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Effect of individual quarter milking with periodic air intake on free fatty acids (FFA) in the milk of dairy cows

Der Einfluss des viertelindividuellen Melkens mit periodischem Lufteinlass auf freie Fettsäuren (FFA) in Milch von Kühen

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Summary

Free fatty acids (FFA) reduce the quality of milk and dairy products by generating a rancid and soapy taste. They are produced by the spontaneous or induced enzymatic hydrolysis of milk fat. Induced lipolysis has its origin in the mechanical stresses produced in milk by pumping the milk, and by the milking process itself. In this study, the effect of the milking system, specifically MultiLactor®, on the resulting concentration of FFA was examined.

The MultiLactor® is an individual quarter-milking system with periodic air intake and sequential pulsation, designed to be used in conventional milking parlours.

The experimental design included testing 40 milk samples at two different flow rates. It was found that milking with the MultiLactor® caused an increase in the concentration of FFA in fresh milk. The FFA increase was higher for a flow rate of 1 l/min compared with a flow rate of 3 l/min. Based on the data obtained, the MultiLactor® generates no higher increase in FFA than that found when using other milking systems.

Keywords: milk quality, MultiLactor®, lipolysis

Zusammenfassung

Freie Fettsäuren (FFA) reduzieren die Qualität von Milch und Milchprodukten durch Hervorrufen eines ranzigen und seifigen Geschmacks. Ihr Ursprung ist die spontane oder induzierte enzymatische Lipolyse von Milchfett. Die induzierte Lipolyse hat ihren Ursprung in der mechanischen Belastung der Milch durch z. B. den Melkvorgang und das Pumpen von Milch. In dieser Studie wurde der Einfluss des Melksystems MultiLactor® auf die Konzentration der FFAs untersucht.

Der MultiLactor® ist ein viertelindividuelles Melksystem mit periodischem Lufteinlass und sequenzieller Pulsation, gebaut für den Einsatz in konventionellen Melkständen.

Der Versuchsaufbau beinhaltete die Analyse von 40 Milchproben bei zwei unterschiedlichen Durchflussraten. Es konnte gezeigt werden, dass das Melken mit dem MultiLactor® zu einem Anstieg der Konzentration der FFAs in der frischen Milch führt. Der FFA Anstieg war höher bei einer Durchflussrate von 1 l/min im Vergleich zu einer Durchflussrate von 3 l/min. Es konnte herausgefunden werden, dass der MultiLactor® im Vergleich zu anderen Melksystemen keinen stärkeren Anstieg der FFA bewirkt.

Schlüsselwörter: Milchqualität, MultiLactor®, Lipolyse

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Introduction

The safety of raw milk is important both for consumers and for companies (Schoder et al., 2010; Franzen and Usleber, 2010). Moderate free fatty acid (FFA) content is natural in milk. Muscle fatty acid composition is important for meat quality, free fatty acids are important for milk quality, and both kinds of quality concern consumers (Samouris et al., 2011). Jensen (1964) finds an average of either 0.2–0.6 mmol FFA/100g or 0.4–0.8 mmol FFA/100g of fat in raw milk, depending on the method used for analysis. An increase in the FFA caused by lipolysis reduces the quality of milk and other dairy products. High FFA can change the flavour of the milk and reduce its suitability for processing (Pillay et al., 1980).

The concentration of FFA is affected by three types of lipolysis, which can be spontaneous, induced or microbial (Suhren et al., 1981). Spontaneous lipolysis is caused by factors affecting individual cows, such as mastitis, stage of lactation, and feeding status. Microbial lipolysis is caused by psychotropic microorganisms, such as *Pseudomonas* (Cogan, 1977). Induced lipolysis has its origin in mechanical stresses affecting milk, such as those involved in the milking process and the pumping of milk (Suhren et al., 1981).

The mechanism for induced lipolysis is damage to the fat droplet's membrane. By destroying the membranes of fat droplets, the barrier which had protected the fat from lipolysis is lost, and lipase is able to bind to triglycerides and begin the process of lipolysis (Suhren et al., 1981). Mechanical stresses and changes in temperature can be reasons for this damage (Slaghuis et al., 2004).

Previous studies indicated that the effect on the FFA content in milk is different for various milking systems (Slaghuis et al., 2004). For automatic milking systems, (AMSs) and also for conventional milking systems, a large increase in FFA, from 0.38 mmol/100 g fat to 0.94 mmol/100 g fat, was observed (Abeni et al., 2005; De Koning et al., 2003; Klungel et al., 2000; Rasmussen et al., 2006; Salovou et al., 2005).

A higher milking frequency could be one important reason for a higher FFA level (Abeni et al., 2005; Klei et al., 1997; Slaghuis et al., 2004; Suhren et al., 1981; Wiking et al., 2006). Slaghuis et al. (2004) found the frequency of milking to be a more important influence on the FFA content in milk than the technical parameters of the milking system. Depending on the management system in AMSs, cows can choose their milking frequency themselves to some extent; this can result in an increase in milking frequency in AMSs (Abeni et al., 2005; Klungel et al., 2000).

Slaghuis et al. (2004) mentioned the higher air intake in AMSs from attaching the cluster, as well as the higher air-milk ratio of between 8 : 1 and 10 : 1, compared to only 3 : 1 in conventional milking systems, as technical factors explaining the higher concentrations of FFA found in those milking systems.

The individual quarter-milking system, MultiLactor®, combines characteristics of both conventional and automatic milking systems. The attachment of the cluster is performed manually in a milking parlour. Each udder-quarter is milked by separate milk tubes as in AMSs. The system works without a claw and is equipped with silicone liners. It provides periodic air intake by employing the Bio-Milker® system, and applies a sequential pulsation. The pulsation ratio is 65/35, and the pulsation rate is 60 pulse/min. MultiLactor® is equipped with a pneumatic arm called an "Actuator". The teat cups can be attached from a milking magazine, and they are attached manually and in pairs to the udder (Rose-Meierhöfer et al., 2010).

In our current study, the influence of the MultiLactor® milking system on the concentration of FFA was analysed. The objective was to investigate the effect of individual quarter milking on FFA in a conventional milking system.

Materials and Methods

Sampling

Our study, designed to investigate the influence of the MultiLactor® as well as the influence of different milk flows on the contents of FFA in milk, was carried out in the milking laboratory of the Leibniz Institute for Agricultural Engineering Potsdam-Bornim e. V. (ATB), located at the teaching and research farm Groß Kreutz, Brandenburg (Germany). The experimental design for the simulated milking process is shown in Figure 1. The FFA analyses were performed according to ICAR Guidelines Section 11.1 (ICAR, 2011). The milk used for the analysis was fresh raw milk harvested directly from Holstein cows being milked in the milking parlour directly connected to the laboratory. The raw milk was then placed directly into a vessel (2), and the first sample (FFA-1) was taken. The vessel (2) was filled completely before every replication to avoid vacuum fluctuations. The vessel was also connected with the MultiLactor®. Then, the milk was sent through the pipes and hoses as though an actual milking were taking place. After starting the simulated milking process, the milk passed through the complete MultiLactor® milking system with the adjusted flow rate. The milk was then collected in a second vessel (milk churn) (8), and sampled for the second time (FFa-2). Duplicate samples were taken for each replication from both vessels (FFA-1 and FFa-2). The MultiLactor® was equipped with long milk tubes with a length of 2100 mm and a diameter of 10 mm. Milk samples were taken at flow rates of 1 l/min and 3 l/min. Twenty replications were made at each flow rate. During the experiment, the machine vacuum was set to 35 kPa, and the air intake was 73 l/min for the four teat cups, with periodic air ingress. The machine vacuum of the MultiLactor® was set at a low level, but it is regularly used this way because of the periodic intake of air.

The experiment was carried out on four consecutive days. The milked cows could have had different characteristics on these days. The raw milk in the tank of the milking laboratory was kept at a temperature of 30 °C and was slowly stirred during the measurements to avoid the separation of fat. The flow of the milk was regulated by a vacuum counter set to 29–30 kPa for a flow rate of 1 l/min and to 21–22 kPa for a flow rate of 3 l/min. The reason for using a vacuum counter was to reduce the mechanical stress compared with pumping or using flow meters.

Five duplicate samples for each flow rate as well as samples taken before and after passing through the milking system were taken per test day. The samples were cooled to 10–12 °C in a running water bath for one hour. After transport to a laboratory, they were stored at 4 °C for 24 hours before analysis.

Milk sample analysis

As a first step, the total fat content was determined for all samples with a butyrometer, according to the method of Gerber (IDF, 2008). For the determination of FFA, the

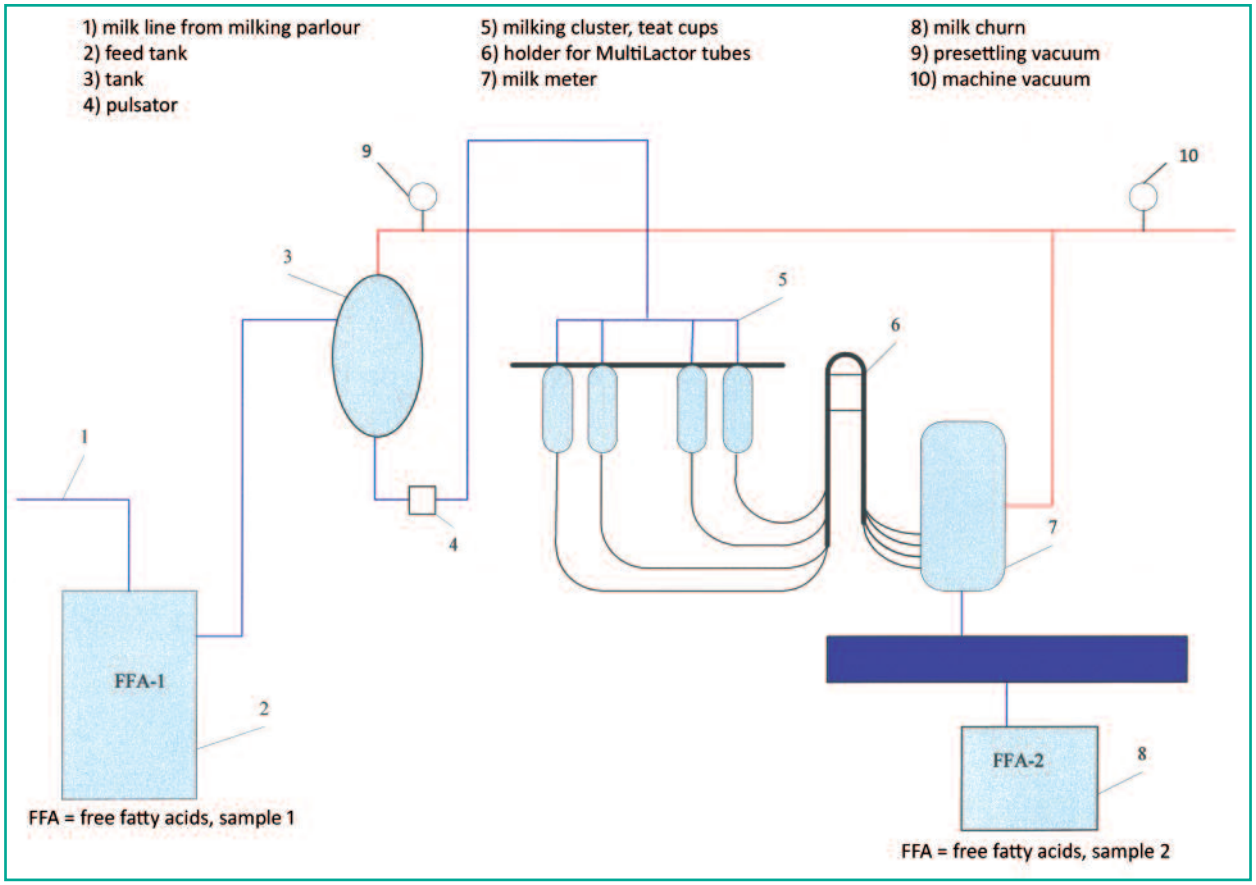

FIGURE 1: *Experimental set up of the milking laboratory.*

method according to Deeth (Greiling, 2000) was used. The FFA concentration is given as mmol per 100 g of fat.

Statistical analysis

The observed FFA values were analysed using the MIXED procedure (SAS Version 9.2, SAS Institute Inc., Cary, NC, USA). A forward stepwise modelling procedure was used, with the HTYPE=1 option in the MODEL statement, for adding terms to the model that contributed to explaining FFA variability. Three fixed effects and their full interactions were considered in this process. The fresh milk used for each replication per day was considered to be randomly assigned to the treatment combinations and thus was modelled as a random effect. Degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997). The significance level was defined as $P < 0.05$. This resulted in a mixed linear model as follows:

$$FFA_{ijkl} = \mu + B_i + MS_j + FLOW_k + (REP * FLOW * B)_{ikl} + e_{ijkl} \quad (1)$$

where FFA_{ijkl} is the observed FFA concentration, μ is the general mean, B_i is the fixed block effect of test day i (1 to 4), MS_j is the fixed effect of the milking system at sampling time j (j = 0: before passing through the milking system; j = 1: after passing through the milking system), $FLOW_k$ is the fixed effect of milk flow rate k (1 l/min vs. 3 l/min), $(REP*FLOW*B)_{ikl}$ is the random effect of milk emptied into the reservoir at B_i at $FLOW_k$ during repetition REP l (1 to 5) and e_{ijkl} is the random error term.

Adjustments made to keep the global significance level for multiple comparisons of factor levels were performed using the ADJUST=SIMULATE option in the LSMEANS statement.

Results

During the modelling process, the test day as block effect (B), milking system (MS), and flow rate (FLOW) were found to have a significant influence ($P < 0.05$) on FFA concentration (Tab. 1). Interactions between the fixed effects did not contribute to explaining FFA variability and thus were excluded from the final model.

The FFA values for the three significant effects are depicted in Figure 2. Mean FFA content was lowest on day 4, with 0.864 mmol/100 g fat, compared to concentrations ranging from 1.097 to 1.113 mmol/100 g fat for days 1 to 3. Only day 4 differed significantly from the other days in this respect; between the other days, no differences were found. Passing through the milking system led to an average

TABLE 1: *Type I Test of Fixed Effects: B_i is the fixed block effect of test day i (1 to 4), MS j is the fixed effect of the milking system at sampling time j (j = 0: before passing the milking system; j = 1: after passing the milking system), $FLOW_k$ is the fixed effect of milk flow rate k (1 l/min vs 3 l/min).*

Effect	Num DF	Den DF	F Value	Pr > F
B	3	18.88	17.29	<.0001
MS	1	127.7	77.21	<.0001
FLOW	1	86.48	4.02	0.0481

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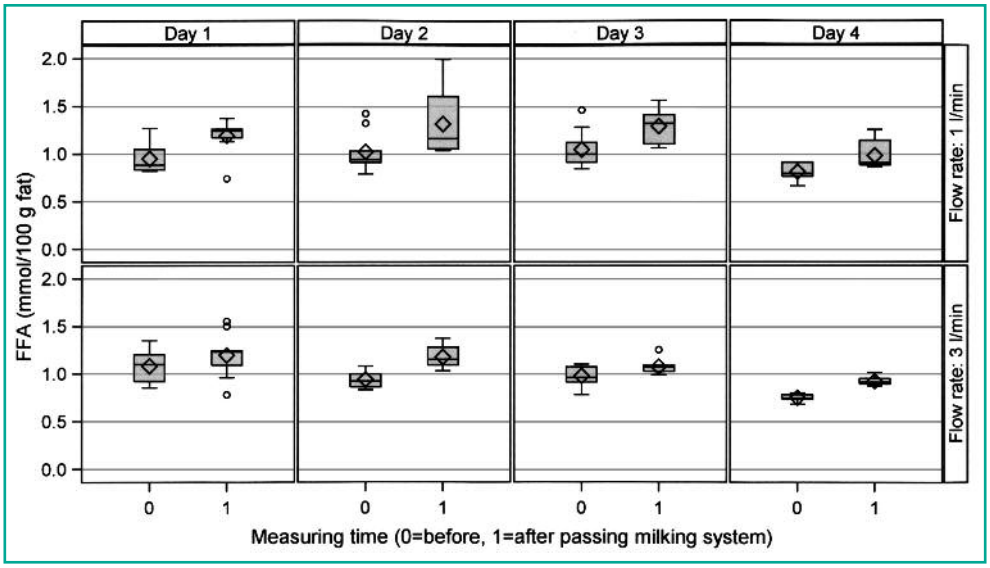

FIGURE 2: *Free fatty acid values by block effect of test day, flow rate and sampling time.*

increase in FFA concentration of 0.196 mmol/100 g fat, compared with the tank milk. The FFA content was about 0.070 mmol/100 g fat higher at a flow rate of 1 l/min than at a flow rate of 3 l/min.

Discussion

Effect of the MultiLactor®

The principal results of the study were as expected. The mechanical stress induced by the milking system itself increased the FFA concentration from an average of 0.946 mmol/100 g fat before passing through the milking system to an average of 1.142 mmol/100 g fat after passing through the milking system. Slaghuis et al. (2004) found an increase of FFA from 0.43 mmol/l to 0.50 mmol/l for a conventional system, and from 0.42 mmol/l to 0.58 mmol/l for an AMS in a laboratory trial. The milk flow was set to 2 kg/min in this study. The air inlet was set at 8 l/min for the conventional and 24 l/min for the automatic system. The vacuum level in both systems was set to 42 kPa.

Based on this study it could not be determined which factor had the most important influence on the increase of FFA caused by the MultiLactor®. Böhlen (1982) found a higher content of FFA after using the Bio-Milker® cluster with periodic air intake, compared with using a conventional two chamber teat cup.

Rasmussen et al. (2006) found a positive correlation between the air intake at the teat cups and the FFA content. They measured a content of 0.77 mmol/100 g fat for a conventional milking system while milking with a blocked air inlet, and 1.02 mmol/100 g fat with an air intake of 7 l/min per teat cup. For another automatic milking system, the FFA content was 1.17 mmol/100 g fat without an air intake and 1.50 mmol/100 g fat with an air intake of 7 l/min. Needs et al. (1986) measured an increase of 21 % in FFA by milking with a high air intake compared to milking with low air bleed. The air intake of 73 l/min for the MultiLactor® was high, even compared to values given in the literature for automatic systems, which normally have an air intake of 15 to 28 l/min (Rasmussen et al., 2006). Although these values are high, the air intake is not fixed in the Bio-Milker® system. Letting in no air during the suckling phase could be a reason for having a moderate increase in FFAs, which is comparable to the increase caused by the other milking systems. Because of the long tubes, and the small diameter of long milk tubes, high vacuum fluctuations can be expected, but because of the combination of periodic air intakes and the lack of the claw, the strong fluctuations we had expected to find were lacking. Problems from fluctuations are much lower than in AMS (Öz et al, 2011; Rose-Meierhöfer et al., 2010). Additionally, a reduced vacuum decreases lipolysis by about 54 % (Needs et al., 1986).

In comparing these studies, it can be concluded that the periodic air inlet milking of the MultiLactor® has an important influence on the increase in FFA.

Effect of the flow rate

Lower flow rates were shown to produce higher values of FFA. This finding agrees with the results of Escobar and Bradley (1990) and Slaghuis et al. (2004) that mixing milk and air may cause damage to the membranes of fat droplets. For lower milk flow rates, the interaction between milk and air is even less favourable, which could be the reason for damage to the fat droplets, and thereby also for higher FFA concentrations.

Effect of the test day

The influence of different test days was indicated by the significant difference between day four and the other test days. The content of FFA in milk was lower on day four. The milked cows for the experiment were chosen depending on the order in which they came to the milking parlour on test day. Therefore, individual animal factors, such as milk yield, fat percentage and fat globule size, which influence the content of FFA, could be reasons for the differences (Rasmussen et al., 2006). A reduced intake of fodder influences not only the content of FFAs in blood serum (Grabherr et al., 2008) but also the FFA content in milk, as found by Thomson et al. (2005) in an experiment about feed restriction.

Conclusions

The results showed that the expected increases in FFA are lowered by using the MultiLactor® rather than by using an automatic or conventional milking system.

In all milking systems, the process of milking increases the concentration of FFA in milk. Milking in a conventional milking parlour with single tube guiding and periodic air intake has no negative influence on the FFA of raw milk.

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