 3= 5 weeks restricted regimen.
Period 4= 1 week realimentation.
a,b,c : Means within columns (in the same period) having different superscripts are significantly different (P<0.01).

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تأثير تحديد الطاقة أو البروتين فى علائق الاغنام على بعض الاستجابات الفسيولوجية فى الاغنام ٢- وزن الجسم وتركيز كل من الجلوكوز واليوريا وهرمون النمو فى البلازما

محمد عبد الفتاح احمد البارودى - فوزى محمود رحيم الفيل - تيرى كلوبفنتين[١]- جيم كندر[١]- عبد المعطى خيرى ابراهيم - سمير توفيق محمد فهمى
قسم الانتاج الحيوانى - كلية الزراعة - جامعة المنيا
[١] قسم الانتاج الحيوانى-جامعة نيبرا سكا-لنكولن- الولايات المتحدة الامريكية٠

اجرى هذا البحث على ١٧ كبش مخصى خليط عمرها ٧ شهور غذيت لحد الشبع على عليقة قياسية لمدة ستة اسابيع (فترة معايرة)٠ وبعد هذه الفترة تم توزيعها عشوائيا لثلاثةمجاميع مجموعة قياسية وعددها خمسة والمجموعة الثانية وعددها سته افراد غذيت على عليقة محددة فى الطاقة اما المجموعة الثالثة وعددها ستة غذيت على عليقة محددة فى البروتين واستمرت فترة التغذية هذه لمدة اسبوع (فترة تحديد قصيرة)٠ ثم استمر نفس النظام الغذائى لفترة اخرى لمدة ٥ اسابيع (فترة تحديد طويلة)٠ الكباش التى غذيت على عليقة محددة فى الطاقة أو البروتين غذيت على عليقة قياسية لمدة اسبوع (فترة اعادة)٠ تم اخذ عينات دم من كل الحيوانات خلال الفترات المختلفة لتقدير تركيز هرمون النمو والجلوكوز واليوريا٠ وزن الجسم وكذلك كمية المادة الجافةالمأكولة تم تقديرها اسبوعيا٠

اوضحت النتائج ان تحديد الغذاء لفترة قصيرة أو طويلة ادى الى انخفاض معنوى (١٪) فى كل وزن الجسم والمادة الجافة المأكولة بالمقارنة بالمجموعة القياسية كما حدث انخفاض فى متوسط تركيز جلوكوز البلازما بإحتمال ١٪ للافراد التى غذيت على عليقة قياسية عن التى غذيت على عليقة منخفضة فى الطاقة ولكنها تساوت فى نفس الوقت مع الحيوانات التى غذيت على عليقة منخفضة فى البروتين٠ على عكس ذلك كان تركيز اليوريا فى البلازما مرتفعا باحتمال ١٪ فى الحيوانات التى غذيت على عليقة قياسية بالمقارنة بالمجموعة التى غذيت على عليقة منخفضة فى البروتين وفى نفس الوقت كان تركيزه منخفضا بمقارنته بالمجموعة التى غذيت على عليقة منخفضة فى الطاقة ٠ ادى تحديد الطاقة والبروتين فى العليقة الى زيادة معنوية ١٪ فى متوسط تركيز هرمون النمو عند مقارنة تلك المجموعات بالمجموعة القياسية كما ادى تحديد البروتين الى زيادة معنوية فى تركيزهرمون النمو عند مقارنة تلك المجموعة بالمجموعة المحددة فى الطاقة٠ عند العودة الى التغذية على العليقة القياسية لمدة اسبوع لم يسبب اختلافات معنوية فى تركيز الجلوكوز أو تركيز اليوريا فى البلازما ٠

العودة الى التغذية على العليقة القياسية لمجموعة الحيوانات التى سبق تغذيتها على عليقة محددة فى البروتين ادى الى نقص معنوى ١٪ فى تركيز هرمون النمو بينما العودة الى التغذية على العليقة القياسية لمجموعة الحيوانات التى سبق تغذيتها على عليقة محددة فى كمية الطاقة لم يؤدى الى تغير فى تركيز هرمون النمو.