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nificant difference of the ratio of the concentrations of 3'- and 6'-sialyllactose to total hexose in milk was observed between dairy and non dairy cows.

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Effects of two different diets on lactoferrin concentrations in bovine milk

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Raw milk contains natural compounds, such as lactoferrin, with anti-microbial activity. These compounds may constitute a form of protection against the proliferation of spoilage and potentially pathogenic microorganisms, as well as being potentially beneficial for health. The objective of this study was to determine the influence of the cows' diet on the concentration of this compound in milk. An experimental herd of 32 Montbéliarde dairy cows was split in two groups, ensuring an equal balance according to the stage and lactation period. The first group grazed natural pasture, and the second was fed dry feed (35% hay and 65% ground barley/soya-bean meal). The results obtained showed that the lactoferrin content was significantly higher in milk from cows fed with hay and meal (292.8±34.0 µg/ml) than from cows fed with grass (159.7±12.2 µg/ml). These results suggest that it may be possible to naturally increase the concentration of lactoferrin in milk through appropriate management of the cows' diet.

Zur Auswirkung von zwei unterschiedlichen Fütterungen auf die Laktoferrin-Konzentrationen in Kuhmilch

Rohmilch enthält natürliche Verbindungen wie Laktoferrin, dir eine antimikrobielle Aktivität besitzen. Diese Verbindungen können einen gewissen Schutz gegen die Vermehrung von Verderbnis- und potentiellen Krankheitserregern bilden. Außerdem werden ihnen günstige gesundheitliche Wirkungen zugeschrieben. Die Zielsetzung dieser Studie war es zu ermitteln, ob die Fütterung der Kühe einen Einfluss auf die Konzentration dieser Verbindungen in der Milch besitzt. Eine Versuchsherde mit 32 Montbéliard-Kühen wurde in zwei Gruppen aufgeteilt, die sich in Laktationsstadium und -zahl entsprachen. Die erste Gruppe hatte natürlichen Weidegang, die zweite Gruppe erhielt Trockenfutter mit 25% Heu und 65% Gerste-Sojabohnen-Schrot. Die Ergebnisse zeigten, dass der Laktoferrin-Gehalt in der Milch von Kühen mit Verfütterung vom Heu und Schrot mit 292,8 ± 34,0 µg/ml deutlich höher lag als bei den Kühen mit Grasfütterung (159,7 ± 12,2 µg/ml). Diese Ergebnisse weisen darauf hin, dass es möglich ist, die natürliche Konzentration von Laktoferrin in Milch durch ein entsprechendes Fütterungsmanagement der Kühe zu erhöhen.

05 Feeding of dairy cows (lactoferrin concentration in milk)

05 Milchkuhfütterung (Laktoferrin-Gehalt der Milch)

1. Introduction

Raw milk is a highly complex matrix that contains nutritional as well as bioactive compounds such as lactoferrin. Lactoferrin is a glycoprotein that is synthesised in the mammary gland (1). Its concentration in bovine milk ranges from 100 to 300 µg/mL (2). Lactoferrin binds iron very tightly and this iron uptake inhibits bacteria by depriving them of iron that is essential for growth. Iron-free lactoferrin has a bacteriostatic, bacteriocidal or fungicidal effect against a wide range of microorganisms (3, 4). Moreover, lactoferrin is known for its immunomodulatory activity and more generally its potential impact on health (5-7). FARNAUD and EVANS (5) detailed the mechanisms of action of lactoferrin.

Different studies have shown that the lactoferrin content fluctuates in milk according to udder health (8), season (9) and level of pasture intake (10). Nevertheless, there is still a lack of knowledge concerning the influence of the diet on lactoferrin concentrations in milk. The aim of this study was to determine the influence of two different diets on lactoferrin concentrations in bovine milk.

2. Materials and methods

2.1 Chemicals

Unless otherwise stated, chemicals were of commercial origin (Sigma Aldrich Chimie, St Quentin Fallavier, France).

2.2 Milk samples

Thirty-two Montbéliarde cows from an experimental herd, 12 primiparous and 20 multiparous, in mid-lactation were divided into 2 groups of 16 cows each with similar parity (2.2±1.7 and 2.4±1.9), calving date (December 14±23d and December 29±23d), milk yield (17.1±2.9 and 17.0±2.8 kg/d/cow) and milk fat (34.3 ±2.2 and 34.6±1.7 g/Kg) and protein content (30.7±1.0 and 30.6±1.3 g/kg). From May 4th, during a 3 week pre-experimental period, these two groups were fed the same diet composed of natural grassland hay distributed *ad libitum* with additional 6 kg of dry feed which included 2nd cut hay supplemented with concentrates (barley and soybean meal mixture), distributed individually according to each cow's requirements. For 5 weeks the cows from group 1 were fed indoors where they consumed 5.7 kg/d of dry hay from natural mountain grassland, 8.8 kg dry ground barley and 0.6 kg dry soybean meal (diet 1). Each component of the diet was distributed in limited quantities fulfilling the cows' energetic and nitrogen requirements (11). Cows from group 2 grazed natural mountain pastures and were given a supplement containing 1.3 Kg dry ground barley and 0.5 Kg dry soybean meal (diet

2). During milking the two groups received identical quantities of minerals and vitamins (80, 140 and 100 g/Kg of phosphorus, calcium and magnesium, respectively, and 200 000, 40 000 and 40 IU/kg of vitamin A, D3 and E). Eleven times over the last 4 weeks of the experimental period, for each of the 2 groups of cows, the milk collected in the evening was held overnight at +4 °C, and then mixed with the un-refrigerated morning milk. The milk samples were taken from this bulk milk over the 5-week experimental period from early June

to July. For each group, a milk sample was collected at the end of the pre-experimental period.

2.3 Compositional analysis.

The lactoferrin in milk was quantified using the ELISA method developed by DUPONT *et al* (12). The true protein content was determined by infrared spectroscopy (Milkoscan 4000, Foss System, Hillerød, Denmark) and the somatic cell count using an epifluorescent method (Fossomatic 5000, Foss System, Hillerød)

2.4 Statistical treatment

The data obtained for the two groups or diet were analysed using a standard comparison of two means arising from unpaired data. Significant differences between means were established using a Student's t test that was carried out using MS Excel 2003, Microsoft Corp.

3. Results and discussion

Table 1 shows the means and standard deviations for each of the compounds measured for the 2 diets.

Table 1: Mean somatic cell count and lactoferrin concentration ± standard error, in milk according to the diet

	Somatic cells 10^3/mL	Protein g/L	Lactoferrin µg/ mL
Diet 1	205 ± 72	34.4 ± .5	292.8 ± 34.0
Diet 2	198 ± 93	33.5 ± 0.7	159.7 ± 12.2
	ns	ns	***

Mean comparison t' test. *** p<0.001. ns: not significant. Diet 1: Dry feed. Diet 2: Pasture fed

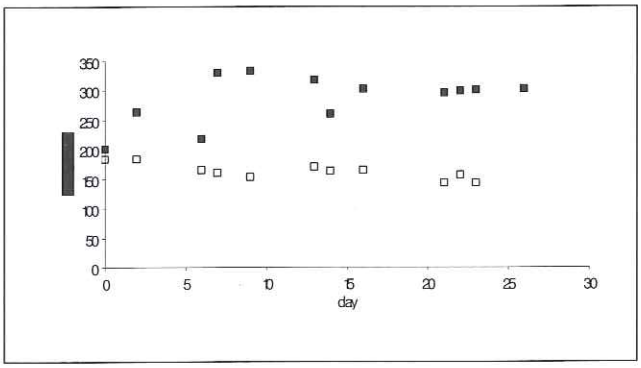

Fig. 1: Evolution of milk lactoferrin concentrations in cows fed with either dry feed, diet 1 (■) or natural pasture, diet 2 (□). Day 0 is the pre-experimental period value.

The diet had a highly significant effect on the concentration of milk lactoferrin ($p<0.001$). Indeed, the lactoferrin concentrations with diets 1 and 2 were respectively 201 and 183 µg/mL before the experiment (Fig. 1). During the experimental period, the lactoferrin concentration in diet 1 dramatically increased to reach levels of 329 µg/mL after 7 d. At the same time, the concentration of lactoferrin in group 2 remained constant or even decreased to reach 159 µg/mL after the same period.

The concentrates-rich diet led to a significant two-fold increase in lactoferrin content compared to that in milk from cows on a grass diet. TURNER *et al* (13) investigated the effect of diet on lactoferrin concentrations in milk. However, the effects of the diet on lactoferrin concentration were inconclusive as they observed that the lactoferrin content in milk from cows fed a total mixed ration was greater than that from cows grazing pasture in April but not in March. Moreover, they noticed that the former diet was significantly correlated to a higher somatic cell count in milk owing to bacteriologically positive udder quarters for mastitis. They also found in their study a positive within-cow correlation between lactoferrin concentration and somatic cell counts.

The lactoferrin content in milk was higher in the colostrum (14) and during the dry period of lactation (15), and increased with mastitis (8). The two groups of cows used in our study were homogenous (similar parity and calving dates) and presented the same level of somatic cells 205.10^3/mL *vs.* 198.10^3/mL (Table 1). Moreover, there was no difference between the levels of protein in the milk for the two diets (table 1). The increase in concentration of lactoferrin in milk from cows fed with concentrates could be explained by the fact that the lactoferrin concentration may be influenced by the iron content in the concentrates. WANG *et al* (16) observed that iron supplementation significantly increased the expression of lactoferrin mRNA in the mammary glands of mice. So, the effects of the diet on the lactoferrin content in milk still need to be investigated further.

4. Conclusions

It may be possible to act on the concentration of lactoferrin in milk naturally via appropriate management of the cows' diet. This could have several impacts in dairy processes. Indeed, the preservation and hygienic quality of dairy products could be improved by limiting the growth of spoilage and pathogenic microorganisms. Moreover, increasing amounts of lactoferrin could enhance health properties of milk and dairy products in terms of prevention.

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Effect of high-pressure treatment on *Pseudomonas fluorescens* DSM 4358 and the action of its proteinase on sodium caseinate

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Species of *Pseudomonas* are among the most important spoilage bacteria in raw milk, due to the production of very heat-stable proteinases, which are able to hydrolyse caseins, even at low temperatures. In this study, the effect of high-pressure (HP) treatment on raw milk microflora and, in particular, *Pseudomonas fluorescens* DSM 4358 and its extracellular proteinase, was examined. An overall reduction of raw milk microflora was obtained by pressure, with yeasts and moulds being most sensitive and enterococci being resistant up to 500 MPa. Complete inactivation of *Ps. fluorescens* inoculated into skim milk was obtained after treatment at 300 MPa. A cell-free supernatant of *Ps. fluorescens* DSM 4358 was prepared and then treated at pressures up to 800 MPa for 10 min at 20°C. The effect of the treated and untreated proteinase in the cell-free supernatant on sodium caseinate during incubation at 37°C for up to 48 h and hydrolysis of caseins was analysed by electrophoresis. While the bacterium was very sensitive to HP, the enzyme was very resistant, continuing to hydrolyse the caseins even after HP treatment at 800 MPa. These results show that the proteinase from *Ps. fluorescens* DSM 4358 is extremely resistant to HP, which may be of significance for dairy products made from HP-treated milk.

Wirkung einer Hochdruckbehandlung auf *Pseudomonas fluorescens* DSM 4358 und der Einfluss seiner Proteinase auf Natriumcaseinat

Pseudomonas-Species gehören zu den häufigsten Verderbniserregern in Rohmilch. Sie produzieren hitzestabile Proteinasen, welche Casein selbst bei niedrigen Temperaturen hydrolysieren können. In dieser Studie wird die Auswirkung von Hochdruck auf die vorhandene Rohmilch-Mikroflora, *Pseudomonas fluorescens* DSM 4358 und seine extrazellulären Proteinasen untersucht. Hochdruck führte zu einer Verminderung der Rohmilchmikroflora, wobei Hefen und Pilze am druckempfindlichsten waren und *Enterococcus* Drücken bis zu 500 MPa standhielt. *Ps. fluorescens* wurde nach einer Druckbehandlung von 300 MPa vollständig inaktiviert. Die Proteinase von *Ps. fluorescens* DSM 4358 wurde mit Drücken von bis zu 800 MPa für 10 min bei 20°C behandelt. Die Wirkung der behandelten und nichtbehandelten Proteinase im zellfreien Überstand auf das Natriumcaseinat während der Inkubation bei 37°C für bis zu 48 h wurde mittels Elektrophorese analysiert. Während das Bakterium sehr druckempfindlich war, war das Enzym selbst nach Drücken von 800 MPa noch in der Lage, Casein zu hydrolysieren. Diese Ergebnisse zeigen, dass die Proteinase von *Ps. fluorescens* DSM 4358 äußerst widerstandsfähig ist, was für Milchprodukte aus hochdruckbehandelter Milch von Bedeutung sein kann.

21 High pressure treatment (survival of *Pseudomonas fluorescens*, resistance of protease)

21 Hochdruckbehandlung (Überleben von *Pseudomonas fluorescens*, Widerstandsfähigkeit der Protease)

1. Introduction

Bovine milk contains fifty to sixty indigenous enzymes, with varying activities depending on breed, diet, stage of lactation and overall health of the animal. In addition to indigenous enzymes, milk also contains endogenous enzymes produced by contaminating bacteria (1), which may originate from the udder of the cow, milking or storage equipment (2).

Raw milk quality has improved significantly over many years due to the introduction of cold-chain storage in the early 1950s; however, this favours the growth of psychrotrophic bacteria which are able to grow in milk at 0-7°C (3,4). In raw milk, approximately 10% of the microflora are *Pseudomonas* spp; however, in raw milk stored at 4°C for 1 week, 70-90% of the isolated psychrotrophs are *Pseudomonas* spp,