Production of raw milk cheese and content of phyto-estrogens from organic produced milk

Acronym: RAWMICHEESE

Project objective and summary in Danish

a)
Produktionen af økologiske oste er hovedsageligt baseret på at fremstille en række ostetyper, som også fremstilles af konventionel mælk. Såfremt at man skal kunne øge markedsandelen af økologisk producerede oste, er det vigtigt at disse er af en kvalitet, der berettiger til øget merpris i forhold til konventionelt producerede oste. Det hævdes ofte at oste baseret på rå mælk kan opnå en rigere sensorisk kvalitet end konventionelle produkter, dette kræver en dokumentation af at disse oste har en anden flavour end pasteuriserede oste.

I Danmark har man en yderst restriktiv politik for produktion af oste baseret på upasteuriseret mælk begrundet i de potentielle problemer med mikrobiologisk sikkerhed. For i fremtiden at kunne vurdere den mikrobiologiske sikkerhed ved ostning af upasteuriseret mælk er det nødvendigt at fastlægge en række kritiske kontrolpunkter i produktionen fra råvare til det færdige produkt samt angive forslag til monitorering af disse. Dette vil kunne gøres i form af en generisk Hazard Analysis Critical Control Point (HACCP) fra stald til bord, som vil kunne give retningslinier for en sikkerhedsmæssig optimal produktion. De mikrobiologiske data genereret i projektet vil indgå i en risiko simuleringsmodel, der inddrager variation i forekomst og niveau af specifikke patogener samt i proces-og produktparametre af betydning for vækst og overlevelse.

Med udgangspunkt i rødkit oste fra et specifikt mejeri er det derfor nærværende projekt mål, at undersøge og sammenligne flavour og textur udviklingen i rød kit oste baseret på rå og pasteuriseret mælk ved hjælp af textur målinger, flavour analyse baseret på høj vacuum distillation, peptid profilering og under anvendelse af elektronisk næse for derigennem at kunne dokumentere upasteuriserede ostes kvalitet samt at udvikle en HACCP model og en specifik risiko simuleringsmodel, der kan understøtte virksomheders og offentlige myndigheders evalueringsprocedurer for etablering af osteproduktion baseret på upasteuriseret mælk.

Mål

Det overordnede mål er at forbedre mulighederne for at producere økologisk højkvalitetsost med acceptabel mikrobiel sikkerhed og af høj sensorisk kvalitet på basis af upasteuriseret mælk.

I denne sammenhæng er de væsentligste mål:

- At undersøge effekten af råvaren på modningsprocessen gennem fastsættelse af sensorisk kvalitet og teksturparametre
- At undersøge niveauet for forurening med specifikke fødevareoverførte patogener tilknyttet ost fra stalden til de færdige oste på et gårdmejeri.
- At evaluere potentialet for vækst eller overlevelse af disse patogener I ostene. At udvikle en HACCP og en risikosimuleringsmodel
- At sammenligne den sensoriske kvalitet af ost lavet af upasteuriseret mælk med den tilsvarende pasteuriserede ost.

b)
I den økologiske mælkeproduktion anvendes i stigende grad bælgplanter som f.eks. kløver, lupin, hestebønner og ærter med henblik på kvælstoffiksering i jorden og forbedret proteinforsyning af koen. F.eks. udgør kløvergræs på energibasis mere end halvdelen af den totale indtagelse i sommerperioden, og i vinterperioden udgør kløvergræs ensilage gennemsnitlig 2/3 af det totale grovfoder. Endvidere udgør cerealier en væsentlig del af foderet i den økologiske mælkeproduktion.

Bælgplanter, men også cerealier, nødder, grøntsager, har et naturligt højt indhold af planteøstrogener også kaldet phytoøstrogener. Phytoøstrogener er en fællesbetegnelse for en stor gruppe af stoffer med østrogenlignende effekter. En række undersøgelser tyder på, at phytoøstrogener kan have en forebyggende virkning på udviklingen af en række velfærdssygdomme.

Phytoøstrogener kan opdeles i tre hovedgrupper, isoflavonoider, coumestans og lignaner. Koncentrationen af isoflavonoider i f.eks. kløver har vist sig at være større end 20 mg/g tørstof. Kløver er derfor en potentiel kilde for phytoøstrogener i komælk og således også i mennesker som konsumenter, specielt hvis den økologiske mælkeproduktion i betydeligt omfang baseres på at udnytte kløvergræsset. Indholdet af phytoøstrogener i komælk er imidlertid kun meget sparsomt belyst. Der findes et enkelt studie fra Finland og ét fra Australien, men der er så vidt vides ingen undersøgelser i mælk fra danske besætninger.

Formålet med nærværende projekt er, at fastlægge indholdet af forskellige phytoøstrogener i mælk og foder fra forskellige økologiske besætninger i relation til rationens sammensætning, specielt indholdet af bælgplanter.

Project summary

a)
The production of organic cheese is mainly based on the production of a number of cheese types, which are also produced from conventional milk. In order to be able to increase the market share of organically produced cheese, it is important that these cheese types are of a quality that justifies an additional price in relation to the conventionally produced cheese. It is often maintained that cheese made from raw milk can obtain a richer sensory quality than conventional products. However, documentation is needed to demonstrate that the flavour of organic cheese is different from cheeses made from pasteurized milk.
In Denmark, the production of cheese based on unpasteurized milk is subject to a very restrictive policy owing to the potential problems with microbiological safety. In order to be able to evaluate the microbiological safety of cheese manufacturing with raw milk, a number of critical control points in the production from raw material to finished product must be established, and suggestions for their monitoring must be made. This can be solved by using a Hazard Analysis Critical Control Point (HACCP) from stable to table which will provide guidelines for safe processing procedures. The specific data generated in the project will further be used in a risk simulation model which will take into account the variations in prevalence and concentration of specific pathogenic bacteria encountered in the production, as well as the variations observed in the process- and product parameters which may influence growth or inactivation.
The present project will – based on the production of red-lead putty cheese made with raw and pasteurized organic milk, respectively, from a specific dairy - compare the flavour and texture development by means of texture measurements, flavour analyses based on high vacuum distillation, peptide profiling and electronic nose measurements. It will further develop a generic

HACCP model and a risk simulation model for the specific cheese which may serve as a tool for public safety managers.

b)
In organic milk production, the use of leguminous plants such as clover, lupin, horse beans and peas has increased because of nitrogen fixation into the ground and as an important energy and protein source for the cow. As an example, clover-grass, on energy basis, has been more than half the total intake in the summer period, and in the winter period, silage from clover-grass covers an average of 2/3 of the total roughage intake. Furthermore, organic dairy cows are given a large amount of cereals.

Especially leguminous plants, but also cereals, nuts, vegetables and berries contain natural high amounts of plant-estrogens, named phyto-estrogens. Phyto-estrogens are a large group of compounds with estrogen-like effects. Recent investigations on the effects of phyto-estrogens on various tissues have suggested that these compounds may improve human health, particularly by protecting against certain chronic diseases.

Phyto-estrogens can be divided in three main groups, isoflavonoids, courmestans and lignans. The concentration of isoflavonoids in clover for example, is more than 20 mg/g dry tissue. Therefore, clover is a potential source of phytoestrogens in cows milk and therefore also in humans as consumers of milk, especially if clovers are used as feed crops in organic milk production. Only a few studies have examined phytoestrogens in milk from cows, including a study from Finland and one from Australia. However, to our knowledge, there are no such studies of the content of phytoestrogens in milk from Danish herds.

The objective of the present project is to determine the concentration of phyto-estrogens in feed- and milk samples collected from different danish organic herds in relation to the ration composition, especially the content of leguminous plants.

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Description of the project:

a)

The production of organic cheese is mainly based on the production of a number of cheese types, which are also produced from conventional milk. If the market share of organically produced cheese is to increase in the future, it is important that these cheese types are of a quality that justifies an additional price in relation to the conventionally produced cheese. Careful and minimal processing are two important parameters in the organic production. Cheese production based on unpasteurized milk is therefore a natural development within organic production. Often gourmets point out that cheese made from raw milk can obtain a richer sensory quality, and several EU countries have a proud tradition within this field. Flavour development of Münster – type cheese based on raw milk has to the applicants knowledge never been performed and the identification of enzymes and mechanisms responsible for the flavour development in the Münster cheese based on raw milk has not been studied.

The major obstacle to production of raw milk cheeses is the restrictive policy of the health authorities. This is due to the recognized, increased risk of foodborne disease associated with raw milk cheeses compared with cheeses made from pasteurized milk. By far the majority of the published cheese-associated outbreaks have been caused by raw or under-pasteurized milk even though the raw milk cheeses constitute a minor part of the market (Institute of Food Science & Technology, 1998). Pasteurisation was specifically designed to destroy bacterial pathogens and it is the simplest means of ensuring safe milk and milk products. However, the bacteriological quality of the milk may vary tremendously between countries and herds as well as the hygiene during handling. Likewise, a number of studies have shown that the fate of the pathogenic bacteria may also vary according to manufacturing processes and from cheese to cheese (Johnson, 1990, Knøchel et al. 1995, Little & Knøchel, 1994, Back et al. 1993) and even specific microbial interactions may also be of importance (Larsen & Knøchel 1997, Ennahar et al.2000). It is understandable that the food safety authorities are reluctant to allow the production of raw milk cheeses in general but it is also recognised that the risks involved will depend on the individual operation. A case-by-case approach is now being adopted in Denmark but in order to be operational some demonstration tools are needed. The present project will allow us to generate, summarize, analyse, and interpret microbiological data develop a HACCP and a risk simulation model for a specific cheese production, where we shall be able to follow the production from the staple environment to the finished cheeses during different seasons and feeding practises.

We will therefore – based on the production of red-lead putty cheese made with raw and pasteurized organic milk, respectively, from a specific dairy – be able to explore the major claimed advantages and disadvantages of raw milk based cheeses; i.e. to compare the flavour and texture development by means of texture measurements, flavour analyses based on high vacuum distillation, peptide profiling and electronic nose measurements and to develop a generic HACCP model and a risk simulation model for the specific cheese which may serve as a tool for public safety managers.

Cheese based on raw milk and produced at farm dairies has the reputation of being of the highest quality (Muir et al., 1997) and is often described as having a more intense flavour than cheese based on pasteurized milk (McSweeney et al., 1993; Roy et al., 1997; Shakeel-Ur-Rehman et al., 1999). The less intense flavour of cheese based on pasteurized milk can be explained by a reduction in the natural bacteria and a partial inactivation of the natural milk enzymes (Urbach, 1997; Grappin & Beuvier, 1997). The microbial flora of unpasteurized milk contributes to the development of flavour, e.g. by production of fatty acid ethyl esters by microbial lipolytic enzymes Dahl et al., 2000). The level of free amino acids has been found to be higher in

unpasteurized ewe's milk cheese compared with pasteurized, which may affect the flavour of the ripened cheese (Mendia et al., 2000). A sensory test of 34 Cheddar cheeses produced from either unpasteurized milk or pasteurized milk showed a larger flavour variation among the unpasteurized cheeses, and the flavour was often described as rancid, bitter and unclean (Muir et al., 1997). Both the flavour and texture or body of the cheese contribute greatly to cheese quality, and texture profile analysis has been used to characterise the texture of semi-hard Italian cheese (Montasio) made from unpasteurized milk (Innocente et al., 2000). The characteristics and ripening conditions of the raw material seem to be important for the sensory quality of the resulting cheese, and decisive to whether it is possible to produce cheese of a quality that exceeds the quality of conventionally produced cheese.

1. Raw milk may contain a number of pathogens but relatively few have been associated with cheese outbreaks. If we look at the globally published outbreaks during the last 25 years most have been caused by *Listeria monocytogenes, Salmonella spp.* and *verotoxin producing E.coli.*. The focus of the project will therefore be on these bacteria (Institute of Food Science & Technology, 1998). All of these bacteria have been associated with soft and semi-soft cheeses such as the Münster cheese. The project will benefit from a number of data through previous projects and surveys re the prevalence of these bacteria in selected Danish herds gathered by The Zoonosis center at the Danish Veterinary Laboratory. Other bacteria potentially relevant will be included on the basis of literature and available data from the Danish Veterinary Laboratory.

Although Denmark and several other countries are known to have a high hygienic standard for milk production pathogens may still be found. A survey conducted in the late 80'es showed a *Listeria monocytogenes* prevalence of 4.2 % of the herds investigated (Jensen et al. 1996) but no quantitative data are available. Similarly, a survey in England and Wales in the early 90'es also found 5% bulk farm samples positive for *Listeria monocytogenes* and 0,36% for Salmonella spp. (O' Donnell, 1995). A recent, still unpublished fresh cheese outbreak in Sweden found up to $>10^6$ cfu *Listeria monocytogenes*/g cheese as well as different pathogenic *E.coli* and *Staphylococcus aureus*. One goat without any clinical signs had $>10^4$ Listeria/ml milk ! (Danielsson-Tham, M-L. personal communication). These findings illustrate that prevalence may vary within regions, herds and individual animals. It also illustrates that the bacteria may sometimes grow to very high numbers in the cheeses. The relative importance of actions directed towards minimisation of contamination at various points or inhibition of growth may be estimated by use of a risk simulation model based on probabilistic distributions and predictive microbiology.

b)

In organic milk production, the use of leguminous plants such as clover, lupin, horse beans and peas has increased because of nitrogen fixation in the soil and as an important energy and protein source for the cow. In a typical organic rotation of crops clover-grass constitutes between 20 and 40 %. Results from case studies over a period from 1990 to 1998 on 20 farms with organic milk production showed that clover-grass was the most important energy and protein source. It was also characteristic, that the organic dairy cows were given a large amount of cereals (Mogensen et al., 1999).

Leguminous plants contain natural high amounts of plant-estrogens, named phyto-estrogens. Phyto-estrogens are a large group of compounds with estrogen-like effects (Bingham et al., 1998). Phyto-estrogens can be divided in three main groups, isoflavonoids, courmestans and lignans. All three groups are phenols that are structurally similar to mammalian estradiols and synthetic estrogens and anti-estrogens (Setchell & Adlercreutz, 1988). Phyto-estrogens are also found in

cereals, nuts, and seeds (Mazur & Adlercreutz, 1998), and in a number of common fruits and vegetables (Price & Fenwick, 1985).
The concentration of isoflavonoids in clover for example, is more than 20 mg/g dry tissue (Francis & Millington, 1965). Therefore, clover is a potential source of phyto-estrogens in milk from cows and therefore also in humans as consumers of milk, especially if clovers are used as feed crops in organic milk production. The knowledge about the content and biological importance of phyto-estrogens in milk are limited. Only a few studies have examined the content of phyto-estrogens in milk from cows, including one study from Finland and one from Australia. However, to our knowledge, there are no such studies in milk from danish cows.

A number of recent investigations on the effect of phyto-estrogens on various tissues suggest that phyto-estrogens may improve health, particularly by protecting against certain chronic diseases including cancer (breast-, prostate- and colon cancer), cardiovascular disease and osteoporosis, as well as other hormone-dependent conditions like menopausal symptoms. Recent epidemiologic evidence and experimental data from animal studies are highly suggestive of beneficial effects on human health (Setchell & Cassidy, 1999). However, as phyto-estrogens in structure are similar to natural and synthetic estrogens and anti-estrogens, their effect might be either positive or negative depending on the type of phyto-estrogen and the time of exposure. A number of studies suggest that the effect of estrogens on for example breast cancer risk depends on the timing of environmental and dietary exposure to estrogens (Hilakivi-Clarke et al., 2001). It is important to note, that although concentrations of single phyto-estrogens in milk might be low, the biological effect for consumers depends on the total amount of phyto-estrogens including additive and synergistic effects. Furthermore, it is known that steroids is low concentrations can have important biological effects in humans.

On this background, it is important to generate more knowledge about the presence of phyto-estrogens in milk and their possible biological effects in consumers. The objective of the present project is to determine the concentration of phyto-estrogens in feed- and milk samples collected from different organic herds in relation to the ration composition, especially the content of leguminous plants. We expect the concentrations of phyto-estrogens in organic milk to be affected by the ration composition, especially the content of leguminous plants.

In a related FØJO II project "Future organic dairy production systems – effect of improved feeding and breeding on production, health and milk quality" (Project II,1), we are currently investigating the quality of organic milk with the focus to investigate the content and the effect of conjugated linoleic acid (CLA). The present project therefore is in immediate continuation of this project and collection of feed and milk samples will be co-ordinated between the two projects.

The quality of milk as food depends mainly on its content of major nutrients including protein, fat and lactose. However, milk also contains a long list of components with biological activity. The list includes enzymes, antibodies and many vitamins, hormones and growth factors. Many of these factors are considered to be essential for the development of the neonate and also of importance for quality of milk for consumers.

Consumers consider organically produced foods to be connected with high quality and health. This is the main motive for their preference for organic products. For milk this statement is supported by the fact that organic milk is guaranteed free of pesticides and that the content of residues of medicine is minimal. Besides that, there are no obvious reasons why the quality of organic milk should be different from conventionally produced milk. However, the limitations in use of imported feedstuffs and the increased reliance on homegrown feeds are likely to influence the composition and possibly the quality of milk. A number of milk components are likely to vary

in organic milk with the elimination of imported feed supplements (i.e. soy) and the inclusion of more clover in the diet. These milk components include phyto-estrogens, conjugated linoleic acid (CLA) and vitamin A, which are all suggested to have biological effects of relevance for human health. The higher reliance of home grown feeds most likely also lead to increased use of untraditional plants, that may have specific effects on the biological activity of the milk. Secondary metabolites in plants grown without the use of pesticides may also affect milk content of bioactive components, as may the variation in feeding level and feeding system.

Description of workpackages

WP1: Flavour and texture development in cheeses made from raw and pasteurised milk

Workpackage number: 1
Start date or starting event:
Responsible person: Jacob Holm Nielsen
Contributing persons: Lotte Bach Larsen

Objectives:
To study whether cheese based on unpasteurized milk has a more complex sensory quality compared with the analogous cheese based on pasteurized milk

Description of work:

Task 1: Cheese production

From well characterised unpasteurized milk are produced a cheese (Münster-type) with a relatively high water content and a short maturation period. The milk used for the cheese production is ob-tained from a single herd of 130 Holstein cows. The production at Hinge Osteri is repeated four times during one season to give variations in feeding and milk quality. As a control the same cheese type is produced from pasteurized milk.

The cheeses will be produced according to the following schedule:

Mid-May 2002
Mid-September 2002
Mid-January 2002
Mid-May 2003

Two batches of cheese are prepared at each time point, one from pasteurized milk and one from unpasteurized. The freshly produced cheeses will be stored at relatively high temperature and humidity for one week and then be in retail for 4 – 5 weeks. Extracts from separate cheeses will be analysed once a week during maturation period as well as the period where the cheeses are normally in retail, giving 5 to 6 samples from each cheese of varying maturation. Salt and pH of each sample will be analysed.

DJF is responsible for distribution of samples and chemical characterisation

Task 2: Enzyme activities and composition of milk and cheese

The raw milk used at each time point (four) of cheese production and extracts prepared (in duplicate, giving a total of 96) from the manufactured cheeses will be characterised by the following parameters:

Protein pct.
Fat pct.
Cell count (only in milk)
Fatty acid composition
Proteolytic enzymes (plasmin/plasminogen)
Casein degradation (γ-casein)
Lipase activity
Xanthine oxidase activity

These parameters describe the gross composition of the milk used as raw material and of the cheeses as well as the level of milk enzyme activities of significance for the development of sensory quality during ripening.

Task 3: Analytical evaluation of sensory attributes

To evaluate and compare the sensory attributes of cheeses made from pasteurized or unpasteurized milk the cheeses will be characterised by the following analytical methods:

Texture analysis
Aroma analyses
HPLC profiling of peptides

The analytical methods are selected to cover development in both texture and aroma. The texture analysis is expected to be carried out as a penetration measurement by texture analyser instrument as a parameter to describe the softening of the cheese. The texture analyses will be carried out on duplicate cheeses (96 samples) 5 to 6 times during storage and retail. Flavour analysis of cheeses are performed by solvent assisted flavour extraction (SAFE) followed by an enrichment on vigreux columns. The enriched extracts are subsequent analysed by GC/MS. The use of the SAFE system makes it possible to quantify semi-volatiles as well as volatiles. The flavour analysis will be carried out on dublicate cheeses (48 samples) 6 times during storage. Aroma analysis will furthermore be carried out on electronic nose in order to classify the cheeses from the aroma profile (6 cheeses of each type will be analysed from each sampling date). The HPLC profiling is carried out to com-pare the ripening process in terms of protein degradation in pasteurized and unpasteurized cheeses. The method involves preparation of extracts from both types of cheeses and the comparison of ob-tained peptide profile after HPLC separation. The production of peptides from milk proteins is the result of proteolytic enzyme activity in the cheeses, and may affect both the texture and flavour attributes through the softening of the protein network and the generation of flavour peptides, re-spectively. The HPLC profiling is relatively time-consuming and expected to be carried out on du-plicate cheeses at 2 to 3 times during storage and retail period (48 samples).

Deliverables:
D1-D4 Production and distribution of Münster cheeses incl. measurements of salt and pH

D5 International papers
D6 End report
D10 Discussion meeting with stakeholders

Milestones:
M1 Method for analysis of peptides in cheeses has been established
M2 Method for analysis of volatiles and semi-volatiles from cheese has been established
M3 Analysis of cheeses has been performed

WP2: Elaboration of a HACCP and a risk simulation model for a specific raw milk cheese production

Workpackage number: 2
Start date or starting event: 02/02
Responsible person: Associate professor Susanne Knöchel.
Dept. Dairy and Food Science
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Contributing persons:
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Objectives:
To evaluate the risks associated with production of raw milk cheese at a farm dairy

Description of work:
Task 4. Literature survey on contamination of raw milk and raw milk cheeses from industrialised countries.

International and national data on the prevalence and concentration of Listeria monocytogenes, Salmonella spp. and E.coli O157and other pathogenic agents in herds, raw milk and raw milk cheeses will be reviewed incl. recent data from a survey conducted by The Danish Veterinary Laboratory.

Task 5. Microbiological investigations at Hinge's dairy farm
Both young animals, milking cows (pooled feces samples) and raw milk will be characterized for Listeria monocytogenes, Salmonella spp. and E.coli O157 3-4 times covering different seasons and feeding patterns before the start of the production batches. The herd investigation will be done in collaboration with The Danish Veterinary Laboratory using standard methodology. If a

sample is positive, individual sampling of the raw milk will be done to obtain a variation in concentration using standard techniques as well as quantitative PCR and samples will also be taken at different stages of production.. All cheeses will be examined at their last day of consumption. A number of samples from the production enviroment post-cleaning will also be examined to validate the pre-requisite programme of the dairy farm.

Task 6. HACCP

A production flowsheet from feed to finished cheese will be elaborated together with the dairy. All data on the developments in temperature, salt and pH gradients of the cheeses during production will be collected and estmations of the variations will be performed. Predictions of growth and sur-vival will be done using predicton modelling tools such as Food Micro Model and results from pre-vious projects. The latter shows that FoodMicroModel tends to overestimate growth rate but also lag phase. A growth simulation study during starter culture acidification will be performed with both pasteurized and raw milk. All the information will be analysed and used to elaborate a generic HACCP programme.

Task 7. Risk simulation model

All the in formation from the three former tasks will be used in a risk simulation model. I terms of risk assessment this simulation model will focus on the exposure-assesment. The model will describe pathways and processes leading up to the exposure and divide these into discrete models. Different approaches will be evaluated in developing an overall model. Estimates of exposure will be made using a probabilistic approach where uncertainty and variability are taken into account by repre-senting parameters as probabilistic distributions. Monte Carlo simulations will then be performed using appropriate software. A sensitivity analysis will be made estimating the effect of changes in the various parameters in order to identify the most efficient ways of inhibiting high level exposure of pathogens. The newly formed risk assessment group at The Danish Veterinary Lab will partici-pate in the discussions.

Deliverables:

WP 2, D7: Review on pathogenic bacteria in raw milk and raw milk cheeses
WP 2, D8: Suggestion for a generic HACCP programme
WP 2, D9: Elaboration of a risk simulation model
WP 2, D10: Discussion meeting with stakeholders
WP 2, D11: Final manuscript

Milestones:

WP 2, milestone 4: Review produced
WP 2, milestone 5: Microbiological analyses performed
WP 2, milestone 6: Generic HACCP programme produced
WP 2, milestone 7: risk simulation model generated

WP3: Phytoestrogens in organic milk

This workpackage includes contributing scientists from two different departments within the Danish Institute of Agricultural Sciences, Department for Animal Nutrition & Physiology and Department for Horticulture.

Description of workpackages:

Workpackage number: 3
Start date or starting event: June 2002
Responsible person: SP
Contributing persons: LPC,KS, MVE, KB

Objectives:

- Collection of feed and milk samples from organic herds with cows fed diets with different ration composition, especially content of leguminous plants.
- Determination of concentrations of selected phyto-estrogens in feed- and milk samples.

Description of work:

The concentrations of phyto-estrogens in organic milk are likely to be affected by the ration composition, especially the content of leguminous plants. We will determine the concentrations of phyto-estrogens in feed and milk samples. The phyto-estrogens will be selected on the basis of the available information in the literature and with respect to possible detection techniques. Development of methods for analysis will be necessary, as only a few assays for phyto-estrogens are available. Focus will initially be on phyto-estrogens included in the group isoflavonoids (genestein, daidzein, etc.) and lignans (matairesinol, secoisolariciresinol, enterolactone, etc.). However, as the analyses proceed, other phyto-estrogens of less knowledge might be included. Milk samples will be collected on individual basis to account for the variation related to content of leguminous plants in the ration. Feed- and milk samples will be collected in different seasons to account for developmental stage of the plants and the amount of clover. For isolation and quantification of phyto-estrogens, column chromatography and HPLC (analytical and preparative), as well as ELISA and TR-FIA, will be used. For identification of isolated compounds, mass spectrometry and NMR spectroscopy, will be used.

Deliverables:

D1.1: Report

Milestones:

M1: Sampling
M2: Laboratory analyses
M3: Evaluation and report

Implementation and time schedule:

The sampling will be coordinated with sampling in FØJO project II,1 "Future organic dairy production systems – effect of improved feeding and breeding on production, health and milk quality" Sampling will start in June 2002.