Body lipid mobilization, acetonemia and hepatic steatosis in the underfed high-yielding dairy cow during early lactation. Y Chilliard [1], A Ollier [1], D Durand [2], R Lefaivre [1], M Tourret [1], D Thomas [1], E Girard [1], G Sauvage [1], D Gruffat [2], JC Robert [3], P Williams [3], D Bauchart [2] *([1] INRA, Laboratoire Sous-Nutrition des Ruminants, [2] INRA, Laboratoire Croissance et Métabolismes des Herbivores, 63122 Saint-Genès-Champanelle, [3] Rhône Poulenc Nutrition Animale, 03600 Commentry, France)*

Relationships between lipid mobilization, ketonemia and hepatic steatosis were studied in 15 high-producing Holstein cows. Animals were fattened before calving (body condition score, BCS, of 3.9 ± 0.5) and received post-partum a low-concentrate (< 25%) corn silage complete diet *ad libitum*. Rump subcutaneous adipose tissue and liver samples were taken by biopsy and blood samples obtained before morning feeding, on d 4, 11, 25 and 80 (71–89) of lactation.

During weeks 2–6 of lactation, milk yield was 34.4 ± 3.3 kg/d and net energy balance was −13.3 ± 2.5 Mcal/d. Peak milk yield occurred between weeks 4 and 9. The losses of estimated body mass were 74 and 32 kg between weeks 1–4 and 4–12, respectively. The corresponding losses of BCS were 0.84 and 0.75, whereas decreases in adipocyte mean volume were 154 and 261 pl. On d 4, 11, 25 and 80 the plasma metabolite values were respectively: 1.4, 1.3, 1.1 and 0.4 mM for non-esterified fatty acids (NEFA); 86, 73, 64 and 58 μM for free glycerol; 1.1, 2.4, 3.0 and 0.5 mM for 3-hydroxybutyrate (3HB); 1.1, 4.3, 5.0 and 0.2 mg/dl for acetone; 0.9, 1.1, 1.6 and 0.8 mM for acetate; 47, 41, 41 and 59 mg/dl for glucose; and 6.2, 6.1, 7.0 and 9.7 μU/ml for insulin. The percentages of C18:1 in milk fat were 32.4, 35.5, 34.9 and 25.7, and values of liver lipids were 78, 131, 104 and 34 mg/g.

For all cows at all lactation stages (n = 59), liver lipids were correlated to NEFA, 3HB, acetone, milk fat content, milk C18:1, glycemia and energy balance (r = 0.55, 0.72, 0.73, 0.68, 0.67, −0.80 and −0.69, respectively), whereas plasma 3HB was correlated to acetone, acetate, milk C18:1 and glycemia (r = 0.94, 0.80, 0.64 and −0.80, respectively).

These results show that maximal lipid infiltration in the liver occurs rapidly following post-partum body lipid mobilization, whereas maximal ketone body and acetate production by the liver is more closely correlated with hypoglycemia occurring at peak milk (lactose) yield.

Plasma and hepatic lipids and lipoproteins in the underfed high-yielding dairy cow during early lactation. D Durand [1], M Martinaud [1], D Gruffat [1], L Leplaix-Charlat [1], J Lefaivre [1], A Ollier [2], JC Robert [3], Y Chilliard [2], D Bauchart [1] *([1] INRA-CHM, [2] INRA–SNUT, 63122 Saint-Genès-Champanelle, [3] RPNA, 03600 Commentry, France)*

In high-yielding dairy cows, intense fat mobilization during early lactation results in severe infiltration of the liver with lipid. Relationships between fatty liver and plasma energy metabolites, apolipoproteins B (apo B) and A–I (apo A–I) and low (LDL) and high (HDL) density lipoproteins have been studied. Further light may be shed on these relationships from data collected in kinetic studies on the same animals and from knowledge of the complete resolution of LDL and HDL occurring in the bovine in the same density range.

Fifteen Hostein cows were overfed during gestation and underfed after parturition to increase fat mobilization and precipitate hepatic steatosis. Samples of blood (80 ml) and liver (200 mg) were simultaneously obtained from each cow at 1, 2, 4 and 12 weeks after calving. Plasma lipids (non-esterified fatty acids (NEFA) and triacylglycerols (TG)), total cholesterol (C), and hepatic lipids (TG, C and phospholipids (PL)), were determined by enzymatic or colorimetric methods. Plasma apo B and A-I were measured by radial immunodiffusion. Plasma LDL and HDL were isolated by gradient density ultracentrifugation and purified by heparin–Sepharose affinity chromatography.

During weeks 1, 2, 4 and 12 post-calving, hepatic TG were 85, 199, 158 and 10 g/10^9 cells, respectively (representing 75 and 14% of total hepatic lipids in weeks 2 and 12, respectively). Over the same period, hepatic C and PL were constant but both plasma LDL and very light HDL increased markedly (18 to 61 and 5 to 83 mg/dl, respectively). Cows with lowest hepatic TG levels in week 1 (42 mg/10^9 cells) developed maximal TG infiltration in week 4 (178 mg/10^9 cells) whereas cows with moderate or severe steatosis in week 1 (80 and 134 mg/10^9 cells) developed

maximal steatosis as early as week 2 (168 and 292 mg/10^9 cells, respectively). Our results showed that NEFA is the best plasma predictor for TG liver infiltration in weeks 1 ($r = 0.68$), 2 ($r = 0.42$) and 4 ($r = 0.57$). Furthermore, plasma LDL, apo B and A-I are other potential predictors with the accuracy of prediction dependent on the time post-partum.

Insulin uptake and effect on glucose utilization by ovine and bovine adipose tissue cultured over 7 days. Y Faulconnier, L Guillon, R Lefaivre, M Tourret, Y Chilliard *(INRA, Laboratoire Sous-Nutrition des Ruminants, 63122 Saint-Genès-Champanelle, France)*

The effect of insulin (2 mU/ml) on glucose utilization was studied on adipose tissue (AT) explants from non-lactating non-pregnant cows ($n = 5$) and ewes ($n = 5$) fed a restricted diet (20–22% of energy maintenance requirement, EMR) for 8-10 d and then overfed (188 or 228% of EMR, for cows or ewes) during 21 (cows) or 10 (ewes) d until slaughter, to induce a rebound in lipogenic activities. The body condition (scale 0–5) of the cows and ewes averaged 2.5 ± 0.6 and 3.0 ± 0.3, and the mean adipocyte diameter 122 ± 3 and 111 ± 9 µm, respectively.

Samples of perirenal AT were cut into 10–15 mg pieces, and cultured over 7 d in sterile conditions in medium 199 supplemented with acetate (7.0 mM). The culture medium was changed daily. Glucose concentration was measured using the glucose dehydrogenase method. The loss of insulin from the cultured medium in the 2 species ranged from 60 (1st d of culture) to approximately 35% (d 2 to d 7) of the initial amount.

In basal conditions, the glucose utilization was similar (33–24 and 16 µmol/24 h/10^6 adipocytes on d 1–2, 3–4 and 5–6–7, respectively) in the 2 species. However, the glucose utilization by bovine AT was higher (+18%) during the 2nd than during the 1st d, in contrast to ovine AT where this utilization decreased (–13%) during this period. In the 2 species, the glucose utilization then progressively decreased until d 7.

The addition of insulin increased ($P < 0.001$) glucose utilization in the 2 species. However, the effect of insulin was different according to the species and the day of culture (interaction species x insulin x day, $P < 0.03$). The effect of insulin on d 1 was greater in bovine (+67%) than in ovine (+20%) AT, whereas on d 3–4 and d 5–6–7 this effect was greater in ovine (+92 and 132%) than in bovine (+64 and 50%) AT.

This study indicates that ruminant AT explants remain metabolically active for at least 7 d when maintained in a suitable medium, with interactions between insulin and animal species that affect glucose utilization.

Hepatic apo B and mRNA apo B levels in the underfed high-producing dairy cow during early lactation. D Gruffat [1], F Duboisset [1], D Durand [1], J Lefaivre [1], A Ollier [2], G d'Onofrio [3], P Williams [4], Y Chilliard [2], D Bauchart [1] *([1] INRA, Laboratoire Croissance et Métabolismes des Herbivores; [2] INRA, Laboratoire Sous-Nutrition des Ruminants, 63122 Saint-Genès-Champanelle; [3] INSERM U 321, Hôpital de la Pitié, 75013 Paris; [4] Rhône Poulenc Nutrition Animale, 03600 Commentry, France)*

In high-producing dairy cows in early lactation, intense mobilization of lipids is often associated with fatty liver syndrome. The limited capacity of the liver to export triglyceride-rich lipoproteins in the form of very low density lipoproteins (VLDL) increases the risk of development of fatty liver. In cows in early lactation, synthesis of apolipoprotein B (apo B), the major apolipoprotein in VLDL, may be a rate-limiting step for hepatic VLDL production and secretion.

Eight multiparous H x F fat cows after calving (body condition score: 3.9/5) were offered a low concentrate (< 25% DM diet) – corn silage complete diet to increase fat mobilization and to induce hepatic steatosis. All cows were liver biopsied (400 mg/cow; samples frozen in liquid nitrogen) at 1, 2, 4, and 12 weeks after calving. Total hepatic RNA was extracted from each biopsy sample using guanidium thiocyanate/phenol/chloroform. Levels of mRNA for apo B were determined by the Dot-blot method using a cDNA human probe and hepatic apo B levels were measured by Western-blot using rabbit specific antibody against bovine apo B. Hepatic DNA was determined by fluorimetric method and was used to express the apo B levels per 10^9 hepatic cells.

Levels of mRNA of apo B remained stable after calving (8.4 ± 1.0, 8.3 ± 1.8, 10.6 ± 1.3, and