Estimation of energy status in non-pregnant and pregnant prolific ewes according to plasma levels of non-esterified fatty acids

Landau S., Madar Z., Nitsan Z., Zoref Z.

in

Purroy A. (ed.).
Etat corporel des brebis et chèvres

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 13

1992
pages 57-62

Estimation of energy status in non-pregnant and pregnant prolific ewes according to plasma levels of non-esterified fatty acids

S. LANDAU*
Z. NITSAN**
Z. ZOREF**
Z. MADAR*

*DEPARTMENT OF BIOCHEMISTRY AND HUMAN NUTRITION, FACULTY OF AGRICULTURE, THE HEBREW UNIVERSITY OF JERUSALEM, POB 12,
REHOVOT 76100, (ISRAEL)

**INSTITUTE OF ANIMAL SCIENCE, AGRICULTURAL RESEARCH ORGANIZATION, THE VOLCANI CENTER, POB 6, BET DAGAN 50250, (ISRAEL)

SUMMARY- The relationship between plasma concentration of non-esterified fatty acids (NEFA) and energy balance in prolific ewes fed daily two meals was studied. The metabolizable energy (ME) requirement was calculated according to body-weight of ewes (BW) and total weight of their fetuses (TWF). It was possible to predict accurately the energy balance of non-pregnant and pregnant ewes when their ME intake was known, with the covariates plasma NEFA, days of pregnancy (DP), BW and TWF (r= 0.98, ESD= 80 kcal ME/d). The accuracy of prediction was also high when TWF was not used as covariate (r= 0.97, ESD= 93 kcal ME/d). In pregnant ewes, regression of body condition score and DP, with TWF (r= 0.78, ESD= 355 kcal ME/d) or without TWF(r= 0.70, ESD= 384 kcal ME/d), as covariates, on energy balance was calculated. NEFA did not improve the prediction of energy balance when ME intake was not included in the regression. A significant decrease in the plasma NEFA was found, 60 minutes after a meal in the late-pregnancy energy-restricted ewes. In non-pregnant and mid-pregnant ewes, this decrease was not significant. It seems that NEFA values should be used carefully as a predictor of energy balance in pregnant ewes until more information on interactions between NEFA and body condition, quality and quantity of feed is available.

RESUME - On a étudié la relation entre la teneur en acides gras non-estérifiés (AGNE) dans le plasma sanguin et le bilan énergétique de brebis recevant deux repas journaliers. Les besoins énergétiques ont été calculés à partir des poids vifs des brebis et de leurs foetus. On a pu déterminer de façon exacte le bilan énergétique quand le niveau d'ingestion était connu, en utilisant comme co-variables la teneur en AGNE, le stade de gestation, avec (r=0,98; ESD= 80 kcal EM/j) ou sans le poids des foetus (r= 0,97; ESD= 93 kcal EM/j). Chez les brebis gestantes, on a pu calculer une régression du bilan énergétique et de la note d'état corporel, avec (r= 0,78; ESD= 355 kcal EM/j) ou sans le poids des foetus (r= 0,70; ESD= 384 kcal EM/j) comme co-variable. La teneur en AGNE n'a pas permis d'améliorer la prévision du bilan énergétique quand le niveau d'ingestion n'a pas été inclus dans la régression. La teneur en AGNE plasmatique a diminué de façon significative 60 minutes après un repas chez les brebis en fin de gestation et limitées en énergie, alors que chez les brebis vides ou en début de gestation cette tendance n'a pas été significative. L'utilisation de la teneur en AGNE pour prédire le bilan énergétique de brebis prolifiques gestantes doit se faire avec prudence, tant que les rapports entre la teneur en AGNE et l'état corporel, la qualité et la quantité de la ration, sont insuffisamment quantifiés.

Introduction

The logarithm of plasma concentration of non-esterified fatty acids (NEFA) is known to be linearly and negatively correlated to feed intake of ewes at maintenance (Russell and Doney, 1969) and during pregnancy (Russell *et al.*, 1967; Stern, 1973). High level of plasma NEFA indicates increased lipolysis consequent to a deficit in energy intake (Russell and Doney, 1969). The level of NEFA in plasma was shown to be a very useful parameter to assess energy balance in dairy goats, with milk production and body weight as co-predictors (R=0.96, Giger and Sauvant, 1982). In pregnant ewes, NEFA levels were poorly correlated with weight of litter (R=-0.43; Stern, 1973) and with energy intake (R=-0.52 to -0.76; Stern, 1973). No attempt has been made to use NEFA as a quantitative predictor of energy balance in prolific ewes. Plasma NEFA reach a maximum concentration before meals and decrease during the first hours after a meal. The amplitude and the frequency of the diurnal NEFA curve are related to the number of meals and to the total energy intake (Bassett, 1974). Although blood for NEFA determination should be sampled before meals, no study has shown if deviations from that procedure have negative effects on the evaluation of energy status in ewes. Such information is important if NEFA is to be used in field studies, where the time of sampling and the time elapsed from last meal cannot always be standardized.

The aim of the present study was to evaluate the contribution of plasma NEFA to the prediction of energy balance in prolific sheep and to provide information on plasma NEFA kinetics.

Materials and methods

Data from 20 non-pregnant (NP) and 13 pregnant Booroola x Assaf and Finn x German Merino ewes were compiled. The pregnant ewes were at mid pregnancy (67 + 0.1 days, n=3, MP) or late pregnancy (116.1 + 0.4 days, n=10, LP). The number of fetuses (mean ± SE) was 2.2 ± 0.12 and 2.1 ± 0.07 in the MP and LP ewes, respectively. All ewes were fed chopped alfalfa hay and corn grain mixtures (1:1) in two daily meals at 12 h intervals. They were housed in individual metabolic cages. Energy content of feeds was established in an in vivo digestibility trial (hay, 1.73 Mcal ME/kg; corn, 3.34 Mcal ME/kg). Daily individual intake of feedstuffs was recorded. The diets were calculated for each sheep according to body-weight (BW), since genotype variation yielded differences between animals. The daily ME requirement for maintenance (M) was assumed to be 100 kcal/kg $BW^{0.75}$, since the same sheep showed no changes in BW and body composition, as assessed by urea dilution when fed the same feeds at the above amount in previous experiments (Landau ,Madar, Zoref and Nitsan, unpublished). In order to allow comparison between ewes of different frame, ME intake (MEI) was expressed as maintenance levels. Ewes of all groups were fed at maintenance level (1 M) or 1.25 M levels, and two sheep of the LP group were given also 1.8 M. All ewes were fitted with permanent cannulas in their jugular vein one week before the sampling day. During this week they were accustomed to the experimental diet and to blood sampling. Time of sampling was before (t_0)and 60 (t_{60}), 120 (t_{120}) and 180 (t_{180}) minutes after food was given. All sheep were weighed and body-scored according to a scale of 0-5 before every sampling period. NEFA were analysed by using a modified Dole procedure, as described by Barash and Akov (1987), based on the linking of 63Ni to the extracted acids and reading radioactivity in a ß-counter. The NEFA kinetics experiment included five assays from NP and ten assays from each group of pregnant sheep, fed 1 M or 1.25 M. All pregnant ewes were aborted after the last sampling period, and the fetuses were weighed. The total weight of fetuses (TWF, grams, mean + SE) was 297 ± 18.1 and 3357 ± 91 in the MP and LP ewes. Energy requirement for pregnancy was established according to the procedure described by Theriez *et al.*(1987), taking into account litter size, and assuming that ME from diets in the present study is utilised at the rate (Kc) of 0.19 for deposition into the uterus and fetuses (Robinson *et al.*, 1980). The same calculation was made using a Kc value of 0.14, which is the mean coefficient suggested by NRC (1985). Energy balance was calculated as energy requirement for maintenance plus pregnancy minus the actual energy intake. The calculations of energy balance included data from 20 NP and 13 pregnant ewes. Prediction equations were evaluated by the coefficient of correlation and the standard deviation error (SDE= √MSE)

Results

Relationships among NEFA, the weight of fetuses and the calculated energy balance

Plasma levels of NEFA (µeq/l, sampled at t_0) were correlated to the exponential of fetus weight (TFW, kg), as follows:

NEFA = 6.738 (± 1.12) * exp(TFW) + 250.2 (SDE=174.8, n=33, r=0.73) in the entire data-base, or:

NEFA = 6.838 (± 1.74) * exp(TFW) + 242.9 (SDE=223, n=13, r=0.73) in the pregnant ewes only. Correlations between NEFA from blood sampled after t_0 and exp(TWF) were not significant.

The Ln(NEFA) and calculated energy balance were poorly correlated (r=-0.57) in the NP ewes. In the pregnant ewes, the coefficient of correlation was lower (r=-0.46, p<0.05, SDE=452 kcal ME/d) when Kc was assumed to be 0.19, than when Kc was assumed to be 0.14 (r=-0.67, p<0.02, SDE=648 kcal ME/d).

The calculated energy balance was well predicted when the individual energy intake, expressed as maintenance levels was one of the predictors both in the whole data-base (Table 1) and in data from pregnant ewes only (Table 2). Including the total weight of fetuses (TWF) as a covariate in the regression improved the prediction of CEB. However, it was possible to fit equations with NEFA, DP, BW and MEI to the same level of accuracy without including TWF in the regression. In pregnant ewes, NEFA, MEI and BW accounted for 96% of the variation in energy balance. Equations could be fitted to predict CEB without including energy intake as a covariate for pregnant ewes only. The transformation of NEFA to Ln(NEFA) did not improve the significance of this parameter in either of the two groups of sheep. Using NEFA values from blood sampled after meals reduced the predictive value of NEFA for CEB estimation.

Postprandial NEFA kinetics

Plasma NEFA levels before, and 60, 120 and 180 minutes after food was given are shown in Table 3. Plasma NEFA levels of NP ewes tended to decrease ($p<0.20$) between 60 and 120 minutes after the meal, and to increase thereafter. In the MP ewes, no decrease was noted between t_0 and t_{120}, and even the subsequent decrease, from t_{120} to t_{180}, was not significant. In the LP ewes, no difference was noted between t_0 and t_{60}, whereas values at t_{120} and t_{180} were significantly lower than at t_0 and t_{60} ($p<0.01$). The NEFA levels were higher in LP than in MP ewes: 421 and 192 µeq/l, respectively. When all data were pooled, only the decrease of NEFA from t_0 to t_{180} was significant. No significant correlation was found between NEFA at t_0 and its values for the other sampling times.

Discussion

It is assumed that energy balance can be predicted well when energy intake and the amount of productive energy (milk, growth or pregnancy) are known. When production is low and ME intake is sufficient, there is probably no net transfer of energy source substances from body depots into product. When energy intake is not sufficient to meet the energy demand, i.e., in high-producing or feed-restricted animals, the extent of lipolysis, determined according to NEFA level and to the body energetic reserve, by body-scoring, should help to determine the energy status. In addition, knowledge of the MEI is theoretically not essential, since MEI and the NEFA levels in plasma are correlated (Russell and Doney, 1969; Stern, 1973). However, this was not the case in our study, since only half of the variation in energy balance of the pregnant ewes could be predicted when the energy intake was not included in the multivariate equations of regression. Moreover, NEFA was not an effective covariate when the energy intake was not included in the regression. Better prediction was achieved when NEFA was replaced by the variable days of pregnancy and body score. In the whole population of sheep (including the non-pregnant ewes), MEI was crucial for the prediction of energy balance. Stern (1973) found in two experiments that the coefficients of correlation between energy intake, corrected for fetus weight and the level of plasma NEFA in ewes at late pregnancy were relatively low (-0.52; -0.76) and nonsignificant. In the same study, 500 µeq/l increase in NEFA was calculated to be equivalent to a 280 kcal deficit of ME. Russell *et al.* (1967) showed significant correlations between Ln(NEFA) and food intake, but did not mention any quantitative relation between Ln(NEFA) and the weight of fetuses. Therefore, no energy balance can be calculated from their data.

It has been shown recently that the metabolic rate of ovine fetuses and of ewe-mothers is very similar (Young *et al.,* 1989). Also, estimates of the chemical composition of fetuses, fetal and uterus growth are well agreed between authors (Theriez *et al.,* 1987). Therefore, the main source of error in the calculation of energy balance in pregnant ewes is Kc, which also affects the accuracy of NEFA as a predictor of energy balance. Although the effect of dietary energy on Kc is known, it is not clear whether and how the quality of concentrates given to ewes at late pregnancy affects this coefficient.

If we assume that the calculation of the energy balance is correct, then the NEFA level is a poor predictor of this balance (Table 2). The concentration of NEFA in plasma expresses an equilibrium between lipolysis and lipogenesis. It should be recalled that many factors affect the utilisation of NEFA by body tissues. The ratio of NEFA to insulin is dependent on the ruminal fermentability of starch. This ratio was more than 300% higher in ewes fed whole corn than in their counterparts fed an isoenergetic ration with extruded corn. The levels of NEFA in ewes fed the whole grain were higher by 180 µeq/l than in those fed extruded corn, both at maintenance level (Landau, Madar, Zoref and Nitsan, unpublished). Insulin and NEFA levels in plasma were found to be negatively and significantly correlated (r=-0.64 and r=-0.81, in two experiments; Bassett, 1974). Similar results were reported by McNiven (1984), who noted, in addition, that thin and fat sheep adjust to an energy shortage very differently: when the MEI was reduced from 4 to 1.6 Mcal/d, the ratio of NEFA to insulin increased by 70% in thin, but decreased by 20% in fat ewes. This finding shows that in order to predict energy balance, NEFA, or another parameter associated with the lipolytic- lipogenic balance and predictors of body composition, such as a space of dilution or body condition score should be available. Other factors affecting the preprandial variation in plasma NEFA levels, such as the number of daily meals, the amount and quality of food offered, may be needed. Since prolific ewes are generally fed whole or processed grain at the end of pregnancy, and since the variation in body condition of ewes at that stage is generally high, the additive influence of all these factors may explain why plasma NEFA levels cannot be used as the sole predictor, and why their contribution in multivariate regression equations on the energy balance is limited.

In the present study no difference was noted between preprandial values and NEFA levels 60 minutes after a meal. Within sub-classes of animals, time of sampling significantly affected the results only from ewes in late pregnancy. It may be assumed that sampling time affects NEFA levels more when the energy supply to animals is inadequate to provide for requirements, than when all requirements are met. In another study (Bassett, 1974), NEFA levels were markedly reduced after meals in ewes given an energy allowance similar to that in our study. A possible explanation for this discrepancy is the use of oats as feed in the study by Bassett and corn grain in our study.

In conclusion, many physiological parameters may affect the plasma NEFA levels in pregnant ewes, especially when fed concentrates. In practical situations, where the individual energy intake is unknown, body condition score is as useful as NEFA in predicting energy balance, especially when the exact pregnancy status is known. If the energy intake is known, NEFA level in plasma is a more valuable predictor of energy balance when blood is sampled before a meal.

References

BARASH, H. and AKOV, S. (1987). Improved 63Ni radiochemical assay of free fatty acids in plasma. Clin. Chem., *33:*176-179

BASSETT, J. M. (1974). Diurnal patterns of plasma insulin, growth hormone, corticosteroids and metabolite concentrations in fed and fasted sheep. Aust. J. Biol. Sci., *27:*167-181.

GIGER, S. and SAUVANT, D. (1982). Utilisation des acides gras non-esterifiés plasmatiques pour la prévision des bilans et besoins énergétiques de la chèvre en lactation. Ann. Zootech., *31*:149-160.

MCNIVEN, M. A. (1984). Effect of body fatness on blood metabolites and insulin insensitivity in adult sheep. Can. J. Anim. Sci., *64:*1049-1053.

NRC. (1985). Nutrient Requirements of Sheep. The National Academy of Sciences, Washington, DC (USA).

ROBINSON, J. J., MCDONALD, I., FRASER, C. and CROFTS, R. M. J. (1980). Studies on reproduction in prolific ewes. 6-The efficiency of energy utilisation for conceptus growth. J. Agric. Sci. Camb., *94:*331-338.

RUSSELL, A. J. F. and DONEY, J. M. (1969). Observations on the use of plasma free fatty acid concentrations in the determination of maintenance requirements of sheep. J. Agric. Sci. Camb.,*72:*59-63.

RUSSELL, A. J. F., DONEY, J. M. and REID, R. L. (1967). The use of biochemical parameters in controlling nutritional state inpregnant ewes and the effect of undernourishment during pregnancy on lamb birth weight. J. Agric. Sci. Camb., *68:*351-358.

STERN, D. (1973). Effects of different nutritional regimes during pregnancy on some metabolic responses of dairy ewes before and after parturition. Ph. D. thesis submitted to the Senate of the Hebrew University of Jerusalem, Israel.

THERIEZ, M., BOCQUIER, F. and BRELURUT, A. (1987). Recommandations alimentaires pour les brebis à l'entretien et en gestation. Bull. Tech. C.R.Z.V. Theix INRA, *70:*185-197.

YOUNG, B. A., BELL, A. W. and HARDIN, R. T. (1989). Mass specific metabolic rate of sheep from fetal life to maturity. Proc. of the 11th Symposium on "Energy Metabolism of Farm Animals", EAAP publication no. 44, ed. by Y. van der Honig and W.H. Close (Pudoc publ., Wageningen, The Netherlands).

Table 1. Multivariate regression of plasma NEFA (μeq/l), days of pregnancy (DP), body condition score (BCS), body weight (BW, kg), total weight of fetuses (TWF, g) and metabolisable energy intake (MEI, levels of maintenance) on the calculated energy balance (CEB, kcal ME/d) in all ewes.

Intercept	NEFA	DP	BW	BCS	TWF	MEI	r	SDE
-2103	-0.011 (0.17)	-0.099 (0.20)	4.27 (0.001)		-0.079 (0.01)	1875 (0.001)	0.98 (0.001)	80.4
-2185	-0.248 (0.01)	-3.12 (0.001)	4.40 (0.01)			1984 (0.001)	0.97 (0.001)	92.6
-1411	-0.482 (0.001)					1478 (0.001)	0.91 (0.001)	147
-2079				185 (0.01)		1430 (0.001)	0.89 (0.001)	162

Numbers in parentheses are probability values.

Table 2. Multivariate regression of plasma NEFA (µeq/l), days of pregnancy (DP), body condition score (BCS), body weight (BW, kg), total weight of fetuses (TWF, g) and metabolisable energy intake (MEI, levels of maintenance) on the calculated energy balance (CEB, kcal ME/d) in pregnant ewes.

Intercept	NEFA	DP	BW	BCS	TWF	MEI	r	SDE
-2708	-0.261 (0.03)	-2.62 (0.18)	8.43 (0.02)			2182 (0.001)	0.98 (0.001)	113
-2927	-0.300 (0.02)		9.08 (0.01)			2126 (0.001)	0.98 (0.001)	119
-2556		18.6 (0.04)		499 (0.07)	-0.19 (0.13)		0.78 (0.03)	355
-2704		9.37 (0.16)		717 (0.001)			0.70 (0.04)	384

Numbers in parentheses are probability values.

Table 3. Plasma NEFA levels (µeq/l) of non-pregnant ewes (NP, n=5), and of ewes at mid (MP, n=10) and late (LP, n=10) pregnancy before and 60, 120 and 180 minutes after a meal (mean ± SE)

t (minutes)	NP	MP	LP
0	327.4 ± 36.5	218.9 ± 10.0	525.9 ± 33.0[a]
60	278.6 ± 62.1	224.1 ± 16.0	582.6 ± 31.8[a]
120	148.4 ± 16.2	201.6 ± 21.3	308.8 ± 13.6[b]
180	283.8 ± 82.4	125.4 ± 6.3	305.7 ± 22.8[b]
Main effects	Pregnancy P < 0.001 Time P < 0.03 P x T NS		

For LP , means with different superscript significantly differ (p<0.05)