The effect of milk production level on host resistance of dairy cows, as assessed by the severity of experimental Escherichia coli mastitis

Esther KORNALIJNSLIJPERa*, Bonne BEERDAb, Ineke DAEMENa, Joop VAN DER WERFb, Tine VAN WERVena, Theo NIEWOLDb, Victor RUTTENC, Elsbeth NOORDHUIZEN-STASSENa

a Department of Farm Animal Health, Ruminant Health Unit, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands
b Division of Animal Sciences, Institute for Animal Science and Health, PO Box 65, 8200 AB Lelystad, The Netherlands
c Immunology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands

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Abstract – This study investigated the possible effects of milk production level on the host resistance of dairy cows. High (n = 18) and low (n = 18) producing cows on a research farm, which respectively produced 11 443 and 7 727 kg milk in their previous lactation, were compared. To enhance the possible differences in host resistance between high and low producing cows, the animals in both groups were metabolically stressed by overfeeding during the dry period or were fed according to requirements, resulting in four groups of nine cows. The metabolic status was monitored from two weeks pre-partum until 2.5–4.5 weeks post-partum. Host resistance was assessed by measuring the severity of experimentally induced Escherichia coli mastitis. Pre-partum blood glucose levels tended to be higher in overfed cows than in cows fed according to requirements. The post-partum energy balance was significantly more negative in high producing cows than in low producers, and tended to be more negative in overfed cows compared to cows fed according to the requirements. Post-partum plasma glucose, NEFA, β-OH-butyrate and urea concentrations were similar in the four groups. Plasma glucose concentrations were significantly lower and liver triacylglycerol concentrations were significantly higher in third than in second parity cows. Host resistance was not affected by the production level or feeding regimen. There were no significant correlations between the metabolic status and the severity of experimental E. coli mastitis, except for the relatively more severe mastitis in the cows with β-OH-butyrate concentrations above 1.4 mmol/L. In conclusion, milk production level did not affect host resistance in dairy cows, as measured by the severity of experimental E. coli mastitis. Even in a situation where cows were metabolically stressed by overfeeding, high producers were as able as low producers to cope with the demands of milk production, without consequences for host resistance.

dairy cow / milk production / host resistance / metabolism / E. coli mastitis

* Corresponding author: j.e.kornalijnslijper@vet.uu.nl
1. INTRODUCTION

Milk production, one of the most important factors contributing to the profitability of a dairy farm, has tremendously increased during the past decades in Western Europe and North America. In the Netherlands, the mean milk production level of dairy cows increased from around 5000 kg in 1970 to 8000 kg in 2000 [16]. It is a growing concern that these high milk production levels are realised at the expense of the dairy cows’ health and well-being. Whether or not high milk production is associated with poor health has mainly been investigated in epidemiological studies. For example, studies from the northeast USA and recently Germany suggest an increased risk of clinical mastitis with increasing milk production [6–8]. However, epidemiological studies are generally not conclusive in terms of causal relations between observed risk factors and disease [21]. To substantiate the hypothesis that increasingly higher milk production negatively affects the dairy cows’ health, experimental studies are necessary. Such studies could also add to the understanding of underlying mechanisms, which are likely to involve host resistance and metabolic status.

Detilleux et al. [4] compared host resistance between high and low producing cows, but did not find negative effects of milk production level on in vitro neutrophil functions and antibody production. However, these host resistance parameters have been measured in a “steady state” situation, and therefore do not give information on the animals’ capacity to respond when challenged. A method to achieve this is to measure induced in vivo humoral and cellular responses after immunisations with a test-antigen such as tetanus-toxoid [27]. Unfortunately, it is not clear to what extent responsiveness to immunisations with test-antigens is related to resistance to infectious diseases. The latter is most reliably assessed when using disease models. A useful disease model is that of Escherichia coli mastitis, a relevant problem in dairy herds [1, 5]. The severity of experimental E. coli mastitis may be used as a parameter of host resistance in dairy cows. The effects of high milk production on host resistance could be mediated by the metabolic status of the animal. The metabolic status reflects the way cows cope with the demands of milk production, e.g. by means of utilizing body fat, and to what extent they manage, e.g. blood levels of ketones and glucose. Ketosis, a situation where coping has been inadequate, has been associated with an increased risk of clinical mastitis in the field [8, 17]. These epidemiological findings have been substantiated by experimental in vivo and in vitro data [13, 22]. It could therefore be of interest, in an experimental setting, to enhance the possible differences in host resistance between high and low producing cows by challenging their metabolism. A well known metabolic stressor, which frequently occurs in the field, is overfeeding during the dry period. This may lead to overcondition at calving and to depression of appetite after calving. As a consequence, cows may go into a more severe negative energy balance post-partum (review by [20]).

The aim of this study was to investigate the possible effects of milk production level on the host resistance of dairy cows, assessed by measuring the severity of experimentally induced E. coli mastitis, in situations where cows were either or not metabolically stressed.

2. MATERIALS AND METHODS

2.1. Experimental design

The main factors in the experiment were milk production level and feeding regimen during the dry period. Two milk production levels were used (“high production” (HP) versus “low production” (LP); see Sect. 2.2). During the dry period the cows were either fed according to recommendations for energy requirements (control = c) or fed
approximately twice such recommendations (overfed = o), and this resulted in four
experimental groups: HP-c, LP-c, HP-o and LP-o (for nutrition: see Sect. 2.3). Within
the production groups, the cows were alternately assigned to a feeding regimen in
order of expected calving date, until a total of 9 cows per group was achieved. Host
resistance was assessed by quantifying the severity of experimental *E. coli* mastitis.
The cows were in week 2.5–4.5 post-partum at the moment of intramammary infu-
sion with approximately 50 cfu *E. coli* (see Sect. 2.5). The cows that calved within a
period of two weeks were tested as a group and this resulted in 11 test groups of 1–
6 animals. The experiment was approved by the Animal Care and Ethics Committee
of the Institute for Animal Science and Health, Lelystad, the Netherlands.

2.2. Animals and housing

A total of 39 animals, including 27 sec-
ond and 12 third parity cows, of the
research farm of the Institute for Animal
Science and Health, Lelystad, the Nether-
lands, were entered in this study. Cows
were 100% Holstein Friesian (HF), except
for three crossbreds (≥ 50% HF). The herd
size of the farm was approximately
400 dairy cows. The animals were selected
and categorized based on 305 days fat and
protein corrected milk production (FPCM)
in the previous lactation. The selection cri-
teria were related to the mean production
level of the farm (approximately 9 500 kg).
The cows in the “high producing (HP)
group” and “low producing (LP) group”
had mean FPCM (± s.d.) in their previous
lactation of 11 443 ± 838 kg (ranging from
9 999 to 13 364 kg) and 7 727 ± 640 kg
(ranging from 6 596 to 8 686 kg), respec-
tively. The animals calved during the
period from October 2000 through April
2001. The animals were housed in a free
stall with cubicles during the dry period
and were moved to a calving pen shortly
before the expected calving. After calving,
the cows were moved to a tie stall. The
cows were milked twice daily in a rotating
milking parlor with milking intervals of
10 and 14 hours. Diseased animals were
treated following the standard procedures
of the research farm.

2.3. Nutrition

During the dry period the cows were fed
a Total Mixed ration (TMR; see Tab. I).
The food was provided to them as a group
in the free stall. During the dry period the
HP-c and LP-c cows were fed around
60 MJ per day (dry period TMR-c). The
HP-o and LP-o cows were fed around
120 MJ per day (dry period TMR-o). Ini-
tially, the cows were fed TMR I. This TMR
was adapted by the manager of the
research farm into TMR II to improve min-
eral supplementation in order to prevent
impending milk fever problems in the
herd. As a consequence, one third of the
animals received TMR I only, part TMR I /
part TMR II and TMR II only, respec-
tively. All animals received TMR-o from
ten days before the expected calving. Post-
partum, all animals were individually fed
ad libitum lactation TMR in the tie stall.
Here, daily feed intakes of the individual
cows were registered.

2.4. Metabolic parameters

Milk production was recorded twice
daily. Composite milk sampling for milk fat
and protein percentages was performed
during four consecutive milkings per week
(Tuesday afternoon until Thursday morn-
ing). Bodyweights were recorded twice
daily after calving. A liver biopsy for liver
triacylglycerol (TAG) determination was
taken as described before [24], when the
cows were between 6–13 days after calving.
Blood sampling for the measurement of
glucose, non-esterified fatty acids (NEFA),
β-OH-butyrate (BHB) and urea started
three weeks before the expected calving
dates. Blood was sampled twice a week
(Mondays and Thursdays) from the tail vein
using 10 mL evacuated tubes containing
EDTA (Vacuette®, Greiner B.V., the Netherlands). Blood sampling continued until experimental *E. coli* mastitis (Wednesdays), with an extra sample taken just prior to infusion. Blood samples were centrifuged for 12 min at 1600 × g at 4 °C and stored at –20 °C until assayed.

### 2.5. Induction of *E. coli* mastitis

The cows were between 18 and 31 days post-partum at the moment of experimentally induced *E. coli* mastitis. An encapsulated strain of *E. coli* O157 (non-verotoxic), isolated from a cow with clinical mastitis, was used for intramammary infusion [14]. The strain was subcultured in brain-heart infusion broth CM 225 (Oxoid; Hampshire, England) at 37 °C starting 2 days before each intramammary infusion. After 22 hours of incubation, the bacterial culture was washed three times in pyrogen-free saline. Subsequently bacterial counts were determined by dilution plating of serial dilutions using a spiral plater (Lameris Laboratory, Breukelen, the Netherlands) and violet red bile glucose agar CM484 plates (Oxoid, Hampshire, England). The bacterial suspension was refrigerated (4 °C) until the next day. Shortly before intramammary infusion, the culture was diluted in pyrogen-free saline to a concentration of approximately 10 cfu/mL. At three time-points pre-infusion (6 days, 2 days and just prior to intramammary infusion), quarter milk samples were taken for bacteriology and SCC. One of the rear quarters free of major pathogens and with SCC < 150 000 cells/mL was used for experimental infusion. Five millilitres of *E. coli* suspension containing approximately 50 cfu was infused aseptically. The actual counts of the inocula were determined by dilution plating, and ranged from 30 to 50 cfu.
Before and after experimental infusion the following parameters were recorded: body temperature, general impression, quarter symmetry and firmness (pre-infusion, hourly up to 24 hours after infusion, thereafter at 30, 36, 42, 48, 60, 72 and 96 hours after infusion), bacterial counts in milk, and milk color and aspect (pre-infusion, every 3 hours until 24 hours after infusion, at 30, 36, 42, 48, 60, 72 and 96 hours after infusion and thereafter every 24 hours until negative, i.e. < 1 cfu/mL). Before each milk sampling, the teat ends were swabbed with cotton swabs drenched in methylated spirits and the first few streaks of milk were discarded. After E. coli infusion, the bacterial counts in the milk were determined using a spiral plater (Lameris) on violet red bile glucose agar CM 484 (Oxoid). In addition, per sample, bacterial counts in 1 mL milk were determined using a pour-plate technique, so that the detection limit in this experiment was 1 cfu/mL. Quarter milk production was measured from six milkings before until six milkings after experimental infusion of E. coli using a quarter milking machine in the tie stall. The milking interval after experimental infusion was 12 hours.

The severity of experimental E. coli mastitis was expressed as: $10^{\log}$ peak bacterial counts in milk, area under the curve (AUC) of bacterial counts in milk in time [14], bacterial clearance time (BCT) and residual milk production (RMP; [26]). AUC ($10^{\log}$ cfu × h) was calculated using the following equation:

$$AUC = \sum \{(t_i - t_{i-1}) f_{i-1} \} + \{0.5(t_i - t_{i-1}) (f_i - f_{i-1})\},$$

where: $t_i =$ time of observation, $t_{i-1} =$ previous time of observation, $f_i =$ $10^{\log}$ bacterial counts at time $i$, $f_{i-1} =$ $10^{\log}$ bacterial counts at time $i-1$.

BCT was defined as the interval between intramammary infusion and the time-point that bacterial counts are below the detection limit (< 1 cfu/mL). RMP was calculated using the mean quarter milk production of the three non-infused quarters during the six pre-infusion milkings as the baseline. The milk production of the non-infused quarters on the second day after infusion (mean of the milking 36 and 48 hours after infusion), was expressed as a percentage of the baseline.

2.6. Calculation of the energy balance

The energy balance (EB), expressed as daily mean per week in lactation, was calculated as the difference between the energy intake and energy requirements for maintenance and milk production, using the following equations:

$$EB = \text{energy intake} - \text{energy requirements}$$

with: energy intake = NE$_l$ intake/day = kg DMI/day × NE$_l$ TMR, energy requirements = (42.4 × BW$^{0.75}$ + 442 × FPCM) × [1 + (FPCM − 15) × 0.00165], where: kg DMI/day = kg feed intake/day × dry matter (g/kg) × 0.001, FPCM = (0.337 + 0.116 × fat content + 0.06 × protein content) × kg milk/milking and: NE$_l$ = net energy for lactation (MJ), DMI = dry matter intake (average per week), TMR = total mixed ration, BW = body weight (average per week), FPCM = fat and protein corrected milk production (four milkings per week).

2.7. Assay procedures

Plasma glucose (Hexokinase method; Gluco-quant®, Roche Diagnostics GmbH, Mannheim, Germany), NEFA (ACS-ACOD enzymatic colometric method; Wako NEFA C test, Wako Chemicals USA Inc. Richmond USA), BHB (Hydroxybutyrate-dehydrogenase method; Roche Diagnostics GmbH) and urea (GLDH method; UR225, Randox Laboratories Ltd., Crumlin UK) were measured enzymatically with commercially available kits. TAG concentration in liver tissue was determined using a commercial kit (kit number 337-A; Sigma Chemical Co.; St. Louis, MO, USA). Bacteriological culture was performed to check the sterility of pre-infusion milk samples, according to the
standard of the National Mastitis Council [9]. Six millilitres of composite milk samples and pre-infusion quarter milk samples were transferred to glass tubes containing Na-azide and were assayed for SCC (Fossomatic, Foss Electric, Hillerød, Denmark) and percentages of fat, protein and lactose (Milcoscan, Foss Electric, Hillerød, Denmark).

2.8. Statistical analysis

All statistical procedures were performed using SPSS 8.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The normality of the data was checked by examination of normal probability plots of unstandardized residuals. If necessary, data were $10^\log$ transformed. The following models (1 to 4) were used:

(1) A general linear model (general factorial) was used with the production level and feeding regimen (including interactions) as fixed factors. The dependent variables in this model were respectively pre-partum blood metabolite concentrations (glucose, NEFA, BHB, urea), $10^\log$ TAG, $10^\log$ peak cfu, $10^\log$ AUC, $10^\log$ BCT or RMP. Pre-partum blood metabolite concentrations were calculated as the mean of the concentrations in the samples taken in the 14 days pre-partum. Possible influences of milk production level on the severity of $E. coli$ mastitis (pre-infusion metabolic status was defined as the mean of values of EB and blood glucose, NEFA, BHB or urea in the week prior to $E. coli$ infusion. For blood metabolite values this was the mean of three samples taken in the week prior to infusion (Thursday, Monday and Wednesday). Partial correlation coefficients were also calculated for associations between $10^\log$ TAG and the severity of $E. coli$ mastitis.

(2) The possible influences of metabolic status on the severity of $E. coli$ mastitis were also analyzed by inclusion of all pre-infusion metabolic parameters in a general linear model (general factorial) as covariates.

For all models, the level of significance was set at $P < 0.05$.

3. RESULTS

3.1. Cows and milk production

During the experiment two cows were excluded from the study. One of these had a non-functional quarter and the other had both rear quarters with SCC > 150 000 cells/mL, and $Staphylococcus aureus$ was isolated from the left rear quarter. A third cow was treated with antibiotics for acute claw disorders at the time of the HPA tests, and excluded from these tests. This animal was included for experimental
E. coli mastitis, and an additional animal was used to complete the 4 × 9 animals for the HPA measurements. Therefore, data on metabolic status are available for 37 cows. Eight cows were treated with antibiotics in the early post-partum period: five cows (1 HP-o, 1 LP-c, and 3 LP-o) for retentio secundinarum and/or endometritis, one of which received additional treatment for ketonuria (LP-o), two cows (1 HP-o and 1 LP-o) for claw disorders and one cow (HP-c) for clinical mastitis in the right front quarter. Three cows (2 HP-c and 1 LP-c) received milk fever treatment. Mean FPCM (± s.d.) in weeks 2 and 3 post-partum are presented in Figure 1. FPCM was significantly higher in HP cows than in LP cows (P < 0.001).

### 3.2. Metabolic status

#### 3.2.1. Energy balance

Mean energy balance (± s.d.) in weeks 2 and 3 post-partum are presented in Figure 2. For all cows, EB was negative during the observation period. In all groups, EB became significantly less negative in week 3 compared to week 2 (P < 0.01). EB was significantly affected by production level (P = 0.026), with more negative EB in high producing cows. EB tended to be affected by the feeding regimen (P = 0.085), with more negative EB in overfed cows. Four cows (2 LP-c and 2 LP-o) were excluded from the repeated measures analysis due to missing body weights in week 3. The results
were, however, similar to those obtained by analyses with all cows (n = 37) for week 2 only.

### 3.2.2. Liver TAG

Individual liver TAG contents are presented in Figure 3. The liver TAG contents of six cows are missing. Due to Foot and Mouth Disease regulations in The Netherlands (in the spring of 2001), the liver biopsies of these animals were not allowed to be transported from the farm to the laboratory. The liver TAG contents of the remainder of the animals were not affected by milk production level and/or feeding regimen. Parity affected liver TAG content significantly, with higher TAG levels in third parity cows (P = 0.004).

### 3.2.3. Metabolites in the blood

Plasma glucose, NEFA, BHB and urea concentrations from 2 weeks before until 3 weeks after parturition are presented in Figures 4, 5, 6 and 7. Pre-partum concentrations of glucose tended to be affected by the feeding regimen (P = 0.055), with relatively higher values in the overfed groups. Pre-partum concentrations of NEFA, BHB and urea were similar in all groups. Post-partum, mean plasma glucose and urea concentrations significantly decreased, and mean plasma NEFA and BHB concentrations significantly increased in all groups (P < 0.01). Post-partum concentrations of blood metabolites were similar in all groups. Post-partum glucose concentrations were significantly affected by parity, with relatively low glucose concentrations in third parity cows (P = 0.007).

### 3.3. Host resistance

#### 3.3.1. Severity of experimental E. coli mastitis

Experimental intramammary infusions with 30–50 cfu E. coli resulted in clinical mastitis in all 36 cows. The cows showed a range of local and systemic signs of disease. Local signs consisted of swelling and increased firmness of the infused quarter and changes in the color and aspect of the milk. Systemic signs consisted of general depression, loss of appetite and elevated body temperatures. Severity of E. coli mastitis varied enormously between cows based both on clinical signs and on bacterial counts in the milk (expressed as peak cfu, AUC and BCT) and quarter milk production (RMP). The bacterial counts in

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**Figure 3.** Scatter plot of individual liver TAG content (mg/g liver) in liver biopsies taken between 6 and 13 days after calving (HP-c: n = 8; HP-o: n = 9; LP-c: n = 6; LP-o: n = 8). HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period. Closed circles depict second parity cows and open circles depict third parity cows.
the milk of the cows with the lowest and highest AUC are presented in Figure 8a. The AUC of bacterial counts in milk for all individual cows are presented in Figure 8b. Different parameters for the severity of E. coli mastitis are summarized in Table II. Correlations between severity parameters ranged from 0.78 to 0.93 (P < 0.001). All cows eliminated the pathogen without therapeutic intervention, except for one cow from the HP-c group that needed treatment for secondary clinical ketosis. The severity of experimental E. coli mastitis was not affected by the production level or feeding regimen during the dry period for any of the measures (see P-values in Tab. II). Also, no effect of milk production level on the severity of experimental E. coli mastitis was seen when the animals’ previous lactation FPCM or mean milk production in the pre-infusion week was included as a covariate.

3.3.2. Associations with metabolic status

Partial correlation coefficients between $10\log$ AUC and parameters of pre-infusion metabolic status are summarized in Table III. Correlation coefficients were low and not significant. No significant effects were seen when all pre-infusion metabolic parameters (EB, $10\log$ TAG, glucose, NEFA, BHB and urea) were included as covariates in the general linear model. When BHB concentrations

Figure 4. Individual weekly average plasma glucose concentrations in the four experimental groups from two weeks before until 3 weeks after parturition. HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period.
were categorized (cut-off 1.4 mmol/L; [13]), the severity of experimental *E. coli* mastitis was significantly affected by BHB concentrations, with higher $10\log$ AUC in cows within the higher category of BHB ($P = 0.041; R^2 = 0.12$). Scatter plots of $10\log$ AUC with BHB concentrations as continuous and categorized values are presented in Figure 9a and b, respectively.

**4. DISCUSSION**

The present experiment is the first to use a disease model to study the possible differences in host resistance between high and low producing dairy cows. The results suggest that milk production level per se does not negatively affect host resistance, as assessed by measuring the severity of experimentally induced *E. coli* mastitis. These findings agree with those of Detilleux et al. [4]. They measured host resistance parameters in a “steady state” situation, comparing cows from high and average genetic merit lines for milk production, and concluded that selection for high milk production does not compromise host resistance. Although the severity of experimental *E. coli* mastitis did not differ between the groups in the present study, large individual variation in severity was seen within groups. The same holds true for metabolic parameters like energy balance, liver TAG content and blood glucose, NEFA, BHB and urea concentrations. It has often been

![Figure 5](image-url). Individual weekly average plasma NEFA concentrations in the four experimental groups from two weeks before until 3 weeks after parturition. HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period.
hypothesised that a negative energy balance (NEB) negatively affects host resistance. The evidence is, however, based mainly upon studies on ketosis, which is not synonymous with negative energy balance. A recent study by Perkins et al. [18] provided no evidence that NEB in itself affects host resistance. They found no relationship between energy balance and the outcome of intramammary endotoxin infusion, used as a mastitis model, in midlactation cows that were either or not feed-restricted. The present study clearly adds to the one by Perkins et al. [18] because of the use of early post-partum cows and a real pathogen in a mastitis model. The early post-partum energy balance was significantly affected by production level, and tended to be affected by feeding regimen, but was not associated with the severity of experimental E. coli mastitis. From our studies too, it can be concluded that NEB per se does not have to be detrimental to the cow. Ketosis, however, has been described as a risk factor for mastitis in the field [8, 17]. Kremer et al. showed that the course of experimental E. coli mastitis is relatively severe in ketonemic cows [13], and Van Werven et al. found a strong positive correlation between pre-infusion BHB concentrations and the severity of experimental E. coli mastitis (r = 0.78 [25]). Ketone bodies, such as BHB, may exert a negative effect on the quality of polymorphonuclear leukocyte (PMN) functions, an important aspect of udder defence (see review by [22]). In the present study, no strong associations were found between pre-infusion blood metabolite concentrations and the severity of E. coli mastitis. Only BHB concentrations

Figure 6. Individual weekly average plasma BHB concentrations in the four experimental groups from two weeks before until 3 weeks after parturition. HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period.
above 1.4 mmol/L were linked to relatively severe experimental *E. coli* mastitis, although only 12% of the variation between cows was explained in this way. A difference with the earlier mentioned studies on ketosis and *E. coli* mastitis is that most cows in this study were able to keep their blood metabolite levels in the normal ranges. In the pre-infusion week, the cows had mean glucose concentrations above 2.5 mmol/L, and mean BHB concentrations between 0.4 and 3.0 mmol/L, i.e. concentrations that differ from those seen in the ketonemic cows in the study by Kremer et al., namely between 1.6 and 2.5 mmol/L and between 2.2 and 4.9 mmol/L, respectively [13]. Also in the study by Van Werven et al., pre-infusion BHB concentrations were around 4 mmol/L in most cows [25]. It seems that blood metabolites affect host resistance only at levels that are associated with metabolic disease.

By means of overfeeding half of the cows in the dry period, it was expected that more animals in this study would have a poorer metabolic status post-partum and show, for example, BHB concentrations in the higher ranges. The increased pre-partum glucose concentrations in the overfed cows (*P* < 0.1) indicate that the feeding treatment was effective to some extent, but post-partum the differences between glucose concentrations disappeared and also other blood metabolites (NEFA, BHB, urea) did not differ between groups, indicating that the cows’ coping abilities after calving were adequate. Parity significantly affected post-partum glucose concentrations and liver TAG content, with lower glucose and higher TAG content in third compared to second parity cows. This experiment was not, however, designed to study the differences between second and third parity cows, and thus does not allow

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**Figure 7.** Individual weekly average plasma urea concentrations in the four experimental groups from two weeks before until 3 weeks after parturition. HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period.
strong conclusions with regards to parity. In other studies, overfeeding during the dry period has caused serious metabolic disturbances such as fatty liver [20], but in these studies overfeeding during the dry period was usually followed by a short period of food deprivation from the onset of parturition [19]. It should be emphasized that in our experiments, overfeeding during the dry period was performed to model suboptimal management conditions in the field and not to induce a pathological state. As a result, the present regimen of overfeeding may have been only moderately effective in straining the animals.

A probably important difference between the feeding conditions in this study as compared to those in the field is that, because of experimental procedures, cows were housed in a tie-stall after calving, while in the field, most cows are moved as soon as possible from the calving pen to a free stall. In free stalls, access to food may be dependent on the animals’ rank in the herd and the number of feeding places [15]. Limited feeding places can result in reduced uptake of food by especially low ranking animals, resembling a “feed restriction model” for some cows in a herd. For example, early post-partum cows housed in groups had higher NEFA concentrations than cows housed in individual box stalls [2]. Post-partum, the cows in this study were moved to a tie stall and were fed individually.
Differences between cows in their ability to cope with the transition to a free stall were ruled out in this way. The results of this study suggest that detrimental effects of suboptimal dry period nutrition can be counteracted by excellent feeding conditions, e.g. unrestricted access to good quality food, after parturition.

Regardless of the effectiveness of the feeding regimen, this study clearly shows that milk production level per se does not affect host resistance as measured by the severity of experimental *E. coli* mastitis. This result seems to be in contrast with the results of epidemiological studies in which correlations have been found between milk production level and the incidence of mastitis [6–8, 17]. The discrepancy between the results of epidemiological and experimental studies might be a pseudo-discrepancy. Gröhn et al. [7] pointed out that the higher incidence of mastitis found in epidemiological studies on commercial dairies could be the result of bias since diseased animals with low production are more likely to be culled than those with high production. This could explain the apparent greater risk of disease in high yielding cows. However, the higher incidence of mastitis found in epidemiological studies could also truly reflect the situation in the field. Experimental studies of mastitis differ from observational epidemiological studies in that the teat duct is surpassed, so only differences between cows at the level of the inflammatory response in the mammary gland (i.e. mammary epithelium, acute phase response, influx of PMN) are evaluated (for review see [12]). Thus the higher incidence of mastitis in high producing cows could be the consequence of differences in the quality of the teat duct. There is circumstantial evidence that the quality of the teat duct might be compromised in high producing animals. High milk production has been described as a risk factor for teat injury [8], and teat injury, in turn, as a risk factor for clinical mastitis [8, 17]. In addition, selection for higher milk production has also

### Table II. Mean values (± s.d.) of different parameters for the severity of *E. coli* mastitis. *P*-values for production and feeding effects on the severity of *E. coli* mastitis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HP-c</th>
<th>HP-o</th>
<th>LP-c</th>
<th>LP-o</th>
<th><em>P</em>-value production</th>
<th><em>P</em>-value feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak cfu (10^log cfu/mL)</td>
<td>5.7 ± 1.6</td>
<td>5.8 ± 1.4</td>
<td>6.1 ± 2.5</td>
<td>5.8 ± 1.8</td>
<td>0.718</td>
<td>0.905</td>
</tr>
<tr>
<td>AUC (10^log cfu × h)</td>
<td>262 ± 199</td>
<td>260 ± 166</td>
<td>322 ± 246</td>
<td>260 ± 178</td>
<td>0.930</td>
<td>0.999</td>
</tr>
<tr>
<td>BCT (h)</td>
<td>129 ± 62</td>
<td>139 ± 68</td>
<td>141 ± 68</td>
<td>122 ± 63</td>
<td>0.649</td>
<td>0.871</td>
</tr>
<tr>
<td>RMP (%)</td>
<td>88 ± 21</td>
<td>84 ± 29</td>
<td>75 ± 34</td>
<td>91 ± 23</td>
<td>0.787</td>
<td>0.364</td>
</tr>
</tbody>
</table>

Abbreviations: AUC = area under the curve for bacterial counts in milk in time, BCT = bacterial clearance time (< 1 cfu/mL), RMP = residual milk production (% of pre-infusion baseline in the three non-infused quarters); HP = high producing, LP = low producing, -c = fed according to requirements during the dry period, -o = overfed during the dry period.

### Table III. Partial correlation coefficients and *P*-values of pre-infusion metabolic status and severity of *E. coli* mastitis expressed as 10^log AUC. Except for 10^log TAG, values for metabolic parameters were the mean of measurements in the week pre-infusion.

<table>
<thead>
<tr>
<th>10^log AUC</th>
<th>Correlation coefficient</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy balance</td>
<td>0.06</td>
<td>0.72</td>
</tr>
<tr>
<td>10^log TAG</td>
<td>-0.02</td>
<td>0.92</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>NEFA</td>
<td>-0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>BHB</td>
<td>0.17</td>
<td>0.34</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.09</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Milk production and host resistance

been a selection on a faster milk flow [11], which, together with increased mechanical pressure due to large volumes of milk, may cause increased leaking of milk in high producing cows [10]. The latter is associated with an increased risk of clinical mastitis [21]. The foregoing stresses the need for comparing the quality of the teat duct, as an important aspect of host resistance, between high and low producing cows. This could be achieved by using exposure of teat ends to an E. coli broth culture as a model, as it has been described by DeHart et al. [3].

In conclusion, milk production level did not affect host resistance in dairy cows, as measured by the severity of experimental intramammary infusion with E. coli in early lactation. Even in a situation that cows were metabolically stressed by overfeeding, high producers were as able as low producers to cope with the demands of milk production. The apparent increased susceptibility for mastitis of high producing cows in the field may be the result of factors such as decreased quality of the teat duct in high producing cows, or feeding management that result in a stronger impact on host resistance under field conditions as compared to the present experimental circumstances.

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