

THE EFFECT OF β -ADRENERGIC AGONIST INJECTION AND FEEDING ENERGY LEVEL ON BLOOD PLASMA NON-ESTERIFIED FATTY ACIDS IN THE LACTATING DAIRY EWE

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ABSTRACT

The lipolytic potential of adipose tissue can be estimated through the plasma NEFA response to β adrenergic. In well-fed lactating ewes, the NEFA response to isoproterenol depended on body lipid mass, but not on energy balance. In underfed ewes however, the NEFA response depended upon energy balance but not on body lipid mass. Most in vitro studies revealed that stimulated lipolytic activity depended on adipose tissue cellularity, whatever the energy status of donor animals. These findings suggest that in vitro results might be incomplete in the case of underfed animals.

(Key words : Lactating ewe, Underfed, Energy balance, Milk production, NEFA, Hormone, Isoprenalin).

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**PENGARUH INJEKSI β -ADRENERGIC AGONIST DAN LEVEL KONSUMSI
ENERGI PADA DOMBA PERAH LAKTASI TERHADAP KADAR
NON-ESTERIFIED FATTY ACIDS PLASMA DARAH**

INTISARI

Potensi lipolisis jaringan lemak tubuh dapat diestimasi dari kadar Asam Lemak Bebas tidak ter-esterifikasi (NEFA: non esterified fatty acids) plasma darah sebagai akibat dari injeksi hormon isoprenalin (β -Adrenergic Agonist). Domba yang kelebihan energi pakan, potensi lipolisisnya tergantung pada tingkat cadangan lemak tubuh tetapi tidak tergantung pada tingkat keseimbangan energi, sedangkan ternak yang kekurangan energi pakan, potensi lipolisisnya tergantung pada tingkat keseimbangan energi bukan pada cadangan lemak tubuh. Hampir semua penelitian yang dilakukan sebelumnya secara *in vitro* menunjukkan bahwa aktivitas lipolisis akibat stimulasi injeksi hormon isoprenalin tergantung pada sensitivitas sel lemak tubuh dan tidak tergantung pada kondisi keseimbangan energi ternak donor. Hasil penelitian *in vivo* ini mengindikasikan bahwa hasil penelitian *in vitro* mungkin kurang lengkap ketika donor sel lemak tubuh diperoleh pada ternak yang kekurangan energi.

(Kata kunci : Domba laktasi, Kekurangan energi, Keseimbangan energi, NEFA, Hormon, Isoprenalin).

Introduction

The ability of animals to support an energy shortage during lactation mainly depends on the amount of non-esterified fatty acids (NEFA) released from adipose tissue (AT). This release depends on numerous factors including the mass of AT (i.e. body lipids: Cowan et al., 1982; Chilliard, 1992) and its responsiveness to lipolytic stimuli (Jaster and Wegner, 1981; McNamara, 1991). Among these lipolytic stimuli, catecholamines (epinephrine and norepinephrine) or syntetic (β -adrenergic compounds (isoproterenol, etc.) are particularly efficient. The lipolytic potential of AT can be estimated (from the NEFA released in the blood plasma by lipolytic agent, either *in vitro* in the culture medium of AT explants or, *in vivo*, in the plasma). The aim of this work was to evaluate *in vivo* the effects of energy balance and body lipid mass (BLIP) on the AT lipolytic potential of the lactating ewe.

Materials and Methods

Twenty-four, lactating, Lacaune dairy ewes, on day 49 of lactation at the start of the 49 days of lactation trial, were divided into two groups: High group and Low group according to the energy supply, for two successive periods (P1-P2) of 4 weeks. Ewes of the High group were fed with excess energy (104 to 111%) of their Total Energy Requirements (TER), whereas ewes in the Low group received 83 to 86% of their TER. Their theoretical protein requirements were fully fulfilled (100%). The feed was given at 08,00. The amount of diets, given were adjusted twice a week according to milk yield (consisted of rye-grass supplemented with concentrate (31% of total DMI)). From the first to second period, the feeding treatments were crossed-over (High-Low or Low-High). Milk production and its composition were measured twice a week. Body composition was estimated *in vivo* by deuterium oxide dilution technique (Bocquier and Theriez, 1984) at the beginning and the end of each period, allowing BLIP to be estimated.

The non-selective β -adrenergic agonist (isoproterenol; ISO) was injected (20,5 nmol/kg BW 0.75) at noon (13.30) into the jugular vein on day 24 (P1) and day 50 (P2). Blood samples were taken for plasma NEFA determination, five minutes before (NEFA-5) and ten minutes after (NEFA+10) the injections. This simplified procedure of blood sampling was chosen after several observations indicated that the NEFA level peaked at about ten minutes after the injection (Chilliard et al., 1994; Ferlay et al., 1996). Morning pre-feeding blood samples were also taken 6h (NEFA-360) before ISO challenge.

Results and Discussion

Underfeeding of ewes (Low vs. High diets) induced the decline of milk yield, the mobilization of BLIP, and the increase of NEFA. All are in good agreement with calculated energy balance (EBAL) (Table 1). The injection of ISO was followed by the increase of NEFA as reported previously in sheep (Basset, 1970) and cow (Blum et al., 1982; Gagliostro and Chilliard, 1991; Chilliard et al., 1994; Ferlay et al., 1996). The mean NEFA response to ISO (NEFA+10 minus NEFA-5) was significantly higher in underfed

ewes (+0.74 mM) than well-fed ewes (+0.45mM). Part of this response can be explained by the pre-injection concentration (NEFA-5) which was already higher (+0.15 mM) in the Low group (Table 1).

There was a positive inter-individual correlation between basal values (NEFA-5) and post-ISO (NEFA+10) levels ($r=+0.65$; $n=48$; $P>0.001$), i.e. between spontaneous and stimulated lipolysis. The correlation with NEFA+10 was even higher ($R=+0.79$; $P<0.001$) when considering pre-feeding morning NEFA levels (NEFA 360). This suggest that basal NEFA concentration (NEFA-5 or NEFA-360) may partly be related to the β -adrenergic component of the lipolytic activity of AT (Blum et al., 1982; Ferlay et al., 1996). NEFA+10 ($n=48$) was positively related to milk energy out put ($r=+0.38$), BLIP ($r=+0.30$) and EBAL ($r=-0.72$). In fact, multiple regression analyses revealed that NEFA +10 depended on animal nutritional status. Ewes of the High group, NEFA+10 was related to BLIP ($P<0.01$) and energy intake (EI; $P<0.01$), but not to EBAL, whereas, for the Low group ewes, NEFA+10 mainly depended on EBAL ($P<0.01$), rather than BLIP.

Table 1. Characteristics of dairy ewes subjected to two feeding levels: consequences on plasma non-esterified fatty acids (NEFA) ($n=12$ ewes per group).

Feeding level 1): Period 2):	Low		High		SEM
	P1	P2	P1	P2	
Body weight (kg)	65.4	66.9	72.6	65.4	1.4
Milk yield (l/d)	1.33 a	1.09 b	1.61 c	0.93 b	0.05
Net energy intake (UFL/d) ³	1.46 a	1.29 c	2.01 b	1.49 a	0.03
Energy balance (UFL/d) ³	-0.29 a	-0.25 a	+0.07 b	+0.08 b	0.02
Body lipids (kg) ⁴	15.4	17.6	17.3	12.8	0.8
Body lipid variation (g/d)	-95 a	-93 a	+8b	+16 b	8
NEFA-360 (mM) ⁵	0.533 a	0.369 b	0.180 c	0.157 c	0.021
NEFA-5 (mM) ⁵	0.257 a	0.229 a	0.114 b	0.090 b	0.016
NEFA+10 (mM) ⁵	1.138 a	0.841 b	0.653bc	0.465 c	0.033

1: Low: 85% and High 111% energy requirements. 2: two periods of 28 days each.

3: UFL = 7.11MJ Net Energy. 4: at the start of each period. 5: see text.

a b c: figure with different letters differ significantly ($P<0.05$). SEM : Standard Error Mean

Previous studies of *in vitro* on lactating ewe AT (Vernon and Finley, 1985) showed close relationship ($r=+0.96$) between stimulated lipolysis and adipose cell size, and this was high correlated to BLIP (Robelin et al., 1989). These *in vitro* results, with ewes presumably in positive EBAL, are in agreement with our *in vivo* results (High diets). For lactating dairy cows mostly in negative EBAL (Gagliostro and Chilliard, 1991), the *in vitro* adrenergic response depended on AT cellularity ($P<0.01$), but not on EBAL. However, *in vivo* response in the same cows was related to EBAL ($P<0.010$) but not cellularity. The latter results was in agreement with underfed lactating ewes results. Hence, it seems that *in vitro* studies on AT lipolytic response illustrated principally the differences in AT cellularity (or BLIP) whatever the energy balance of the donor animal. Our results show that this might be also true *in vivo* for animals having a positive EBAL, but not for underfed animals. In underfed animals, the *in vivo* approach is more likely to take into account other factors that are known to modulate the expression of lipolytic potential, such as endocrine control (Vernon, 1992; Chilliard et al., 1995).

Adrenergic challenge is also useful in explaining the differences in inter-individual adaptation strategies to underfeeding in the ewe. In the underfed ewes (Low diets), we observed that the relative variation in milk yield was negative correlated to NEFA+10 ($r=-0.51$; $P<0.01$, $n=23$): ability to sustain lactation was related to ability to mobilise BLIP. It would be interesting to study the effect of the milk genetic potential on the ability of AT to respond to adrenergic stimuli as it has been observed in dairy cows (McNamara, 1991).

Conclusions

In vivo estimation of AT lipolytic potential in the lactating ewe revealed that the roles of body lipid mass and energy balance depended on nutritional status. In underfed

animals, when lipolytic activity was of particular importance for animal survival or for maintenance of lactation, this lipolytic potential is strongly related to the energy balance, which is quite logical. In well-fed animals, however, the AT lipolytic activity was animal and limited, and the NEFA response to adrenergic stimulation appeared to be proportional to the amount of adipose tissue present.

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