K. Barth, K. Aulrich, H. C. Haufe, U. Müller, D. Schaub, F. Schulz / Landbauforschung - vTI Agriculture and Forestry Research 4 2011 (61)307-316

307

Metabolic status in early lactating dairy cows of two breeds kept under conditions of organic farming – a case study

Kerstin Barth*, Karen Aulrich*, Helge Christiane Haufe*, Ute Müller**, Dagmar Schaub*, and Franz Schulz***

Abstract

The transition period is the key for the health and performance of the lactating dairy cow: An increasing demand of energy due to the foetal growth, lactogenesis and the beginning lactation is accompanied by a restricted feed intake and leads to a negative energy balance. Organic farming aims to produce forage based milk – a goal which might increase the risk of metabolic disorders during that time. Aim of this study was to compare a dual-purpose (German Red Pied – GRP) and a dairy breed (German Holstein – GH) kept under the same management conditions and a comparison of two herds consisting of the same dairy breed (GH) but managed differently to test the effect of the breed or the management on the metabolic status of cows during the first five weeks of lactation. Records of 179 cows (84 GH at Farm1, and 46 GH and 49 GRP at Farm2) were included in the study. Energy requirements (EB%) were better fulfilled at Farm1 than at Farm2. Week of sampling and farm had an effect on the ratio of fat to protein (FPR) and β-hydroxybutyric acid (BHBA) in milk, nonesterified fatty acids (NEFA) and glutamate dehydrogenase (GLDH) in blood, respectively. Breed affected FPR, BHBA and GLDH significantly but not NEFA, and the EB% showed a significant effect on FPR, BHBA and NEFA. Season affected FPR and GLDH with the tendency of higher readings in winter. Our study confirmed that breeds with a higher genetic merit for milk yield suffer a higher metabolic load when the feeding management in the periparturient period is suboptimal but under conditions better fulfilling their demands, these differences could not be observed. Thus, there is no need to prefer dual-purpose breeds in organic dairy farming as long as the management is appropriate for high yielding cows.

Keywords: organic farming, metabolism, ketosis, German Red Pied, German Holstein

Zusammenfassung

Stoffwechselstatus von zwei Rassen in der Frühlaktation unter Bedingungen des ökologischen Landbaus – eine Fallstudie

Die Transition Periode ist entscheidend für die laktierende Kuh: Eine reduzierte Futteraufnahme steht dem steigenden Energiebedarf für das Wachstum des Fötus sowie der Laktogenese gegenüber und bedingt eine negative Energiebilanz. Das Ziel der Bio-Milchviehhaltung, Milch raufutterbasiert zu erzeugen, erhöht das Risiko für Stoffwechselstörungen in dieser Zeit und es stellt sich die Frage, ob weniger milchbetonte Rinderrassen besser für diese Haltungsform geeignet sind. Um den Effekt der Rasse bzw. des Managements auf den Stoffwechselstatus der Kühe während der ersten Laktationswochen zu testen, wurden zum einen eine Doppelnutzungs- (Deutsche Rotbunte – DRB) und eine milchbetonte Rasse (Deutsche Holstein – DH) unter den gleichen Bedingungen und zum anderen zwei unterschiedlich gemanagte Herden gleicher Rasse (DH), verglichen. 179 Kühe (Herde 1: 84 DH, Herde 2: 46 DH und 49 DRB) konnten ausgewertet werden. Der Energiebedarf (EB%) der Tiere wurde in Herde 1 besser gedeckt als in Herde 2. Die Probenwoche sowie die Herde hatten einen Effekt auf den Fett-Eiweiß-Quotienten (FEQ) und den β-Hydroxy-Buttersäure-Gehalt (BHBA) in der Milch, die nichtveresterten freien Fettsäuren (NEFA) und die Glutamadehydrogenase-Aktivität (GLDH) im Blut. Die Rasse beeinflusste FEQ, BHBA und GLDH und EB% zeigte einen Effekt auf FEQ, sowie BHBA und NEFA. Es bestand ein saisonaler Effekt bei FEQ sowie GLDH mit höheren Messwerten im Winter. Es bestätigte sich die stärkere metabolische Belastung von Tieren der milchbetonten Rasse in der Frühlaktation aufgrund suboptimaler Bedingungen während der Transition Periode. Bei einer adäquaten Fütterung trat dieser Unterschied nicht mehr auf.

Schlüsselwörter: Ökologischer Landbau, Stoffwechsel, Ketose, Deutsche Rotbunte, Deutsche Holstein

* Johann Heinrich von Thünen-Institut (vTI), Federal Research Institute for Rural Areas, Forestry and Fisheries, Institute of Organic Farming, Trenthorst 32, 23847 Westerau, Germany

** University Bonn, Institute of Animal Science, Physiology and Hygiene Group, Katzenburgweg 7 – 9, 53115 Bonn, Germany

*** Justus Liebig University Giessen, Experimental Station Gladbacherhof, 65606 Villmar, Germany

Corresponding author: Dr. Kerstin Barth, E-mail: kerstin.barth@vti.bund.de

Introduction

Organic dairy farming aims to "feed no food" and thus, reduce the amount of concentrates fed to cows and produce forage-based milk. Therefore the European Commission Council Regulation on Organic Production (EC, 2007) requires at least 60 % roughage in dairy cow rations. To fulfil the energy requirements of the early lactating cow, this limit might be reduced to 50 % at the beginning of lactation. It is well known that the transition period, starting with the third week prior to calving and lasting until the third week *post partum*, is the key period for the health and performance of the lactating dairy cow (Drackley, 1999). During that time, an increasing demand of energy due to the foetal growth, lactogenesis and the beginning lactation is accompanied by a restricted feed intake and leads to a negative energy balance (e.g., Bell, 1995; Grummer, 1993; Goff & Horst, 1997). Due to homeorhetic regulation the nutrients are partitioned to support pregnancy and lactation, and especially the onset of lactation is associated with metabolic changes in body tissues (Bauman & Currie, 1980). To overcome the deficit of energy, body fat is mobilized resulting in an increase of nonesterified fatty acids (NEFA) concentration in blood. NEFA are used to provide energy by complete oxidisation to carbon dioxide and partial oxidisation to ketone bodies; they are incorporated into lipoproteins or stored as triglycerides in the hepatic tissue. Increasing NEFA concentrations in blood are followed by an increasing NEFA uptake rate of the liver resulting in a higher production of ketone bodies and an accumulation of triglycerides in the hepatic tissue, which might cause fatty liver syndrome in the animal (Goff & Horst, 1997). As a consequence of fatty liver syndrome, liver cells will be damaged and release the enzyme glutamate dehydrogenase (GLDH) (Wemheuer, 1987; West, 1990), primarily located in mitochondria of liver cells and required for the urea synthesis. Of all ketone bodies, β-hydroxybutyric acid (BHBA) is seen as predominant (Duffield et al., 2009) and can be found in blood, milk and urine (Nielsen & Ingvartsen, 2004). Elevated NEFA and BHBA concentrations are considered to have a depressing effect on the bovine immune system and thus, to increase the risk of diseases (Hoeben et al., 2000; Hachenberg et al., 2007). Although mastitis as one of the most important diseases of dairy cows has to be seen as caused by various factors, Leslie et al. (2000) found that increased rates of mastitis also were associated with subclinical ketosis.

Besides the sustainability of food production organic farming targets the maintenance of animal health by encouraging the natural immunological defence of the animal, as well as the selection of appropriate breeds and husbandry practices. As a consequence, the EU regulations ban the prophylactic use of "chemically synthesised allopathic veterinary medicinal products including antibiotics" (EC, 2008), and according to the standards of the American National Organic Program, the treated animal itself as well as its products are not allowed to be labelled organic. Among other things, disease prevention shall be based on breed selection, husbandry management practices and meeting the animal's nutritional requirements at the various stages of its development by providing organic feed of high quality (EC, 2008).

Although one might expect a higher ketosis incidence due to the limitations of concentrate feed, Hardeng and Edge (2001), evaluating treatment records found a much lower risk of ketosis in cows kept under organic farming conditions in Norway, where the maximum limit for concentrates is 30 % of the ration. This is in contrast to Hardarson (2001) who expected cows with high genetic merit for milk yield to be at greater risk to develop metabolic disorders in organic farming.

Hardeng & Edge (2001) explained their results with a tastier while diversified diet and consequently a higher feed intake. However, analyses of veterinary treatment records are always biased by the fact that only treated cases were reported, which means that the records are limited to clinical cases.

Nevertheless, organic cows produced 22 % less milk than the conventional cows in 305 days (Hardeng & Edge, 2001), and this cannot be explained by the use of lower yielding breeds, because high performance breeds like Holsteins are dominant in organic as well as in conventional dairy farming (Rahmann & Nieberg, 2005). According to the calculations made by Knaus et al. (2001), cows kept under the rules of organic farming would be able to produce up to 7,000 kg milk without exceeding the tolerable restrictions for suboptimal energy supply if the quality of the forage were high enough and the management were optimal. However, the current genetic potential of dairy breeds is much higher and the lower performance of organic cows suggests that the farms face difficulties in fulfilling the nutrient requirements of their cows.

EU legislations of organic farming demand the choice of breeds be based on their capacity to adapt to local conditions, their vitality and their resistance to health problems (EC, 2008).

Crossbreeds are seen as advantageous under suboptimal management conditions (Freyer et al., 2008), and organic dairy farming might benefit from using such animals instead of purebred high yielding cows. Another approach would be the use of dual-purpose breeds. Distl et al. (1989) found lower incidences of metabolic disorders in the dual-purpose German Simmental than in the German Brown Swiss, but in the study by Fall et al. (2008) comparing early lactating cows under organic and conventional farming conditions, the dual purpose Swedish Red breed

K. Barth, K. Aulrich, H. C. Haufe, U. Müller, D. Schaub, F. Schulz / Landbauforschung - vTI Agriculture and Forestry Research 4 2011 (61)307-316

309

had significantly higher NEFA readings than the Swedish Holstein breed.

However, the number of studies comparing the metabolic status of dual-purpose and dairy breed cows at the beginning of lactation is still limited, and especially comparisons carried out under conditions of organic farming are lacking.

Thus, our study aimed for comparing of a dual-purpose and a dairy breed kept under the same management conditions and a comparison of two herds consisting of the same dairy breed but managed differently to test the effect of the breed or the management on the metabolic status of the cows during the first five weeks of lactation. NEFA and GLDH in blood, BHBA in milk, and the ratio of fat to protein (FPR) were used as indicators of the metabolic status.

Material and Methods

Animals, housing and feeding

The study was carried out from October 2007 to January 2009 on two research farms working according to the rules of organic farming. The experimental station "Gladbacherhof" of the University Giessen, Germany (Farm1) has been following the rules of the German Bioland organization for more than 20 years and keeps 90 German Holstein black and white cows (Farm1-GH) at a free stall barn.

Since 2004, the Institute of Organic Farming of the Johann Heinrich von Thünen-Institut, Trenthorst, Germany (Farm2), keeps two herds of two different breeds allowing breed comparisons under the same management conditions. Therefore, the 50 German Holstein black and white (Farm2-GH) and the 50 dual purpose German Red Pied (Farm2-GRP) cows are kept separately in two identically designed compartments of a free stall barn.

Cows were milked twice daily with milking intervals of 11:13 and 10:14 hours at Farm1 and Farm2, respectively. Milk yield was automatically recorded during each milking (Metatron®, GEA, Bönen, Germany). During withdrawal periods (colostral period, medical treatment) the milk was collected in a bucket and manually weighed after milking. Based on the daily records per sampling week the mean milk yield per cow and day was calculated.

At Farm2 the herd was divided into two feeding groups: a group consisting of high yielding and cows in early lactation and a group including dry cows and cows in late lactation. At Farm1 dry cows formed an additional third group. At both farms the cows were fed a mixed ration *ad libitum* and received additional concentrate feed according to the yield and stage of lactation at an automatic feeding station. The mixed ration varied throughout the year and consisted of grass silage, clover grass silage and corn silage in both farms but was supplemented with potatoes, legumes and grain in various parts (Farm1) and 2 kg cow^{-1} day^{-1} of the concentrate mixture (Farm2). The composition of the used ingredients was analysed by commercial laboratories ("Hessisches Landeslabor", Kassel, Germany and "LUFA NRW", Köln-Auweiler, Germany). The amount of additional concentrate feed was limited to 8.0 and 6.5 kg cow^{-1} day^{-1} at Farm1 and Farm2, respectively. Feedstuff provided by the farms differed in energy, protein and fibre content (Table1).

Table 1:

Mean nutrient contents of the mixed rations and the concentrates fed during the experimental period

		Farm1		Farm2	
		Mean	SD	Mean	SD
Mixed ration					
Dry matter	[%]	41.1	4.0	43.2	3.9
Energy	[MJ NEL kg^{-1}]	6.5	0.2	6.2	0.2
Crude protein	[g kg^{-1}]	151	11	143	27
Utilisable crude protein	[g kg^{-1}]	141	3	136	4
Ruminal-Nitrogen-Balance		1.5	1.6	1.1	4.2
Crude fibre	[g kg^{-1}]	209	12	231	14
Concentrate feed					
Energy	[MJ NEL kg^{-1}]	8.4	0.1	7.9	0.1
Crude protein	[g kg^{-1}]	150	23	173	1
Utilisable crude protein	[g kg^{-1}]	171	3	167	3
Ruminal-Nitrogen-Balance		-2.8	3.3	0.9	1.8
Crude fibre	[g kg^{-1}]	40	16	61	23

Three weeks before the expected date of calving, cows at Farm1 received the dry cows' ration supplemented by 10 kg maize silage per cow and day. Heifers were integrated at the same time. Farm2 did not separate the dry cows from the late lactating ones but moved the cows in late pregnancy into the group of early lactating cows. Thus, three weeks ante partum these cows, as well as the new integrated heifers had access to the ration of early lactating and high yielding cows. Between May and October all cows of Farm1 were pastured about 3 hours per day. Animals at Farm2 did not have access to pasture but had outdoor access over the whole time period.

The daily consumption of concentrate feed per cow was recorded on both farms. The mean feed intake per group and day was calculated as difference between the amounts of mixed ration provided (recorded daily) and the remains (recorded once per week). Daily feed intake per cow was then roughly estimated by dividing the feed intake per group by the number of cows belonging to that group in the regarded week.

To get an impression about the nutrient supply despite lacking records of the individual feed intake per cow, the fulfilment of the energy needs was estimated by comparing the individual demand of energy and the energy provided by the ration. At Farm1 maintenance requirements were 35.5 MJ NEL and 39.9 MJ NEL for primiparous (estimated body weight: 650 kg) and pluriparous (estimated body weight: 750 kg) cows, respectively. At Farm2 individual body weight of each cow was recorded twice per day by an automatic walk-through weight scale (GEA, Bönen, Germany). Based on the readings per week, the mean body weight at each lactation week was calculated. The median for primiparous cows was 582 kg (from 448 to 803 kg) and for pluriparous cows 684 kg (from 496 to 866 kg). If less than five readings per week were available, data were rejected due to unreliability and a body weight of 650 kg and 700 kg was assumed for primiparous and pluriparous cows, respectively. The daily requirement for milk production was calculated (Spiekers & Potthast, 2004):

$$MJ\ NEL = milk\ yield\ [kg\ day^{-1}] \times (0.38\ fat\ content\ [\%] + 0.21\ protein\ content\ [\%] + 1.05)$$

The total requirement of energy for maintenance and milk production was related to the energy provided by the ration and the concentrate feed, and the individual coverage of the energy needs (EB%) was calculated.

Body condition of the studied animals was scored weekly by trained technicians according to Edmonson et al. (1989) using 0.25 increments on a 1 to 5 scale. BCS loss (BCSDiff) during the experimental period was calculated as difference between BCS on Day 5 and Day 1.

Usually cows at both farms were dried off 6 to 8 weeks before the expected calving. Based on cyto-bacteriological analyses, Farm1 used different antibiotics together with an internal teat sealer (Orbeseal®, Pfizer, New York, USA) whereas Farm2 treated when necessary the animals with Orbenin® (Pfizer, New York, USA). Due to an increased incidence of parturient paresis all cows at Farm2 received a Ca- and P-bolus post calving (Bovikalc, Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany). Farm1 applied such a prophylactic treatment only on six older cows.

Sampling and analyses

During the first five weeks post calving cows were sampled once per week on a fixed day. Due to the various calving dates, cows differed in the DIM at the sampling day. On average the first sample was taken at day 5 (range: 1 to 8) and the last at day 35 (range: 27 to 37) of the lactation.

Duplicated composite milk samples were automatically gained at morning milking. One sample was stored at -20 °C until analysis of the fat and protein content was carried out at the laboratory of the "Hessischer Verband für Leistungs- und Qualitätsprüfungen in der Tierzucht e. V." (Alsfeld, Germany, samples of Farm1) and at the laboratory of the "Landeskontrollverband Schleswig-Holstein e. V." (Kiel, Germany, samples of Farm2). The FPR was calculated based on these analyses.

Trichloroacetic acid (TCA, 50 %) was added to the second composite milk sample (ratio: 1 ml TCA to 10 ml milk). Samples were cooled and centrifuged. The supernatant was stored at -20 °C until its analysis for the BHBA content at the laboratory of the University of Applied Science and Arts Hanover (Germany). BHBA was measured using an AutoAnalyzer (Traacs 2000 System®, SEAL Analytical GmbH, Norderstedt, Germany). The analysis based on the oxidation of BHBA to acetoacetate while the enzyme BHBA-dehydrogenase and the coenzyme NAD+ are present. The generated NADH was measured photometrically ($\lambda = 340nm$).

Following the morning milking, trained technicians took blood samples from the jugular vein (Farm1) and by tail venipuncture (Farm2). Serum samples were prepared by centrifugation (15 min, 3000 x g, 4 °C) and aliquoted for analyses of NEFA and GLDH.

Serum aliquots for analyses of NEFA were stored at -20 °C until analysed at the laboratory of the Institute of Animal Science (University of Bonn, Germany). NEFA were measured using an enzymatic test kit (kit nr. 1383175, Roche Diagnostics, Mannheim, Germany), which was adapted for use on microtitre plates (Oliver et al., 1995). Intraassay and interassay coefficients were 6.3 % and 5.6 % plus 8.3 % and 8.2 %, respectively. The test was

K. Barth, K. Aulrich, H. C. Haufe, U. Müller, D. Schaub, F. Schulz / Landbauforschung - vTI Agriculture and
Forestry Research 4 2011 (61)307-316

311

based on the enzymatically catalysed reaction of NEFA
to Acetyl-CoA resulting in hydrogen peroxide which was
measured photometrically (λ = 490 nm).

The second serum aliquot was cooled and transported
to a commercial laboratory (synlab.vet, Geesthacht, Ger-
many) for analysis of GLDH on the sampling day. GLDH
was measured photometrically (λ = 340 nm, AU 2700®,
Olympus, Hamburg, Germany).

Statistical analyses were done using the program
PASW®Statistics 18.0 (IBM, 2009). The procedure Linear-
MixedModels was used to analyse the effects of the fixed
explanatory variables farm (Farm1 or Farm2), breed (GH
or GRP), status (primiparous or pluriparous), season (sum-
mer = May to October or winter = November to April)
and EB% (continous) and BCSDiff (continous), the effect
of the repeated sampling (week of sampling 1 to 5) and
their interactions on FPR, BHBA, NEFA and GLDH. The cow
was included as a random effect. Level of significance was
determined at $P < 0.05$. Variables without a significant ef-
fect were excluded from the model following a step-wise
backward method. If necessary, the response variables
were log-transformed to meet statistical assumptions con-
cerning the distribution of the residuals. These assump-
tions were checked again graphically.

Due to different numbers of cows per group, and thus
different sample sizes Hochberg's GT2 procedure (Field,
2009) was used to test the differences between the three
groups (Farm1-GH, Farm2-GH and Farm2-GRP) in each
week of sampling.

Results

185 animals were sampled and records of 179 cows
could be included in the analyses. 21 primiparous and
63 pluriparous GH cows belonged to Farm1. From Farm2
46 GH (19 primiparous/ 27 pluriparous) and 49 GRP (18
primiparous/ 31 pluriparous) cows were included. The
average number of lactations did not differ between the
breeds at Farm2: 2.7 (range: 1 to 6) and 2.6 (range: 1 to
5) for GH and GRP, respectively, but was much lower than
on Farm1 (3.8, range: 1 to 12).

Mean milk yield (given as ECM) was equal in the three
groups at the beginning of lactation but started to dif-
fer significantly between the Farm2-GH and Farm2-GRP at
the second week, and in Week 5 both GH herds produced
significant ($P < 0.05$) more milk than the GRP (Figure 1). As
expected, GRP had higher BCS than GH but also the GH
at Farm1 and Farm2 differed significantly ($P < 0.001$) over
the experimental period (Figure 2). BCSDiff was highest in
Farm2-GH (Mean: -0.3, SD: 0.32) and significantly differ-
ent ($P < 0.01$) from Farm1-GH (Mean: -0.22, SD: 0.31) but
not from Farm2-GRP (Mean: -0.24, SD: 0.30).

Calculation of EB% revealed that the energy require-
ments of the tested animals were significantly better ful-
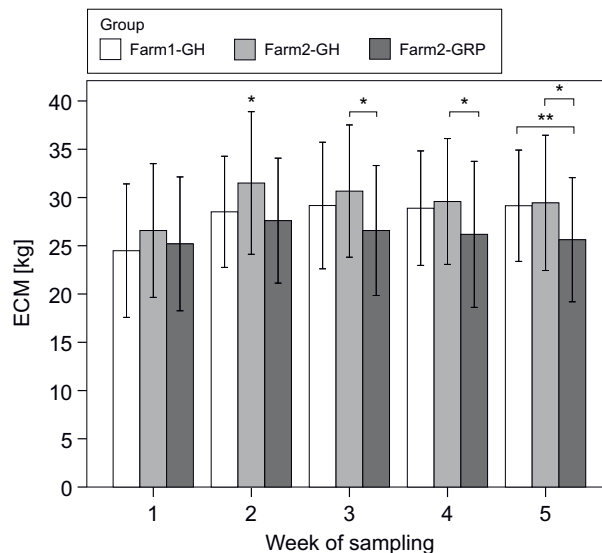filled at Farm1 than at Farm2 (Figure 3).

Figure 1:

Mean, SD and significant differences of the daily milk yield given as
energy corrected milk (ECM) of the three studied groups during the
five sampling weeks (GH = German Holstein, GRP = German Red
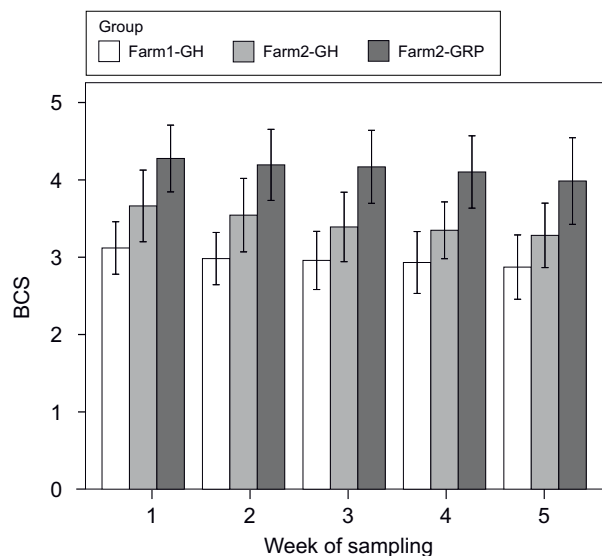Pied, signed bars differed at *$P < 0.05$, **$P < 0.01$)

Figure 2:

Mean and SD of the body condition score (BCS) of the three stu-
died groups during the five sampling weeks (GH = German Holstein,
GRP = German Red Pied, all groups significantly differed at $P < 0.001$)
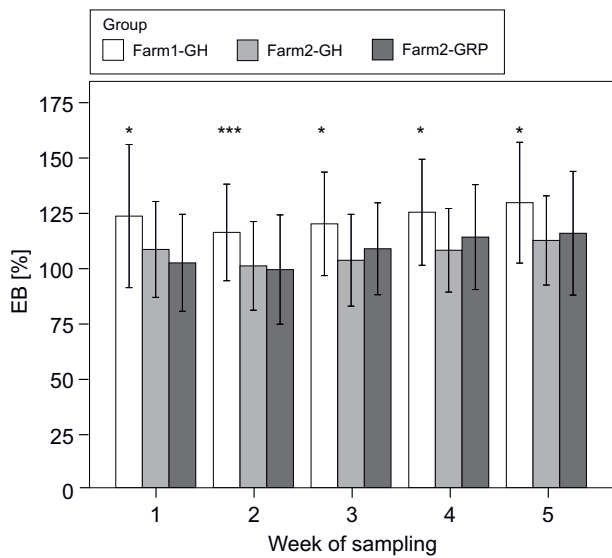
Figure 3:

Mean and SD of the energy needs coverage (EB%) of the three studied groups during the five sampling weeks (GH = German Holstein, GRP = German Red Pied, signed bars differed at *$P < 0.05$, ***$P < 0.001$)

Effects of the tested variables on the outcome variables FPR, BHBA, NEFA and GLDH differed in the models. Farm and the week of sampling affected all studied variables, while breed affected only FPR, BHBA and GLDH (Table 2). An increasing EB% predicted lower readings for FPR ($b = -0.012$, $t = -14.34$, $P < 0.001$), BHBA ($b = -0.003$, $t = -3.33$, $P < 0.01$) and NEFA ($b = -0.004$, $t = -4.66$, $P < 0.001$) but no effect on GLDH was found.

Milk samples

The final model showed that FPR was significantly lower at Farm1 (1.09 ± 0.04) than Farm2 (1.27 ± 0.02, $t = -4.28$, $P < 0.001$), and GRP (1.11 ± 0.04) had significantly lower readings than GH (1.24 ± 0.02, $t = -2.75$, $P < 0.01$). During the summer (1.11 ± 0.03) FPR was significantly lower than in winter (1.25 ± 0.03, $t = -3.93$, $P < 0.001$) and first lactating heifers (1.26 ± 0.04) had higher FPR than older cows (1.09 ± 0.02, $t = 4.35$, $P < 0.001$). FPR was lowest at the beginning of lactation. In all statistical models the farm effect was more dominant than the breed effect which was confirmed by multiple comparisons of the three groups of cows carried out on data of each sampling week. FPR of milk from Farm1-GH was always significantly lower than from Farm2-GH and Farm2-GRP, respectively. The latter differed only at the last sampling day (Figure 4). If an FPR threshold of 1.4 was applied (Dirksen, 1994), 22.6 % (19 out of 84), 76.1 % (35 out of 46) and 59.2 % (29 out of 49) of the sampled Farm1-GH, Farm2-GH and Farm2-GRP, respectively, overstepped this threshold at least once during the sampling period.

BHBA increased from week to week, too. Farm1 (13.33 ± 1.06 µmol l^{-1}) had significantly lower BHBA contents than Farm2 (19.88 ± 1.04 µmol l^{-1}, $t = -5.80$, $P < 0.001$) and GH (18.54 ± 1.03 µmol l^{-1}) significantly higher BHBA readings than the dual-purpose breed GRP (14.30 ± 1.06 µmol l^{-1}, $t = -3.40$, $P < 0.01$). Primiparous and pluriparous cows did not differ, and also the season did not significantly affect BHBA. Farm2-GH showed always the highest BHBA contents in milk and always differed significantly from the GH kept on Farm1 (Figure 5). GRP at Farm2 had also higher BHBA readings than Farm1-GH but the differences were not significant until the third week of sampling. In week 4 and 5 also the BHBA of GH and GRP at Farm2 differed

Table 2:

Effects of the explanatory fixed variables on the investigated indicators of metabolic disorders gained in milk (Fat-Protein-Ratio - FPR), β-hydroxybutyric acid - BHBA) and in blood (nonesterified fatty acids – NEFA, glutamate dehydrogenase – GLDH)

	FPR		lnBHBA		lnNEFA		lnGLDH	
	F	*P*	F	*P*	F	*P*	F	*P*
Farm	18.28	< 0.001	33.66	< 0.001	7.27	< 0.01	21.34	< 0.001
Breed	7.57	< 0.01	11.54	< 0.01		n.s.	14.29	< 0.001
Parity status	18.95	< 0.001		n.s.		n.s.		n.s.
Season	15.48	< 0.001		n.s.		n.s.	4.73	< 0.05
Week of sampling	18.81	< 0.001	7.23	< 0.001	43.46	< 0.001	20.49	< 0.001
EB%[a]	205.57	< 0.001	11.07	< 0.01	21.69	< 0.001		n.s.
BCSDiff[b]		n.s.		n.s.	11.76	< 0.01		n.s.

[a]EB%= coverage of the energy needs,
[b]BCSDiff= body condition score loss from sampling week 1 to sampling week 5

K. Barth, K. Aulrich, H. C. Haufe, U. Müller, D. Schaub, F. Schulz / Landbauforschung - vTI Agriculture and Forestry Research 4 2011 (61)307-316

313

significantly. Number of cows which showed BHBA readings higher than 70 µmol l⁻¹ (according to Enjalbert et al., 2001) reflected these findings: 1, 9 and 3 cows sampled in group Farm1-GH, Farm2-GH and Farm2-GRP indicated a subclinical ketosis by BHBA contents in milk.
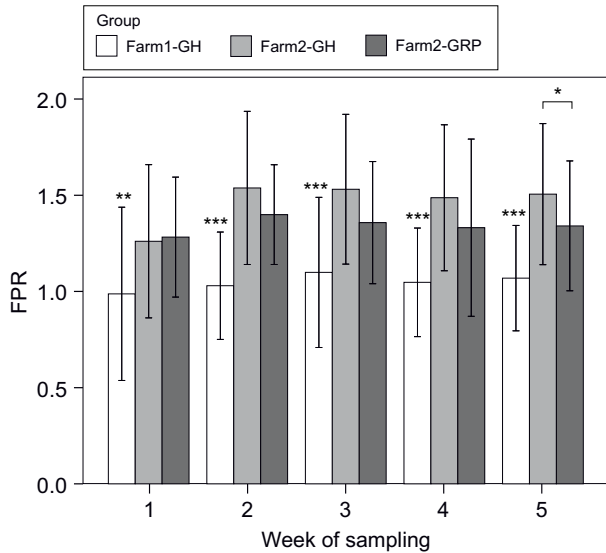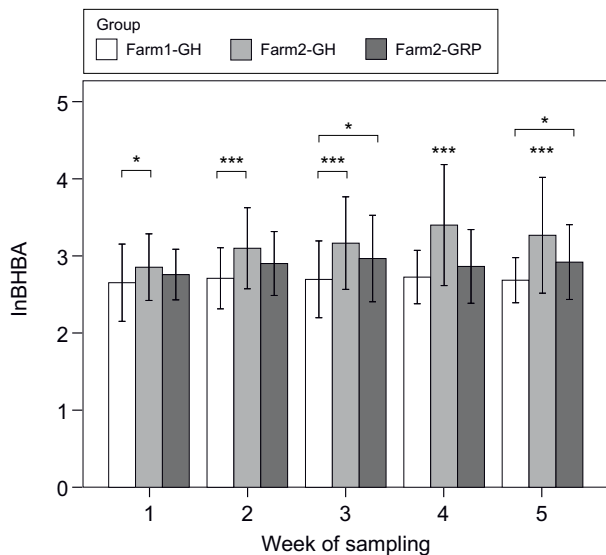

Figure 4:
Mean and SD of the Fat-Protein-Ratio (FPR) of the three studied groups during the five sampling weeks (GH = German Holstein, GRP = German Red Pied, signed bars differed at *P < 0.05, **P < 0.01, ***P < 0.001)

Blood samples

NEFA contents in blood were highest at the beginning of lactation and decreased over the weeks. Animals of Farm1 (185 ± 1.05 µmol l⁻¹) had significantly lower NEFA readings than animals of Farm2 (215 ± 1.04 µmol l⁻¹, t = -2.70, P < 0.01). A negative BCSDiff predicted higher NEFA concentrations in blood (b = -0.35, t = -3.43, P < 0.01). Farm1-GH had always lower NEFA contents in blood but only at Week 2 and 5 were the differences to cows at Farm2 significant. NEFA did not differ between the breeds kept at Farm2 (Figure 6). A total of 9, 8 and 8 animals of Farm1-GH, Farm2-GH and Farm2-GRP, respectively, had NEFA contents higher than 500 µmol l⁻¹ according to the threshold recommended by Hachenberg et al. (2007) for the first week of lactation.

GLDH activity increased over the sampling period and was significantly higher in cows from Farm2 (11.12 ± 1.06 U l⁻¹) than from Farm1 (7.09 ± 1.08 U l⁻¹, t = -4.62, P < 0.001). The GLDH of GRP cows (7.24 ± 1.09 U l⁻¹) was significantly lower than that of GH cows (10.89 ± 1.05 U l⁻¹, t = -3.78, P < 0.001). There was a slight but significant difference between GLDH activity in summer (8.11 ± 1.07 U l⁻¹) and winter (9.73 ± 1.06 U l⁻¹, t = -2.18, P < 0.05). In Farm2-GH, activity of GLDH was always highest and always differed significantly from GH on Farm1 (Figure 7). Farm2-GRP and Farm1-GH never showed significant differences of GLDH activity. GLDH readings higher than 40 U l⁻¹ indicating damages of the hepatic tissue (Fürll, 2005) showed at least once during the sampling period 9, 9 and 2 animals of Farm1-GH, Farm2-GH and Farm2-GRP, respectively.

Figure 5:
Mean and SD of the β-hydroxybutyric acid concentration in milk (BHBA) of the three studied groups during the five sampling weeks (GH = German Holstein, GRP = German Red Pied, signed bars differed at *P < 0.05, **P < 0.01, ***P < 0.001)
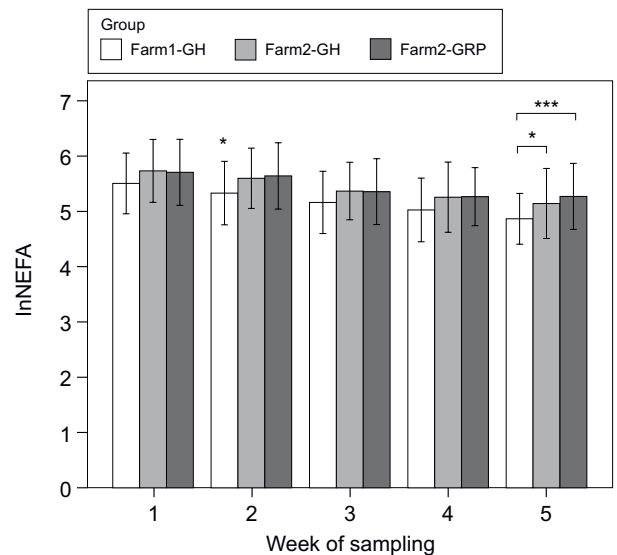

Figure 6:
Mean and SD of the blood concentration of nonesterified fatty acids (NEFA) of the three studied groups during the five sampling weeks (GH = German Holstein, GRP = German Red Pied, signed bars differed at *P < 0.05, **P < 0.01, ***P < 0.001)
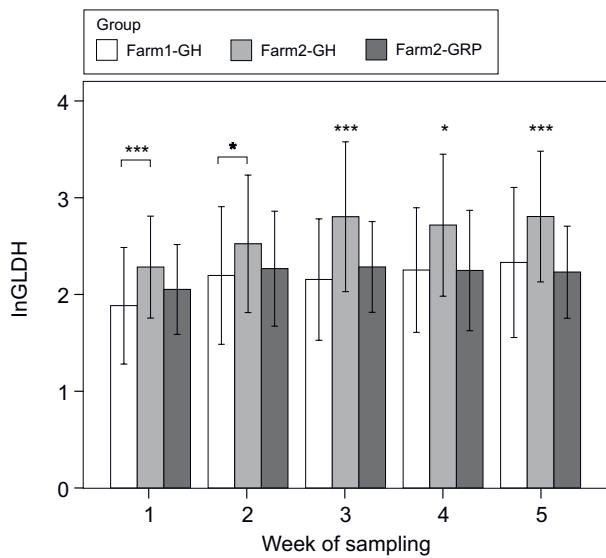
Figure 7:

Mean and SD of the glutamate dehydrogenase activity (GLDH) in blood of the three studied groups during the five sampling weeks (GH = German Holstein, GRP = German Red Pied, signed bars differed at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Discussion

As expected, milk yield of GH was higher than of GRP, and the milk yield of the GH at the two farms differed only once significantly in the second week of sampling. The average milk yield of Farm2-GH was highest in this week and indicates that the acceleration in milk yield from Week 1 to Week 2 was greater than in Farm1-GH. Milk yield acceleration reflects trends for higher stress in high yielding cows (Hansen et al., 2006), and the decrease of ECM in Farm2-GH after the second week might be explained by non-fulfillment of the energy requirements of the cows at Farm2 leading to a depression in milk yield although the partitioning of nutrients in dairy breeds prefers the mammary tissue (Veerkamp et al., 2003).

Besides the lower energy density of the feed provided at Farm2, the dry cow management has perhaps influenced the performance of the cows after calving, too. The BCS support this assumption. BCS ranging from 2.6 to 3.0 are seen as optimal for Holstein breeds (Bernabucci et al., 2005). Due to the different type of nutrient accretion, dual-purpose breeds show higher scores, but a limit of 3.5 is recommended to avoid ketosis (Gillund et al., 2001). Compared to these thresholds the cows at Farm2 were clearly overfed during the dry period. Though BCS is subject to individual effects of the assessors (Edmonson et al., 1989), in this case this does not explain the significant differences between the GH at the farms. All technicians who gained BCS were trained at the beginning of the study and again in the middle of the whole sampling period. Thus, the ob-

served differences have to be a result of the differences in the dry cow management on the two farms.

Adipose tissue of cows overfed during the dry period shows lower basal lipolytic rates and the livers of these cows seem to be less adapted for the metabolization of fatty acids around parturition (Rukkwamsuk et al., 1998). Thus, a higher metabolic load was to be expected in animals at Farm2. NEFA concentration in blood was higher for Farm2 indicating a higher body fat mobilization of cows kept there. This was confirmed by a significantly higher BCS loss of these cows. However, the NEFA concentration only differed significantly in sampling Weeks 2 and 5, and there was no difference at all between GH and GRP at Farm2. This is in contrast to Kronschnabl (2010) who found higher NEFA readings (+ 100...200 µmol l^{-1}) in GH compared to the dual-purpose breed German Simmental. According to Hachenberg et al. (2007), NEFA readings ≥ 500 µmol l^{-1} during the first week of lactation signal a limited adaptive performance in dairy cows. When this threshold was applied, the frequency of cows showing this limitation was twice as much at Farm2 than at Farm1 – again without a difference between GH and GRP at Farm2. The fulfilment of the energy requirements at Farm2 as well as the BCS loss did not significantly differ between these two breeds. Thus, either of them had to mobilize body fat to cope with the accelerating milk production. The revealed significant effect of EB% on NEFA concentrations supports these findings which are in accordance with Reist et al., (2002) who found a highly significant and relevant correlation between NEFA concentration in blood and EB ($r = -0.685$, $P < 0.001$). Energy balance profiles through lactation are genetically driven (Friggens et al., 2007) but differences observed between breeds do not point in the same direction: in the study by Friggens et al. (2007) Holstein dairy cows mobilized significantly more body energy at the beginning of lactation than the dual-purpose Danish Red and Jersey breeds kept under the same dietary treatment. This is in contrast to Fall et al. (2008) who found opposite results for Swedish Red and Swedish Holstein cows kept under organic farming conditions.

Unlike NEFA in blood, BHBA concentration in milk was clearly affected by the management conditions on both farms as well as by the breed. Sources of BHBA in milk are the hepatic metabolism resulting from fat mobilization (Nielsen et al., 2003), feedstuff (Ingvartsen, 2006) and the rumen epithelial cells (Nielsen et al., 2003). Although Nielsen et al. (2003) found it difficult to determine an absolute threshold of BHBA in milk as an indicator of ketosis, they recommended using this criterion instead of fat or citrate due to the fact that BHBA responded with a higher increase to feed restrictions in early lactating cows. Previously, Enjalbert et al. (2001) defined a threshold of 70 µmol l^{-1} for BHBA as a sign of an increased risk of keto-

K. Barth, K. Aulrich, H. C. Haufe, U. Müller, D. Schaub, F. Schulz / Landbauforschung - vTI Agriculture and
Forestry Research 4 2011 (61)307-316

315

sis. Applying this value to our readings, 9 out 45 of Farm2-GH were at a higher risk compared to 1 out of 84 GH cows at Farm1 and 3 out of 46 Farm2-GRP cows. BHBA in milk is significantly highly correlated to BHBA in serum independent of the metabolic status (Nielsen et al., 2003). Thus, results gained on serum BHBA concentration may be extrapolated – but with care – to BHBA in milk. Rukkwamsuk et al. (1998) found no significant differences of BHBA concentrations in serum of cows either overfed or fed restricted amounts before and after parturition. Therefore, the difference between GRP and GH at Farm2 might not be caused by the overfeeding during the dry period but by the higher body fat mobilization of the GH cows to transfer more energy to the udder. The grade of nutrient partitioning towards the mammary gland is genetically determined (Veerkamp et al., 2003). This was confirmed for serum BHBA concentration by Hammon et al. (2010) who studied Charolais x Holstein F_2 families and revealed sex chromosomal effects causing higher BHBA levels in cows descending from Charolais grandfathers with a higher genetic merit for milk yield.

Cows with serum BHBA concentrations > 1mM show higher fat and lower protein contents in milk, resulting in a higher FPR (Kessel et al., 2008). FPR is a sensitive indicator of changes in nutritional variables, and a useful predictor of the energy status of the cow (Grieve et al., 1986; Heuer, 2004). In our model, EB% showed the strongest influence on FPR of all studied variables and the comparison with the FPR threshold (Dirksen, 1994) revealed an energy deficit in three quarters of all GH cows at Farm2 in contrast to Farm1 where only 20 % of the GH suffered from deprivation. First lactating cows had significantly higher FPR than pluriparous cows, which confirms the results of Meikle et al. (2004) that primiparous cows show a more unbalanced metabolic profile than pluriparous cows. The seasonal effect might indicate a better fulfilment of energy requirements during summer. However, from a practitioner's point of view the easy to gain and fast reacting FPR can be seen as a useful tool to identify shortcomings in dry cow management as well as in feeding of cows in early lactation.

The higher metabolic load also caused elevated GLDH readings in GH cows at Farm2. Although the direct comparison between the three groups at the sampling days did not reveal significant differences between the Farm2-GRP and the Farm1-GH, more Farm1-GH cows showed GLDH readings higher than 40 U l^{-1} at least once, confirming the significant effect of breed in our model. As in the study by Hachenberg et al. (2007), mean GLDH activity increased over the weeks but the significant difference in the first sampling week between the GH at the two farms might be explained by a compromised liver function prepartum (Burke et al., 2010) maybe caused by overfeeding during

the dry period (Rukkwamsuk et al., 1998). The seasonal effect on GLDH showed the same tendency as on FPR and is in accordance with Distl et al. (1989) who found higher incidences of ketosis during the winter time compared to the summer.

Conclusion

The direct comparison of a dairy and a dual purpose breed kept under the same conditions confirmed the finding that breeds with a higher genetic merit for milk yield suffer a higher metabolic load when the feeding management in the periparturient period is suboptimal. Under conditions better fulfilling the demands of the dairy breed, these differences could not be observed. Thus, there is no need to prefer dual-purpose breeds in organic dairy farming as long as the management is appropriate for high yielding cows.

Acknowledgements

References

Bauman DE, Currie WB (1980) Partitioning of nutrients during pregnancy and lactation : a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci 63:1514-1529

Bell AW (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci 73:2804-2819

Bernabucci U, Ronchi B, Lacetera N, Nardone A (2005) Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J Dairy Sci 88:2017-2026

Burke CR, Meier S, McDougall S, Compton C, Mitchell M, Roche JR (2010) Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J Dairy Sci 93:5363-5373

Dirksen G (1994) Kontrolle von Stoffwechselstörungen bei Milchkühen an Hand von Milchparametern. In: Proceedings : XVIII World Buiatrics Congress, XXVI Congress of the Italian Association of Buiatrics, August 29 – September 2, 1994, Bologna, Italy. Bologna, pp 35-45

Distl O, Wurm A, Glibotic A, Brem G, Kräußlich H (1989) Analysis of relationships between veterinary recorded production diseases and milk production in dairy cows. Livest Prod Sci 23:67-78

Drackley JK (1999) ADSA Foundation Scholar Award Biology of dairy cows during the transition period : the final frontier? J Dairy Sci 82:2259-2273

Duffield TF, Lissemore KD, McBride BW, Leslie KE (2009) Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci 92:571-580

EC (2007) Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91 (online). To be found at <http://europa.eu/legislation_summaries/agriculture/food/f86000_en.htm> [quoted 08.11.2011]

EC (2008) Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with

regard to organic production, labelling and control [online]. To be found at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:250:0001:0084:EN:PDF> [quoted 08.11.2011]

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G (1989) A body condition scoring chart for Holstein dairy cows. J Dairy Sci 72:68-78

Enjalbert F, Nicot MC, Bayourthe C, Moncoulon R (2001) Ketone bodies in milk and blood of dairy cows : relationship between concentrations and utilization for detection of subclinical ketosis. J Dairy Sci 84:583-589

Fall N, Gröhn YT, Forslund K, Essen-Gustafsson B, Niskanen R, Emanuelson U (2008) An observational study on early-lactation metabolic profiles in Swedish organically and conventionally managed dairy cows. J Dairy Sci 91:3983-3992

Field A (2009) Discovering statistics using SPSS. Los Angeles : Sage Publ, 821 p

Freyer G, König S, Fischer B, Bergfeld U, Cassell BG (2008) Invited review : crossbreeding in dairy cattle from a German perspective of the past and today. J Dairy Sci 91:3725-3743

Friggens NC, Berg P, Theilgaard P, Korsgaard IR, Ingvartsen KL, Lovendahl P, Jensen J (2007) Breed and parity effects on energy balance profiles through lactation : evidence of genetically driven body energy change. J Dairy Sci 90:5291-5305

Fürll M (2005) Spezielle Untersuchungen beim Wiederkäuer. In: Kraft W, Dürr UM (eds) Klinische Labordiagnostik in der Tiermedizin. Stuttgart : Schattauer, pp 30

Gillund P, Reksen O, Gröhn YT, Karlberg K (2001) Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J Dairy Sci 84:1390-1396

Goff JP, Horst RL (1997) Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci 80:1260-1268

Grieve DG, Korver S, Rijpkema YS, Hof G (1986) Relationship between milk composition and some nutritional parameters in early lactation. Livest Prod Sci 14:239-254

Grummer RR (1993) Etiology of lipid-related metabolic disorders in periparturient dairy cows. J Dairy Sci 76:3882-3896

Hachenberg S, Weinkauf C, Hiss S, Sauerwein H (2007) Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period. J Anim Sci 85:1923-1932

Hammon HM, Metges CC, Schulz A, Junghans P, Steinhoff J, Schneider F, Pfuhl R, Bruckmaier RM, Weikard R, Kühn C (2010) Differences in milk production, glucose metabolism, and carcass composition of 2 Charolais × Holstein F2 families derived from reciprocal paternal and maternal grandsire crosses. J Dairy Sci 93:3007-3018

Hansen JV, Friggens NC, Hojsgaard S (2006) The influence of breed and parity on milk yield, and milk yield acceleration curves. Livest Sci 104:53-62

Hardarson GH (2001) Is the modern high potential dairy cow suitable for organic farming conditions? Acta Vet Scan Suppl 95:63-67

Hardeng F, Edge VL (2001) Mastitis, ketosis, and milk fever in 31 organic and 93 conventional Norwegian dairy herds. J Dairy Sci 84:2673-2679

Heuer C (2004) The use of test day information to predict energy intake of dairy cows in early lactation. J Dairy Sci 87:593-601

Hoeben D, Monfardini E, Opsomer G, Burvenich C, Dosogne H, De Kruif A, Beckers JF (2000) Chemiluminescence of bovine polymorphonuclear leucocytes during the periparturient period and relation with metabolic markers and bovine pregnancy-associated glycoprotein. J Dairy Res 67:249-259

Ingvartsen KL (2006) Feeding- and management-related diseases in the transition cow : physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim Feed Sci Tech 126:175-213

Kessel S, Stroehl M, Meyer HHD, Hiss S, Sauerwein H, Schwarz FJ, Bruckmaier RM (2008) Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. J Anim Sci 86:2903-2912

Knaus WF, Steinwidder A, Zollitsch W (2001) Energy and protein balance in organic dairy cow nutrition : model calculations based on EU regulations. In: Hovi M, Baars T (eds) Breeding and feeding for animal health and welfare in organic livestock systems : proceedings of the fourth NAHWOA Workshop, Wageningen, 24-27 March 2001. Reading : Univ, pp 141-154

Kronschnabl C (2010) Ermittlung von laktationsspezifischen Prognosebereichen zur Beurteilung der Konzentration von Blutparametern beprobter Milchkühe. München : Ludwig-Maximilians-Univ, 229 p

Leslie KE, Duffield TF, Schukken YH, LeBlanc SJ (2000) The influence of negative energy balance on udder health [online]. To be found at <http://www.nmconline.org/articles/ketosis.pdf> [quoted 08.11.2011]

Meikle A, Kulcsar M, Chilliard Y, Febel H, Delavaud C, Cavestany D, Chilibroste P (2004) Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reprod 127:727-737

Nielsen NI, Ingvartsen KL (2004) Propylene glycol for dairy cows : a review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Anim Feed Sci Tech 115:191-213

Nielsen NI, Ingvartsen KL, Larsen T (2003) Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. J Vet Med 50:88-97

Oliver MH, Harding JE, Breier BH, Evans PC, Gallagher BW, Gluckman PD (1995) The effects of ovine placental-lactogen infusion on metabolites, insulin–like growth-factors and binding-proteins in the fetal sheep. J Endocrin 144:333-338

Rahmann G, Nieberg H (2005) New insights into organic farming in Germany : empirical results of a survey in 218 farms. Landbauforsch Völkenrode 55(3):193-202

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N, Blum JW (2002) Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J Dairy Sci 85:3314-3327

Rukkwamsuk T, Wensing T, Geelen MJH (1998) Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J Dairy Sci 81:2904-2911

Spiekers H, Potthast V (2004) Erfolgreiche Milchviehfütterung. Frankfurt a M : DLG-Verl, 448 p

Veerkamp RF, Beerda B, van der Lende T (2003) Effects of genetic selection for milk yield on energy balance, levels of hormones, and metabolites in lactating cattle, and possible links to reduced fertility's. Livest Prod Sci 83:257-275

Wemheuer W (1987) Evaluation of blood values in dairy herds with fertility disorders. Tierärztl Prax 15:353-360

West HJ (1990) Effect on liver-function of acetonaemia and the fat cow syndrome in cattle. Res Vet Sci 48:221-227