Forskningsrådet för miljö, areella näringar och samhällsbyggande
The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning

Formas Direct ID
2006-4759-7317-45

Ansökan om forskningsbidrag

2006

COPY

1.

Utlysning:	Bidragsform:
CORE Organic - Pilot Call	ERA CORE

2.

Huvudsökande, efternamn	Förnamn	Personnummer	Kön
Gustavsson	Anne-Maj	561127-8960	X K ☐ M

Telefon	E-post	Anställning	Titel	År-mån-dag för dr.examen
+46907868717	Anne-Maj.Gustavsson@njv.slu.se	Research leader, S	Associate prc	1994-09-12

Organisation (universitet/högskola/institut/företag)	Värdhögskola
Sveriges Lantbruksuniversitet	Sveriges Lantbruksuniversitet

Institution	Faxnr
Institutionen för norrländsk jordbruksvetenskap	+46907868704

Avdelning
Avd för växtodling

Gata, Box etc.	Postnummer	Ort
Box 4097	90403	Umeå

3.

Projekttitel, svenska (högst 200 tecken)
Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation

Project title, English (200 characters at the most)
Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation

Projektbeskrivning svenska(högst 1500 tecken inklusive mellanslag)

Dairy milk and milk products have been challenged due to their relatively high concentration of saturated fatty acids (SFA) and, therefore, are considered to be associated with cardio-vascular disease and cancer. However, recent research has revealed a number of ingredients in milk that may be salutary. Among these bioactive components are phytoestrogens (e.g. lignans and isoflavenoids), fatty acids (FA, e.g. c9t11 C18:2 (CLA)), vitamins (e.g. vitamin A and E), and endogenous hormones and growth factors (e.g. estradiol, IGF-I and TGF-â). Due to a higher proportion of forage in the ration, with high proportion of legumes and other herbs, organic milk quality is more and differently affected by the forage than conventionally produced milk that often is based on grass silage. There is limited knowledge regarding chemical and sensory characteristics of organic dairy milk internationally that has focused on organic grassland management and the milk salutary properties. The objective of proposed project is to: - investigate how different forage species affect the fatty acid composition and the content of bioactive components such as tocopherols, carotenoids, selenium (Se) and phytoestrogenes of organic dairy milk. - investigate the biological activity of the collected dairy milk samples from the Nordic countries on normal and cancer cells.

Projektbeskrivning engelska (högst 1500 tecken inklusive mellanslag)

Dairy milk and milk products have been challenged due to their relatively high concentration of saturated fatty acids (SFA) and, therefore, are considered to be associated with cardio-vascular disease and cancer. However, recent research has revealed a number of ingredients in milk that may be salutary. Among these bioactive components are phytoestrogens (e.g. lignans and isoflavenoids), fatty acids (FA, e.g. c9t11 C18:2 (CLA)), vitamins (e.g. vitamin A and E), and endogenous hormones and growth factors (e.g. estradiol, IGF-I and TGF-â). Due to a higher proportion of forage in the ration, with high proportion of legumes and other herbs, organic milk quality is more and differently affected by the forage than conventionally produced milk that often is based on grass silage. There is limited knowledge regarding chemical and sensory characteristics of organic dairy milk internationally that has focused on organic grassland management and the milk salutary properties. The objective of proposed project is to: - investigate how different forage species affect the fatty acid composition and the content of bioactive components such as tocopherols, carotenoids, selenium (Se) and phytoestrogenes of organic dairy milk. - investigate the biological activity of the collected dairy milk samples from the Nordic countries on normal and cancer cells.

Projektstart	Beräknat projektslut	Sökt 2006 tkr	Sökt 2007 tkr	Sökt 2008 tkr	Sökt 2009 tkr	Sökt 20010 tkr	Sökt 2011 tkr
2007-07-01	2010-07-01	243	344	335			

Formas Direct ID
2006-4759-7317-45

2006

Sida
2

COPY

Namn		Projekttitel
Gustavsson	Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer		
561127-8960		

4.

[X] nytt bidrag [ ] fortsatt bidrag Ange diarienummer:	Ansökan har även ingivits till annan finansiär Identisk till: Liknande till: Samfinansieras med:	Del av samarbetsprojekt: [ ] Ämnesövergripande projekt: [ ]

5.

Nyckelord
fatty acids, phyto oestogenes, antioxidants, grassland, legumes

Klassificering (SCB) i prioriteringsordning	Ämnesområde/ Målområde
173201 | 173301 |	Agriculture

Förslag till beredning/beredningsgrupp
brg9406, brg9406

6.

Bifogade bilagor [X] A [X] B [X] C [ ] D [ ] E [ ] F [ ] G [ ] H [X] I

7. Projektperiod

Projektstart	Projektslut
2007-07-01	2010-07-01

8. Medsökande

Efternamn	Förnamn	Personnummer	Titel
See Appendix B		-	
Kön [ ] K [ ] M / År-mån-dag för dr.examen	Organisation (universitet/högskola/institut/företag)	Institution	
Efternamn	Förnamn	Personnummer	Titel
		-	
Kön [ ] K [ ] M / År-mån-dag för dr.examen	Organisation (universitet/högskola/institut/företag)	Institution	
Efternamn	Förnamn	Personnummer	Titel
		-	
Kön [ ] K [ ] M / År-mån-dag för dr.examen	Organisation (universitet/högskola/institut/företag)	Institution	
Efternamn	Förnamn	Personnummer	Titel
		-	
Kön [ ] K [ ] M / År-mån-dag för dr.examen	Organisation (universitet/högskola/institut/företag)	Institution	

Formas Direct ID
2006-4759-7317-45

2006

Sida
3

Namn	Projekttitel
Gustavsson Anne-Maj Personnummer 561127-8960	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation

9. Human/djurförsök

Humanförsök. Godkänt av regional etisk kommitté:	Djurförsök. Godkänt av djurförsöksetisk nämnd:
☐ Ja, datum . . . . . Dnr	☐ Ja, datum . . . . . Dnr . . . . .
☐ Ansökan inlämnad (bifogas) ☐ Ämnar ansöka	Djurslag: 1 . . . . . 2 . . . . . 3 . . . . .
	☐ Ansökan inlämnad (bifogas) ☒ Ämnar ansöka

10. Etiska överväganden

Etiska överväganden avseende persondata redovisas i forskningsprogrammet (bilaga A) under särskild rubrik på sidan . . . . . .

11. Dyrbar och medeldyr vetenskaplig utrustning till en kostnad över 500 tkr

Utrustning avses användas i följande projekt:	Finansiär	Projekttitel
☐ Pågående projekt ☐ Sökt projekt Dnr:		
Utrustning avses användas i följande projekt:	Finansiär	Projekttitel
☐ Pågående projekt ☐ Sökt projekt Dnr:		
Utrustning avses användas i följande projekt:	Finansiär	Projekttitel
☐ Pågående projekt ☐ Sökt projekt Dnr:		

12. Sektorrelevans

The project will give increased knowledge about the nutritional and salutary quality of organic milk. The proposal specially meets the request in the thematic area fiQulity of organic food Œ heath and safetyfl in the pilot call for proposal and particularly the sub theme: fiOrganic farming practices and their impacts on food quality, health and safetyfl.

The project will also give increased knowledge of the relationship between production system and environmental condition and milk properties, which might serve as a basis for both farming management as well as human dietary recommendations.

Formas Direct ID
2006-4759-7317-45

Budget

Namn	Projekttitel
Gustavsson Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer	
561127-8960	

2006

Sida 4

Inga indirekta kostnader skall anges i ansökan.

Sökta belopp (tkr)

13. Personal/löner (inklusive sociala avgifter)

	2006	2007	2008	2009	2010	2011
Huvudsökande: namn, arbetsinsats i projektet i % av heltid						
Övrig personal: namn, kön, födelseår, år för doktorsexamen, månadslön idag, arbetsinsats i projektet (% av heltid) samt andel forskning i nuv. anställning (% av heltid).						
Total personal costs	172	206	192			
(See Appendix I)						
(*1000 EURO)						
Summa löner	172	206	192			

14. Andra kostnader (specificerade)

Total consumable costs	33	84	104			
(See Appendix I)						
Total travel costs	15	27	16			
Overhead, Finland	3	7	3			
Overhead, Denmark	20	20	20			
(*1000 Euro)						
Summa andra kostnader	71	138	143			

15. Dyrbar och medeldyr vetenskaplig utrustning till en kostnad över 500 tkr

Summa utrustning						
Summa sökta belopp till sidan 1, punkt 4.	243	344	335			

Formas Direct ID
2006-4759-7317-45

Totala forskningsresurser

Namn		Projekttitel
Gustavsson	Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer		
561127-8960		

2006

Sida
5

COPY

16. Huvudsökandens beviljade och /eller sökta forskningsresurser

Belopp i tkr

Bev. samfinans. detta projekt		2006	2007	2008	2009	2010	20011
Finansiär							
Andra Formas-projekt	Projekttitel				Ev. beviljat 2006	Beviljat 2007	Sökt 2007
Pågående (dnr) och sökta							
Projekt med annan finansiär	Projekttitel				Totalt beviljat	Projektperiod	Sökt 2007
Finansiär							

Formas Direct ID
2006-4759-7317-45

Obligatorisk populärvetenskaplig beskrivning

Namn	Projekttitel
Gustavsson Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer	
561127-8960	

2006

Sida 6

Texten skall ha en populärvetenskaplig rubrik (skrivs i ruta 17.) och vara på svenska. Den skall vara skriven så att även den som inte alls är insatt i ämnet har möjlighet att förstå vad projektet handlar om eller vad utrustningen skall användas till. Använd inte inomvetenskaplig terminologi eller oförklarade begrepp i texten eller rubriken. Texten skall disponeras enligt följande:

För projekt

- Projektets bakgrund
- Projektets frågeställning och mål
- Projektets innehåll; lyft fram projektets grundvetenskapliga innehåll
- Projektets nytta: förklara syftet och varför den nya kunskapen blir viktig. Om möjligt, diskutera hur resultaten kan leda till tillämpningar eller till fortsatt forskning.

För utrustning

- Beskriv hur utrustningen skall användas och vilka nya möjligheter och förbättringar som den erbjuder forskningen.

Det är viktigt att beskrivningen är informativ. Den används både i Formas interna arbete och för information utåt. Beviljade projekts beskrivningar kommer att ingå i den projektkatalog som finns på Formas webbplats samt användas i populärvetenskapliga sammanhang på Internet.

17. Populärvetenskaplig rubrik och beskrivning (högst 2 500 tecken)

Dairy milk and milk products have been challenged due to their relatively high concentration of saturated fatty acids (SFA) and, therefore, are considered to be associated with cardio-vascular disease and cancer. However, recent research has revealed a number of ingredients in milk that may be salutary. Among these bioactive components are phytoestrogens (e.g. lignans and isoflavenoids), fatty acids (FA, e.g. c9t11 C18:2 (CLA)), vitamins (e.g. vitamin A and E), and endogenous hormones. Due to a higher proportion of forage in the ration, with high proportion of legumes and other herbs, organic milk quality is more and differently affected by the forage than conventionally produced milk that often is based on grass silage. There is limited knowledge regarding chemical and sensory characteristics of organic dairy milk internationally that has focused on organic grassland management and the milk salutary properties.

The objective of proposed project is to: - investigate how different forage species affect the fatty acid composition and the content of bioactive components such as tocopherols, carotenoids, selenium (Se) and phytoestrogenes of organic dairy milk. - investigate the biological activity of the collected dairy milk samples from the Nordic countries on normal and cancer cells.

18.

Webbadress/adresser	E-post till kontaktperson (om annan än huvudsökande)

Namn		Projekttitel
Gustavsson	Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer		
561127-8960		

Detta försättsblad syftar till att underlätta kansliets hantering av ansökan. Då den sökande markerat i ansökningsblanketten att en bilaga skall bifogas kommer sökandes namn, personnummer och projekttitel att automatiskt fyllas i på försättsbladet. På varje sida av bilagan skall det högst upp anges namn, personnummer och bilagans bokstavsbeteckning.

Formas

Bilaga: A

Forskningsprogram

Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation

The objective of the proposed project is to:
- investigate how different forage species affect the fatty acid composition and the content of bioactive components such as tocopherols, carotenoids, selenium (Se) and phytoestrogens of organic dairy milk
- investigate the biological activity of the collected dairy milk samples from the Nordic countries on normal and cancer cells.

State of the art

General. Dairy milk and milk products have been challenged due to their relatively high concentration of saturated fatty acids (SFA) and, therefore, are considered to be associated with cardio-vascular disease (Pedersen et al., 2004) and cancer. Nordic Council of Ministers (2004) states that the relatively high fat content in the diets in the Nordic countries may have contributed to the high prevalence of cardiovascular diseases, certain types of cancer, obesity and gallstones. However, recent research has revealed a number of ingredients in milk that may be salutary. Among these bioactive components are phytoestrogens (e.g. lignans and isoflavenoids), fatty acids (FA, e.g. *c*9*t*11 C18:2 (CLA)), vitamins (e.g. vitamin A and E), and endogenous hormones and growth factors (e.g. estradiol, IGF-I and TGF-β) (Kris-Etherton et al., 1999; Parodi, 1999; Williams, 2000; Larsson et al., 2005)**.** The nutritionists focus also on the dietary balance between n-6 and n-3 polyunsaturated (PUFA), and that the n-6/n-3 ratio is too high in Western diets (Simopoulos, 2002). It would therefore be beneficial to enhance the proportion of n-3 FA in milk fat as a mechanism for enhancing PUFA intake in the diet.

Organic milk Legumes play an essential role in organic forage production. Legumes are the main input of nitrogen to the farming systems, provide high dry matter yields with high nutritive quality and are therefore important for the productivity of the farming system (Steinshamn, 2001). However, organic grasslands due to low nutrient input and the avoidance of herbicide may also contain high proportion of unsown species, particularly herbs in long term swards (Govasmark et al., 2005a). Due to a higher proportion of forage in the ration, with high proportion of legumes and other herbs, organic milk quality is more and differently affected by the forage than conventionally produced milk based on grass silage. There is limited knowledge regarding chemical and sensory characteristics of organic dairy milk internationally. In general, only small differences have been found between organic and conventional dairy milk gross composition, fatty acids, iodine, and β-carotene (Toledo et al., 2002;Toledo & Andren, 2003;Nicholas et al., 2004). Others have found higher content of some few or several parameters like protein, vitamin C, C18:3n-3, *t*11C18:1 (TVA) and CLA, tocopherol and carotene in organic milk and dairy foods (Lund, 1991;Jahreis et al., 1996;Bergamo et al., 2003;Nielsen & Lund-Nielsen, 2004; Ellis et al., 2006). Recent studies in Denmark show that the content of both FA composition and phytoestrogens in bovine milk is markedly affected by the diet of the animals, and milk from cows fed grass silage contains a significantly higher concentration of alpha-tocopherols, carotenoids and unsaturated fatty acids, than milk from cows fed maize silage (Sejrsen et al. 2001; Weisbjerg et al. 2001; Purup et al 2002; Purup et al. 2004; Nielsen et al. 2004; Purup et al. 2005; Purup et al. 2007). However, to our knowledge very few studies have focused on organic grassland management and the milk salutary properties. As there are and will be limitations to supplementation in organic milk production, dietary factors related to the roughage are likely of vital importance.

Milk fatty acid composition affected by grassland botanical composition. Milk fat and the fatty acid composition is influenced by several factors, such as feeding of grazed vs. conserved forages, plant species and varieties (Palmquist et al., 1993 , Chilliard et al., 2000, Dewhurst et al., 2006, Elgersma et al., 2006a). Grassland species contain high proportion of PUFA in their lipids, especially C18:3n-3 (Hawke, 1973). In the rumen, extensive biohydrogenation results in extensive loss of PUFA, yielding SFA, which together with *de novo* synthesis of SFA results in a high proportion of SFAs in ruminant products (Chilliard et al., 2000). However, some PUFA passes to the duodenum and some beneficial intermediates (CLA and t11C18:1(TVA)) of the hydrogenation pathway may also escape further hydrogenation and may be absorbed, desaturated in the mammary gland (TVA) and excreted in milk (Griinari et al., 2000). Several factors affect the content of C18:3n-3 in the forage, such as species, variety, phenology, nutrient supply and weather (Dewhurst et al., 2001; Boufaied et al., 2003). Preservation, such as silage and hay making, reduces the concentration considerably, and the content

declines with advancing maturity of the plants (Dewhurst & King, 1998; Dewhurst et al., 2001). Therefore, it is generally found that milk produced on pasture has a higher content of C18:3n-3 and CLA than milk produced on conserved forage (Chilliard et al., 2000; Dewhurst et al., 2003b;Lock & Garnsworthy, 2003;Elgersma et al., 2006b). Increased concentrate to forage ratio usually decreases the content of C18:3n-3 and CLA in milk (Dewhurst *et al.* 2003a; Steinshamn *et al.* 2006), and milk fat content of CLA and C18-3n-3 decreases with maturity of the plants during grazing (Ferlay et al., 2002).

Higher content of CLA and PUFA has been found in milk produced by cows grazing on botanical diverse pasture in the highlands than on grass-based pastures in the Alp lowlands (Collomb et al., 2001), and the milk FA composition was correlated with pasture plant families or species (Collomb et al., 2002). Grazing birdsfoot trefoil (*Lotus corniculatus)* resulted in increased concentrations of C18:2n-6 and C18:3n-3, and decreased concentrations of c9C18:1, CLA and TVA in milk compared to grazing ryegrass (Turner et al., 2005). Effect of plant species on milk FA composition is also found on silage based rations. It has been found higher content of PUFA, C18:3n-3 and n-3/n-6 FA ratio on clover than on grass silage and higher on red clover (*Trifolium pratense*) than on white clover (*Trifolium repens*) silage (Dewhurst et al. 2003; Steinshamn et al. 2006). The botanical effect is not necessarily directly linked to the plants' content or animals' intake of C18:3n-3. Lourenco *et al.* (2005) found similar C18:3n-3 content and higher CLA content in milk fat from feeding silages prepared from species-rich semi-natural than from intensively managed grasslands despite reduced dietary supply of C18:3n-3, and a sharp increase in milk C18:3n-3 content has been found when moving dairy cows from lowland pasture to highland pasture without any increase in the intake of C18:3-n3 (Leiber et al., 2005). Thus, the botanical effect may be due to differences in the supply of precursor FA, higher rumen outflow rates of FA or the presence of inhibitory compounds. It is hypothesized that the reduced biohydrogenation for some forages may be due to effects of specific metabolites (e.g. polyphenol oxidase and tannins) on lypolysis, microbial biohydrogenation activity, or both (Lee et al., 2003; Leiber et al., 2005; Turner et al., 2005).

Milk vitamin content and oxidative stability. Fresh herbage has high contents of vitamin E and carotenoids that act as antioxidants and protect the milk and milk products from oxidation. Preservation, such as ensiling in round bales and particularly hay making reduce the content of vitamin E (Bernhoft et al., 2002), whilst ensiling in silo may maintain the content (Nadeau et al., 2004). Milk produced by grazing cows usually has higher content of vitamin E than milk produced indoor (O'Brien et al., 1999) but a higher oxidative stability of milk produced on pasture is only partly explained by a higher content of vitamin E (Martin et al., 2004).There are also secondary plant metabolites, like polyphenolic compounds, that may improve the oxidative stability of milk (O'Connell & Fox, 2001; Acamovic & Brooker, 2005). However, Al Mabruk et al., (2004) found rapid loss of tocopherol and reduced oxidative stability of milk from diets based on red clover and lucerne silages in comparison with diets based on grass silage. Thus, it might be that organic diets yielding milk with high levels of PUFA is more susceptible to oxidation. It is also observed very low content of Se in milk (Kuusela & Okker, unpublished) and in blood (Govasmark et al. 2005b) from organically managed cows, and Govasmark et al., (2005b) recommended supplementation with Se and vitamin E.

Phytoestrogens. In ruminants, intake of phytoestrogens by feeding legumes has usually been associated with reduced fertility (Seested et al., 2000), but high intake of red clover has in some cases also improved fertility (Austin et al., 1982). Leguminous plants, such as clover, but also grains contain high amounts of phytoestrogens (Cornwell et al., 2004). Phytoestrogens in leguminous crops are isoflavones, which all have been found to be estrogenic (Aoki et al., 2000). The content of phytoestrogens in dairy milk and products is very little investigated, but it is likely influenced by intake of legumes and is higher in organic than in conventional produced milk (Antignac et al., 2004; Purup et al., 2005), and for some specific estrogens much higher in milk from cows fed red clover-grass silage than white clover-grass silages (Steinshamn et al. unpublished).

Objectives of the proposed project - Hypothesis

- Milk production based on herbage from organically managed long term grasslands with high proportion of non-clover herbs yields milk with lower content and proportion of n-3 FA and CLA, lower content of phytoestrogens and higher oxidative stability than milk produced on herbage from organically managed clover rich leys

- The effect of non-clover herbs in grass silage on the FA composition of milk fat is due to partly inhibition of rumen biohydrogenation
- Tocopherols and carotenoids can balance milk oxidative stability despite of low Se concentration
- Organic Se supplementation will increase milk Se concentration, decrease somatic cell count and improve milk oxidative stability
- The fatty acid composition and phytoestrogen concentration of organically managed forage grass and legumes are affected by latitude and weather conditions
- The choice of silage preservation methods will affect the fatty acid composition and carotene and tocopherol content of the silage
- There is biological activity of the collected dairy milk samples from the Nordic countries on normal and cancer cells.

Project description

Task 1. Pasture and silage botanical composition - effect on milk quality. Two feeding experiments will be carried out at the Animal Production Centre, University of Life Science, Norway (APC-UMB) and one at Röbecsdalen, SLU, Umeå.

Grazing experiment APC-UMB. A continuous grazing trial will be carried out during the grazing season 2008, with the first 2 weeks as pre-experimental period where the cows graze the same pasture. Two groups of 8 cows (Norwegian Red Cattle) each, in mid lactation, will be formed on the basis of their performance before the grazing season, calving date and lactation number. After the 2-week adaptation to grazing, three 4-week experimental periods will follow; early (May), mid (June/July) and late (August) summer, during which each group will graze one of the two organic pasture management treatments; botanical diverse long term pasture and clover-rich short term pasture. The long term pasture has a relatively high botanical diversity and high proportion of other herbs than red clover (Steinshamn et al., 2001), whilst the short term managed pasture will be dominated by red clover sown with timothy (*Phleum pratense)* and Meadow fescue *(Festuca pratensis*). The cows will receive small and equal amounts of concentrate, a mineral additive but no vitamin supplements. The groups will as far as possible receive the same herbage allowance, by adjusting the area, so that the cows will have the same energy intake.

Silage feeding experiment (APC-UMB and SLU-Umeå). Two silage feeding experiments will be carried out during 14 weeks of the housing period 2008/ 2009, with the first 2 weeks as a transition period where all cows receive the same forage. The number of cows, stage of lactation and allocation to treatments will be similar to and carried out in the same way as in the grazing experiment. The experiment will be conducted using a change-over design with three 4-week periods. Two weeks for adaptation to the experimental feed and two weeks for measurement and sampling. The silage will be fed *ad libitum* with a small and equal amount of concentrate supplementation. The cows will receive no vitamin supplements but a mineral additive. In the experiment conducted at APC-UMB, the treatments will be round bale silage prepared from two contrasting organically managed grasslands; DS) Grassland with high proportion of non red clover herbs like *Taraxacum officinale* and *white clover* and RC) short term rotational ley with high proportion of sown species rich in red clover. In the experiment at SLU-Umeå the treatments will be silage from short term rotational leys with high proportion of red clover and grass mixture (RC), and birdsfoot trefoil and grass mixture (BG). The same varieties will be used in RC at the two sites to investigate effects of site on milk quality.

Task 2. Silage botanical composition – effect on rumen FA hydrogenation and milk FA composition (Department of Animal and Aquacultural Sciences – IHA/UMB). The experiment will be carried out during the housing period 2008/2009. Four types of silages ensiled in round bales will be prepared from the first cut in 2008: *Lolium perenne* (LP); *timothy* and meadow fescue mixture (GS); Red clover-grass mixture (RC); non red clover herb-grass mixture (DS). The LP and GS are included as control treatments The RC and DS silages will be the same as the silage used in Task 2, and the varieties used in the GS and RC treatments will be the same as the one used in the field trials in Task 4 and 5. The silages will be offered *ad libitum* with small and equal amount of concentrate supplementation. The experimental diets will be fed to 4 cannulated dairy cows in a Latin square design with 4 periods of 24 days (14 days adaptation to the diet and 10-day measurement period for milk sampling and collection of faeces, rumen and duodenal fluid. Markers will be used in order to estimate digesta flow at the duodenum. Faecal output will be measured on days 15 to 20, and duodenal digesta will be collected every 4 h on days 21 and 22. Samples of rumen fluid will be taken every 2h

over a 24-h period on day 23. Silage will be sampled daily during the measurement period. Sub-samples of silage, digesta and faeces will be stored frozen for chemical analysis. Rumen fluid will be analysed for pH, ammonia and volatile fatty acids. Digesta flow and apparent digestibility of fatty acids in the rumen will be calculated after mathematical reconstitution of true digesta, and the biohydrogenation of C:18 PUFAs will be estimated by simple difference between feed and duodenal flow.

Task 3. Effects of Se supplementation on milk quality

Current situation. A survey will conducted in Eastern Finland in cooperation with the local ProAgria and local organic farms. In the beginning of year 2008 tank milk samples will be collected from 50 organic farms (25 practicing certified organic animal production and 25 farms practising only organic field farming). Representative samples of silage and concentrate mixture will be taken from each farm. Number of cows and average daily milk yield will be recorded. Farmers will be interviewed for their current feeding and Se and vitamin supplementation.

Improved Se situation. All farmers will be introduced to Se yeast. Twenty voluntary farms will switch to Se yeast supplementation (3 mg Se/cow) for 4 weeks. Tank milk samples will be collected in the end of experimental period.

Task 4. Effects of latitude and harvest time on FA composition and phytoestrogen concentrations on four organically managed forage species. In an ongoing collaboration, the seasonal dynamics of herbage tocopherols, carotenoids, lignin and ash content and herbage carbohydrate and crude protein fractions and digestibility in timothy, meadow fescue, red clover and birdsfoot trefoil are investigated together with plant development and plant morphology investigations on three sites (SLU-Umeå, Sweden), (SLU, Skara, Sweden) and (DIAS-Foulum, Denmark). From this experiment, already collected samples can be used in the proposed study to investigate site and harvest time effects on FA composition and concentration of phytoestrogens. From each site we have freeze dried plant material from two years harvested at 9 different harvest times (3 in first cut and 2*3 in regrowth) that are separated in pure species.

Task 5. Effects of storage time and silage preservation methods on FA composition and concentrations of tocopherols and carotenoids on four organically managed forage species. In the same experiment as in Task 4, a silage experiment will be conducted at one site. First cut of a birdsfoot trefoil/timothy mixture and a red clover/timothy mixture is harvested at the heading stage of timothy in 2007. Each mixture is replicated three times in the field. Forages are wilted to 30% dry matter before ensiling, without an additive or with an addition of a bacterial inoculants or an acid, in laboratory silos. The silos are opened after 1.5, 3, 6 and 9 months. Forages will be analysed for FA composition, α-tocopherol and carotenoids before and after wilting and after each storage period. Chemical composition of wilted forage before ensiling and of silage at three months of storage (incl. fermentation characteristics) will be assessed according to the NorFor system.

Task 6 Bioactive components in organic milk with suggested health beneficial effects. The milk samples that will be collected from the Nordic countries in the project will represent a repository of milk samples collected from cows at different breed and lactation- and pregnancy stages, and from cows fed rations high in clover, grass, birdsfoot trefoil, corn etc. This repository of complex mixtures containing naturally occurring bioactive components is suitable for studies of cellular effects of bioactive components with suggested health beneficial importance. *In vitro* cell-based models will be used for assessing the biological activity in specific human tissues. Relevant cell-based models include normal- and cancer cell lines of gastro-intestinal, mammary or prostate origin exposed to different concentrations of milk samples or specific bioactives for different time-intervals. Cellular endpoints such as proliferation, viability and apoptosis as well as estrogenic or anti-estrogenic activity of milk will be evaluated. Milk samples having the ability to inhibit proliferation of human breast, prostate and intestinal cancer cells will receive a high priority. The milk samples for testing will be selected mainly based on the content of bioactive components.

Analytical methods to be applied within task 1-5

Sampling in the controlled experiments (Task 1-2). Milk yield and feed intake (housing) will be recorded daily during the experimental periods. Consecutive milk samples will be collected at three morning and three evening milkings in the last week of each measurement period. Samples will be taken of each round bale and merged into three samples per period of each silage type. Sward samples will be collected daily during the grazing period and merged to three samples per period. Both the

pasture and the ley botanical composition will be recorded before harvesting and during the whole grazing period by the means of a dry weight rank method (Mannetje & Haydock, 1963). Pre- and postgrazed forage will be clipped and weighed to determine yield and utilization of the pasture. The cows' species preference during grazing will be monitored by counting shoots/tillers before and after grazing in plots.

Milk analysis (Task 1-3). The milk samples will according to standard procedures be analysed for the content of protein, fat, lactose, urea, volatile fatty acids, somatic cell count and bacteria. Milk fatty acid composition will be analysed by gas chromatography (GC) as fatty acid methyl esters, tocopherols and carotenoids will be determined by high pressure liquid chromatography (HPLC) and phytoestrogens (flavenoids and non-flavenoids) will be analysed by LC-MS/MS technique at the Danish Institute of Agricultural Sciences, Dept. of Animal Health, Welfare and Nutrition (DIAS). Oxidative stability will be assessed by TBARS determination and Se according to standard procedure

Plant samples, feed and digesta analysis (Task 1-5). Feed and digesta will be analysed for dry matter, ash, crude protein, neutral detergent fibre, ammonia (silage), fermentation products (silage) according to the recommendations in the NORFOR feed evaluation system. Analysis of fatty acids will be done by GC as fatty acid methyl esters according to (Sukija & Palmquist, 1988), and tocopherols and carotenoids will be determined by HPLC (Jensen et al., 1999) and phytoestrogens by LC-MS/MS at DIAS.

Statistical analyses. The data will be processed by analysis of variance.

Project organisation

The project will be lead by Anne-Maj Gustavsson , SLU-Umeå. In Sweden, SLU is project owner. A PhD student will be recruited (supervisor will be Anne-Maj Gustavsson). Experiments will be done in SLU-Umeå (Anne-Maj Gustavsson and Kjell Martinsson) and in SLU-Skara (Elisabet Nadeau). In Norway, the research institutions Bioforsk- Organic Food and Farming Division and IHA/UMB will collaborate. A PhD student (Steffen Adler), financed by the Bioforsk project "Organic grassland management and milk quality", will be connected to the project. Project owner is Bioforsk. Project leader is Håvard Steinshamn (Bioforsk) and responsible at IHA is Odd Magne Harstad who also is the main supervisor for Adler. In Finland, MTT Agricultural research in Finland (Animal production, Chemistry laboratory), University of Helsinki (Research centre for animal welfare) and University of Kuopio (Department of Biosciences) will collaborate. Project owner is University of Joensuu, Faculty of Biosciences. Project leader is Eeva Kuusela. In Denmark the project is performed at and owned by the Danish Institute of Agricultural Sciences, Research centre Foulum. Søren Krogh Jensen will be responsible for analyses of fatty acids and vitamins, and Stig Purup will be responsible for analyses of phytoestrogens and bioactivity in cell-based assays. To ensure that project milestones and integration of the different tasks (1-5) are achieved and to facilitate joint publication, a project management group, consisting of project national project leaders, will be formed and coordinated by Anne-Maj Gustavsson.

Collaboration benefits

The proposed project is a collaboration between scientists from different disciplines; crop, animal and chemistry. The collaboration makes it possible to utilize research expertise and facilities more efficiently and creates increased knowledge out of limited research funding. Organic dairy production is important in all Nordic countries, and the milk is produced under very different environmental condition and production systems. The collaboration makes it possible to increase the knowledge of the relationship between production system and environmental condition and milk properties, which might serve as a basis for both farming management as well as human dietary recommendations.

Plans for scientific publications

- Botanical composition of pasture and quality of organically produced milk – Effect on fatty acid composition and vitamin and phytoestrogen content and oxidative stability
- Botanical composition of silage and quality of organically produced milk - Effect on fatty acid composition and vitamin and phytoestrogen content and oxidative stability (2 papers)
- Milk fatty acid composition and associated fatty acid hydrogenation when feeding organically produced silage with different botanical composition
- Effects of weather and latitude on the dynamics of fatty acid composition and phytoestrogens in organically managed legumes and grasses.

- Effects of storage time and preservation method on fatty acid composition and vitamins in organically managed silage.
- Biological activity in organic milk with suggested health beneficial effects
- Effect of current and improved selenium supply on tank milk quality – a farm study
- Effect of varying milk selenium and vitamin concentrations on oxidative stability of organic milk

Plan for communication and knowledge transfer

Dissemination of results will be done through meetings for farmers, papers, journals and existing organic agricultural websites in the participating countries.

Milestones (quarter of year)

M1. Workshop planning animal production experiments and survey Task 1-3 and Task 6 (02-2007). *M2*. Establish grassland task 1-2 (02-03 2007). *M3*. Plant analysis Task 4. *M4*. Silage experiment and chemical analysis Task 5 (02 2007- 02 2008). *M5*. Survey including milk sampling and feed and questionnaire data collection Task 3 (01 2008 – 01 2009). *M6*. Grazing experiment Task 1 (02-03 2008) *M7*. Silage feeding experiment, including sampling at harvesting and conservation, Task 1 (03 2008-02 2009). *M8*. Rumen biohydrogenation study Task 2. (01-02 2009). *M9*. Bioassay experiment on in vitro cell cultures (03 2008 – 03 2009). *M10*. Workshop facilitating joint publication (02 2009). *M11*. Scientific publication (Task 1-2 02 2009- 04 2009, Task 3 02 2009, Task 4 04 2007, Task 5 04 2008, Task 6 04 2009). *M12*. Dissemination on websites, meeting, papers etc (04 2007-04 2008).

References

Acamovic, T. & Brooker, J. D. 2005. Proc. Nutr. Soc. 64, 403-412. Al Mabruk, R. M., Beck, N. F. G. & Dewhurst, R. J. 2004. J. Dairy Sci. 87, 406-412. Antignac, J. P., Cariou, R., Le Bizec, B. et al.. 2004. Food Chem. 87, 275-281. Aoki, T., Akashi, T. & Ayabe, S. 2000. J. Plant Res. 113, 475-488. Austin, A. R., Aston, K., Drane, H. M. et al. 1982. Grass Forage Sci. 37, 101-106. Bergamo, P., Fedele, E., Iannibelli, L. et al. 2003. Food Chem. 82, 625-631. 2003. Bernhoft, A. et al. . 2002. In: Kaurstad, E. (ed.) Husdyrforsøksmøtet 2002. pp. 209-212. Boufaied, H., Chouinard, P. Y., Tremblay, G. F. et al.. 2003. Can. J. Anim. Sci. 83, 501-511. Chilliard, Y., Ferlay, A., Mansbridge, R. M. et al. 2000. Ann. Zootech. 49, 181-205. Collomb, M., Butikofer, U., Sieber, R., et al. 2001. J. Dairy Res. 68, 519-523. Collomb, M., Butikofer, U., Sieber, et al. O. 2002. Int. Dairy J. 12, 661-666. Cornwell, T., Cohick, W. & Raskin, I. 2004. Phytochem. 65, 995-1016. Dewhurst, R. J., Fisher, W. J., Tweed, J. K. S. et al. 2003. J. Dairy Sci. 86, 2598-2611. Dewhurst, R. J. & King, P. J. 1998. Grass Forage Sci. 53, 219-224. Dewhurst, R. J., Scollan, N. D., Youell, S. J., et al.. 2001. Grass Forage Sci. 56, 68-74. Dewhurst, R. J., Shingfield, K. J., Lee, M. R. F. et al.. 2006. Anim. Feed Sci. Techn. 131, 168-206. Elgersma, A., Tamminga, S. & Ellen, G. 2006. Anim. Feed Sci. Techn. 131, 207-225. Ellis, K. A., Innocent, G., Grove-White, D., et al.. 2006. J. Dairy Sci. 89, 1938-1950. Ferlay, A., Martin, B., Pradel, Ph., et al.. 2002. Grassl. Sci. Eur. 7: 556-557. Govasmark, E., Steen, A., Bakken, A. K., et al.. 2005a. Acta Agricult. Scand. B, 55, 131-142. Govasmark, E., Steen, A., Strom, T., et al. 2005b. Acta Agricult. Scand. A, 55, 40-46. Griinari, J. M., Corl, B. A., Lacy, S. H. et al.. 2000. J. Nutr. 130, 2285-2291. Hawke, J. C. 1973. In: Butler, U. W. & Bailey, R. W. (eds.) Chemistry and Biochemistry of Herbage. Academic Press, London, pp. 213-263. Jahreis, G., Fritsche, J. & Steinhart, H. 1996. Lipid 98, 356-359. Jensen, S. K, Johannsen, A. K. B. & Hermansen, J. E., 1999. J. Dairy Res. 66. 511-522. Kris-Etherton, P. M., Pearson, T. A., Wan, Y., et al.. 1999. Am. J. Clin. Nutr. 70, 1009-1015. Larsson, S. C., Bergkvist, L. & Wolk, A. 2005. Am. J. Clin. Nutr. 82, 894-900. Lee, M. R. F., Harris, L. J., Dewhurst, R. J. et al.. 2003. Anim. Sci. 76, 491-501. Leiber, F., Kreuzer, M., Nigg, D. et al.. 2005. Lipids 40, 191-202.
Lock, A. L. & Garnsworthy, P. C. 2003. Liv. Prod. Sci. 79, 47-59. Lourenco, M., Vlaeminck, B., Bruinenberg, M., et al. 2005. Anim. Res. 54, 471-484. Lund, P. 1991. Milchwissenschaft-Milk Science International 46, 166-169. Mannetje, L. & Haydock, K. P. 1963. J. Brit. Grassl. Soc. 18, 268-275. Martin, B., Fedele, E., Ferlay, A. et al.. 2004. Grassl. Sci. Eur. 9, 876-886. Nadeau, E., Johansson, B., Jensen, S. K. et al. 2004. Grassl. Sci. Eur. 9, 891-893. Nielsen, T.S., Sejrsen, K., Andersen, H,R, et al.. 2004. J. Anim. Feed Sci. 13, Suppl. 1, 697-700. Nicholas, P. K., Padel, S., Cuttle, S. P. et al. 2004. Biol. Agric. Hort. 22, 217-249. 2004. Nordic Council of Ministers 2004. Nordic Nutrition Recommendations 2004. 4th edition. Copenhagen. O'Brien, B., Lennartsson, T., Mehra, R. et al. 1999. Ir. J. Agr. Food Res. 38, 75-85 O'Connell, J. E. & Fox, P. F. 2001. Int. Dairy J. 11, 103-120. Palmquist, D. L., Beaulieu, A. D. & Barbano, D. M. 1993. J. Dairy Sci. 76, 1753-1771. Parodi, P. W. 1999. J. Dairy Sci. 82, 1339-1349. Pedersen, J. I., Tverdal, Aa. & Kirkhus, B. 2004. Tidsskr. Nor. Lægeforen. 124, 1532-1536. Purup, S., Vestergaard, M., Weisbjerg, M.R. et al. 2002. J. Dairy Sci. 85, Suppl.1, 9-10. Purup S, DeNichilo M & Sejrsen K, 2004. 7th joint EAAP/ASAS workshop on Biology of Lactation in Farm Animals, Bled, Slovenia, 5-9 September 2004, 30. Purup, S., Hansen-Møller, J., Sejrsen, K. et al.. 2005. http://www.darcof.dk/enews/jun05/milk.html. Purup, S., Vestergaard, M., Pedersen, L.O. et al. 2007. J. Dairy Sci. 74 (in press). Seested, S., Norgaard, P. & Ranvig, H. 2000. Dansk Veterinaertidsskrift 83, 6-12. Sejrsen, K., Pedersen, L.O., Vestergaard, M. et al. 2001. Livest. Prod. Sci. 70, 79-85. Simopoulos, A. P. 2002. Biomed. Pharmacother. 56, 365-379. Steinshamn, H. 2001. Acta Agricult. Scand. B, 51, 113-124. Steinshamn, H., Grønmyr, F. & Tveit, H. 2001. Grassl. Sci. Eur. 6, 161-163. Steinshamn, H., Thuen, E., Brenøe, U.T. 2006. Proc. Joint Organic Congres, Odense, Denmark. pp. 532-533. Sukhija, P. S. & Palmquist, D. L. 1988. J. Agric. Food Chem. 36, 1202-1206. Toledo, P. & Andren, A. 2003. Food Agric. Environ. 1, 122-125. Toledo, P., Andren, A. & Bjorck, L. 2002. Int. Dairy J. 12, 75-80. Turner, S.-A., Waghorn, G. C., Woodward, L. S. et al. 2005. N. Z. Soc. Anim. Prod. 65, 283-288. Weisbjerg, M.R., Purup, S., Vestergaard, M. et al. 2001. DJF rapport nr. 25. 39 pp. Williams, C. M. 2000. Annales de Zootechnie 49, 165-180.

Namn	Projekttitel
Gustavsson Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer	
561127-8960	

Detta försättsblad syftar till att underlätta kansliets hantering av ansökan. Då den sökande markerat i ansökningsblanketten att en bilaga skall bifogas kommer sökandes namn, personnummer och projekttitel att automatiskt fyllas i på försättsbladet. På varje sida av bilagan skall det högst upp anges namn, personnummer och bilagans bokstavsbeteckning.

Formas

Bilaga: B

CV

Appendix B

APPLICANTS

Surname	First name	Date of birth	Sex	Academic title	Year of dissertation	University/corresponding
Gustavsson	Anne-Maj	561127	Female	Associate professor	1994	SLU, Sweden
Steinshamn	Håvard	591015	Male	Senior researcher	1997	Bioforsk, Norway
Jensen	Søren Krogh	591202	Male	Senior scientist	1991	DIAS, Denmark
Kuusela	Eeva	571224	Female	Lecturer	2004	Faculty of Biosciences, University of Joensuu, Finland
Purup	Stig	581206	Male	Senior scientist	1995	DIAS, Denmark
Nadeau	Elisabet	620201	Female	phD	1995	SLU, Sweden
Harstad	Odd Magne	520529	Male	Proffessor	1984	Norwegian Univ. Of Life Sciences (UMB)
Martinson	Kjell	461120	Male	Associate professor	1983	SLU, Sweden

Namn		Projekttitel
Gustavsson	Anne-Maj	Potential improvement of the salutary effect of organic dairy milk by forage species and by supplementation
Personnummer		
561127-8960		

Detta försättsblad syftar till att underlätta kansliets hantering av ansökan. Då den sökande markerat i ansökningsblanketten att en bilaga skall bifogas kommer sökandes namn, personnummer och projekttitel att automatiskt fyllas i på försättsbladet. På varje sida av bilagan skall det högst upp anges namn, personnummer och bilagans bokstavsbeteckning.

Formas

Bilaga: I

Valfri bilaga

Appendix I: Detailed financial plan

Total cost for all participants (*1000 Euro)

		2007	2008	2009	Sum	Norway	Sweden	Finland	Denmark
Personnal costs	Norway	45	63	63	170	170			
Personnal costs	Sweden	55	55	55	164		164		
Personnal costs	Finland	15	30	15	60			60	
Personnal costs	Denmark	57	59	60	176				176
Consumable costs	Norway	12	36	86	133	133			
Consumable costs	Sweden	2	15	2	20		20		
Consumable costs	Finland	1	16	1	18			18	
Consumable costs	Denmark	18	17	16	50				50
Travel costs	Norway	5	15	6	26	26			
Travel costs	Sweden	3	3	3	9		9		
Travel costs	Finland	2	4	2	8			8	
Travel costs	Denmark	5	5	5	14				14
Overhead Finland	Finland	3	7	3	12			12	
Overhead Denmark	Denmark	20	20	20	60				60
Overhead Sweden	Sweden	21	25	21	67				
Total costs		**262**	**369**	**355**	**987**	**328**	**192**	**98**	**300**
Overhead Sweden							**67**		
Total cost Sweden							**260**		

Total cost for all participants (*1000 Euro)

		*1000 Euro	*1000 Euro	*1000 Euro
Personnal costs		172	206	192
Consumable costs		33	84	104
Travel costs		15	27	16
Overhead Finland		3	7	3
Overhead Denmark		20	20	20
Overhead Sweden*				
Total costs in application		**242**	**343**	**334**
Overhead Sweden*		21	25	21
Total costs		**262**	**369**	**355**

* Not included in the application

Financial cost Norway (applied funding) (Euro)

Cost type	Specification	2007	2008	2009	sum
Personal*	PhD Steffen Adler	0	0	0	
	Senior Researcher Håvard Steinshamn	26786	35714	35714	98214
	Professor Odd Magne Harstad	17857	26786	26786	71429
Consumable cost	Analalysis Task1	0	13237	13237	26475
	Analalysis Task2	0	0	17486	17486
	Experimental Cost Task1	11726	17928	12160	41814
	Experimental Cost Task2	0	4762	42619	47381
Travel Cost	Project meeting, experimetal work, overseas stay PhD	4762	14881	5952	25595
Total		**61131**	**113308**	**153955**	**328394**

*Included overhead

Financial cost Denmark (Euro)

						Percent of time			
		2007	**2008**	**2009**		**2007**	**2008**	**2009**	
SKJ	Month	2	2	2		16.7	16.7	16.7	
		12000	12400	13067					
ELP	Month	5	5	4.5		41.7	41.7	37.5	
		16800	16520	16053					
	Consumeable	7200	7080	6880					
	Travel	1333	1333	1333					
	Overhead	9333	9333	9333					
	Total	46667	46667	46667					
SKJ = Søren Krogh Jensen									
ELP = Elsebeeth Lyng Pedersen, Lab technician									
						Percent of time			
		2007	**2008**	**2009**		**2007**	**2008**	**2009**	
SPU	Month	2	2	3		16.7	16.7	25.0	
		12000	12400	19599					
AKN	Month	5	5	3		41.7	41.7	25.0	
		16667	17507	11027					
	Consumeable	10667	9427	8707					
	Travel	3333	3333	3333					
	Overhead	10667	10667	10667					
	Total	53333	53333	53333					
SPU = Stig Purup									
AKN = Annette Kamgaard Nielsen, Lab technician									
Total costs Denmark		**=100010**	**100010**	**100010**					
SPU = Stig Purup									

AKN = Annette Kamgaard Nielsen, Lab technician						

Financial cost Sweden (Euro)

				2007	**2008**	**2009**
Personnal costs	Anne-Maj Gustavsson	16,7	80	11500	11500	11500
Personnal costs	PhD-student	100	100	43017	43017	43017
Personnal costs	Elisabet Nadeau			0	0	0
Personnal costs	Kjell Martinsson			0	0	0
Consumable costs	Experiment (Task 2)			0	12971	0
Consumable costs*	Experiment (Task 4 and 5)			0	0	0
Consumable costs**	Analysis (Task 2, 4 and 5)			0	0	0
Consumable costs	Analysis (Task 2, 4 and 5)			2206	2206	2206
Travel costs	(4 persons*7000)			3088	3088	3088
Overhead (35 %)				20934	25474	20934
Total costs				**80746**	**98258**	**80746**

* Finansed in another project

** Analyses made by DIAS, Denmark

Financial costs Finland (Euro)

						Percent of time		
		2007	**2008**	**2009**		**2007**	**2008**	**2009**
Eeva Kuusela*	Months	1	2	2		8.8	16.7	16.7
	Euros	0	0	0				
Laura Okker	Months	6	12	6		50	100	50
	Euros	15 000	30 000	15 000				
	Consumeable** sampling analysis experimental feed	1 000	16 000	1 000				
	Travel	2 000	4 000	2 000				
Overhead 14.5% (not included)		2 610	7 250	2 610				
	Total	**20 610**	**57 250**	**20 610**	**98 470**			

* Finansed by lectorership

** Part of analyses made by DIAS, Denmark