Composition of major proteins in cow milk differing in mean protein concentration during the first 155 days of lactation and the influence of season as well as short-term restricted feeding in early and mid-lactation

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ABSTRACT: A variety of proteins contributes greatly to the unique nutritional and functional quality of dairy cow milk. Particularly, milk casein content and composition have substantial influence on the processing capabilities. In the present study, milk of 23 multiparous Holstein-Friesian cows, grouped as high- (3.49 ± 0.05%; n = 11) and low-protein (3.03 ± 0.05%; n = 12) cows, was sampled approximately weekly during the first 155 days of lactation to determine the course of relative milk protein composition (α-lactalbumin; β-lactoglobulin; α-, β-, and κ-casein). Furthermore, feed restrictions by 30% of dry matter intake in early and mid-lactation as well as experimental tissue biopsies were conducted to observe their effect on milk protein composition. Milk protein composition was relatively stable and displayed similar concentration patterns throughout the experimental period between both groups. Mean relative concentrations of α-, β-, κ-casein, α-lactalbumin, and β-lactoglobulin were 34.2, 31.4, 16.0, 2.1, and 9.7% of total protein, respectively. Feed restrictions did not alter milk protein composition, whereas the season influenced α- and β-casein as well as α-lactalbumin. Further, effects were observed in both groups at times of unfamiliar stressful situations caused by taking liver or muscle biopsies. As a result, the relative concentration of β-casein increased. Therefore, acute stress factors may lead to a deviation in milk protein composition and should be avoided.

Keywords: dairy cattle; casein; feed restriction; milk proteins; seasonal changes; stress

During recent years, the production of milk protein in high-yielding dairy cows has received more emphasis as component pricing based on units of fat and protein has become more established in the dairy factories. In 2011, 61% of skimmed milk collected in Germany was devoted to the protein-dependent production of cheese, milk powder, butter milk, and caseins (Bundesministerium fuer Ernaehrung, Landwirtschaft und Verbraucherschutz, 2012). A further growth in the demand for milk protein in comparison with the other milk components such as fat and lactose is anticipated due to the expected change in consumer habits. Milk protein yield is mainly dependent on milk yield (correlation 0.83), but also on milk protein concentration (correlation 0.06) (Teepker and Swalve, 1988). However, increases in milk yield are not only associated with increased milk protein production, but in general also with the energy-demanding production of

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milk fat and lactose in the mammary gland. Due to the well-known relationship of high milk yields and production disease occurrence, particularly during early lactation (Fleischer et al., 2001; Ingvartsen et al., 2003), increase in milk protein yields should be accomplished by increasing milk protein concentration with concomitant constant milk yield. Milk protein accounts for approximately 3.2–3.8% of milk. It consists of about 20% whey proteins with major components α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and 80% caseins, divided into major subclasses α- (α_{S1}- and α_{S2}-), β-, and κ-casein (-CN), which are arranged in micelles (Swaisgood, 1982; Rodriquez et al., 1985). The reported composition of respective major proteins in milk partly depends on the applied measuring method. In mid-infrared spectroscopy α-LA accounts for 3% of milk protein, β-LG for 9%, and the caseins for 31, 10, 37, and 10% (αS1-, αS2-, β-, and κ-CN, respectively) (De Marchi et al., 2009), whereas in polyacrylamide gel electrophoresis, α-LA and β-LG relate to 5 and 15% of milk protein and α-, β-, and κ-CN for 40, 29, and 11% (Ng-Kwai-Hang and Kroeker, 1984). Furthermore, minor constituents such as proteolyzed fragments, bovine serum albumin, free amino acids, and immunoglobulins add to the total protein concentration of milk (Maas et al., 1997; Elgar et al., 2000). Caseins, α-LA, and β-LG are synthesized in the epithelial cells of the mammary gland from primary blood constituents, which serve as precursors. The yield and composition of major bovine milk proteins determine the value of the product, depending on how the milk will be used. For cheese making a higher casein content, particularly higher κ-CN, correlates to increased curd yield, stronger curd firmness, and less casein loss in whey (Hallen et al., 2010). The composition of milk and milk proteins is influenced by many factors. With the increasing age, casein concentration decreases and whey concentration increases, whereas with the increasing lactation, casein concentration increases after its nadir in the 2nd month (Ng-Kwai-Hang et al., 1982). Protein composition is also influenced by genotype (Cerbulis and Farrell, 1975; Heck et al., 2009) and diseases (Hogarth et al., 2004). However, it is not known if milk protein composition varies in cows which differ in total milk protein concentration and milk yield and information could be used for further breeding strategies.

Amino acid supply of mammary gland is elevated due to feeding higher amounts of rumen-non-degradable protein and roughage which is metabolized by rumen bacteria (Jouany, 1994; Pop et al., 2001). Furthermore, feeding plays an important role in milk protein composition, particularly during the time of the undesirable nadir in milk protein concentration during early lactation (Auldist et al., 2000). Yet, scarce information is available on the influence of energy restriction, season or stress on the composition of all major milk proteins. It was therefore the aim of this study to measure potential differences in the composition of major milk proteins in multiparous Holstein-Friesian cows differing in milk protein concentration and milk yield, but not in milk protein yield during the first 155 days of lactation. Also, the influence of short-term feed restrictions at different time points of lactation and the effect of acute stress were assessed.

MATERIAL AND METHODS

Cows, housing, and feeding. The present study was approved by the animal welfare committee of the government of Upper Bavaria, Germany (AZ 55.2-1-54-2531-110-09) and federal guidelines were followed throughout the experimental period. Twenty-three multiparous Holstein-Frisian cows were included in the study which took place from August 2009 to January 2011 at the research farm Veitshof in Freising, Germany. The housing and feeding of the multiparous Holstein-Friesian cows are described in Sigl et al. (2013). Animals were retrospectively grouped according to mean milk protein concentration during the first 155 days of lactation as high-protein cows (HP-cows; $n = 11$) and low-protein cows (LP-cows; $n = 12$) (Table 1).

Milk protein genotypes of cows. Sanger sequencing from hepatic tissue (DNA extraction with peqGOLD TriFast®; PEQLAB Biotechnologie GmbH, Erlangen, Germany) was performed in the laboratories of the Centre for Molecular Biosciences (Christian-Albrechts-University Kiel) with a 3730xl DNA Analyzer (Applied Biosystems, Darmstadt, Germany). After performing a Z-test (SigmaPlot, Version 12.0, 2011), proportions of genotypes in LP- and HP-cows showed no significant differences except for β-lactoglobulin AB (42 and 90% in LP- and HP-cows; $P < 0.05$) (Table 1).

Feed restriction. The cows were subjected to a feed restriction in early lactation (days 26–28 postpartum (pp)) and in mid-lactation (days 141–143 pp). From day 23 until day 31 pp and from day 138 until day 146 pp, the cows were moved to a tie-stall with eye contact to the herd. They had free access to

Table 1. Mean milk yield, protein concentration, protein yield, and proportions of genotypes of high-protein (HP) and low-protein (LP) cows during the first 155 days of lactation

Group	LP-cows				HP-cows			
n	12				11			
Parity of animals	2.5 ± 0.2				2.6 ± 0.2			
Milk yield (kg/day)[1]	37.8 ± 0.9*				33.8 ± 1.0			
Milk protein (%)[1]	3.03 ± 0.05*				3.49 ± 0.05			
Milk protein (g/day)[1]	1146 ± 24				1167 ± 26			
Genotype (%)	AA	AB	BB	unknown	AA	AB	BB	unknown
α_{S1}-Casein[2]	100				100			
α_{S2}-Casein	67	17		17	64	9		27
β-Casein	92		8		91		9	
κ-Casein	92	8	0		64	27	9	
α-Lactalbumin	92			8	82			18
β-Lactoglobulin	42	42*	17		9	90	0	

[1]LSM ± SEM over the first 155 days of lactation

[2]proportion of HP- or LP-cows showing respective genotype

*differences between means of the groups ($P < 0.05$)

water. In the first three days (days 23–25 pp and days 138–140 pp), the cows were fed *ad libitum* with a lactation diet (LD) and additional concentrates (6 and 4.5 kg, respectively) in separated feeding troughs (Sigl et al., 2013). In days 26–28 pp (feed restriction period 1; FR1) and in days 141–143 pp (feed restriction period 2; FR2) the cows received a restriction diet (RD) containing 56.4% corn silage, 21.6% grass silage, 3.8% hay, 11.3% concentrates, 0.9% mineral mix, and 6.0% straw with no additional concentrates (Sigl et al., 2013). Cows were fed 70% of their previous *ad libitum* feed intake. Fresh feed was mixed daily and the cows were fed half of their daily allotment of RD at 07.00 and 17.00 h, respectively. For the following three days (29–31 pp and 144–146 pp) the animals were fed again with LD *ad libitum* and with 6 or 4.5 kg of additional concentrates. The amount of feed offered and refused was weighed and recorded daily for the calculation of dry matter intake (DMI).

Sampling and analysis

Milk. Milk yield was recorded with electronic milk meters Metatron P21 (GEA WestfaliaSurge GmbH, Boenen, Germany). Approximately 500 ml of milk were obtained as proportional subsamples of total milk during each morning and evening milking depending on the total amount of milk and milk flow rate. Milk yield data were stored electronically using DairyPlan C21 (GEA WestfaliaSurge GmbH). Milk samples for the analysis of milk components and protein fractions were taken during lactation at days 8, 15, 20, 22, 36, 43, 57, 64, 78, 92, 106, 113, 120, 127, 134, and 155 pp. Additional samples were taken the day before (days 25 and 140 pp), on the third day of (days 28 and 143 pp), and three days after (days 31 and 146 pp) the restricted feeding.

To obtain a representative sample, aliquots of morning and evening milk were composited according to the morning and evening milk yield and an 11 ml aliquot was stored at –20°C until the analysis of protein fractions. For the analysis of milk fat, protein, lactose, and urea concentration as well as somatic cell count (SCC) and pH, milk samples were stored using acidiol as the preserving agent at 4°C until analysis (for maximally 7 days) in the laboratories of Milchpruefring Bayern e.V. (Wolnzach, Germany). The analysis of total protein and fat was performed using a infrared-spectrophotometer MilkoScan-FT-6000 (FOSS GmbH, Rellingen, Germany).

*Tissue biopsies***.** To characterize the metabolic differences in cows with high and low milk protein yield, liver biopsies were obtained on the day of parturition within 24 h after calving (day 1 pp) and on days 15 and 57 pp after morning milking and

before feeding. The biopsy procedure and results on the characterization of metabolic differences between cows with high and low protein yields are described in Sigl et al. (2013). Muscle tissue was collected after morning milking and before feeding at day of parturition within 24 h after calving (day 1 pp) and at days 43 and 113 pp as described in Wiedemann et al. (2013). Two pea-sized samples (approximately 600 mg) of semitendinous muscle as an easily accessible example of skeletal muscle were removed and subjected to further analyses (not published data).

Analysis of major milk proteins. Analyses of α-LA, β-LG, α-, β-, and κ-CN were conducted using a microfluidic electrophoresis (Agilent 2100 Bioanalyzer; Agilent Technologies, Waldbronn, Germany). The provided Protein 80 kit contained chips and all reagents (gel matrix, dye concentrate, sample buffer with upper (95 kDa) and lower (1.6 kDa) marker and molecular mass ladder (Agilent Technologies). According to the manufacturer's protocol, a gel-dye mix was prepared by spin filtration (2500 *g*, 15 min) of 650 µl gel matrix and an addition of 25 µl dye concentrate. A destaining solution was obtained solely by spin filtration of the gel matrix (650 µl). A reducing denaturing solution was prepared by addition of 1M dithiothreitol solution (7 µl, 3.5%) to 200 µl sample buffer. After thawing the milk samples (37°C, 20 min), skimmed milk was obtained by centrifugation (3000 *g*, 4°C, 15 min) and diluted in deionized water (1 : 20). The protein mix (200 µg/ml of each α-LA, β-LG, α-, β-, κ-CN; all Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), milk samples, and ladder were prepared according to the manual: the ladder (6 µl) or sample (4 µl) with denaturing solution (2 µl) were placed in a 0.5 ml tube and heated (95°C, 5 min). After cooling, the tubes were shortly centrifuged and 84 µl of deionized water were added to a total volume of 90 µl. For calculation of variability and to ensure comparability among chips, the protein mix was measured on every chip.

The chip was primed by pushing 12 µl gel-dye mixture into the channels of the chip with air pressure produced by a syringe. Subsequently, the wells were filled with either gel-dye mix (12 µl), destaining solution (12 µl), prepared ladder (6 µl) or prepared samples, and/or the prepared protein mix (6 µl).

The chip was placed into the bioanalyzer, the electrodes were inserted into each well by closing the lid, and measurement was started immediately. The electrophoresis procedure and simultaneous automatic integration took approximately 30 min. For standardization of peak area and migration time, the upper and lower markers were used as internal standards. For standardization of molecular mass, the molecular mass of the ladder proteins was used. If required, automatic integration could be corrected manually by using Agilent 2100 Expert software. The chips were discarded after completed runs. The electrodes were cleaned after each run with the provided cleaning chip and fresh deionized water.

Data were provided by the software as the percentage amount of total protein concentration by calculation of areas under the curves. To obtain comparable results among chips, each protein fraction was corrected by multiplication with a correction factor (CF) on the basis of the results of the respective proteins in the protein mix sample as follows:

$$CF = 20\ (\%\ \text{of protein fraction in protein mix})^{-1}$$

After correction, the relative amount of each protein fraction within the sum of all protein fractions was determined.

Based on 55 measurements, inter-assay variations were 6.5, 6.1, 5.8, 8.0, and 10.8% for α-LA, β-LG, α-, β-, and κ-CN, respectively. Repeated measurements of the same sample on one chip revealed intra-assay variations of 1.7, 4.3, 1.4, 0.0, and 0.0% for α-LA, β-LG, α-, β-, and κ-CN.

Statistical analysis. The energy balance (EB) was calculated as described by Kamphues et al. (2004):

$$EB = (DMI\ diet \times NE_L\ diet) + (DMI\ concentrates \times \times NE_L\ concentrates) - (0.293 \times body\ weight^{0.75}) - - (0.38 \times milk\ fat\ concentration) - (0.21 \times milk\ protein\ concentration) + 0.95) \times milk\ yield)$$

where:

DMI = dry matter intake

NE_L = net energy for lactation

The energy-corrected milk yield (ECM) was calculated as

$$ECM = (milk\ yield \times 0.327) + (milk\ fat\ yield \times 12.86) + + (milk\ protein\ yield \times 7.65)$$

A statistical analysis was performed with data of those days on which milk protein fractions were determined. Data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, Version 9.0, 2002). The statistical model

with the Akaike Information Criterion closest to zero included "day of test", "season" (March–May, spring; June–August, summer; September–November, autumn; December–February, winter) and "mean milk protein concentration of cows" (high or low protein) as fixed effects. In this model, "animal" was used as a random effect. For analyses of influence of biopsy procedure on milk protein composition results on days 15, 43, 57, and 113 pp were compared to results of respective previous and subsequent test days. If an overall significant effect was found for a fixed effect, a subsequent Bonferroni post hoc analysis was performed. All data are presented as Least Squares Means (LSM) ± standard error of means (SEM) and were considered to differ significantly at $P < 0.05$.

RESULTS

Milk yield and composition. The milk yield displayed the typical lactation curve with a steep increase at the onset of lactation, a maximum around day 45 pp and a subsequent decrease (Walsh et al., 2007) (Figure 1). Although mean milk yield varied between groups over the course of the first 155 days of lactation ($P < 0.01$) (Table 1), no differences between HP- and LP-cows were observed at any specific sampling time point (Figure 1), whereas season of sampling ($P < 0.01$) as well as day of lactation ($P < 0.001$) influenced milk yield. As a result of restricted feeding, the milk yield decreased in early (by 7.4 kg; $P < 0.001$) and in mid-lactation (by 5.0 kg; $P < 0.001$) in LP-cows, but no significant decrease was measured in HP-cows.

The milk protein concentration showed the characteristic lactation course with a nadir around day 36 pp and a subsequent increase until the end of the experiment in all cows (Figure 1). During lactation, HP-cows showed higher milk protein concentration compared to LP-cows (Table 1). Solely during early feed restriction (FR) (2.89 ± 0.07 and 3.15 ± 0.07% in LP- and HP-cows, respectively) and three days after early FR (2.81 ± 0.07 and 3.07 ± 0.07% in LP- and HP-cows, respectively), the milk protein concentration was comparable between the groups. The sampling season also affected milk protein concentration ($P < 0.001$).

Feed restriction. No differences were observed between HP- and LP-cows regarding DMI, energy intake, and energy balance (Table 2). Feed restriction in early (FR 1) and mid-lactation (FR 2) reduced DMI in all cows by 5.1 kg (30%) and 6.3 kg (32%; $P < 0.001$) resulting in reduced energy intake by 58 and 70 MJ NE_L ($P < 0.001$). The energy balance in all cows was not significantly decreased in both FR 1 and FR 2.

Composition of casein. During the first 155 days of lactation and also after three days of restricted feeding, relative concentrations of α-CN between 33 and 35% of total protein were measured in both LP- and HP-cows (Figure 2A). Next to the effect of lactation day ($P < 0.001$) and season ($P < 0.05$), the effect of group was observed ($P < 0.05$).

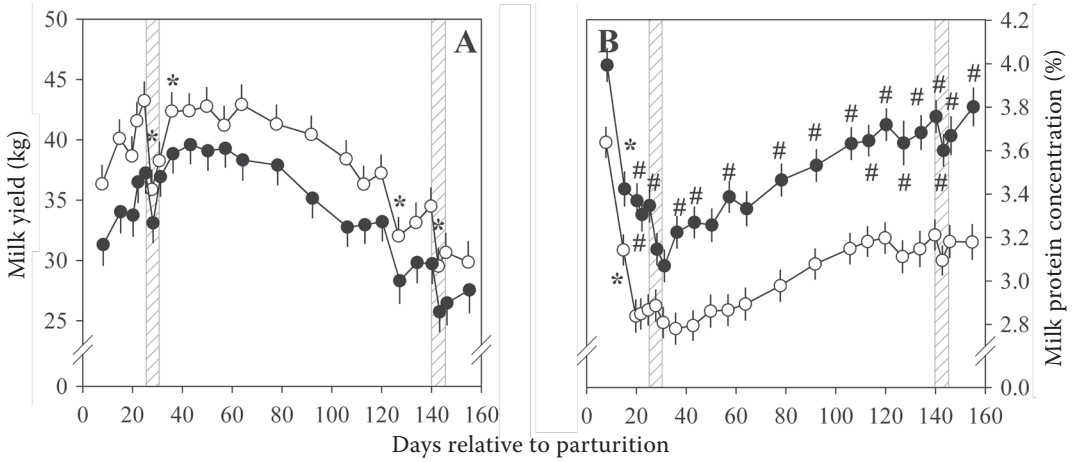

Figure 1. Mean milk yield (**A**) and milk protein concentration (**B**) (LSM ± SEM) during the first 155 days of lactation in cows differing in milk protein concentration

cows with low milk protein concentration (3.03 ± 0.05%) are shown as empty circles (○), cows with high milk protein concentration (3.49 ± 0.05%) as filled circles (●), shaded areas show feed restrictions

*differences to previous sampling day within groups ($P < 0.05$)

#differences between groups at the same sampling time point ($P < 0.05$)

Table 2. Effect of short-term feed restriction (FR) in early and mid-lactation on feed and energy intake as well as energy balance (LSM ± SEM)

Parameter	Day relative to FR	LP-cows[1]	HP-cows[1]
Dry matter intake (kg/day)	last day before FR1	16.5 ± 0.9^{ac}	17.5 ± 0.9^{ac}
	last day of FR1	12.0 ± 0.9^{b}	11.9 ± 0.9^{b}
	3 days after FR1	15.9 ± 1.3^{abc}	18.0 ± 1.1^{ac}
	last day before FR2	19.7 ± 0.8^{c}	20.4 ± 0.9^{c}
	last day of FR2	13.4 ± 0.8^{ab}	14.1 ± 0.9^{ab}
	3 days after FR2	18.5 ± 0.9^{c}	18.0 ± 1.1^{c}
Energy intake (MJ NE_L/day)	last day before FR1	186 ± 18^{ac}	189 ± 18^{ac}
	last day of FR1	134 ± 18^{bd}	125 ± 18^{bd}
	3 days after FR1	178 ± 23^{ab}	196 ± 21^{ac}
	last day before FR2	217 ± 16^{ab}	205 ± 18^{ab}
	last day of FR2	146 ± 16^{cd}	136 ± 18^{cd}
	3 days after FR2	199 ± 17^{ab}	177 ± 20^{abc}
Energy balance (MJ NE_L/day)	last day before FR1[2]	–62.5 ± 11.9^{abc}	–61.1 ± 12.7^{ab}
	last day of FR1	–86.1 ± 12.3^{ac}	–89.3 ± 12.0^{a}
	3 days after FR1	–112 ± 15.0^{a}	–73.2 ± 12.7^{a}
	last day before FR2	–12.2 ± 11.4^{b}	–0.9 ± 12.3^{b}
	last day of FR2	–44.0 ± 10.9^{bc}	–35.3 ± 12.3^{ab}
	3 days after FR2	–5.5 ± 13.4^{b}	1.6 ± 15.9^{b}

NE_L = net energy for lactation, FR1 = feed restriction in early lactation over three days, FR2 = feed restriction in mid-lactation over three days

[1]low-protein (LP-) cows showed mean milk protein yield during the first 155 days of lactation 3.03 ± 0.05 kg/day and high-protein (HP-) cows 3.49 ± 0.05 kg/day

[a–d]differences between time points within groups ($P < 0.05$)

Relative concentrations of β-CN varied only slightly between 30 and 32% in both LP- and HP-cows during the first 155 days of lactation and during feed restrictions (Figure 2B). Over the whole experimental period, group had a strong influence on the results ($P < 0.001$), but not on individual time points. Distinct elevations of β-CN relative concentrations were observed at day 43 pp in both groups (by 4.1 and 3.3% to 37.0 ± 0.6 and 34.5 ± 0.7% of total protein in LP- and HP-cows, respectively; $P < 0.05$) and at day 57 pp in HP-cows (by 4.6% to 34.2 ± 0.7% of total protein; $P < 0.001$), whereas in LP-cows the increase was only numerical (by 3.0% to 36.4 ± 0.7% of total protein; $P = 0.16$). An elevated relative β-CN concentration was also observed at day 113 pp in both groups (by 4.1 and 3.5% to 36.0 ± 0.7 and 33.6 ± 0.7% of total protein in LP- and HP-cows, respectively; $P < 0.01$). Also, day of lactation ($P < 0.001$) and season ($P < 0.05$) had an effect on β-CN concentration.

Relative concentrations of κ-CN revealed no significant differences between groups during the whole trial and days of lactation. Values levelled around 14 and 18% of total protein for LP- and HP-cows (Figure 2C). In HP-cows, the relative concentration of κ-CN at day 57 pp was lower (15.0 ± 1.1% of total protein) compared to days 120 and 134 pp (19.2 ± 1.1 and 18.9 ± 1.2% of total protein; $P < 0.05$). In LP-cows, the κ-CN concentration was higher at day 120 pp (15.4 ± 1.1% of total protein) compared to days 43 and 57 pp (11.6 ± 1.1 and 11.7 ± 1.1% of total protein; $P < 0.05$).

Composition of whey protein. No differences between the groups were observed in the relative concentration of α-LA (Figure 2E). In HP-cows, α-LA concentrations were higher at days 22 (2.4 ± 0.1% of total protein), 31 (after FR1, 2.4 ± 0.1% of total protein), and 78 pp (2.4 ± 0.1% of total protein) compared to days 140 (during FR2, 1.7 ± 0.1% of total protein) and 113 pp (1.8 ± 0.1% of total protein;

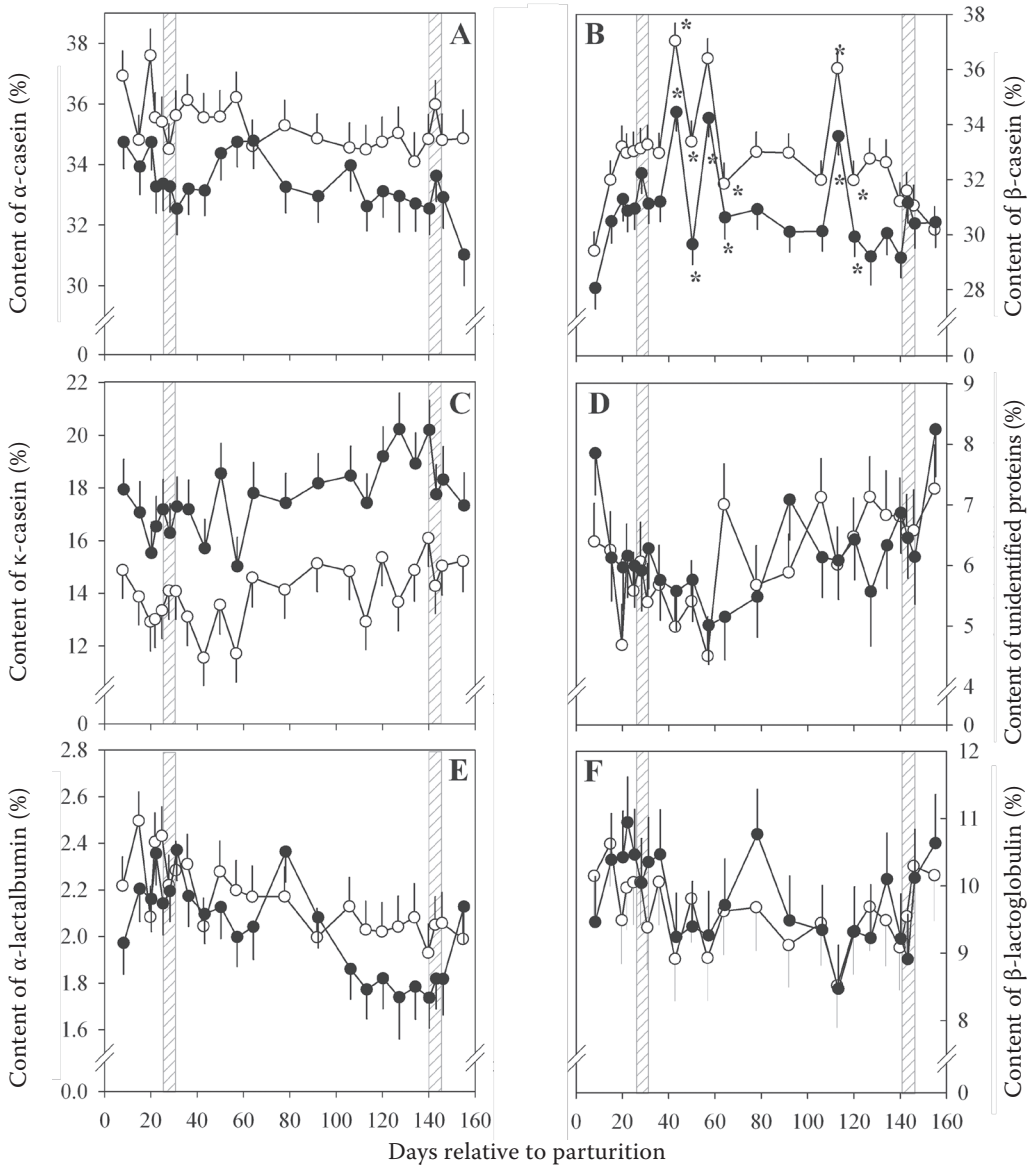

Figure 2. Composition of milk protein (LSM ± SEM) during the first 155 days of lactation in cows differing in milk protein concentration

(**A**) α-lactalbumin, (**B**) β-lactoglobulin, (**C**) α-casein, (**D**) β-casein, (**E**) κ-casein, (**F**) unidentified proteins
cows with low milk protein concentration (3.03 ± 0.05%) are shown as empty circles (○), cows with high milk protein concentration (3.49 ± 0.05%) as filled circles (●), shaded areas show feed restrictions
*differences to previous sampling day within groups ($P < 0.05$)

$P < 0.05$). Day of lactation ($P < 0.001$) and season ($P < 0.001$) affected the relative concentration of α-LA.

The relative concentration of β-LG did not differ between groups (Figure 2F). In HP-cows, the relative β-LG concentration was lower at day 113 pp (8.5 ± 0.7%) compared to days 22 (11.0 ± 0.7%; $P < 0.001$), 36 (10.5 ± 0.7%; $P < 0.05$), and 78 pp (10.8 ± 0.7% of total protein; $P < 0.01$). In LP-cows, levels decreased at day 113 compared to day 15 pp (8.5 ± 0.6 and 10.6 ± 0.6% of total protein; $P < 0.01$). Only day of lactation had an effect on the relative concentration of β-LG ($P < 0.001$).

The relative concentration of unidentified proteins was not different between days and groups during the first 155 days of lactation and both feed restrictions (Figure 2D). Day of lactation ($P < 0.001$) and season ($P < 0.05$) had an influence on the concentration of unidentified proteins.

Influence of season on milk protein composition. In summer, the α-CN concentration displayed lower

Table 3. Mean concentrations of major milk proteins (LSM ± SEM) in spring, summer, autumn, and winter

Concentrations of major milk proteins, of total protein	Season[1]			
	spring	summer	autumn	winter
α-Casein	34.1 ± 0.5	33.8 ± 0.5	34.8 ± 0.4	34.7 ± 0.4
β-Casein	31.8 ± 0.3[ab]	32.6 ± 0.4[a]	31.7 ± 0.3[ab]	31.2 ± 0.3[b]
κ-Casein	16.2 ± 0.7	15.5 ± 0.7	15.9 ± 0.7	15.8 ± 0.7
α-Lactalbumin	2.1 ± 0.1[ab]	2.2 ± 0.1[a]	2.0 ± 0.1[b]	2.1 ± 0.1[ab]
β-Lactoglobulin	9.9 ± 0.4	9.6 ± 0.4	9.6 ± 0.4	9.8 ± 0.4
Unidentified proteins	6.5 ± 0.4[a]	6.2 ± 0.4[ab]	5.6 ± 0.4[b]	6.3 ± 0.4[a]

[1]spring (March–May), summer (June–August), autumn (September–November), winter (December–February)
[a,b]differences between seasons ($P < 0.05$)

values in tendency compared to those in autumn (33.8 ± 0.5 and 34.8 ± 0.4% of total protein; $P < 0.1$) (Table 3), whereas the β-CN content was higher in summer compared to winter (32.6 ± 0.4 and 31.2 ± 0.3% of total protein; $P < 0.05$). Moreover, concentrations of α-LA and unidentified proteins were lower in autumn compared to summer (2.0 ± 0.1 and 2.2 ± 0.1% of total protein; $P < 0.001$) and compared to spring and winter (5.6 ± 0.4, 6.5 ± 0.4, and 6.30 ± 0.4% of total protein; $P < 0.05$), respectively.

DISCUSSION

The milk protein composition of HP- and LP-cows was analyzed applying the microfluidic chip technology adopted from Anema (2009). Despite large differences in total milk protein concentrations and differences in relative casein concentrations over the course of the experimental period, the pattern of individual milk protein curves was for the most part similar over the first 155 days of lactation in HP- and LP-cows, suggesting comparable adaptation as well as strong genetic effects. Consistent with previous results, relative concentrations of α- and β-CN accounted for approximately one third each and of κ-CN roughly for one sixth of all proteins in both groups (Mackle et al., 1999; Bobe et al., 2007). In the less milk-producing HP-cows, relative concentrations of α- and β-CN were lower over the course of the experimental period: this is not seen in cows differing in genetic merit for milk production (Bobe et al., 2007). However, differences in total milk protein concentration were larger in our study compared to that of Bobe et al. (2007) (max. 0.1% difference) which could explain the deviating results. Higher relative concentrations of κ-CN in HP-cows compared to LP-cows could be of importance for dairy processing as well as for nutrition. The milk of HP-cows had a more favourable κ-CN content particularly towards mid-lactation as higher κ-CN contents are associated with increased curd yield and firmness (Ng-Kwai-Hang et al., 1987; Heck et al., 2009). As no previous study revealed a genetic correlation between milk yield and milk protein composition, selection for higher κ-CN might also be considered in future breeding processes without compromising economic efficiency (Bobe et al., 2007).

Surprisingly, concentrations of β-CN were higher in both groups at days 43, 57, and 113 pp compared to previous values. At these days, biopsies of liver (day 57 pp) and muscle (days 43 and 113 pp) tissue were conducted. Although the tissue was anaesthetized (Sigl et al., 2013), the biopsy procedure seemed to be a stressor for the cows. Acute stress situations lead to increased values of adrenaline and noradrenaline and might alter nutritive blood flow to the mammary gland (Linzell, 1960; Prosser et al., 1996). However, the underlying reason warrants further investigation as relative concentrations of other milk proteins were not significantly influenced.

Regarding the concentration of individual milk proteins independently of group affiliation, summer resulted in higher concentrations of β-CN and α-LA compared to winter and autumn, respectively. Ng-Kwai-Hang et al. (1982) show that calendar month influences protein, casein, and whey protein concentration. Szijarto et al. (1973) observe the highest casein concentrations in winter (77.1% of total protein) coinciding with the lowest serum protein (17.9% of total protein). As calving was not seasonal, all cows were housed in a stall meaning similar feeding during all seasons; no obvious explanation for the effect of season on milk protein composition in this study can be given. However, it can be speculated that effects of heat stress in ruminants reported by Silanikove et al.

(2000) could also lead to a change in milk protein composition as temperatures were generally high during summer months in the experimental period.

As intended, feed restriction during early and mid-lactation led to a reduced energy intake, but resulted in no significant change in energy balance in both LP- and HP-cows. Milk yield was slightly lower compared to previous results after a 50% restriction of DMI over 5 days in mid-lactation (–11 kg; Carlson et al., 2006) and a 40% nutrient restriction during 4 days (–10 kg; Bjerre-Harpøth et al., 2012). In LP-cows, the decrease in milk yield was significant due to higher milk yields before the restricted feeding in early and mid-lactation. Total milk protein concentration was different between cows before FR1, but not during or after it. It seems that in HP-cows milk protein synthesis was decreased more than milk yield, leading to a decrease in milk protein concentration. Auldist et al. (2000) found significant changes in all individual milk proteins during restricted pasture allowance over 8 days in dairy cows. Bobe et al. (2009) observed slightly decreased whey protein concentrations with declining α-LA and increasing β-LG and constant casein concentrations with slightly increased α_{S1}-CN and decreased α_{S2}-, β-, and κ-CN concentrations during energy restriction of 20% over 14 days. In the present study, no obvious differences were observed in concentrations of individual proteins between LP- and HP-cows. It can be assumed that the feed restriction in the present study lasted for a too short time or was not severe enough to change the metabolism of rumen microbial flora, which is known to supply the main milk protein precursors (Pop et al., 2001; Brun-Lafleur et al., 2010).

CONCLUSION

Increasing milk protein concentration towards the end of the experimental period at day 155 pp seems to be caused by an increasing concentration of κ-CN. Furthermore, the milk protein composition was relatively stable and not altered by feed restrictions in early or mid-lactation. Variation in milk protein composition did not explain the differences in milk yields produced by LP- and HP-cows. Nevertheless, stressors should be avoided in dairy cows. Biopsies under local anaesthesia changed milk protein composition towards an increased content of β-CN and decreased concentrations of κ-CN.

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