Concentrate Feeding Strategy in Lactating Dairy Cows: Metabolic and Endocrine Changes with Emphasis on Leptin^{1,2}

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ABSTRACT

This study aimed to evaluate metabolic and endocrine adaptations to energy intake in multiparous Holstein cows ($n = 90$; mean 9434 kg energy-corrected milk yield/305 d) over the first 20 wk postpartum and to assess the association of leptin with metabolic, endocrine, and zootechnical traits. Concentrates were fed automatically for 24 h at 30% (C30) or 50% (C50) of total dry matter intake (DMI) from wk 1 to 10 postpartum and at linearly reduced amounts thereafter. Roughage was fed for ad libitum intake. The DMI was measured over 24 h; milk yield and body weight (BW), twice/d; milk composition, 4 times/wk; and milk acetone, weekly. Blood samples for determination of metabolite, hormone, and electrolyte concentrations and enzyme activities were obtained at wk 2 prepartum, and at wk 1 to 16 and at wk 20 postpartum from 0730 to 0900. Body condition scores (BCS) and backfat thickness were measured postpartum and during wk 1, 4, 8, 12, 16, and 20. Energy balance (EB) was considerably lower, but milk yield only slightly lower, in C30 than C50. Metabolic stress was more marked in C30 than C50, expressed by lower, glucose, insulin, insulin-like growth factor-1 (IGF-1), triiodothyronine, milk

protein, and lactose concentrations, higher nonesterified fatty acid, β -hydroxybutyrate, growth hormone, and milk acetone concentrations, and an accelerated decrease in BCS and backfat thickness. Nevertheless, C30 adapted successfully and thus maintained high milk yields despite negative EB. Leptin concentrations were lower in C30 than in C50 over the first 20 wk postpartum and were positively associated with BCS, EB, BW, cholesterol, albumin, insulin, and IGF-1; negatively associated with DMI and triiodothyronine; and were higher in cows calving in spring than in fall. Leptin is one among several factors involved in the regulation of energy metabolism and may be important for overall homeostatic and homeorhetic control of metabolism and thus for maintenance of performance.

(**Key words:** concentrate, dairy cow, leptin, metabolic and endocrine status)

Abbreviation key: AP = potentially intestinally absorbable protein, AST = aspartate amino transferase, BFT = back-fat thickness, C30 = cows fed 30% of total DMI as concentrate, C50 = cows fed 50% of total DMI as concentrate, CF = crude fiber, EB = energy balance, ECM = energy-corrected milk yield, FD = fat depth, GH = growth hormone, GLDH = glutamate dehydrogenase, L₄ = 4th loin vertebra, LDH = lactate dehydrogenase, MD = longissimus dorsi muscle diameter, T₃ = 3,5,3'-triiodothyronine, T₄ = thyroxine, Th₁₂₋₁₃ = 12th to 13th thoracic vertebra.

INTRODUCTION

In high-yielding postpartum dairy cows the amount of energy and protein required for maintenance of body tissue functions and milk production exceeds the amounts of energy and protein which cows can obtain from dietary sources, i.e. in early lactation cows are in a negative energy and protein balance. Body fat,

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protein, and mineral stores have to be mobilized to meet requirements for milk production (Bauman et al., 1988). These processes are orchestrated by complex interactions between numerous endocrine and metabolic signals and express homeostatic and homeorhetic control of metabolism (Blum et al., 1983; Kunz et al., 1985; Chilliard et al., 1998a). Leptin, the product of the *ob* gene, is a 16-kDa protein secreted predominantly in white adipose tissue and acts primarily on the central nervous system (especially the hypothalamus), but is also active in peripheral tissues (Houseknecht et al., 1998; Harris, 2000; Smith and Sheffield, 2002). Numerous studies have confirmed leptin's involvement in metabolic homeostasis in rodent species, e.g. regulation of fat metabolism, energy balance (EB), insulin sensitivity, and appetite, but also in regulating processes critically dependent on energy supply such as reproduction, stress responses, and immune functions (Harris, 2000; Block et al., 2001; Spicer, 2001). In ruminant species, such evidence is incomplete, because specific radioimmunoassays to measure plasma leptin in these species have only recently been developed (Delavaud et al., 2000; Ehrhardt et al., 2000; Chilliard et al., 2001).

Dairy cows can produce up to 7000 kg energy-corrected milk (ECM) per 305-d standard lactation with very low quantities of concentrates if fed high-quality roughage (Jans, 1989). Thus, ad libitum roughage and limited concentrate intake is advocated in Switzerland and is a goal in organic farming. However, roughage intake, and thus total DMI, is limited at least in part by anatomical constraints (Gill et al., 1988). Therefore, in high-yielding dairy cows the degree of energy and protein deficiency can only be reasonably reduced by feeding concentrates. However, energy density and the protein to energy ratio of the total ration are limited, too, and the supply of crude fiber and the structure of the feed should not fall below a certain limit to maintain functionality of the ruminal processes and to avoid rumen acidosis. Furthermore, roughage intake should be maximized to keep feed costs low.

We have tested the hypothesis that from wk 2 prepartum to wk 20 postpartum high yielding multiparous dairy cows, fed roughage for ad libitum intake, exhibit an improved metabolic status if fed concentrate at 50% versus 30% of total DMI and that leptin is closely associated with metabolic, enzymatic, and endocrine traits and thus is crucial for the maintenance of metabolic homeostasis and for high milk yields despite expected negative EB in early lactation.

MATERIALS AND METHODS

Animals, Husbandry, Feeding Plan, and Ration Composition

The experimental procedures followed the Swiss Law on Animal Protection and were approved by the

Committee for the Permission of Animal Experiments of the Canton of Zug, Zug, Switzerland.

Multiparous dairy cows ($n = 90$; 86 Holstein-Friesian and 4 Red Holstein) of parities 2 to 7 were studied from wk 2 prepartum to wk 20 postpartum at the Chamau research farm of the Swiss Federal Institute of Technology, Switzerland. The herd's mean 305-d milk production was 9434 ± 1067 kg (mean $\pm$ SD) ECM. The animals were housed in a free-stall barn. Calving seasons were spring (April and May) and fall (November and December). Cows calved in 4 groups of equal size from fall 1998 to spring 2000. Ambient temperature, relative humidity, and intensity and duration of light exposure (photoperiod) were continuously measured (1 sample every 30 min over the entire study period) using HOBO data loggers (HOBO TEMP, RH, and LIGHT INTENSITY, Bakrona AG, Zurich, Switzerland) fixed at 2 locations in the barn.

The feeding plan is summarized in Table 1. During the dry period, cows were fed a roughage mix containing 5 MJ NE_L/kg DM, corresponding to 1.19 Mcal NE_L/kg DM. From wk 4 prepartum until calving, cows received the same roughage mix as postpartum containing 6 MJ NE_L/kg DM or 1.43 Mcal NE_L/kg DM, respectively, and, additionally, concentrate in increasing quantities from 0 to 40% of the calculated amount postpartum. After parturition, cows were fed a roughage mix ad libitum (6 MJ or 1.43 Mcal NE_L/kg DM), which was freshly provided 4 times per day at 0730, 1100, 1500 and 1745, respectively. Mangers for roughage were fixed on electronic balances to measure the amount of forage consumed on an individual cow basis over 24 h over the entire study period. One manger was available per cow. The concentrate was provided in restricted amounts by automatic feeders. Cows had access to the concentrate up to 20 times per day over 24 h. The shorter the interval between two visits of the automatic feeder, the less concentrate was provided per visit. Two groups (C30 and C50) of equal size were formed based on differences in the total amount of concentrate provided. The cows were assigned to treatment C30 or C50 using stratified randomization to ensure good balance of the cows' characteristics such as parity, 1st lactation milk yield, BW prepartum, and BCS prepartum across the two groups. From wk 1 to 10 postpartum C30 cows ($n = 45$) received concentrate in amounts corresponding to 30% of their individual DMI of the previous week and C50 cows ($n = 45$) in amounts corresponding to 50%, respectively. From wk 11 to wk 34 postpartum, the concentrate was lowered linearly to 2.4% for C30 and to 4% for C50 of the total DMI. The cows had access to feed during 24 h. Individual roughage and concentrate intake of each animal were continuously recorded over 24 h during the whole

Table 1. Experimental feeding plan.

Stage of lactation	Roughage	Concentrate		AP ³ /NE _L ratio
		C30 ¹	C50 ²	
Drying off—wk 5 prepartum	5 MJ NE _L ad libitum	...	...	...
wk 4—1 prepartum	6 MJ NE _L ad libitum	0 to 40 % of quantity postpartum in increasing amounts	0 to 40% of quantity postpartum in increasing amounts	...
wk 1—10 postpartum	6 MJ NE _L ad libitum	30% of total DMI	50% of total DMI	gradually changed from 17 to 15 g/MJ
wk 11—34 postpartum	6 MJ NE _L ad libitum	gradually changed from 30% to 2.4% of total DMI	gradually changed from 50% to 4% of total DMI	gradually changed from 15 to 14 g/MJ

¹C30 = 30% of total dry matter intake as concentrate from wk 1–10 postpartum.

²C50 = 50% of total dry matter intake as concentrate from wk 1–10 postpartum.

³AP = Potentially intestinally absorbable protein.

study period. Minerals and vitamins were fed according to calculated needs (RAP, 1999) and sodium chloride was provided for ad libitum intake.

The composition of the rations consumed are shown in Table 2. The roughage mix was composed of grass silage, whole plant corn silage and grass hay, and was kept at approximately 6 MJ or 1.43 Mcal NE_L/kg DM. The concentrate contained 8 MJ or 1.91 Mcal NE_L/kg DM and its protein components were individually mixed according to lactation week and concentrate-feeding group (C30 or C50) in order to attain the desired ratio between potentially intestinally absorbable protein components (AP) and NE_L in the total ration for each individual cow (Table 2). Feed was sampled on a weekly basis and the DM content was determined. Feed was analyzed for CP, crude fiber (CF), AP and NE_L at the Swiss Federal Research Station for Animal Production, Posieux, Switzerland, on a monthly basis (RAP, 1999).

BW, BCS, Ultrasonic Measurements, and EB

The BW was determined twice daily by weighing cows on an electronic balance after milking. Evaluations of BCS according to Edmonson et al. (1989) and ultrasonic measurements of longissimus dorsi muscle diameter (MD), backfat thickness (BFT), and fat depth (FD) in the pelvic area according to Bruckmaier et al. (1998) and Schwager-Suter et al. (2000) were implemented in wk 4 and 1 prepartum and in wk 1, 4, 8, 12, 16 and 20 postpartum. Real time linear array ultrasound (B-mode) cross-section measurements were performed of the MD, BFT, and FD. The MD and BFT were measured on the left side perpendicular to the spinal chord between the 12th and the 13th rib (Th₁₂₋₁₃) and at the 4th loin vertebra (L₄) and FD in the pelvic area midway between tuber coxae (hooks) and tuber ischii (pins). The positions for ultrasonic measurement were shorn, rapeseed oil was added to

Table 2. Least squares means $\pm$ SD of compositions of rations consumed from wk 1–20 postpartum.

	Roughage	Concentrate		Total ration	
		C30 ¹	C50 ²	C30 ¹	C50 ²
CP, g/kg	125.7 $\pm$ 15.7	232.2 $\pm$ 30.9	208.3 $\pm$ 22.6	155.9 $\pm$ 13.5	161.1 $\pm$ 12.8
CF, ³ g/kg	228.0 $\pm$ 11.9	83.8 $\pm$ 5.8	84.9 $\pm$ 6.1	187.6 $\pm$ 8.9	166.1 $\pm$ 11.9
AP, ⁴ g/kg	77.4 $\pm$ 6.4	140.0 $\pm$ 14.7	129.5 $\pm$ 10.4	95.0 $\pm$ 5.8	99.9 $\pm$ 5.9
NE _L , MJ/kg	5.8 $\pm$ 0.3	7.9 $\pm$ 0.1	8.0 $\pm$ 0.1	6.4 $\pm$ 0.3	6.8 $\pm$ 0.3
AP ⁴ /NE _L , g/MJ	13.2 $\pm$ 0.5	17.6 $\pm$ 2.0	16.3 $\pm$ 1.4	14.8 $\pm$ 0.6	14.8 $\pm$ 0.7

¹C30 = 30% of total dry matter intake as concentrate from wk 1–10 postpartum.

²C50 = 50% of total dry matter intake as concentrate from wk 1–10 postpartum.

³CF = Crude fiber.

⁴AP = Potentially intestinally absorbable protein.

the skin surface to remove air and viscous ultrasonic gel (Provet AG, Lyssach, Switzerland) was added to couple the ultrasound probe to the skin surface. Ultrasonic measurements were determined with an Aloka SSD-650 ultrasound device (Aloka Co., Ltd., Tokyo) with a 5-MHz linear array transducer of 7.5 cm. The transducer was put lightly on the skin and perpendicular to the interface to avoid compression of the fat layer. The following recordings were obtained by measuring on screen the layer of subcutaneous fat including the skin and the muscle diameter at the 3 different points: BFT Th₁₂₋₁₃, MD Th₁₂₋₁₃, BFT L₄, MD L₄ and FD.

According to Bell et al. (1995) and the Swiss Federal Research Station for Animal Production (RAP, 1999), EB [MJ NE_L] was calculated as [NE_L-intake - 0.460 × kg BW^{0.75}] from wk 2 to 1 prepartum and as [NE_L-intake - (ECM × 3.14 + 0.293 × kg BW^{0.75})] from wk 1 - 20 postpartum, AP balance [g] was calculated as [AP-intake - (3.253 × kg BW^{0.75} + 780)] from wk 2 - 1 prepartum and as [AP-intake - (ECM × 50 + 3.253 × kg BW^{0.75})] from wk 1 - 20 postpartum, and CP balance [g] was calculated as [CP-intake - (5.860 × kg BW^{0.75} + 1100)] from wk 2 to 1 prepartum and as [CP-intake - (ECM × 62.8 + 5.860 × kg BW^{0.75})] from wk 1 - 20 postpartum.

Blood Measurements

Blood samples were taken from the jugular vein between 0730 and 0900 using evacuated tubes containing dipotassium-EDTA (1.8 g/L) or no anticoagulant, in wk 2 prepartum, weekly from wk 1 to 16 postpartum, and in wk 20 postpartum. Blood samples with the anticoagulant were immediately put on ice, whereas tubes without anticoagulant (for recovery of serum) were left at room temperature until clotting was finished (within 30 min). Tubes were then centrifuged for 20 min at 1500 × g. Serum for determination of BHBA, sodium, potassium, chlorine, calcium, phosphorus, magnesium, lactate dehydrogenase (LDH), glutamate dehydrogenase (GLDH), and aspartate aminotransferase (AST), and plasma for the determination of glucose, NEFA, cholesterol, creatinine, albumin, urea, insulin, IGF-1, growth hormone (GH), 3,5,3'-triiodothyronine (T₃), and thyroxine (T₄) were stored at -20°C until assayed as described by Bruckmaier et al. (1998) and Aeberhard et al. (2001). For the determination of plasma leptin concentrations in duplicates the specific double-antibody radioimmunoassay described by Delavaud et al. (2000) was followed, with slight modifications. Instead of anti-ovine Ab 7137 leptin antiserum Ab 8172 was used in a final dilution of 1:15,000. After addition of ¹²⁵I-ovine leptin,

the incubation continued for an additional 44 h instead of 20 h. As second antibody goat anti-rabbit IgG was used instead of ram anti-rabbit IgG. It was diluted 1:50 in horse serum for standard curves, non-specific binding tubes, and blanks and 1:50 in incubation buffer for the unknown plasma samples. In wk 4 of lactation, i.e. when maximal metabolic stress could be expected, metabolic and endocrine 8-h profiles were studied in 10 cows of C30 and C50, respectively. Blood samples were taken hourly from the tail vein between 0730 and 1530 using evacuated tubes containing dipotassium-EDTA (1.8 g/L). Blood samples were processed and concentrations of glucose, NEFA, cholesterol, creatinine, urea, BHBA, insulin, glucagon, IGF-1, GH, T₃, T₄ and cortisol were determined as described (Bruckmaier et al., 1998; Aeberhard et al., 2001).

Milk Measurements

Milk yield was measured twice daily. Time of milking was between 0430 and 0630 and between 1530 and 1730. Milk samples for determination of acetone (AC) were taken once per week (Tuesday morning) in wk 1 to 16 and in wk 20 postpartum, frozen immediately after milking, and stored at -20°C until analyzed (Miettinen, 1994). Concentrations of AC were determined by flow injection analysis (Marstorp et al., 1983). Milk samples for the determination of milk fat, protein, lactose, and urea were taken 4 times per week (Monday and Wednesday evening, Tuesday and Thursday morning) and analyzed at the laboratory of the Swiss Brown Cattle Breeders' Federation, Zug, Switzerland. The ECM was calculated as [(0.038 × g crude fat + 0.024 × g crude protein + 0.017 × g lactose) × kg milk] ÷ 3.14 (RAP, 1999). The 305-d standard lactations were calculated using the coefficients of the Holstein Association of Switzerland, Posieux, Switzerland.

Statistical Analyses

Values of blood, milk and zootechnical traits are expressed as means ± SEM. Level of significance was set at *P* < 0.05. The S-PLUS 2000 Professional Release (MathSoft, 1999) statistical software was used. Mixed-effects models were fitted using the NLME version 3.4 program library for S-PLUS of Pinheiro and Bates (2000).

Descriptive statistical analysis revealed that a considerable number of traits were not normally distributed. Therefore, NEFA, creatinine, BHBA, leptin, insulin, IGF-1, GH, T₄, cortisol, glucagon, LDH, GLDH, AST, milk AC, milk fat, milk protein, BFT Th₁₂₋₁₃, BFT L₄ and FD were logarithmically transformed and

total DMI, NE_L , AP and CP intake were subjected to a quadratic transformation prior to analysis. Although BCS represents a discrete variable, normality was assumed based on graphical evaluation of BCS distribution using histograms and quantile-quantile plots.

To evaluate the differences of blood, milk, and zootechnical traits between concentrate-feeding groups from wk 1 to 20 postpartum, linear mixed-effects models were used of the form $y_i = X_i \beta + Z_i b_i + \varepsilon_i$, where y_i is the response vector, β is the vector of fixed effects, b_i is the vector of random effects, X_i and Z_i are the fixed-effects and random-effects regressor matrices, and ε_i is the within-group error vector. Models were fitted by backward elimination procedures (type I-error $P < 0.05$) starting from a basic model with the blood, milk or zootechnical traits as response variables and concentrate-feeding group, week of sampling, concentrate-feeding group $\times$ week of sampling interaction, calving season (spring, fall) and parity (dichotomized as 2 and ≥ 3) as explanatory variables (fixed effects). The week of sampling was modeled as an orthogonal second-class polynomial. The animal was the repeatedly studied subject and was modeled as random effect. The models were crossvalidated in order to test for collinearity and the residual plots of the final models were used as criterion for the model fit regarding homogenous variance assumption.

The relationship between plasma leptin and the different metabolic, enzymatic, endocrine and zootechnical traits was evaluated by linear mixed-effects models of the same form as described above. Models were also fitted by backward elimination procedures (type I-error $P < 0.05$) starting from a basic model with leptin as response variable and calving season (spring, fall), parity (dichotomized as 2 and ≥ 3), and the zootechnical, metabolic, endocrine and enzymatic traits as explanatory variables (fixed effects). The animal was the repeatedly studied subject and was modeled as random effect. The correlation structures of the fixed effects were examined and the models were validated as described above. Differences between C30 and C50 of traits in wk 2 prepartum were localized by Student's t -test. Correlation coefficients were calculated as Pearson's correlation.

RESULTS

Feed Intake

Total, roughage, and concentrate DMI and NE_L , AP and CP intake of C30 and C50 cows from wk 2 prepartum to wk 20 postpartum are shown in Figure 1. Total DMI was lower and roughage DMI was higher in C30 than C50 cows over the first 20 wk of lactation. Concentrate DMI paralleled total DMI and, according to the

feeding plan, was considerably lower in C30 than C50 cows throughout the studied period. The NE_L intake paralleled total DMI and was lower in C30 than C50 cows from wk 1 to 20 postpartum, as well as CP and AP intake.

EB, BCS, and Ultrasonic Measurements

The EB, BCS, BFT Th_{12-13} , and MD Th_{12-13} of C30 and C50 cows from wk 2 prepartum until wk 20 postpartum are presented in Figure 2. The EB was positive prepartum, reached a nadir in wk 2 postpartum (-46.0 and -29.6 MJ NE_L in C30 and C50, respectively) and then increased up to wk 20 postpartum. The EB became positive in wk 12 postpartum in C30 and in wk 8 postpartum in C50 cows, respectively. The EB was lower in C30 than C50 cows from wk 1 to 20 postpartum. The AP balance and CP balance were closely correlated (Pearson's correlation) with EB ($r = 0.96$ and 0.91 , respectively; $P < 0.001$). There were no differences in BCS and in ultrasonic traits between C30 and C50 cows prepartum. However, BCS, BFT, and MD decreased more in C30 than C50 cows from wk 1 to 20 postpartum, as evidenced by the significant concentrate-feeding group $\times$ week of sampling interaction. The FD at the pelvic area and the ultrasonic measurements at L_4 (data not shown) paralleled those at Th_{12-13} .

Blood Measurements

There were no differences in concentrations of blood traits between C30 and C50 cows prepartum. The profiles of plasma glucose, NEFA, and urea, and of serum BHBA of C30 and C50 cows in the course of lactation are presented in Figure 3. Glucose concentrations were lower and NEFA and BHBA concentrations were higher in C30 than C50 over the studied period postpartum. Urea concentrations were nonsignificantly lower in C30 than in C50 cows. Cholesterol, creatinine, and albumin concentrations (data not shown) were not different in C30 than in C50 over the studied period postpartum. Cholesterol was low prepartum (2.27 and 2.33 mmol/L in C30 and C50), reached a nadir in wk 1 postpartum (1.99 and 2.00 mmol/L), increased until wk 12 postpartum (6.08 and 5.64 mmol/L) and, thereafter, remained at a high level until wk 20 postpartum. Creatinine concentration was highest prepartum (102.3 and 99.6 μ mol/L in C30 and C50), decreased rapidly until wk 4 postpartum (85.2 and 83.3 μ mol/L) and then remained at this level until wk 20 postpartum. Albumin concentrations were lowest around parturition (38.5 and 38.1 g/L in wk 2 prepartum and 38.2 and 38.2 g/L in wk 1 postpartum in C30 and C50,

Figure 1. Total, roughage, and concentrate DMI and NE_L, potentially intestinally absorbable protein (AP), and CP intake from week 2 prepartum through week 20 postpartum in C30 (—○—) and C50 (—●—) cows. Statistically significant ($P < 0.05$) effects of group, week postpartum and group $\times$ week postpartum interactions over the first 20 wk postpartum are given in the figure.

respectively) and then increased towards wk 20 postpartum (40.8 and 40.2 g/L in C30 and C50, respectively).

The profiles of blood hormones of C30 and C50 cows in the course of lactation are presented in Figure 4. There was no difference in leptin concentration between C30 and C50 cows prepartum. Leptin concentrations were highest prepartum (7.3 μ g/L in both C30 and C50), reached a nadir in wk 1 postpartum (4.9

and 5.5 μ g/L in C30 and C50, respectively), and then increased until wk 20 postpartum (5.9 and 6.5 μ g/L in C30 and C50, respectively). Leptin concentrations were lower in C30 than in C50 cows over the studied period postpartum. Insulin, IGF-1, and T₃ concentrations were higher and GH concentrations were lower in C30 than C50 cows from wk 1 to 20 postpartum. The T₄ concentrations were nonsignificantly lower in C30 than in C50 cows.

Figure 2. Energy balance (EB), BCS, backfat thickness (BFT Th₁₂₋₁₃), and longissimus dorsi muscle diameter between the 12th and the 13th rib (MD Th₁₂₋₁₃) from week 2 prepartum through week 20 postpartum in C30 (—○—) and C50 (—●—) cows. Statistically significant ($P < 0.05$) effects of group, week postpartum and group $\times$ week postpartum interactions over the first 20 wk postpartum are given in the figure.

The activities of serum enzymes (data not shown) were higher in C30 than C50 over the first 4 (AST) to 8 (LDH and GLDH) wk postpartum, however, over the entire sampling period the difference between C30 and C50 was not statistically different. The activities of LDH were lowest prepartum (31.8 and 31.9 $\mu\text{kat/L}$), increased markedly from wk 2 prepartum to wk 1 postpartum (37.6 and 38.0 $\mu\text{kat/L}$), remained high in C30 and decreased transiently in C50, but remained above prepartum levels until wk 20 postpartum (37.2 and 37.1 $\mu\text{kat/L}$) in both C30 and C50. The AST activities were lowest prepartum (0.84 and 0.83 $\mu\text{kat/L}$), peaked in wk 1 postpartum (1.38 and 1.28 $\mu\text{kat/L}$), decreased until wk 6 and 4 postpartum in C30 (1.10 $\mu\text{kat/L}$) and C50 (1.08 $\mu\text{kat/L}$), respectively, and then remained at this level. The GLDH activities were lowest prepartum (0.15 and 0.14 $\mu\text{kat/L}$), peaked in wk 3 postpartum (0.47 and 0.32 $\mu\text{kat/L}$), and decreased to 0.25 and 0.24 $\mu\text{kat/L}$ in C30 and C50 cows in wk 20 postpartum.

Data of serum electrolyte concentrations are not shown. Sodium, potassium, and phosphorus concentrations were stable from wk 2 prepartum through wk

20 postpartum and were not different in C30 than C50 cows. However, chlorine concentrations were lower in C30 than C50 over the studied period postpartum. Chlorine concentrations were highest prepartum (102 and 103 mmol/L in C30 and C50), decreased postpartum, reached a nadir in wk 6 postpartum in C30 (98 mmol/L) and in wk 7 postpartum in C50 (99 mmol/L), then increased up to wk 20 postpartum (101 and 101 mmol/L). Calcium concentrations were 2.4 mmol/L (C30 and C50) in wk 2 prepartum, reached a nadir in wk 1 postpartum (2.2 mmol/L in C30 and C50), rose rapidly to 2.3 (C30) and 2.4 (C50) mmol/L in wk 2 postpartum, and then increased slightly in C30, but remained at this level in C50 cows. Magnesium concentrations were not different in C30 than in C50 cows. They decreased from wk 2 prepartum (0.96 and 0.92 mmol/L in C30 and C50) to wk 1 postpartum (0.90 and 0.88 mmol/L), transiently increased thereafter, peaked in wk 5 postpartum (1.04 mmol in C30 and C50) and then slightly decreased until wk 20 postpartum (0.98 and 0.99 mmol/L).

Figure 3. Glucose, NEFA, Urea, and BHBA concentrations from week 2 prepartum through week 20 postpartum in C30 (—○—) and C50 (—●—) cows. Statistically significant ($P < 0.05$) effects of group, week postpartum and group $\times$ week postpartum interactions over the first 20 wk postpartum are given in the figure.

Milk Yield, Milk Components, and Milk Acetone

Profiles of milk traits of C30 and C50 cows from wk 2 prepartum until wk 20 postpartum are presented in Figure 5. Mean ECM over the first 20 wk postpartum was 1.28 kg ECM/d lower in C30 than in C50 cows. Milk fat concentrations were higher in C30 than C50 cows over the first month postpartum, whereas milk protein and lactose concentrations were lower in C30 than C50 from wk 1 to 20 postpartum. Milk urea concentrations paralleled plasma urea concentrations and did not show differences between groups. Milk AC concentrations were higher in C30 than C50 cows over the studied period postpartum.

Eight-hour Profiles

Eight-h profiles in wk 4 postpartum of C30 and C50 cows are presented in Tables 3 and 4, including information on effects of concentrate-feeding group (γ), sampling hour (τ) and concentrate-feeding group $\times$ sampling hour interactions (ι). Glucose concentrations slightly decreased and were lower in C30 than C50.

Concentrations of NEFA were highest between 0730 and 0930, lowest between 1230 and 1530, and were nonsignificantly higher in C30 than C50. Cholesterol concentrations were stable. Creatinine concentrations decreased slightly from 0730 through 1530 and were nonsignificantly higher in C30 than C50. Urea concentrations increased slightly in C30, slightly decreased in C50, and were nonsignificantly lower in C30 than C50 from 0730 to 1530. Concentrations of BHBA increased slightly from 0730 to 1530 and were markedly higher in C30 than C50. Leptin concentrations decreased slightly from 0730 to 1530 and were nonsignificantly lower in C30 than in C50. Insulin concentrations increased slightly from 0730 to 1530 and were nonsignificantly lower in C30 than C50. Glucagon concentrations were stable and did not differ between groups. The GH concentrations were nonsignificantly higher in C30 than C50. The IGF-1 concentrations were stable, but lower in C30 than in C50. Concentrations of thyroid hormones increased from 0730 to 1530. Concentrations of T_3 were nonsignificantly lower in C30 than in C50 cows. Cortisol concentrations were stable and did not differ between groups.

Figure 4. Leptin, insulin, IGF-1, growth hormone (GH), triiodothyronin (T_3), and thyroxine (T_4) concentrations in plasma from week 2 prepartum through week 20 postpartum in C30 (—○—) and C50 (—●—) cows. Statistically significant ($P < 0.05$) effects of group, week postpartum and group $\times$ week postpartum interactions over the first 20 wk postpartum are given in the figure.

Relationships Between Leptin and Metabolic, Enzymatic, and Endocrine Traits

The regression coefficients, 95% confidence intervals, P -values and coefficients of determination (R^2) of the mixed-effects model for assessment of the relationship between leptin and the various zootechnical, metabolic, enzymatic and endocrine traits are presented in Table 5. Because the correlation of BCS to the ultrasonic measurements (MD, BFT, and FD) was too strong, only BCS, which was closer related to leptin

than MD, BFT, or FD, could be included into the basic model, in order to avoid colinearity.

Leptin was positively related to BCS, EB, BW, cholesterol, albumin, insulin and IGF-1, and negatively to total DMI, AST and T_3 . Furthermore, leptin concentrations were higher ($P < 0.001$) in cows calving in spring (blood sampling from March to September) than in fall (blood sampling from October to April). Leptin concentrations were positively correlated ($P < 0.001$) to the length of photoperiod ($r = 0.26$), to mean light intensity ($r = 0.33$) and to mean ambient temperature

Figure 5. Milk traits from week 2 prepartum through week 20 postpartum in C30 (—○—) and C50 (—●—) cows. Statistically significant ($P < 0.05$) effects of group, week postpartum and group $\times$ week postpartum interactions over the first 20 wk postpartum are given in the figure. ECM = energy corrected milk yield.

($r = 0.33$). The R^2 of the complete model was 0.846 and the R^2 of the fixed effects was 0.232.

DISCUSSION

General Aspects, Feed Intake, EB, BCS, and Ultrasonic Measurements

In this study, multiparous dairy cows were classified into 2 groups (C30, C50) according to the amount of concentrate provided in relation to the total DMI. Both

concentrate feeding strategies were reasonable with respect to practical applicability, i.e. C30 cows were not explicitly undernourished. Groups were balanced for parity, BW, BCS prepartum, calving season and potential for milk production. The facilities at the research station allowed to realize this study with a large number of cows under highly standardized conditions.

Total DMI followed a typical curve (Ingvarthsen and Andersen, 2000). As a consequence of the feeding strategy concentrate DMI was considerably lower in C30

Table 3. Metabolic 8-h profiles in wk 4 postpartum of 10 C30² and 10 C50³ cows.

Parameter	Group	Time									SE ³	ANOVA		
		7:30	8:30	9:30	10:30	11:30	12:30	13:30	14:30	15:30		I ⁴	T ⁵	I ⁶
Glucose, mmol/L	C30 ¹	2.6	2.6	2.6	2.5	2.5	2.4	2.5	2.5	2.5	0.12	*	*	NS
	C50 ²	3.3	3.3	3.2	3.2	3.2	3.1	3.2	3.1	3.2	0.16			
NEFA, mmol/L	C30	0.32	0.31	0.37	0.34	0.32	0.23	0.23	0.19	0.25	0.52	NS	*	NS
	C50	0.32	0.18	0.25	0.21	0.19	0.16	0.16	0.15	0.18	0.40			
Cholesterol, mmol/L	C30	3.4	3.4	3.4	3.5	3.4	3.5	3.5	3.5	3.5	0.19	NS	NS	NS
	C50	3.9	3.8	3.8	3.8	3.8	3.8	3.8	3.9	3.8	0.18			
Creatinine, μ mol/L	C30	83	82	82	83	82	81	81	82	82	3.35	NS	*	NS
	C50	80	78	77	77	78	76	76	77	78	1.86			
Urea, mmol/L	C30	3.4	3.5	3.5	3.6	3.6	3.6	3.5	3.6	3.6	0.15	NS	*	*
	C50	3.7	3.8	3.9	3.9	3.8	3.8	3.7	3.7	3.7	0.17			
β -hydroxybutyrate, mmol/L	C30	2.22	2.30	2.18	2.28	2.39	2.45	2.48	2.36	2.36	0.35	*	*	NS
	C50	0.94	1.07	1.09	1.08	1.15	1.15	1.19	1.13	1.16	0.23			

¹C30 = 30% of total dry matter intake as concentrate from wk 1–10 postpartum.²C50 = 50% of total dry matter intake as concentrate from wk 1–10 postpartum.³SE = Pooled standard error.⁴ γ = Effect of group.⁵ τ = Effect of time.⁶ ι = Effect of group $\times$ week interaction.* $P < 0.05$.

than in C50 cows. Although roughage was provided ad libitum, C30 compensated their lower claim for concentrate only partly with an increase in roughage DMI. Therefore, total DMI was substantially smaller in C30 than C50, i.e. concentrate replaced roughage only partly. Consequently, differences in NE_L, CP, and AP intakes between C30 and C50 were even more pronounced.

As expected, calculated EB was positive prepartum and there were no differences between C30 and C50. After parturition, EB followed a typical pattern (Kunz et al., 1985; Ronge et al., 1988). A negative EB early postpartum could be expected as feed intake does usually not meet requirements for milk production and maintenance in high yielding dairy cows in this period. The EB was markedly more negative in C30 than in

Table 4. Endocrine 8-h profiles in wk 4 postpartum of 10 C30² and 10 C50³ cows.

Parameter	Group	Time									SE ³	ANOVA		
		7:30	8:30	9:30	10:30	11:30	12:30	13:30	14:30	15:30		I ⁴	T ⁵	I ⁶
Leptin, μ g/L	C30 ¹	5.9	5.7	5.7	5.8	5.6	5.7	5.7	5.6	5.5	0.21	NS	*	NS
	C50 ²	5.3	5.3	5.2	5.4	5.1	5.3	5.2	5.3	5.3	0.26			
Insulin, μ g/L	C30	0.43	0.40	0.36	0.34	0.44	0.47	0.52	0.51	0.53	0.06	NS	*	NS
	C50	0.56	0.56	0.47	0.59	0.54	0.54	0.67	0.62	0.67	0.08			
Glucagon, ng/L	C30	171	153	160	152	159	152	154	156	154	21.09	NS	NS	NS
	C50	158	150	146	151	152	151	155	154	162	9.97			
Growth hormone, μ g/L	C30	5.1	5.9	5.4	7.5	6.7	6.8	7.3	6.6	7.1	1.14	NS	NS	NS
	C50	4.7	4.1	5.3	4.7	5.2	4.4	4.9	4.3	4.5	0.70			
IGF-1, μ g/L	C30	103	104	103	99	102	100	100	99	99	7.38	*	NS	NS
	C50	149	143	144	145	144	142	135	145	141	15.55			
3,5,3'-triiodothyronine, nmol/L	C30	1.2	1.2	1.3	1.3	1.4	1.5	1.5	1.5	1.5	0.12	NS	*	NS
	C50	1.4	1.3	1.5	1.5	1.5	1.5	1.6	1.6	1.6	0.10			
Thyroxine, nmol/L	C30	35	35	35	37	39	41	42	44	45	3.29	NS	*	NS
	C50	35	33	36	35	38	39	42	42	42	3.02			
Cortisol, nmol/L	C30	6.3	3.7	6.3	8.2	5.5	5.1	5.1	3.4	6.7	1.15	NS	NS	NS
	C50	7.3	5.3	7.6	6.2	7.6	5.1	7.5	6.1	6.3	1.72			

¹C30 = 30% of total dry matter intake as concentrate from wk 1–10 postpartum.²C50 = 50% of total dry matter intake as concentrate from wk 1–10 postpartum.³SE = Pooled standard error.⁴ γ = Effect of group.⁵ τ = Effect of time.⁶ ι = Effect of group $\times$ week interaction.* $P < 0.05$.

Table 5. Regression coefficients of the final linear mixed-effects model¹ obtained by backward elimination procedures, for evaluation of the relationship between leptin² and the various metabolic, enzymatic, endocrine, and zootechnical traits.

Parameter	Coefficient	95% Confidence interval	P-value
Constant	0.2205	0.0119, 0.4290	0.039
Season			
Spring	0		
Fall	-0.1608	-0.2375, -0.0842	<0.001
BCS	0.0838	0.0468, 0.1208	<0.001
DMI, kg, squared	-0.0001	-0.0001, -0.0001	<0.001
Energy Balance, MJ NE _L	0.0013	0.0009, 0.0016	<0.001
Body Weight, kg	0.0012	0.0010, 0.0015	<0.001
Cholesterol, mmol/L	0.0270	0.0210, 0.0330	<0.001
Albumin, g/L	0.0108	0.0077, 0.0139	<0.001
AST ³ , μ kat/L, ln	-0.0523	-0.0866, -0.0180	0.003
Insulin, μ g/L, ln	0.0426	0.0303, 0.0549	<0.001
IGF-1, μ g/L, ln	0.0357	0.0118, 0.0597	0.004
T ₃ ⁴ , nmol/L	-0.0477	-0.0679, -0.0276	<0.001

¹R² of the complete model was 0.846 and R² of the fixed effects was 0.232.

²Leptin, μ g/L, ln = Response variable of the mixed-effects model, cow as random effect.

³AST = Aspartate aminotransferase.

⁴T₃ = 3,5,3'-triiodothyronine.

C50 throughout the postpartum study period, because mean milk production was only slightly smaller, whereas NE_L intake was considerably lower in C30 than C50. The very strong correlation of EB to CP and AP balance can be attributed to the fact that the concentrate composition postpartum was individually formulated with the objective of equal ratios between AP and NE_L intake in the total ration in C30 and C50. The BCS, ultrasonic measurements of BFT, MD and FD, and BW behaved similarly in both C30 and C50 prepartum, revealing the fact that groups have been carefully balanced. However, BCS, BFT, MD, and FD decreased more in C30 than C50 postpartum, indicating that more body fat and protein stores had to be mobilized in C30 than in C50 in order to meet energy and protein requirements for milk production. Although BCS, BFT, MD and FD basically mirrored EB as shown previously (Bruckmaier et al., 1998), they reacted much more sluggishly to insufficient energy intake, i.e. BCS, MD and FD were able to differentiate between C30 and C50 only with a considerable delay after reaching the EB nadir. That BW is not a sensitive indicator of energy intake is well known.

Blood Measurements

The recorded metabolic, endocrine and enzymatic parameters reflected the well-known changes in energy metabolism during early lactation. Importantly, there were no differences in metabolic, endocrine and enzymatic status between C30 and C50 cows prepartum, as evidenced by equal concentrations of metabolites and hormones and activities of enzymes. There-

fore, differences in these traits postpartum between groups can be attributed to differences in metabolic adaptations upon onset of lactation.

Glucose concentration reached its nadir at the same moment as EB and equaled prepartum levels at approximately the week when EB became positive. Although concentrations of glucose were considerably lower in C30 than in C50, concentrations were still in the normal range, i.e. glucose homeostasis was maintained in both groups. As expected, NEFA concentrations were considerably higher in C30 than in C50. Cholesterol concentrations were typically lowest around parturition and then increased concomitantly with DMI, but with a lag in response to lactation. Cholesterol concentrations expressed mainly the marked changes in lipid metabolism (Blum et al., 1983; Bruckmaier et al., 1998). The decrease of creatinine concentrations was typically most marked over the first weeks of lactation (Bruckmaier et al., 1998) and reflected skeletal muscle breakdown (Van Niekerk et al., 1963), i.e. the mobilization of protein as energy source for milk production (Botts et al., 1979). Changes of albumin concentrations were similar to those described previously (Ronge et al., 1988). Under conditions of normal liver and kidney function, plasma urea concentrations are well-known to be influenced by protein and energy intake (Oltner and Wiktorsson, 1983). Plasma urea concentrations increased transiently during lactation, possibly reflecting enhanced tissue protein breakdown, but values ranged within normal limits, indicating that protein intake relative to energy intake was adequate. There were no differences in urea concentrations between C30 and C50, reflecting

that the concentrate composition postpartum was individually formulated with the objective of equal ratios between AP and NE_L intake in the total ration in C30 and C50. Transiently elevated concentrations of BHBA could be expected and indicated enhanced ketogenesis as a consequence of enhanced fat mobilization and glucose shortage (Herdt et al., 1981). The increase in BHBA was more marked and prolonged in C30 than C50, explaining the time $\times$ group interaction. Especially in C30, there was a lag of 1 to 3 wk between EB and glucose nadir and NEFA peak on the one hand and maximal BHBA concentrations on the other hand, as reported previously (Kunz et al., 1985; Ronge et al., 1988). Although available only in limited amounts, especially from labile proteins resulting from skeletal muscle breakdown and involution of the uterus (Botts et al., 1979; Zurek et al., 1995; Chilliard et al., 1998a), free amino acids used for gluconeogenesis may have permitted the maintenance of a functioning tricarboxylic acid cycle very early in lactation, partly explaining why the concentrations of ketone bodies in the first 2 wk postpartum were rather low. Furthermore, limited amounts of free fatty acids might have been stored in the liver after parturition. The higher concentrations of BHBA in C30 than in C50 could be expected (Kunz et al., 1985; Ronge et al., 1988) and indicated that metabolic stress was more marked in C30 compared to C50 cows.

The abrupt decrease of plasma leptin concentrations at parturition was in agreement with Block et al. (2001), who reported a reduction in plasma leptin concentrations by 50% after calving in dairy cows. A decrease just before parturition was also reported in rats (Chien et al., 1997) and 3 d after delivery in humans (Schubring et al., 1998). In sheep, plasma leptin concentrations were lower in early lactation than in mid pregnancy. However, differences between late pregnancy and early lactation were not significant (Ehrhardt et al., 2001). Whereas in the study of Block et al. (2001) leptin concentrations remained depressed during lactation despite improvement in EB, leptin concentrations increased towards wk 20 postpartum in the present study, as described in humans (Schubring et al., 1998). Leptin concentrations paralleled EB, insulin, and IGF-1, concentrations, but behaved inversely to GH.

In agreement with Chilliard et al. (2001), Delavaud et al. (2000), and Block et al. (2001), who reported positive relationships between plasma leptin levels, plane of nutrition, energy intake, and EB in bovine and ovine species, leptin concentrations were lower in C30 than in C50 cows throughout the studied postpartum period. Importantly, lowest leptin concentrations in wk 1 of lactation coincided with highest NEFA con-

centrations, i.e. leptin concentrations were negatively associated with fat mobilization and positively with fat mass, as expected (Houseknecht et al., 1998).

Low concentrations of insulin were typical for negative EB during early lactation in dairy cows and thus permitted the direct use of most glucose for milk synthesis, while glucose utilization and oxidation in extramammary tissue is reduced (Bauman et al., 1988; Chilliard et al., 1998a). The lower insulin concentration in C30 than in C50 could be expected, because insulin is positively associated with EB (Kunz et al., 1985; Ronge et al., 1988). Changes in plasma IGF-1 and GH were similar to those described previously (Ronge et al., 1988; Spicer et al., 1990; Bruckmaier et al., 1998). It is well recognized that appropriate evaluation of the GH status is only possible if its pulsatile secretion is respected. Nevertheless, the high GH concentrations after parturition, measured only once per d, typically reflected negative energy balance and a catabolic status of metabolism, i.e. high GH concentrations likely allowed enhanced mobilization of depot fat and may have favored partitioning of absorbed nutrients to the mammary gland to provide sufficient substrates for milk synthesis (Bauman et al., 1988). Thyroid hormone concentrations in plasma in the course of lactation were similar to those described previously (Blum et al., 1983; Kunz et al., 1985; Gueorguiev, 1999). Low thyroid hormone concentrations after parturition were typical for a marked negative EB. Therefore, lower T₃ and, at least partly lower T₄ concentrations in C30 compared to C50 cows could be expected. Similar to Ronge et al. (1988) effects of low energy intake on T₃ were more marked and longer lasting than on T₄.

Enzyme activities increased after parturition and were sporadically higher in C30 than C50 over the first 8 wk of lactation, indicating that hepatic metabolism was more stressed and tissue catabolism was more pronounced in C30 than in C50 over this period (Mills et al., 1986). However, because enzyme activities were within the normal range and because milk production and albumin synthesis persisted, integrity and functionality of liver tissue was obviously maintained in both groups.

Plasma electrolyte concentrations were in the normal range and did not indicate nutritional deficiencies or marked metabolic stress throughout the sampling period. The transient decrease of Cl concentrations coincided with peak milk production and may have reflected loss of Cl with milk. Although C30 produced less milk, and salt (NaCl) was fed ad libitum, Cl concentrations were lower in C30 than in C50. The importance of this finding is not clear. The transient decrease of Ca concentrations at wk 1 could be expected (Blum et al., 1972) and reflected loss of this electrolyte

with milk. However, Ca concentrations recovered quickly, indicating that Ca homeostasis was maintained. There were 4 cases of parturient paresis in C30 and 2 cases in C50 which needed treatment. In accordance with Blum et al. (1972), Mg concentration increased transiently after parturition.

Milk Yield, Milk Components, and Milk Acetone

The two groups were balanced for potential of milk production. The milk yield curve showed a characteristic pattern with a peak at wk 4 postpartum, followed by a steady decrease towards the end of the sampling period. It typically preceded the curve of DMI, which followed with a lag of 6 to 8 wk, in accordance with Ingvarlsen and Andersen (2000). Although EB was considerably lower in C30 than in C50 cows, C30 produced only slightly less ECM than C50 cows, indicating that whole-body homeostasis was maintained. However, the milk yield curve behaved differently in C30 and C50 cows, as shown by the significant time $\times$ group interactions.

Changes in milk fat, protein, lactose and urea concentrations during the first 20 wk of lactation were typical (Thomas and Martin, 1988). Higher milk fat concentrations in C30 than C50 over the first month after parturition indicated a greater mobilization of fat stores in C30 than C50 at the start of lactation. Milk protein concentrations were at the same level in C30 and C50 over the first 4 wk postpartum, but then segregated and were lower in C30 than in C50 cows, indicating a better energy supply in C50 than C30. Surprisingly and contradictory to Thomas and Martin (1988) and Kennelly et al. (1990) lactose concentrations were affected by concentrate feeding, i.e. C30 had lower lactose concentrations than C50 cows, especially from wk 2 to 15 postpartum. Milk urea paralleled blood urea concentrations, as expected. Transiently elevated concentrations of milk AC could be expected and indicated enhanced ketogenesis and enhanced fat mobilization (Holtenius and Holtenius, 1996). As seen for blood BHBA, the transient increase in milk AC was more marked and prolonged in C30 compared to C50 and a lag between EB nadir and peak of ketone body concentration could be observed. The higher concentrations of milk AC in C30 than in C50 indicated that metabolic stress was more marked in C30 than C50 cows.

Eight-hour Profiles

In wk 4 of lactation, i.e. the time of greatest metabolic stress in response to energy deficiency when considering ketogenesis, 8-h profiles were realized in 10

C30 and 10 C50 cows to study the stability of metabolic and endocrine traits and to judge whether the weekly samples taken between 0730 and 0900 were representative for the range of 8-h variations. Although in approximately half of the studied traits the effect of sampling hour was statistically significant, it was small and therefore not relevant, apart from NEFA concentrations, which decreased considerably from 0730 until 1530, and thyroid hormones, which increased slightly from 0730 until 1530. Hence, weekly samples of blood traits taken between 0730 and 0900 reflected mean concentrations during daytime, except NEFA and thyroid hormones. The marked decrease of NEFA from 0730 to 1530 can be attributed to a higher feed intake—especially of concentrate—during daytime and to a concomitantly decreased adipose tissue lipolysis and increased adipose tissue reesterification (Fröhli and Blum, 1988), besides better oxidation of NEFA in the tricarboxylic acid cycle when glucose precursors are available in greater amounts. Changes of T_3 and T_4 from 0730 to 1530 were typical (Blum et al., 2000). The increase in thyroid hormone concentrations during day-time might have been due to elevated feed intake. The slightly lower leptin concentrations in 8-h-profiles at 1530 than at 0730 were in agreement with Delavaud et al. (1999) and Ingvarlsen and Boisclair (2001), who concluded that leptin is secreted in a pulsatile manner in ruminants, but without a marked diurnal rhythm. Where existent, differences between C30 and C50 in 8-h patterns generally reflected the differences of the weekly samples taken at wk 4 postpartum between 0730 and 0900.

Relationships Between Leptin and Metabolic, Endocrine and Zootechnical Traits

The R^2 of the mixed-effects model for assessment of the association of leptin with blood, milk and zootechnical traits was high ($R^2 = 0.846$). However, the individual cow, which was the repeatedly studied subject in the model, accounted for most of the variance of leptin, whereas the fixed effects (blood and zootechnical traits) surprisingly accounted only for an R^2 of 0.232. A seasonal effect on plasma leptin concentrations in bovine is here to our knowledge reported for the first time.

Higher leptin concentrations of cows calving in spring, whose sampling period lasted from March to September, can be explained with the positive correlation of plasma leptin with the photoperiod, light intensity and ambient temperature and is in agreement with Bocquier et al. (1998), who reported a decreased plasma leptin concentration and leptin gene expres-

sion in adipose tissue of ovariectomized ewes exposed to short compared to long days (8 versus 16 h light/d).

A closer association of plasma leptin with BCS than with MD, BFT, or FD is supported by Schwager-Suter et al. (2000) who reported that Edmonson's BCS chart (Edmonson et al., 1989) can be applied to different dairy types, is valid over the whole lactation, and can thus substitute expensive ultrasonic measurements of subcutaneous fat or time consuming weighing of cows. The positive association of plasma leptin concentration with BCS could be expected and was in accordance with Chilliard et al. (1998b), who described a close relationship between leptin concentrations and adipocyte volume in Holstein and Charolais, and with Ehrhardt et al. (2000), who reported a linear relationship of BCS with plasma leptin concentrations.

A negative association of leptin concentrations with total DMI could be due to a decreased availability of neuropeptide Y, a potent stimulator of appetite in rodents and a main target of leptin for binding to the dorso- and ventromedial nuclei of the hypothalamus and the arcuate nucleus, as described in ovine (Dyer et al., 1997; Henry et al., 1999). The roles of leptin in EB regulation in rodents is well documented (Houseknecht et al., 1998). The positive relationship between plasma leptin concentrations and EB in our study was also reflected by the higher leptin concentrations in C50 than in C30, although total DMI was higher in C50 than in C30.

Thus, in bovine too, leptin is not only associated with BCS and DMI, but also with energy status and might therefore play a role in regulation of feed intake, body stores and energy homeostasis in this species. The positive association of BW with leptin can be explained with an increase in body fat mass concomitant with weight gain, resulting in increased leptin secretion (Chilliard et al., 1998b).

The positive relationship between plasma leptin and cholesterol concentrations might reflect endocrine and paracrine regulation of body stores by leptin (Houseknecht et al., 1998; Chilliard et al., 2001). Positive associations between cholesterol and leptin were reported previously in humans (Liuzzi et al., 1999) and in rodents (Roden et al., 2000). Both plasma leptin and albumin concentrations are indicators of the nutritional status and were therefore likely to be correlated. A negative association of leptin with high AST activities, which indicate a high lipid mobilization rate and storage of lipids in the liver, was not surprising. However, causal relationships between leptin and albumin or AST, respectively, have not been reported previously.

Because insulin regulates leptin levels in rodents and humans (Saladin et al., 1995; Utriainen et al.,

1996), a positive association of plasma leptin with insulin concentrations could be expected. Our data were also in agreement with Amstalden et al. (2000a, 2000b), who described fasting-mediated decreases in leptin gene expression and circulating leptin to be associated with reductions in secretion of insulin and IGF-I in prepubertal heifers and dairy cows, and with Houseknecht et al. (2000), who reported that leptin abundance was highly correlated with adipose tissue IGF-1 mRNA in GH-treated animals. Thus, interactions between leptin and insulin or IGF-1 might be of importance in whole-body homeostasis in the bovine, too. An association between thyroid hormones and leptin in cattle is here reported to our knowledge for the first time. In accordance with results in rodents (Syed et al., 1999), high concentrations of plasma T_3 were associated with low leptin concentrations. The effect of thyroid hormones on leptin concentrations might be indirectly through the regulation of EB and fat mass.

CONCLUSIONS

Although metabolic stress was more marked in C30 than C50 cows, expressed by lower blood plasma concentrations of glucose, insulin, IGF-1, T_3 , milk protein, lactose, higher concentrations of NEFA, BHBA, GH and milk AC, and a faster decrease of BCS, backfat thickness and muscle diameter in C30 than C50, C30 cows were able to adapt successfully to the metabolic stress after parturition and to maintain metabolic homeostasis. This was evidenced by only slightly lower milk production in C30 than in C50 cows. The relationships between leptin and the various metabolic, endocrine and zootechnical traits in the present study suggest that leptin plays a role in whole-body homeostasis in bovine, too. Leptin is one among several factors involved in the regulation of energy metabolism and may be important for overall homeostatic and homeorhetic control of metabolism and thus for maintenance of performance. It remains to be investigated if it is a key player or not.

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