Differentiation between organic and conventionally produced milk in pasture based farming systems.

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Abstract

Consumer perception of organic cow's milk is associated with the assumption that organic milk differs from conventionally produced milk. The value associated with this difference, justifies the premium retail price. It includes the perception that organic dairy farming is kinder on the environment, animals and people; that organic milk products are produced without the use of antibiotics, added hormones, synthetic chemicals and genetic modification and may have potential benefits for human health. Controlled studies investigating the chemical differences between organic and conventionally produced milk have so far fallen short of a conclusion as to whether or not these exist. Reasons for this are many folds, caused principally by the complexity of the research problem. A main complication is that farming practices and their impacts differ depending on country, region, year and season between and within organic and conventional systems. Factors influencing milk composition (e.g. diet, breed, and stage of lactation) have been studied individually, while interactions between multiple factors have been largely ignored. Studies fail to consider that factors other than the farming system (organic versus conventional) could have caused or contributed to, the reported differences in milk composition. These omissions make it impossible to determine whether there is a system related difference between organic and conventional milk, or not. The present study investigated the chemical differences between organic and conventionally produced milk in a pasture based farming system. Milk samples have been collected on two farm sets each comprised of one organic and one conventional farm. All farms applied year-round pasture grazing. Milk samples were collected from individual animals on Farm Set 1 and throughout the milking season on both farm sets. Milk samples have been analysed for fatty acid, free oligosaccharides, major casein and whey proteins, and milk fat volatiles, as well for a limited set of milk metabolites using a non-targeted NMR method. Considering the known influence factors on milk composition and the differences observed between the farms on the farm sets in our study, we postulated that fatty acids were influenced by breed and fertilizer application. Oligosaccharides differed between farming systems, with causes presently unknown. The farm set was the dominant influence factor on protein composition, while none of the compounds identified using NMR show any trend. Thus, the major conclusions from this study were that the factors influencing milk composition are not exclusive to either farming system, and pasture feeding conventional cows will most probably remove differences previously reported in other organic and conventionally produced milk studies.

List of Publications

Schwendel, B. H., P. C. H. Morel, T. J. Wester, M. H. Tavendale, C. Deadman, B. Fong, N. M. Shadbolt, A. Thatcher, and D. E. Otter. 2015. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. J. Dairy Sci. 98:1411-1425. http://dx.doi.org/10.3168/jds.2014-8322.

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List of Presentations

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Chapter 1

1.1. IDEOLOGY AND BACKGROUND

Any researcher investigating organically produced products cannot help but become exposed to the inconsistent regulative framework of certified organic food production, the 'mob mentality' of consumer perception, the commercial interest of organic food producers, and the rather variable definition of the term 'organic' itself.

When starting this thesis, I trusted the precision of the research question 'Is there a difference between organic and conventional milk?' Unexpectedly, the struggle of answering this was not based on scientific results, but the attempt to understand the organic ideology, which often appears to be completely removed from science.

Since the industrialisation of agriculture starting with introduction of synthetic fertilizer in the 1920's and the 'Green Revolution' coinciding with availability of synthetic pesticides in the 1940's, there has always been a small group of people who objected to their use and advocated so called 'organic' farming ideals. During the first half of the 20th Century, the Demeter Certification Program in Germany and the Soil Association in the UK were formed to oppose intensive farming methods and promote biodynamic agriculture. In the following decades, support for organic farming practices grew slowly and steadily worldwide, marked in 1972 with formation of the International Federation of Organic Agriculture Movements (IFOAM). Since 1990, markets for organic products have grown rapidly with annual increases of up to 25% resulting in an overall food market share of 1-7%. This happened in conjunction with a loss of consumer confidence in commercially produced food. Outbreaks of Bovine Spongiform Encephalopathy (BSE) and mistrust in genetically modified products (GMO) are just two of a multitude of reasons consumers have, to turn towards organically produced food. For a century, the assumption that food produced without use of synthetic products (e.g., fertilizer, pesticides, antibiotics, growth hormones) is safer (Hasimu et al., 2017), more nutritious and, therefore, more beneficial for human health (Rembiałkowska et al., 2008; Huber et al., 2011; Sirieix et al., 2011) has been promoted by advocates of organic farming. These beliefs are reflected in consumer surveys. Further assumptions are that organic farming practices are kinder on the animal, environment (Liu et al., 2013), and people raising the animals, thus, allowing consumers to feel more altruistic and environmentally friendly (Sirieix et al., 2011; Zagata, 2012). Presently, the main consequence for consumers purchasing organically produced products is payment of premium prices (Zagata, 2012; Rödiger et al., 2016; Hasimu et al., 2017). In addition, consumer identification with organic labels is shown

to be based on subjective assumptions rather than objective knowledge of production standards and control regimens (Janssen and Hamm, 2012; Schleenbecker and Hamm, 2013).

Based on claims made by organic food producers, the scientific community has become divided in their research approach and objectives. One faction is investigating whether there are measurable differences in chemical composition between organic and conventionally produced food (Jensen et al., 2013; Srednicka-Tober et al., 2016), while the others focus on analytical methods and identification of unique markers to enable authentication of conventional and organic products (Molkentin, 2009). The latter is due to risk of fraud in consequence of the premium price paid for organic products.

In regard to dairy products, scientific studies have shown that composition of cow's milk is influenced by a multitude of factors, e.g., feed, genetics, season, breed, and stage of lactation. Differences in composition between organic and conventional milk, especially when considering milk fatty acids (FA), are largely explained by differences in diet. Health benefits claimed by organic milk proponents are commonly linked to an increase in poly-unsaturated FA or fat soluble vitamins in milk (Srednicka-Tober et al., 2016). This increase can generally be attributed to higher forage intake by cows in organic systems compared to most conventional systems, as organic regulations specify a minimum requirement for access to pasture. While intensive conventional dairy systems feed high concentrate rations, extensive low-input conventional cows rely on high forage intake. Milk produced in this latter system is largely identical to milk from organic cows (I have confirmed this in the NZ all-year-round pasture grazing system in this thesis). This highlights the role of diet in milk composition and underscores where most differences in organic and conventionally produced milk arise. Furthermore, all approaches that have attempted to authenticate organic milk have relied on this common difference between diets of organic and conventionally farmed dairy cows. A fact, seemingly ignored by organic producers, is that no dietary component (e.g., ingredient, feed-type) is fed exclusively to organic cows; pasture and forage feeding is not limited to cows in organic systems. As such, current authentication methods are applicable only when comparing milk from forage-fed organic cows to concentrate-fed conventional cows. These methods are consequently unable to differentiate between forage-based organic and extensive, pasture-based conventional farming systems. Furthermore, actual impacts on human health resulting from observed chemical differences between (forage-based) organic and (concentrate-fed) conventionally produced milk have recently been questioned (Givens and Lovegrove, 2016), demonstrating that a statistical difference between two products does not invariably lead to biological significance.

Problematic for both scientific approaches is that milk composition varies enough, albeit by small amounts, to prevent a universal statement on the concentration and profile of constituents unique to organic or conventional milk. Furthermore, invalid comparisons as a result of not taking into account all influence factors or simply the comparison of different variables (e.g., sampling time, breed) have been a major shortcoming of previous research.

The New Zealand dairy industry is based on all-year-round pasture grazing for both organic and conventional milk production. It is therefore questionable whether published conclusions of differences made between organic and conventional milk in overseas studies using different farming practices can be assumed to occur in New Zealand milk. Furthermore, it is hypothesised that an experiment designed in such a way to exclude as many of the known influence factors as possible, while monitoring all other variables, would provide a conclusive statement regarding inherent differences between organic and conventionally produced cow's milk.

The aim of this thesis was to identify whether there are differences in the chemical composition between organic and conventionally produced milk in a pasture-based production system in which major production variables were recognised and mitigated. As diet has been shown to produce the largest differences between the two different farming systems, ensuring that diets are similar for cows in both the organic or conventional farming systems will enable a more definitive answer on previously reported differences between organic and conventional milk composition resulted from an holistic, inherent 'organic effect', or whether differences were simple caused by differences in what the cows were fed.

1.2 QUESTIONS AND EXECUTION

This thesis focussed on raw milk from individual animals, as well as pooled milk samples from four herds taken from bulk-milk vats on-farm. Samples were collected from two sets of dairy farms in New Zealand. Both farm sets had a certified organic farm directly adjacent to one that was managed conventionally, with farms in each set operated similarly under the same management structures. Both organic farms were managed according to Organic Foods Production Act Provisions 2014 (US Government Printing Office, 2014). Farm Set 1 belonged to Massey University, Palmerston North (38.23° S, 175.86° E), while approximately 320 km north near Tokoroa, Farm Set 2 was privately owned (40.38° S, 175.61° E). In both sets, organic and conventional herds were made up of Friesian × Jersey crossbreed cows farmed on an all-year-round pasture grazing system as is commonly practiced in New Zealand.

I initially conducted an extensive review of the literature to determine what factors influence composition of milk and to draw attention to other studies that have been published comparing organic to conventionally produced milk (Chapter 1). This review was selected as a highlighted invited review in the Journal of Dairy Science (Schwendel, B. H., T. J. Wester, P. C. H. Morel, M. H. Tavendale, C. Deadman, N. M. Shadbolt, and D. E. Otter. 2014. Invited review: Organic and conventionally produced milk-An evaluation of influence factors on milk composition. J. Dairy Sci. http://dx.doi.org/10.3168/jds.2014-8389).

Leading from this review, my first aim was to investigate milk FA profile (Chapter 2). FA represent by far the most investigated class of compound, most likely because they show a large degree of variability in milk. Differences observed for FA between organic and conventional milk have predominantly been caused by differences in pasture intake. These observations lead to our first research question.

1.2.1 Are there differences in milk FA composition between organic and conventional milk when organic and conventionally farmed cows both consume a diet based on pasture?

I analysed and processed milk FA data from samples which I helped collect four times from individual cows from Farm Set 1 (n = 45 organic, n = 50 conventional) throughout the milking season, with one sample each from morning and afternoon milking collected during one day in New Zealand spring (November 2010) and one day in New Zealand autumn (March 2011). Both herds originated from a single herd which was divided in 2001 after taking into account breeding value, production value, somatic cell count, age, and parity of each individual animal in order to create two matching herds. Both herds were grazed and managed similarly on different paddocks at adjacent locations under the same management, which was representative of organic and conventional dairy herds for this geographical area in New Zealand. No supplemental feed was provided to either herd in the six weeks leading up to and on the day of sampling, with pasture growth being sufficient to feed the animals. The results of this study were published in Schwendel, B. H., P. C. H. Morel, T. J. Wester, M. H. Tavendale, C. Deadman, B. Fong, N. M. Shadbolt, A. Thatcher, and D. E. Otter. 2015. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. J. Dairy Sci. 98:1411-1425. http://dx.doi.org/10.3168/jds.2014-8322.

Bovine milk contains 3-5% lipid representing a variety of different classes (e.g., phospholipids, cholesterol, free fatty acids), with triacylglycerol most dominant (Jensen, 2002). When investigating milk FA composition in organic and conventionally produced milk, research studies commonly present FA results

obtained after hydrolysis of triacylglycerol into three FA. The FA are then derivatised to create FA esters which are volatile enough to be analysed with gas chromatography.

My first step was to determine which method to use to prepare milk samples for FA analysis. Sample preparation methods for FA analysis vary in chemicals used, however, the chemical reactions are similar. They usually involve a step to separate the fat from the milk, which is done either by extraction with a variety of solvent combinations (e.g., methanol and chloroform (Slots et al., 2009), or isopropanol and hexane (Toledo et al., 2002)) or by centrifugation. This is commonly followed by a methylation step in which the glycerol part of triglycerides is exchanged by a methyl group for each individual FA via trans-esterification, which results in FA methyl esters (FAME). Alternatively to methyl groups, various types of amide and picolinyl (3-hydroxymethyl-pyridine) (Christie, 1987) ester derivatives of FA can be used as they give distinctive mass spectra from which many functional groups, as well as double bonds, can be located. A range of different methylation reagents have been described in the literature with sodium methoxide (CH_3NaO), potassium hydroxide (KOH) in methanol, or boron trifluoride (BF_3) in methanol the most commonly used. Each approach has specific characteristics and advantages with studies published comparing different methods on diverse sample sets (Juárez et al., 2008; Böcking et al., 2010). An *in situ* method with a combined extraction and methylation step has been described by Sukhija & Appelqvist, (1988) with a variation reported by Butler et al. (2011). For analysis of FA in our milk samples, I considered three different methods: 1) *in situ* method described by Butler et al. (2011); 2) method after (Havemose et al., 2007) as described by (Slots et al., 2009); and, 3) method from Nourooz-Zadeh & Appelqvist, (1988) as described by (Toledo et al., 2002).

I considered the *in situ* method the most favourable as it is the quickest and consumes the least amount of solvent, but in practice results for FA proved to be rather variable, which was mainly due to instantaneous violent reactions of acetyl chloride (CH_3COCCl) with the water in the samples, often causing the loss of sample. The method reported by Slots et al. (2009) could not be tested because I was not able to source CH_3NaO within the timeframe considered for method development. Therefore, after adjusting to a smaller sample size, the method described by Toledo et al. (2002) was chosen to prepare to samples for FA analysis.

Without any preliminary consideration regarding the number or type of FA of interest, my aim was to separate as many FA as possible, without focussing on a specific group (e.g., branched chain FA). Due to the large range of abundance, the focus on minor FA or a certain group of FA would have required a more targeted approach, potentially including the use of multiple derivatisation reagents. The column (SGE BTX70, ID 0.25

mm, film 0.25 µm, L 60 m) selected has a stationary phase developed especially for the analysis of FAME. The separation of FAME was achieved using a maximum run time of the gas chromatography–mass spectrometer (GC-MS) of 60 min for each individual sample. A longer runtime might have enabled a better separation of certain FAME, however, it would have affected peak shape of later eluting compounds and general sample throughput time.

Somewhat surprisingly, I observed that 28 of 51 analysed FAME were affected by the farming system, even when sampling time and sampling season were taken into account. The main points of interest were the significant increase of 13 of 17 odd and branched chain FA (OBCFA), as well as increases of vaccenic acid (VA) and conjugated linoleic acid (CLA) in conventionally produced milk. These FA had previously been reported to be increased in organic milk.

This study showed that the results were not distorted by a few animals that influenced the average result for each herd. The weakness of this first study (Chapter 2), however, was that only four time-points were investigated from one farm set during one lactation period. Consequently, our second research question needed to look across different farm sets. To be able to make general conclusions regarding differences of organic and conventionally produced milk in a pasture based system, I needed to investigate whether any of the observations made in this first data set, could be replicated throughout the lactation period, and furthermore, could be observed on a separate, independent farm set.

1.2.2 Are previous findings observed throughout lactation and when another farm set is included?

To answer this question, two sets of dairy farms in New Zealand were selected where a certified organic farm was directly adjacent to one that was managed conventionally, with farms in each set operated similarly under the same management structures. We collected milk samples twice a week on farms belonging to Massey University described in the previous study as Farm Set 1. Researchers from Fonterra Research and Development Centre in Palmerston North, NZ supported us in locating Farm Set 2 near Tokoroa, NZ, with the weekly sample collection conducted by the owner. Both organic farms were managed according to Organic Foods Production Act Provisions 2014 (US Government Printing Office, 2014). Farm Set 1 was located in Palmerston North, while privately owned Farm Set 2 was approximately 320 km north near Tokoroa. In both sets, both organic and conventional herds used the all-year-round pasture grazing system commonly practiced in New Zealand. The cows in each respective herd in Farm Set 1 originated from one single herd that was split in 2001 and two

matching herds were achieved by taking into account breed value, production value, somatic cell count, age, and parity of each individual cow (Schwendel, et al., 2015). The organic and conventional herds in Farm Set 2 were originally from different farms, with both farms coming under the same management in 2007. Milk samples collected throughout the lactation period from bulk milk vats on Farm Sets 1 and 2 were analysed for milk FA using the same GC-MS method as described above to facilitate comparisons to the previous trial (Chapter 2). The results for the bulk milk samples from Farm Set 1 were in agreement with the results for the individual milk samples collected in the previous trial. Therefore, the smaller dataset of bulk milk samples proved representative of the more complex set of individual milk samples. The differences for OBCFA we observed between the organic and conventionally produced milk with Farm Set 1 were not repeated on Farm Set 2. This could have been a consequence of drenching of the organic herd with garlic cider vinegar on Farm Set 1. However, we did observe the same increase in VA and CLA in conventionally produced milk on Farm Set 2 that we saw on Farm Set 1. Knowledge of individual farms and farm sets lead us hypothesise that differences in nitrogen fertilizer application between organic and conventional farms was the most probable reason VA and CLA were elevated in conventionally produced milk.

The rather philosophical question of whether one can claim that one milk variety differs from another when the difference lies in small changes in concentrations of minor FA, lead to my next research question.

1.2.3 Do other milk compounds vary between organic and conventionally produced milk?

Working with the same milk samples taken from cows on pasture based systems in Farm Sets 1 and 2 described above, I investigated a further three milk compound groups: free oligosaccharides (OS), milk protein, and milk fat volatiles (Chapter 3). As described above, use of these samples allowed me to either minimise or accounted for the influence of a large number of farming variables, including breed, climate, and pasture composition, which have been shown to influence milk composition. Results of this study have been published as Schwendel, B. H., Wester, T. J., Morel, P. C. H., Fong, B., Tavendale, M. H., Deadman, C., Shadbolt, N. M., & Otter, D. E. (2017). Pasture feeding conventional cows removes differences between organic and conventionally produced milk. Food Chemistry, 229, 805-813.

I focussed on milk free-OS because they represent a growing research area, especially in regard to their importance in infant nutrition and gut health. As this is an emerging research area, especially in regard to bovine milk which contains much lower concentrations of OS compared to human milk, no standard analytical method

had been established, when conducting my research. Also, relative abundance of minor OS in bovine milk appears to be highly variable. Unavailability and prohibitively high cost of OS standards further complicated this research area when conducting this study. To date there are two approaches to analyse free OS. The first is based on identification of compounds by accurate mass, using a MS with a high mass resolution as detector, while the second uses either high-performance anion exchange chromatography-pulsed amperometric detection (HPAE-PAD) or a diode-array detector. The first approach enables identification and structural analysis of OS, however, matrix effects may affect quantification and, therefore, need to be considered (Lee et al., 2015). The second approach enables quantification of OS with high-resolution separation and high detection limits (Lee et al., 2015), however, it requires use of standards for identification. I used the first approach as the second approach was not available as I did not have access to the required instrumentation or standards.

Previous studies using MS to investigate milk OS applied various sample preparation methods, I investigated two of these approaches to use for my bovine milk samples. The first method uses sodium borohydride ($Na_2B_4O_7$) to reduce OS to an alditol form (Tao et al., 2009) to enhance signal intensity. I found that the standard deviation in control samples using the alditol method was larger than I could accept and, consequently, used a different method despite it having reduced sensitivity for minor OS. In the second method, defatted and deproteinised milk was filtered through a 10 kDa molecular weight cut-off filter, with the OS and other low-molecular weight compounds appearing in the filtrate (Liu et al., 2014). OS were analysed using high performance liquid chromatography (HPLC) with a MS as detector. Because reference standards were not available and the current HPLC-MS methods were not robust enough, absolute quantification of OS were not possible. Compounds were tentatively identified based on their accurate mass and presented as peak areas with relative abundance. Eleven chromatographic features of the correct calculated m/z values observed in all milk samples and were putatively assigned as the corresponding bovine milk OS. All OS were affected by sampling date, while four OS concentrations were increased in organic milk (independent of the farm set.

Bovine milk contains approximately 3.5% protein, with four caseins (αS1-CN, αS2-CN, β-CN, and κ-CN) and two whey proteins (α-LA and β-LG) representing approximately 90% of the bovine protein fraction (Walstra, 1999). Variations in the major milk proteins is predominantly caused by underlying genetic polymorphism (Heck et al., 2009), with stage of lactation and parity (Poulsen et al., 2016) as further influence factors. Only a few studies have compared protein composition in organic and conventionally produced milk, probably because it is recognised that milk protein is less susceptible to changes in diet. However, this lack of

data, the uniqueness of our study, and the desire to present a complete picture of the differences between organic and conventional milk produced in a pasture based system, lead me to investigate protein composition of the two milk varieties obtained from Farm Sets 1 and 2. For this study, I prepared, and analysed the samples according to the method of Day et al. (2015) using HPLC and UV/Vis detector. I processed the data by quantifying individual milk proteins using peak area ratios of identified peak areas in milk samples compared to external milk protein standards. I observed differences in the total casein percentage between the two farm sets, which are presumably related to the differences in clover content in pastures from Farm Set 1 and 2. Overall, no system effect for any major protein could be observed between organic and conventionally produced milk, with effects of breed and changes in lactation period investigated.

Additionally, I investigated milk fat volatiles predominately to see whether it was possible to detect volatile secondary plant metabolites or traces of the garlic drench in the milk samples. Both secondary plant metabolites (Collomb et al., 2008) and garlic have been shown to affect rumen microbiota, which in turn can influence FA originating from the rumen into milk. Either of these may have caused the differences in OBCFA composition observed in organic and conventionally produced milk from Farm Set 1. I analysed milk fat volatiles using a GC-MS method after simultaneous distillation-extraction. This method was based on one used previously to analyse aromatic heterocyclic compounds (e.g., skatole and indole), but which I modified to identify specific compounds using MS in selected ion mode for compounds expected in milk fat, and in SCAN mode which captures all ions generated. There is no single method routinely used for volatile metabolite analysis and the methodology reported by other studies varies widely. I did not have access to the different extraction technologies (e.g., dynamic headspace solid phase micro extraction) and consequently was not able to compare our results with those derived using other methods. Each methodology has its own characteristics and should be able to detect differences in volatile composition if they exist. One disadvantage of our methodology was that I was not able to identify compounds commonly associated with secondary plant metabolites. Overall, I did not observe any trends for any of the detected compounds, with neither system nor sampling date affecting milk fat volatile composition.

After considering individual milk compounds analysed with the methods described above, I decided to use a non-targeted metabolomic methodology to investigate a variety of milk metabolites. These could provide potential bio-markers from a variety of biological pathways, which may enable the authentication of organic and conventionally produced milk from pasture based systems.

1.2.4 Are there differences in milk metabolite composition using a non-targeted analytical method?

The first method of choice for analysis of the metabolite composition from organic and conventionally produced milk was the use of a high resolution MS. This methodology enables the separation of potentially thousands of mass features (putative metabolites), with the ability to chromatographically separate the same sample using different stationary phases, run solvents, and electrical charge stages. However, the bottleneck of MS metabolomics analysis does not lie in the preparation and analysis of the samples, but in the data processing. To be able to process the vast number of features (not all of them representing actual compounds), while also accounting for shifts in retention time, loss of sensitivity, and run and batch order effects, one needs a robust set of data processing tools, which were not available to me.

Consequently, I chose an alternative method using Nuclear Magnetic Resonance (NMR) spectroscopy metabolomics analysis, which allows identification and quantification of a multitude of compounds per sample. The advantage of NMR methodology is its standardised methodology, and the possibility to analyse a large number of samples without loss of signal, and no run order or batch effects (Chapter 4). NMR samples were prepared by myself with the help of a technician at the Institute of Fundamental Sciences at Massey University, put on the instrument by the technical director responsible for NMR instrumentation, with the resulting data processed by me. The NMR analysis is less practical, however, for complex samples such as milk. Lactose is dominating the spectra and only allows for the identification of smaller compounds outside its chemical shift. I was able to quantify 33 compound, none of which showed any system effect across both farm sets.

1.3 CONCLUSION

The results of the present thesis indicated that in pasture-based farming systems where many of the factors known to influence milk composition (e.g., breed) have been controlled, there is very little or no difference in chemical composition between milk produced organically and that produced conventionally.

REFERENCES

Böcking, C., W. A. Nockher, M. Schreiner, H. Renz, and P. I. Pfefferle. 2010. Development and validation of a combined method for the biomonitoring of omega-3/-6 fatty acids and conjugated linoleic acids

in different matrices from human and nutritional sources. Clinical Chemistry and Laboratory Medicine 48:1757-1763. http://dx.doi.org/10.1515/cclm.2010.350.

Butler, G., S. Stergiadis, C. Seal, M. Eyre, and C. Leifert. 2011. Fat composition of organic and conventional retail milk in northeast England. J. Dairy Sci. 94:24-36. http://dx.doi.org/10.3168/jds.2010-3331.

Christie, W.W., Brechany, E.Y. & Holman, R.T. (1987) Mass spectra of the picolinyl esters of isomeric mono- and dienoic fatty acids. Lipids 22(4), 224-228.

Collomb, M., W. Bisig, U. Buetikofer, R. Sieber, M. Bregy, and L. Etter. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. Int. Dairy J. 18:976-982.

Day, L., R. P. W. Williams, D. Otter, and M. A. Augustin. 2015. Casein polymorphism heterogeneity influences casein micelle size in milk of individual cows. J. Dairy Sci. 98:3633-3644. http://dx.doi.org/10.3168/jds.2014-9285.

Givens, D. I., and J. A. Lovegrove. 2016. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: A systematic literature review and meta- and redundancy analyses. Br. J. Nutr. 116:1-2. http://dx.doi.org/10.1017/s0007114516001604.

Hasimu, H., S. Marchesini, and M. Canavari. 2017. A concept mapping study on organic food consumers in Shanghai, China. Appetite 108:191-202. http://dx.doi.org/10.1016/j.appet.2016.09.019.

Havemose, M. S., P. Justesen, W. L. P. Bredie, and J. H. Nielsen. 2007. Measurement of volatile oxidation products from milk using solvent-assisted flavour evaporation and solid phase microextraction. Int. Dairy J. 17:746-752. http://dx.doi.org/10.1016/j.idairyj.2006.09.008.

Heck, J. M. L., H. J. F. van valenberg, J. Dijkstra, and A. C. M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. J. Dairy Sci. 92:4745-4755. http://dx.doi.org/10.3168/jds.2009-2146.

Huber, M., E. Rembiałkowska, D. Średnicka, S. Bügel, and L. P. L. Van De Vijver. 2011. Organic food and impact on human health: Assessing the status quo and prospects of research. NJAS Wageningen J. Life Sci. 58:103-109. http://dx.doi.org/10.1016/j.njas.2011.01.004.

Janssen, M., and U. Hamm. 2012. Product labelling in the market for organic food: Consumer preferences and willingness-to-pay for different organic certification logos. Food Qual. Preference 25:9-22. http://dx.doi.org/10.1016/j.foodqual.2011.12.004.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Jensen, M. M., H. Jørgensen, and C. Lauridsen. 2013. Comparison between conventional and organic agriculture in terms of nutritional quality of food - A critical review. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 8. http://dx.doi.org/10.1079/pavsnnr20138045.

Lee, H., V. L. D. MeloSilva, Y. Liu, and D. Barile. 2015. Short communication: Quantification of carbohydrates in whey permeate products using high-performance anion-exchange chromatography with pulsed amperometric detection. J. Dairy Sci. 98:7644-7649. http://dx.doi.org/10.3168/jds.2015-9882.

Liu, Z., C. A. Kanter, K. D. Messer, and H. M. Kaiser. 2013. Identifying significant characteristics of organic milk consumers: A CART analysis of an artefactual field experiment. Appl. Econ. 45:3110-3121.

Liu, Z., P. Moate, B. Cocks, and S. Rochfort. 2014. Simple liquid chromatography-mass spectrometry method for quantification of major free oligosaccharides in bovine milk. Journal of Agricultural and Food Chemistry 62:11568-11574. http://dx.doi.org/10.1021/jf5037849.

Molkentin, J. 2009. Authentication of organic milk using delta¹³C and the alpha-linolenic acid content of milk fat. Journal of Agricultural and Food Chemistry 57:785-790.

Nourooz-Zadeh, J. & Appelqvist, L.Å. (1988) Cholesterol oxides in swedish foods and food ingredients: Butter and cheese. Journal of the American Oil Chemists' Society 65(10), 1635-1641.

http://dx.doi.org/10.1017/s0007114516000349.

Poulsen, N. A., H. B. Jensen, and L. B. Larsen. 2016. Factors influencing degree of glycosylation and phosphorylation of caseins in individual cow milk samples. J. Dairy Sci. http://dx.doi.org/10.3168/jds.2015-10226.

Rembiałkowska, E., R. Kazimierczak, D. Średnicka, K. Bieńko, and M. Bielska. 2008. Different aspects of organic and conventional food consumers' lifestyle. New Med. 12:16-19.

Rödiger, M., S. Plaßmann, and U. Hamm. 2016. Organic consumers' price knowledge, willingness-to-pay and purchase decision. Br. Food J. 118:2732-2743. http://dx.doi.org/10.1108/bfj-04-2016-0164.

Schleenbecker, R., and U. Hamm. 2013. Consumers' perception of organic product characteristics. A review. Appetite 71:420-429. http://dx.doi.org/10.1016/j.appet.2013.08.020.

Sirieix, L., P. R. Kledal, and T. Sulitang. 2011. Organic food consumers' trade-offs between local or imported, conventional or organic products: A qualitative study in Shanghai. Int. J. Consum. Stud. 35:670-678. http://dx.doi.org/10.1111/j.1470-6431.2010.00960.x.

Slots, T., G. Butler, C. Leifert, T. Kristensen, L. H. Skibsted, and J. H. Nielsen. 2009. Potentials to differentiate milk composition by different feeding strategies. J. Dairy Sci. 92:2057-2066. http://dx.doi.org/10.3168/jds.2008-1392.

Srednicka-Tober, D., M. Barański, C. J. Seal, R. Sanderson, C. Benbrook, H. Steinshamn, J. Gromadzka-Ostrowska, E. Rembiałkowska, K. Skwarło-Sońta, M. Eyre, G. Cozzi, M. K. Larsen, T. Jordon, U. Niggli, T. Sakowski, P. C. Calder, G. C. Burdge, S. Sotiraki, A. Stefanakis, S. Stergiadis, H. Yolcu, E. Chatzidimitriou, G. Butler, G. Stewart, and C. Leifert. 2016. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: A systematic literature review and meta- and redundancy analyses. Br. J. Nutr. 115:1043-1060.

Sukhija, P.S. & Palmquist, D.L. (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry 36(6), 1202-1206.

Tao, N., E. J. DePeters, J. B. German, R. Grimm, and C. B. Lebrilla. 2009. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. J. Dairy Sci. 92:2991-3001. http://dx.doi.org/10.3168/ jds.2008-1642.

Toledo, P., A. Andrén, and L. Björck. 2002. Composition of raw milk from sustainable production systems. Int. Dairy J. 12:75-80. http://dx.doi.org/10.1016/s0958-6946(01)00148-0.

Walstra, P. 1999. Casein sub-micelles: Do they exist? Int. Dairy J. 9:189-192. http://dx.doi.org/10.1016/s0958-6946(99)00059-x.

Zagata, L. 2012. Consumers' beliefs and behavioural intentions towards organic food. Evidence from the Czech Republic. Appetite 59:81-89. http://dx.doi.org/10.1016/j.appet.2012.03.023.

Chapter 2

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Review: Organic and conventionally produced milk — An evaluation of factors influencing milk composition

Summary

Consumer perception of organic cow's milk is associated with the assumption that organic milk differs from conventionally produced milk. Controlled studies investigating the differences between organic and conventional produced milk have so far fallen short of a conclusion as to whether or not these exist. Factors influencing milk composition have been studied individually, while interactions between multiple factors have been largely ignored. The effect of the farming practices rather than the farming system (organic vs conventional) determines milk composition, and current regulations for organic milk production do not allow for a distinct separation from conventionally produced milk.

ABSTRACT

Consumer perception of organic cow's milk is associated with the assumption that organic milk differs from conventionally produced milk. The value associated with this difference, justifies the premium retail price. It includes the perception that organic dairy farming is kinder on the environment, animals and people; that organic milk products are produced without the use of antibiotics, added hormones, synthetic chemicals and genetic modification and may have potential benefits for human health. Controlled studies investigating the differences between organic and conventionally produced milk have so far fallen short of a conclusion as to whether or not these exist. Reasons for this are many fold, caused principally by the complexity of the research problem. A main complication is that farming practices and their impacts differ depending on country, region, year and season between and within organic and conventional systems. Factors influencing milk composition (e.g., diet, breed, and stage of lactation) have been studied individually, while interactions between multiple

factors have been largely ignored. Studies fail to consider that factors other than the farming system (organic versus conventional) could have caused or contributed to, the reported differences in milk composition make it impossible to determine whether there is a system related difference between organic and conventional milk, or not. Milk fatty acid composition has been a central research area when comparing organic and conventional milk. This can be explained by the fast response of the fatty acid profile to changes in the diet. Consequently, the effect of the farming practices (high input versus low input) rather than the farming system (organic versus conventional) determines milk fatty acid profile, and rather similar results are seen between low input (LI) organic and LI conventional milk. This confounds our ability to develop an analytical method to distinguish organic from conventionally produced milk and provide product verification. Lack of research on interactions between several influential factors and differences in trial complexity and consistency between studies (e.g., sampling period, sample size, reporting of experimental conditions) complicate data interpretation and prevent us from making unequivocal conclusions. The first part of this review provides a detailed summary of individual factors known to influence milk composition. The second part presents an overview of studies which have compared organic and conventionally produced milk and discusses their findings within the framework of the various factors presented in part one.

2.1 INTRODUCTION

Composition of bovine milk is influenced by a multitude of factors which are either related to the individual animal or to the environment the animal is in. Elements such as diet (Ferlay et al., 2008, Larsen et al., 2010), breed (Soyeurt et al., 2006, Palladino et al., 2010), individual animal genetics (Soyeurt et al., 2008), stage of lactation (Craninx et al., 2008, Stoop et al., 2009), management (Coppa et al., 2013) and season (Heck et al., 2009), as well as the interactions between them (Macdonald et al., 2008, Piccand et al., 2013, Stergiadis et al., 2013), affect milk composition with many mechanisms behind these effects not fully understood. Therefore, when attempting to study the effect of one specific factor (e.g., diet) on cow's milk composition, it is necessary to eliminate other influences. Those factors that cannot be eliminated must be accounted for and their effects considered and minimized.

Currently, there is no evidence that the consumption of organic food leads to meaningful nutritional benefits for human health (Forman et al., 2012, Załecka et al., 2014). Studies purportedly comparing organic and conventionally produced milk are rife with complications. To be able to determine whether organic milk differs from conventional produced milk, all factors which influence milk composition must be identical, except the for

the factors which specifically define the farming system (organic or conventional). If more than the system factor varies between compared milk samples, it is difficult to determine whether results derive from the differences between the farming systems, or are the consequence of other elements. Recent reviews (Magkos et al., 2003, Dangour et al., 2010, Guéguen & Pascal, 2010, Smith-Spangler et al. 2012) remarked on the lack of 'true' comparison in studies evaluating organic and conventionally produced foods (including milk and dairy products). Many studies comparing organic and conventionally produced milk are inadequate in their discussion of what has actually been causing the results they present. Commonly, factors which could have contributed to the reported differences (between organic and conventional milk), have not been considered (e.g., differences in diet, breed, and animal health). Most studies proclaiming the comparison of organic and conventional milk used diets which varied in their amount of fresh forage and concentrate for organic and conventional cows, respectively. Consequently, presented results are most likely related to the effect of the differences in diet, rather than to the fact that cows consumed organic or conventionally produced feed. On the contrary, studies which identify specific production differences for organic and conventional milk (e.g., higher amount of pasture in the diet of organic cows), fail to consider which influence the farming system (organic or conventional) had on their results (Palupi et al., 2012). Additionally, comparisons among studies are problematic as it is difficult to account for any number of variables, including sampling conditions (e.g., frequency of sampling, time of sampling, samples taken from individual cows vs. bulk milk vs. multiple farms), inherent differences in farming systems between regions, levels of input, and even regulatory differences in conventional and organic production between nations.

Regulations regarding organic dairy farming, although similar in principle, vary in detail (Table 2.1) between countries (e.g., pasture access and use of antibiotics). Therefore, heterogeneity of organic regulations may contribute to the variation in organic milk composition between countries.

The problems outlined above account for the inability of previous studies to reach a consensus on whether there are compositional differences between organic and conventionally produced dairy foods. Consequently, comparison of research studies should be undertaken with the awareness that study-specific factors can have a significant impact on animal production and milk composition, and might have contributed to reported differences.

This review focusses on the chemical composition of bovine milk and summarizes the variety of different milk components that have been analysed in regard to their quantitative and qualitative presence in organic and conventionally produced milk. It also aims to show how different milk components are influenced

by a variety of individual factors and their interactions, and how the resulting variations can be perceived as differences between organic vs. conventional milk. It reinforces that these factors need to be considered when evaluating existing studies or designing comparative experiments. Variations within organic and conventional production methods have also created differences that have so far prevented development of a method to test authenticity of organic milk products. A brief discussion of proposed tests to identify organically produced products is also included.

Table 0.1 Country specific regulations for organic dairy farming in regard to pasture access, forage feeding and use of antibiotics

Country	Pasture access	Forage feed	Antibiotics use	Regulation
USA	Grazed for 120 days per year	During grazing season 30% of total forage intake must come from pasture	Producer must not: Sell, label, or represent as organic any edible product derived from any animal treated with antibiotics	Organic foods production act provisions 2014[C] (US Government Printing Office, 2014)
Canada	Pasture access during grazing season	During grazing season 30% of total forage intake must come from pasture 60% of dry matter in daily rations consists of hay, fresh/dried fodder or silage	Milk withdrawal time[A] Animals that require more than two treatments[B] shall undergo a 12-month transition period.	Organic Production Systems General Principles and Management Standards 2011[C] (Canadian General Standards Board, 2011)
European Union	Pasture access for grazing whenever conditions allow	60% of dry matter in daily rations consists of hay, fresh/dried fodder or silage A reduction to 50% for a maximum period of three months in early lactation is allowed	Milk withdrawal time[A] When animals that require more than three treatments[B], or more than one course of treatment if productive lifecycle is < 1 year, the or produce derived from the animal, may not be sold as organic products	Guidance document on European union organic Standards 2010 [C] (Department for Environment Food and Rural Affairs, 2010)
Japan	Pasture access, no less than twice a week	Feeds other than fresh or dried fodder or silage are less than 50% of the average feed intake, in dry weight.	Prescribed drugs or antibiotics are used only when therapy with veterinary drugs other than these is not effective.	Japanese Agricultural Standard for Organic Livestock Products, 2005[D] (Ministry of Agriculture Forestry and Fisheries)

New Zealand	Ruminants must be grazed for throughout the grazing season 150 days	For herbivores a minimum of 50% of feed must come from pasture	Use of synthetic allopathic veterinary drugs or antibiotics will cause the animal to lose its organic status	AsureQuality Organic Standard For Primary Producers, 2013 [E]
Australia	Grazing of animals in natural/rangeland areas is considered part of an organic production system		After treatment with allopathic veterinary drugs or antibiotics, the products can be marketed as organic or bio-dynamic after a minimum management period of 180 days	National standard For Organic and bio-dynamic produce, 2013 [C] (Organic Industry Standards and Certification Committee, 2013)1

[A] Milk withdrawal time = at least 30 days or two times the specific medication's withdrawal period, whichever is longer

[B] Treatments = combined parasiticides and antibiotics per year

[C] Organic livestock standards for producers are compulsory

[D] Organic livestock standards for producers are voluntary

[E] Several organic livestock standards, which are voluntarily and chosen by farmer according to their organic production style

2.2 FACTORS THAT INFLUENCE MILK COMPOSITION

There are numerous and varied factors that influence milk yield and composition which, ideally, should be controlled when conducting a trial examining factors that may change milk composition. These factors can seem relatively minor, but they could account for a significant amount of variation. A study conducted by Roche et al. (2009) between 1995 and 2001 showed that the combined influence of weather, herbage quality, and herbage mineral concentration explained up to 22% of the variation in dairy cattle production. In a different trial, Roesch et al. (2005) compared cow performance from organic and integrated farming systems and found that milk yield positively correlated with breed (especially Holstein), concentrate feeding, routine teat dipping, and greater outdoor access during winter independent of the system. They concluded that lower milk yields (in organic and integrated cows) are a result of the individual animal and on-farm level factors such as breed, nutrition, management, and udder health. A study by Waiblinger et al. (2002) investigating 30 small, family-run dairy farms suggested that milk production was lower on farms where management had negative attitudes towards interacting with cows during milking. Various factors that influence milk yield, as well as fat, protein and lactose concentrations on farm and individual animal levels have been compiled in Table 2.2.

The factors considered most influential, however, vary depending on study conditions and aims. Stage of lactation, for example, can be neglected when bulk milk samples are collected from a farm with an all-year-round calving system, but it becomes significant when milk samples of individual animals are taken, or when block calving is practiced (Nantapo et al., 2013). As major influences are accounted for and controlled (e.g., cows in one trial are all of one breed, with similar genetics, at the same stage of lactation, fed similar diets, etc.), previously minor factors (e.g., pasture composition) become more important.

Analysis and (potential) alteration of milk fatty acid (FA) composition are key areas of dairy research. This can be explained by the fast response of the FA profile to changes in the diet. Other factors influential for milk FA composition are breed, energy status, stage of lactation, udder health, and season. The latter predominantly reflects alterations in diet, especially when these are rich in forage. Chemical and botanical composition of fresh forages varies throughout the seasons, and conservation for hay or silage affects the nutritional value of forages. Seasonal transition of dairy cows from outdoor grazing to indoor housing and the accompanying change in diet can be observed in milk composition (Larsen et al., 2010, Kuczyńska et al., 2012). The effects of breed and season on milk fat composition are summarized in Table 2.3, while the effects of different forages on milk FA are listed in Table 2.4.

Table 0.2 Summary of Factors influencing milk yield, fat, protein, and lactose concentration

Factor	Milk Yield	Reference	Fat %	Reference	Protein %	Reference	Lactose %	Reference
Altitude			Higher in Highland vs. Lowland	(Bartl et al., 2008)				
Breed	Higher in Holstein vs. Simmental	(Roesch et al., 2005)	Higher in Jersey vs. DF, MRY and GWH	(Maurice-Van Eijndhoven et al., 2011)	Highest in Jersey, lowest in DF	(Maurice-Van Eijndhoven et al., 2011)	Higher in Brown Swiss vs. Jersey	(Carroll et al., 2006)
	Higher in HF vs. Jersey	(Palladino et al., 2010)	Higher in Minhota vs. HF	(Ramalho et al., 2012)	Higher in Jersey vs. HF	(Palladino et al., 2010)		
	Higher in HF vs. Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Jersey vs. HF	(Palladino et al., 2010)	Higher in Jersey vs. Holstein	(Croissant et al., 2007)		
			Higher in Jersey vs. Holstein	(Croissant et al., 2007)	Higher in Brown Swiss vs Holstein	(Carroll et al., 2006)		
Fertilizer					Lower with higher nitrogen application	(Hermansen et al., 1994) (Mackle et al., 1996)		
Grazing allocation (frequency)	Higher if allocation every day vs. every fourth day	(Abrahamse et al., 2008)	Higher for allocation every fourth day vs. every day	(Abrahamse et al., 2008)	Higher if allocation every fourth day vs. every day	(Abrahamse et al., 2008)	NS[C]	(Abrahamse et al., 2008)

Grazing high sugar grasses	Positively correlated	(Miller et al., 2001)			Positively correlated	(Roche et al., 2009)		
Grazing pasture	Lower vs. Concentrate	(Coleman et al., 2010)	NS	(Coleman et al., 2010)	NS	(Coleman et al., 2010), (Croissant et al., 2007)	unknown	(Coleman et al., 2010)
			Lower vs. TMR	(Croissant et al., 2007)				
Genotype	[A] Higher in HighNA vs. HighNZ and LowNA	(Coleman et al., 2010)	[A] Higher in HighNZ vs. HighNA and LowNA	(Coleman et al., 2010)	[A] Higher in HighNZ vs. HighNA and LowNA	(Coleman et al., 2010)	[A] Higher in HighNZ vs. HighNA and LowNA	(Coleman et al., 2010)
	[B] Higher in NA90 than NZ90	(Macdonald et al., 2008)	[B] Higher in NZ90 than NA90	(Macdonald et al., 2008)	[B] Higher in NZ90 than NA90	(Macdonald et al., 2008)	[B] Higher in NZ90 than NA90	(Macdonald et al., 2008)
Heritability	Correlated	(Soyeurt et al., 2007)	Correlated	(Soyeurt et al., 2007)	Correlated	(Soyeurt et al., 2007)		
Management attitude	Positively correlated	Waiblinger et al. (2002)						
Parity	Higher	(Roesch et al., 2005, Craninx et al., 2008)	Higher	(Craninx et al., 2008)				
Season			Minimum in Summer	(Heck et al., 2009) Larsen et al., 2010) (Stergiadis et al., 2013)	Minimum in Summer	(Heck et al., 2009)	Minimum in Autumn	(Heck et al., 2009)

					NS	(Larsen et al., 2010) (Stergiadis et al., 2013)		
Somatic cell count	Negatively correlated	(Maréchal et al., 2011)			Negatively correlated	(Ballou et al., 1995)	Negatively correlated	(Auldist et al., 1998)
Stage of lactation	Correlated	(Craninx et al., 2008) (Palladino et al., 2010)	Correlated	(Craninx et al., 2008) (Palladino et al., 2010)	Correlated	(Palladino et al., 2010)		
Sunlight hours	Positively correlated	(Roche et al., 2009)						
Teat dipping	Positively correlated	(Roesch et al., 2005)						

DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; GWH = Groningen White Headed HF = Holstein-Friesian

[A] Low NA = national average-genetic-merit North American Holstein-Friesian, High NA = high-genetic-merit North American Holstein-Friesian, High NZ = high-genetic merit New Zealand Holstein-Friesian

[B] NZ90 = a 1990s high Breeding worth Holstein-Friesian of New Zealand origin; NA90 = a 1990s high Breeding worth Holstein-Friesian of North American origin.

[C] NS = not significant

Table 0.3 Effect of breed and season on individual milk fatty acids.

Fatty acid	Breed Effect	Reference	Seasonal Effect	Reference
Even-chain saturated FA				
C4:0 Butyric acid	Higher for DF than MRY, GWH, and Jersey	(Maurice-Van Eijndhoven et al., 2011)	NS	(Palladino et al., 2010)
	Higher for Brown Swiss than Jersey	(Carroll et al., 2006)	NS, for Herbage	(Larsen et al., 2010)
			Higher in Winter	(Revello Chion et al., 2010)
			NS, Spring or Winter	(Rego et al., 2008)
			Lower in Winter with Maize silage and by-products, NS with pasture	(Larsen et al., 2010)
C6:0 Caproic acid	Higher for DF and MRY than GWH and Jersey	(Maurice-Van Eijndhoven et al., 2011)	NS	(Palladino et al., 2010)
	Higher for Jersey than Brown Swiss	(Carroll et al., 2006)	NS, Spring or Winter	(Rego et al., 2008)
			Higher in Winter	(Revello Chion et al., 2010)
C8:0 Caprylic acid	Holstein lower than Jersey	(Croissant et al., 2007)	Lower in Summer	(Palladino et al., 2010)

			NS, for Herbage	(Larsen et al., 2010)
	NS between Minhota and HF	(Ramalho et al., 2012)	NS, Spring or Winter	(Rego et al., 2008)
	Higher for DF and MRY than GWH and Jersey	(Maurice-Van Eijndhoven et al., 2011)	Higher in Winter	(Revello Chion et al., 2010)
C10:0 Capric acid	Holstein lower than Jersey	(Croissant et al., 2007)	Lower in Summer	(Palladino et al., 2010)
	Minhota lower than HF	(Ramalho et al., 2012)	Higher in Spring than Winter	(Rego et al., 2008)
	Lowest for GWH; highest for DF	(Maurice-Van Eijndhoven et al., 2011)	Higher in Winter	(Revello Chion et al., 2010)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)		
C12:0 Lauric acid	Holstein lower than Jersey	(Croissant et al., 2007)	Lower in Summer	(Palladino et al., 2010)
	Minhota lower than HF	(Ramalho et al., 2012)	Higher in Spring than Winter	(Rego et al., 2008)
	Higher for DF and MRY than GWH and Jersey	(Maurice-Van Eijndhoven et al., 2011)	Higher in Winter	(Revello Chion et al., 2010)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)		

C14:0 Myristic acid	Higher for DF and MRY than GWH and Jersey	(Maurice-Van Eijndhoven et al., 2011)	Lower in Summer	(Palladino et al., 2010)
	Minhota lower than HF	(Ramalho et al., 2012)	Higher in Winter	(Revello Chion et al., 2010)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Highest in Winter, lowest May till July	(Kliem et al., 2013)
C16:0 Palmitic acid	HF lower than Jersey	(Palladino et al., 2010)	NS	(Palladino et al., 2010)
	NS between Minhota and HF	(Ramalho et al., 2012)	NS	(Revello Chion et al., 2010)
	Lowest for GWH highest for Jersey	(Maurice-Van Eijndhoven et al., 2011)	May higher than August when lower content of lucerne	(Larsen et al., 2010)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Winter than Spring	(Rego et al., 2008)
			Highest in Winter, lowest May till July	(Kliem et al., 2013)
C18:0 Stearic acid	NS between Minhota and HF	(Ramalho et al., 2012)	Higher in Summer	(Palladino et al., 2010, Revello Chion et al., 2010)

	No difference for DF, MRY, GWH and Jersey	(Maurice-Van Eijndhoven et al., 2011)	May and August lower than June when lower content of chicory and lucerne	(Larsen et al., 2012)
	NS between HF and Jersey	(Palladino et al., 2010)	NS, spring or winter	(Rego et al., 2008)
			Highest in June lowest in October	(Kliem et al., 2013)
Odd-chain saturated FA				
C13:0	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Lower in Summer	(Palladino et al., 2010)
C15:0	Minhota higher than HF	(Ramalho et al., 2012)	Lower in Summer	(Palladino et al., 2010)
	HF higher than Jersey	(Palladino et al., 2010)	Higher in Spring than winter	(Rego et al., 2008)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Summer	(Revello Chion et al., 2010)
C17:0	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	NS	(Palladino et al., 2010)
			Higher in Spring than Winter	(Rego et al., 2008)
Branched-chain FA				
C13:0 iso			Lower in Summer	(Palladino et al., 2010)

C14:0 iso			Higher in Spring than Winter	(Rego et al., 2008)
C15:0 iso	Minhota higher than HF	(Ramalho et al., 2012)	Higher in Spring than Winter	(Rego et al., 2008)
C15:0 anteiso	NS between Minhota and HF	(Ramalho et al., 2012)	NS, Spring or Winter	(Rego et al., 2008)
C16:0 iso	Minhota higher than HF	(Ramalho et al., 2012)		
C17:0 iso			NS, Spring or Winter	(Rego et al., 2008)
C17:0 anteiso			Higher in Spring than Winter	(Rego et al., 2008)
Unsaturated FA				
C14:1 c9 Myristoleic acid	NS between Holstein and Jersey	(Croissant et al., 2007)	Higher in Winter	(Revello Chion et al., 2010)
	NS between Minhota and HF	(Ramalho et al., 2012)		
	HF higher than Jersey	(Palladino et al., 2010)		
C16:1 c9 Palmitoleic acid	Holstein higher than Jersey	(Croissant et al., 2007)	Higher in Winter than Spring	(Rego et al., 2008)
	Minhota higher than HF	(Ramalho et al., 2012)	Higher in Winter	(Revello Chion et al., 2010)
C18:1 t9 Elaidic acid	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Winter than Spring	(Rego et al., 2008)

C18:1 t10			NS Spring or Winter	(Rego et al., 2008)
C18:1 t11 Vaccenic acid	NS between Holstein and Jersey	(Palladino et al., 2010)	NS	(Palladino et al., 2010)
	Higher in Holstein than Brown Swiss	(Carroll et al., 2006)	Total trans 18:1 highest Aug/Sep/Oct	(Dunshea et al., 2008)
			Higher in Spring than Winter	(Rego et al., 2008)
			Higher in Summer	(Revello Chion et al., 2010)
C18:1 c9 Oleic acid	Holstein higher than Jersey	(Croissant et al., 2007)	NS	(Palladino et al., 2010)
	HF higher than Jersey	(Palladino et al., 2010)	Higher in Winter than Spring	(Rego et al., 2008)
	Higher in Brown Swiss than Jersey and Holstein	(Carroll et al., 2006)	Lowest in May/ lowest content of chicory and lucerne	(Larsen et al., 2012)
			Higher in Summer	(Revello Chion et al., 2010)
C18:2 c9,12 Linoleic acid LA	Holstein higher than Jersey	(Croissant et al., 2007)	Higher in Summer	(Palladino et al., 2010)
	NS between Holstein and Jersey	(Palladino et al., 2010)	Highest in May/ highest concentration of red clover	(Larsen et al., 2012)

	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Summer	(Revello Chion et al., 2010)
C18:2 c9t11	HF higher than Jersey	(Palladino et al., 2010)	NS	(Palladino et al., 2010)
Conjugated linoleic acid CLA	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Total cis/trans 18:2 highest Aug/Sep/Oct	(Dunshea et al., 2008)
			Higher in Spring than Winter	(Rego et al., 2008)
			Higher in Summer	(Revello Chion et al., 2010)
C18:3 c9,12,15 α-Linolenic acid ALA	NS between Holstein and Jersey	(Palladino et al., 2010)	NS	(Palladino et al., 2010)
	NS between Holstein, Jersey and Brown Swiss	(Carroll et al., 2006)	Higher in Spring than Winter	(Rego et al., 2008)
			Highest in May/ highest concentration of red clover, lowest in white clover	(Larsen et al., 2012)

DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; GWH = Groningen White Headed HF = Holstein-Friesian

Table 0.4 Effect of different forages on individual milk fatty acids.

Fatty acid	Increased in	R
Even-chain saturated FA		
C4:0 Butyric acid	Alpine pasture vs. Pasture	(Collomb et al., 1999)
	Pasture Hay vs. Grass silage	(Baars et al., 2011)
C6:0 Caproic acid	Pasture Hay vs. Grass silage	(Baars et al., 2011)
C16:0 Palmitic acid	NS, WC or RC silage	(Steinshamn and Thuen, 2008)
	Hay or Grass silage vs. Pasture	(Villeneuve et al., 2013)
	Maize silage vs. Pasture	(Croissant et al., 2007)
C18:0 Stearic acid	Pasture vs. Grass silage	(Elgersma et al., 2004)
	NS, WC silage or RC silage	(Steinshamn and Thuen, 2008)
	RC silage vs.WC silage	(Wiking et al., 2010)
Odd-chain saturated FA		
C13:0	Pasture Hay vs. Grass silage	(Baars et al., 2011,Villeneuve et al., 2013)
C17:0	Maize silage vs. Grass silage	(Vlaeminck et al., 2006)
Branched-chain FA		
C14:0 iso	Pasture or Hay vs. Grass silage	(Villeneuve et al., 2013)
C15:0 iso	Grass silage vs. Maize silage	(Vlaeminck et al., 2006)
C15:0 anteiso	Grass silage vs. Maize silage	(Vlaeminck et al., 2006)
	Pasture Hay vs. Grass silage	(Baars et al., 2011)
C16:0 iso	Grass silage vs. Maize silage	(Vlaeminck et al., 2006)
	Pasture or Hay vs. Grass silage	(Baars et al., 2011, Villeneuve et al., 2013)
C16:0 anteiso	Pasture Hay vs. Grass silage	(Baars et al., 2011)

C17:0 iso	Maize silage vs. Grass silage	(Vlaeminck et al., 2006)
	Pasture vs. Hay	(Villeneuve et al., 2013)
C17:0 anteiso	Maize silage vs. Grass silage	(Vlaeminck et al., 2006)
	Pasture or Hay vs. Grass silage	(Villeneuve et al., 2013)
C18:0 iso	Grass silage vs. Maize silage	(Kliem et al., 2008)
Unsaturated FA		
C18:1 t9 Elaidic acid	Pasture vs WC silage	(Wijesundera et al., 2003, Elgersma et al., 2004)
	Grass silage vs. WC and RC silage	(Wiking et al., 2010)
	Pasture vs Grass silage or Hay	(Villeneuve et al., 2013)
	Maize silage	(Wijesundera et al., 2003, Kliem et al., 2008)
C18:1 t10	Maize silage vs. Pasture	(Kliem et al., 2008)
	Pasture vs Grass silage or Hay	(Villeneuve et al., 2013)
C18:1 t11 Vaccenic acid	Pasture vs. Maize silage	(Elgersma et al., 2004, Slots et al., 2009)
	Pasture vs Grass silage vs. Hay	(Villeneuve et al., 2013)
	WC and RC pasture vs. Maize silage	(Wiking et al., 2010)
	NS, Grass or Maize silage	(Kliem et al., 2008)
C18:1 c9 Oleic acid	Pasture	(Ellis et al., 2006, Croissant et al., 2007, Heck et al., 2009)
	Pasture vs. Grass silage	(Chilliard et al., 2001, Elgersma et al., 2004)
	Pasture vs Hay	(Villeneuve et al., 2013)
	NS, Grass silage vs. Maize silage	(Kliem et al., 2008)

C18:1 c11	WC pasture	(Ellis et al., 2006)
C18:2 c9,12 Linoleic acid, LA	Maize silage vs. Fresh pasture	(Kliem et al., 2008, Slots et al., 2009)
	Pasture vs. Grass silage or Hay	(Villeneuve et al., 2013)
C18:2 c9t11 Conjugated linoleic acid, CLA	Pasture	(Chilliard et al., 2001, Elgersma et al., 2004,Croissant et al., 2007, Slots et al., 2009, Heck et al., 2009, Prandini et al., 2009)
	Pasture vs Grass silage	(Ellis et al., 2006, Villeneuve et al., 2013)
	Pasture or Grass silage vs. Hay	(Villeneuve et al., 2013)
	RC and WC pasture vs. Maize silage	(Wiking et al., 2010)
	Hay	(Prandini et al., 2009)
C18:3 c9,12,15 α-Linolenic acid, ALA	Pasture	(Chilliard et al., 2001, Lourenço et al., 2008, Prandini et al., 2009, Slots et al., 2009, Schröder et al., 2011)
	RC pasture	(Lourenço et al., 2008, Butler et al., 2011)
	RC pasture or WC silage	(Steinshamn and Thuen, 2008, Slots et al., 2009,)
	WC pasture or WC silage	(Ellis et al., 2006, Slots et al., 2009,)
	RC silage	(Dewhurst et al 2003, Ellis et al., 2006, Slots et al., 2009)
	Pasture vs.Hay vs. Grass silage	(Villeneuve et al., 2013)
	Hay	(Slots et al., 2009)

NS – Not Significant, WC – White clover, RC – Red clover

2.3 CONVENTIONAL VERSUS ORGANIC MILK – MAIN COMPONENTS

2.2.1 Milk yield

Despite existence of highly specialised, grassland based, organic farms with cows producing more than 9,000 kg fluid milk per annum (Muller-Lindenlauf et al., 2010), on average milk production from organically reared cows is lower than from conventional cows (Sundberg et al., 2009). These differences are significant, with organic herds achieving 85% (range 72 - 91%) of the yields recorded for conventional herds (Bilik and Lopuszanska-Rusek, 2010, Müller and Sauerwein, 2010, Stiglbauer et al., 2013). Decreased production under organic management can be traced to lower energy intake, either through less concentrate feeding (Garmo et al., 2010, Stiglbauer et al. 2013) or lower energy content in forages from organic systems. This is exemplified by Gruber et al. (2001) who conducted a six-year study with nearly identical diets for organic and conventional cows. They demonstrated that milk yield per cow and year was identical for both herds, but milk production per area grazed was reduced in the organic herd. This was caused by lower dry matter yields from organic pasture and, therefore, lower stocking rate per hectare. Consequently, diets similar in composition and metabolizable energy content had the same effect on milk production, independent of whether the farming system was organic or conventional.

2.2.2 Milk fat content

Results of research studies examining the fat content in organic and conventional milk are ambivalent. Zagorska and Ciprovica (2008) and Anacker (2007) found increased fat content in organic milk, while trials undertaken by Sundberg et al. (2009), Hanus et al. (2008a) and Kuczyńska et al. (2012) observed higher fat percentage in conventional milk. Samples of retail milk collected during October and November 2006 in the USA found no significant difference for fat percentage between the two milk varieties (Vicini et al., 2008). This result might be due to the federal standards for butterfat content for fluid milk products. Müller and Sauerwein (2010) analysed bulk milk samples of 35 organic and 33 conventional farms during 2002 and 2004 reported similar amounts in milk fat between the two farming systems. Reasons for the reported differences can be diverse, with only a few publications mentioning potential causes. Higher fat concentration in milk from organic compared to conventional farms could have been caused by a preference for non-Holstein breeds in organic herds (Nauta et al., 2009) resulting in a higher number of Jersey and other breeds (Palladino et al., 2010). An increase in starch-based concentrates has been associated with a decline in milk fat concentration. Higher amounts of starch-based concentrates are commonly associated with diets of conventionally farmed dairy cows, compared

to organic cows (Rosati and Aumaitre, 2004), as organic farming regulations restrict the usage of concentrates. Alternatively, an increase in milk fat percentage in milk from conventional farms may indicate a diet enriched with fat supplements (Vyas et al., 2012, Lock et al., 2013). A negative energy balance, predominantly found during the early stages of lactation, and the winter period in LI farmed organic cows (Trachsel et al., 2000), might also affect the fat percentage in milk (Gross et al., 2011). Additionally, a higher parity average (Craninx et al., 2008), variations in heritability (Soyeurt et al., 2007), and genotype (Coleman et al., 2010) can be reflected in milk fat percentage. A result of inadequate descriptions of experimental trials is that conclusions from these studies need to be interpreted cautiously. Table 2.5 compiles several studies where organic and conventionally produced milk have been compared with regard to their fat, protein and lactose content, and lists the reported causes for any differences as proposed by the authors.

2.2.3 Milk fat – Individual fatty acids

The effect of bovine milk fat on human health cannot generally be described as favourable or unfavourable, and the biological function of each FA should be considered separately (Arnold and Jahreis, 2011). However, much research is currently engaged in trying to alter milk FA composition to create a FA profile which is considered more desirable for human health. Two common approaches are to either influence milk FA composition through dietary changes, or to genetically select cows with a more preferable milk FA profile (Bilal et al., 2012). A low ratio of ω-6 to ω-3 FA, for example, is beneficial for human health. Typically, the amount of ω-6 FA in western diets is too high, with possible negative consequences (e.g., cardiovascular disease, cancer, and inflammatory and autoimmune diseases) (Simopoulos, 2003). Current recommendations regarding the dietary ratio of ω-6/ω-3 FA target 1:1 or 2:1, but even a 4:1 ratio was found to have a positive effect on asthma patients (Simopoulos, 2003), and decreased mortality in patients with a previous myocardial infarction (Simopoulos, 2010). The ω-6/ω-3 ratio in bovine milk essentially describes the concentrations of linoleic acid (LA) versus α-linolenic acid (ALA), as they represent the most abundant ω-6 and ω-3 FA. Forage is rich in ALA, while cereals (e.g., barley, maize, oats, and soybean) contain higher amounts of LA (Khiaosa-Ard et al., 2010). A lower ω-6/ω-3 ratio is therefore, indicative of a forage-based diet.

The concentrations of individual FA in milk fat are influenced by cow breed (Croissant et al., 2007), stage of lactation (Craninx et al., 2008, Nantapo et al., 2013), genetics (Soyeurt et al., 2008), and diet. Diet is especially relevant when comparing concentrate-fed Table 0.5 Differences in milk composition between organic and conventional produced milkand pasture-based systems. Milk FA composition in pasture-based systems is,

additionally, subjective to seasonal variations which influence quantity and quality of available forages. Specific characteristics of forage diets have been widely studied.

Adler et al. (2013), for example, compared long-term (LT) and short-term (ST) grassland management. The pasture composition on LT organic farms showed a lower amount of legumes (*Fabaceae*) and a higher proportion of other dicotyledon families, compared with ST organic farms. Differences in FA composition in milk from two organic systems were found for C9:0 to C12:0 and explained by the differences in pasture composition. Similarly, Baars et al. (2011) observed significant differences for C4:0 – C11:0 FA milk samples from cows fed hay of pasture or hay of ley. This exemplifies how minor dietary differences can affect the milk FA composition. Variation in milk FA composition between different breeds has been documented by a number of researchers. Maurice-Van Eijndhoven et al. (2011) compared four cattle breeds (Dutch Friesian, Meuse-Rhine-Yssel, Groningen White Headed, and Jersey) in the Netherlands and found significant differences in total fat percentage as well as in the concentration of short- and medium-chain FA (SMCFA), vaccenic acid (VA) and conjugated linoleic acid (C18:2 c9t11, CLA). Similar variations between breeds for milk fat concentration and composition have been observed by Ramalho et al. (2012) and Carroll et al. (2006). Soyeurt et al. (2007) analysed data from 7,700 milk samples, from 25 herds, representing seven cow breeds including Holstein-Friesian and Jersey. They observed that heritability for milk yield, milk protein, and fat percentages were 18, 28 and 32%, respectively. In addition, 20% of the variability seen in milk fat composition, especially with regards to the most abundant FA in milk, was caused by genetics. A summary of studies comparing milk FA composition from different breeds are listed in Table 2.3. For most conventional dairy farms, the effect of breed on milk composition might be considered negligible as Holstein is the dominant breed for dairying; however, strain and genetic merit affect milk composition and performance under a specific farming system differently (Auldist et al., 2000, Nauta et al., 2009). Organic dairy farmers have a preference for non-Holstein and mixed breeds, and generalisations are, therefore, less appropriate (Nauta et al., 2006, Honorato et al. 2014).

Table 0.5 Differences in milk composition between organic and conventional produced milk

Milk Compound	Literature	Reported causes for differences in composition between organic and conventional milk
Fat %		
Increased in Organic	(Zagorska and Ciprovica, 2008)	No comment on breed or diet specifics
	(Anacker, 2007)	No comment on breed or diet specifics, higher amount of green fodder in the diet and use of clover silage in winter for organic herd.
	(Butler et al., 2011)	Differences in diet but no specifics [A]
Increased in Conventional	(Hanus et al., 2008)	Diet differences, all year round TMR for conventional cows, pasture grazing for organic cows during summer
	(Sundberg et al., 2009)	Preference for non-Holstein and mixed breeds in organic herds, lower replacement rates in organic herds
	(Kuczyńska et al., 2012)	Higher fibre intake intake
NS[1]	(Vicini et al., 2008)	No comment on breed or diet specifics
	(Müller and Sauerwein, 2010)	No comment on breed or diet specifics
	(Nauta et al., 2006)	No comment on breed or diet specifics[B]
Protein %		
Increased in Organic	(Vicini et al., 2008)	No comment on breed or diet specifics
	(Partida et al., 2007)	Diet differences, longer grazing time for organic, peas instead of soy.
Increased in Conventional	(Bilik and Lopuszanska-Rusek, 2010)	Better energy balance in conventional cows, different fermentation processes in rumen.

	(Kuczyńska et al., 2012)	Sugar-rich juicy feed for conventional cows, which stimulates production of butyric acid used for protein synthesis[C]
	(Anacker, 2007)	No comment on breed or diet specifics, higher amount of green fodder in the diet and use of clover silage in winter for organic herd.
	(Hanus et al., 2008)	Diet differences, all year round TMR for conventional, pasture grazing for organic during summer, energy and protein deficiency in organic herd
	(Sundberg et al., 2010)	Preference for non-Holstein and mixed breeds in organic, lower replacement rates in organic herds. The interaction between system and breed was found to significantly affect all milk yield traits. Lower energy density in organic rations caused by limited concentrate content.
	(Müller and Sauerwein, 2010)	No comment on breed or diet specifics [D]
NS	(Butler et al., 2011)	Differences in diet but no specifics [A]
	(Nauta et al., 2006)	No comment on breed or diet specifics [C]
Lactose %		
Increased in Organic	(Zagorska and Ciprovica)	Higher concentrations of sugars in grasses feed from organic farms.
Increased in Conventional	(Kuczyńska et al., 2012)[E]	
NS	(Roesch et al., 2005) (Nauta et al., 2006) (Bilik and Lopuszanska-Rusek, 2010)	

A Retail milk

B Data from 188 organic and 152 conventional dairy farms in the Netherlands, collected between 1990 and 2004

C During late pasture season

D Data from 35 organic and 33 conventional dairy farms from North Rhine–Westphalia in West Germany, collected between 2002 and 2004

E During early indoor season

[1] NS = not significant

Individual FA in cow's milk derive from different sources (e.g., diet, rumen, and mammary gland). Better understanding of the origin of FA may help to explain the variations observed between different milk samples. Even-chain saturated FA with chain length from C4 to C16 are produced *de novo* in the mammary gland from acetic and butyric acids (Lindmark Månsson, 2008). Odd- and branched-chain FA (OBCFA) are synthesised by ruminal bacteria and are influenced indirectly by the diet, while long-chain FA (including C16:0) and polyunsaturated FA (PUFA) originate directly from feed. A large proportion of PUFA is bio-hydrogenated in the rumen, with up to 99% of ALA partially, or completely hydrogenated (Leiber et al., 2005). Conversely, a large proportion of FA are de-saturated in the mammary gland by Δ-9-desaturase (Vlaeminck et al., 2006). Long chain PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are converted endogenously from ALA in the mammary gland, but the conversion rate is low (Tu et al., 2010). Small amounts of FA in milk can be derived from adipose tissue of the animal. This occurs predominantly when the animal has a negative energy balance, and can be observed by an increased concentration of oleic acid (C18:1 c9) in milk (Gross et al., 2011, Loften et al., 2014)

2.2.4 Milk protein content

Protein concentration and composition in milk are largely unresponsive to variations in nutrition and management (Walker et al., 2004), while individual cow genetics, stage of lactation, and breed significantly influence the concentration of protein in milk (Maurice-Van Eijndhoven et al., 2011). Increased amounts of protein in conventional milk were observed by Bilik and Lopuszanska-Rusek (2010), Kuczyńska et al. (2012), as well as in trials conducted by Hanus et al. (2008a) and Sundberg et al. (2010). Müller and Sauerwein (2010) reported a tendency for conventional milk to contain higher protein concentration. In contrast Vicini et al. (2008) reported significantly increased protein concentration in organic milk, compared to conventional and rbST-free milk (3.22 vs. 3.14 vs. 3.15% protein, respectively). Anacker (2007) similarly observed higher protein concentrations in organic milk, during monthly recordings on two conventional and one organic farm over a two year period (3.39% compared to 3.20% ($P < 0.001$) in organic and conventional milk, respectively). Milk protein concentration is positively correlated with metabolizable energy (ME) and, to a lesser extent, metabolizable protein intake. Dietary starch and crude protein interaction affect milk and protein yield and concentration (Cabrita et al., 2007). Consequently, supplementation with starch based concentrates can increase the rate of protein synthesis in the mammary gland (Rius et al., 2010). Organic farming regulations limit the use of supplements; therefore, lower protein concentration could be expected in milk from organic farms. Higher

protein concentration in milk can be expected in herds with NZ Friesian as the dominant breed, compared to US Holstein cows on a similar diet (Auldist et al. 2000). Different types of forage or grains and fertilizer application rate can also affect milk protein concentration. Moorby et al. (2009) observed a decrease in milk protein concentration when red clover silage was replaced with ryegrass silage, while Vanhatalo et al. (2006) reported a reduction in milk protein concentration when feeding oats rather than barley. Lower concentrations of milk protein have been reported when higher amounts of N fertilizer (240 kg N/ha compared to none, and 150 compared to 25 kg N/ha) were applied (Hermansen et al., 1994, Mackle et al., 1996). Consequently, differences in intensification of grassland cultivation can impact on milk protein concentration.

2.2.5 Milk lactose content

Lactose, the major carbohydrate of milk, maintains the osmolarity of milk and is positively correlated with milk volume (Shahbazkia et al., 2010). Mechanism and biology of lactose synthesis and regulation are subjects of on-going research (Jenkins and McGuire, 2006). The concentration of the two proteins making up lactose synthase, α-lactalbumin and β1,4-galactosyltransferase, are positively correlated with milk protein, fat, and lactose concentration, and stage of lactation (Bleck et al., 2009). Dietary starch and crude protein interaction can affect lactose concentration and yield (Rius et al., 2010). Nevertheless, changes in lactose concentration caused by dietary changes are less common and occur only in extreme circumstances (Jenkins and McGuire, 2006). Studies on human milk showed no relationship between lactose concentration and maternal nutrition (Lonnerdal et al., 1976, Emmett and Rogers, 1997). Lemosquet et al. (2009) found no link between whole body glucose rate of appearance and milk lactose yield in dairy cows after duodenal infusion of glucose. Similarly, the level of metabolizable protein in the diet has reportedly no effect on milk lactose percentage (Wang et al., 2007). Stage of lactation (Walker et al., 2004), and SCC (Forsbäck et al., 2010) affected the lactose content in milk, while no difference in concentration of lactose was detected between Holstein, Jersey, Brown Swiss, and Ayrshire breeds (Bleck et al., 2009). A number of publications reported no significant difference in lactose content between organic and conventionally produced milk (Roesch et al., 2005, Nauta et al., 2006, Bilik and Lopuszanska-Rusek, 2010), however, Kuczyńska et al. (2012) observed differences in lactose concentration between the two milk varieties after cows transitioned to an indoor diet. No cause for this change was suggested. Zagorska and Ciprovica (2008) reported a variation in lactose concentration between systems, suggesting that differences in diets were a possible cause.

2.2.6 Summary of Main Components

Results reported for milk yield, fat, protein, and lactose concentration are inconclusive if considered solely from an organic versus conventional point of view. Seemingly contradicting results (as listed in Table 2.5) can be expected when individual trial results are not put into context. Factors which influence milk fat and protein concentrations need to be considered before drawing conclusions on whether organic and conventional milk are different in their chemical composition or not. Individual trials need to report basic information on cow breed and diet, along with any additional influence factors which could be responsible for reported results (age, SCC, stage of lactation, and fertilizer application). Unfortunately, many authors fail to provide any indication of diet or breed used in their trials, although both factors are well known to influence milk composition (Toledo et al., 2002). Sundberg et al. (2010) demonstrated that interactions between system and breed were significant and affected all milk production traits including milk fat and protein yield, while (Cabrita et al., 2007) observed significant interactions between dietary starch and crude protein, affecting milk, protein and lactose yield and protein and lactose concentration. It is therefore rather difficult to achieve a general conclusion in regard to differences between organic and conventionally produced milk for the main milk components if these factors are unknown.

2.3 CONVENTIONAL VERSUS ORGANIC MILK – FATTY ACIDS

2.3.1 Milk from retail and dairy plants

Fatty acids are the most widely studied components when comparing organic and conventional milk, with a considerable amount of research focusing on FA composition. For this review, studies which analysed milk samples from retail outlets and dairy plants were considered separately from those which observed individual farms or animals. Retail samples represent a mixture of milk from a wide variety of individual cows and farms. This consequently 'dilutes' the effect of each individual cow and specific farm practices (Table 2.6). Extremes are, therefore, less significant (e.g., genetics, health, parity and stage of lactation from individual cows; farm specifics in diet, breed, drenching, and fertilizer application). Nevertheless, regulations (e.g., food standards and subsidies), geographical features (e.g. climate and altitude) and regional characteristics (e.g., local breeds, and agricultural practices) have an effect on milk FA composition, independent from the individual farm and the impact of diet and cow breed utilized in a specific region. Kliem et al. (2013) analysed conventional milk samples purchased monthly from five different retail outlets in an eight kilometre radius in the UK between November 2008 and October 2009. Significant differences in the FA profiles between the supermarkets were observed and explained by the different pools of milk suppliers, which most likely originated from different geographical areas

(Kliem et al., 2013). Not surprisingly, the comparison of several studies on organic and conventional retail milk, as listed in Table 2.6, showed inconsistent results for a majority of even-chain saturated FA. Therefore, no conclusions can be drawn in regard to whether or not a specific FA is more likely to be increased, or decreased, in any of the two milk varieties (organic or conventional). Results for odd-chain and branched-chain FA display greater agreement, but the number of studies considering these is limited. Results for monounsaturated FA (MUFA) and PUFA show greater consistency and higher concentrations of VA, CLA, ALA and EPA in organic milk have been reported independently from the country of origin (USA, UK, Denmark, Germany, Switzerland, and Italy), sampling season (January - December) and year (2003 to 2011). This result might suggest, that independent of origin, organic cows consume a different diet (higher amount of pasture and other forages) than conventional cows. This can be seen as a direct result of regulations mandating that organic dairy cows in the United States and European countries (Department for Environment, Food and Rural Affairs, 2010) have access to pasture and outdoor areas.

Some studies have suggested that these FA impart health benefits to the consumer (Givens, 2005, Nagpal et al., 2007). One could therefore conclude that organic retail milk may be advantageous for human consumption. However, the comparison of actual amounts of individual FA (Table 2.7) and the ratios of ω-6/ω-3 FA (Table 2.8) showed large variation between countries and supports the claim from Schönfeldt et al. (2012) for country specific milk data. Ratios of PUFA and SFA (saturated fatty acids) are rather similar by comparison with only Collomb et al. (2008) reporting significantly higher values than the other studies. This can be explained by the specific study conditions in the mountainous area of Switzerland which relied on a high forage diet for both organic and conventional cows. Kuczynska et al. (2011) reported similar high PUFA/SFA ratios for cows on a high forage diet during the summer season.

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Table 0.6 Ratio of ω-6/ω-3 FA and PUFA/SFA in organic and conventional retail milk from different countries

Country	ω-6/ω-3		PUFA/SFA		Reference
	org	conv	org	conv	
Denmark	4	8	-	-	(Slots et al., 2008)
Sweden	1.88	2.11	0.06	0.05	(Fall & Emanuelson, 2011)
Switzerland	1.37	1.61	0.08	0.08	(Collomb et al., 2008)
UK	1.51	2.54	0.06	0.05	(Ellis et al., 2006)
UK	2.63	3.76	0.05	0.04	(Butler et al., 2011)
USA	3.24	7.12	0.06	0.08	(O'Donnell et al., 2010)
USA	2.28	5.77	0.05	0.06	(Benbrook et al., 2013)

Causes for the different trends of individual FA (e.g., SFA) between studies are variable, but are largely influenced by country and regional characteristics. O'Donnell et al. (2010) reported higher amounts for all SFA in organic retail milk, except for C18:0 in the USA during October and November 2006. Benbrook et al. (2013) investigated milk FA composition in retail milk with monthly collections from January 2011 till July 2012 in the USA. The results for all FA match those from O'Donnell et al. (2010), but no significant difference between the milk varieties was observed for C4:0, C10:0 and C18:0. The study from O'Donnell et al. (2010) was undertaken before USDA standards came into effect in 2010, which mandate that organic cows must have access to pasture for 120 days per year, and must consume 30% of their DMI from pasture while grazing. The change in milk FA between the two American studies might be an effect of the change in standards, but the significant difference in study length also needs to be considered. Butler et al. (2011) reported that samples collected in England between Aug 2006 and Jan 2008 had lower amounts of C12:0 and C16:0 in organic milk and no significant difference between the two milk varieties for C4:0. The UK register of Organic Food Standards (UKROFS) states that herbivores should have a DMI consisting of at least 60% roughage, fresh or dried fodder, or silage. This difference in standards might explain the difference in FA composition between organic and conventional milk in the US and the UK. Organic and conventional bulk tank milk from three dairies in Switzerland showed no significant difference for short and medium chain FA (SMCFA), except for C12:0, which was increased in organic milk, and C4:0, which was higher in conventional milk (Collomb et al., 2008). Slots et al. (2008) sampled milk from Danish dairy plants and observed SMCFA either increased in organic milk or not significantly different between the two milk types, with C16:0 as exception.

Collomb et al. (2008) reported that while diets between organic and conventional cows were not identical, both groups had more than 80% of their DM intake (DMI) from grass (fresh grass, grass silage, or hay) which might explain why no differences for most SMCFA were found. This diet differs significantly from the USDA and UK standards.

Table 0.7 Fatty acid composition of organic and conventional retail milk

Fatty acid	Increase in organic	Decrease in organic	NS
Even-chain saturated FA			
C4:0 Butyric acid	(O'Donnell et al., 2010)	(Collomb et al., 2008)	(Slots et al., 2008) (Butler et al., 2011) (Benbrook et al., 2013)
C6:0 Caproic acid	(O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)		(Slots et al., 2008) (Collomb et al., 2008)
C8:0 Caprylic acid	(O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)		(Collomb et al., 2008)
C10:0 Capric acid	(Slots et al., 2008) (O'Donnell et al., 2010) (Butler et al., 2011)		(Collomb et al., 2008) (Benbrook et al., 2013)
C12:0 Lauric acid	(Collomb et al., 2008) (Slots et al., 2008) (O'Donnell et al., 2010)	(Butler et al., 2011)	(Benbrook et al., 2013)
C14:0 Myristic acid	(Slots et al., 2008) (O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)		(Collomb et al., 2008)
C16:0 Palmitic acid	(O'Donnell et al., 2010) (Benbrook et al., 2013)	(Slots et al., 2008) (Butler et al., 2011)	(Collomb et al., 2008)
C18:0 Stearic acid	(Butler et al., 2011)	(Collomb et al., 2008) (O'Donnell et al., 2010)	(Slots et al., 2008) (Benbrook et al., 2013)[A]
Odd-chain saturated FA			
C15:0	(Collomb et al., 2008) (O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)		

C17:0	(Collomb et al., 2008) (O'Donnell et al., 2010) (Benbrook et al., 2013)		
Branched-chain FA			
C14:0 iso	(Collomb et al., 2008)		
C15:0 iso	(Collomb et al., 2008)		
C17:0 iso	(Collomb et al., 2008)		
C17:0 anteiso	(Collomb et al., 2008) (Vetter and Schröder, 2010)		
Unsaturated FA			
C14:1 c9 Myristoleic acid	(O'Donnell et al., 2010) (Benbrook et al., 2013)		(Collomb et al., 2008) (Slots et al., 2008) (Butler et al., 2011)
C16:1 c9 Palmitoleic acid		(Slots et al., 2008) (Collomb et al., 2008) (O'Donnell et al., 2010) (Butler et al., 2011)	(Benbrook et al., 2013)
C18:1 t10	(Collomb et al., 2008)	(O'Donnell et al., 2010)	
C18:1 t11 Vaccenic acid	(Bergamo et al., 2003) (Collomb et al., 2008) (Prandini et al., 2009) (O'Donnell et al., 2010) (Butler et al., 2011)		
C18:1 c9 Oleic acid		(Collomb et al., 2008) (Slots et al., 2008) (O'Donnell et al., 2010) (Benbrook et al., 2013)	(Butler et al., 2011)
C18:2 c9,12 Linoleic acid LA	(O'Donnell et al., 2010)	(Bergamo et al., 2003) (Prandini et al., 2009) (Butler et al., 2011)	(Collomb et al., 2008) (Slots et al., 2008)
C18:2 c9t11	(Bergamo et al., 2003) (Collomb et al., 2008)		(Molkentin, 2009)

Conjugated linoleic acid CLA	(Prandini et al., 2009) (O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)
C18:3 c9,12,15 α-Linolenic acid ALA	(Bergamo et al., 2003) (Molkentin and Giesemann, 2007) (Collomb et al., 2008) (Slots et al., 2008) (Prandini et al., 2009) (O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)
C20:5 n3 EPA	(Molkentin and Giesemann, 2007) (Collomb et al., 2008) (O'Donnell et al., 2010) (Butler et al., 2011) (Benbrook et al., 2013)

Table 0.8 Concentration of selected FA in organic and conventional retail milk from different countries

Country	Vaccenic acid		Conjugated linoleic acid		α-Linolenic acid		Eicosapentaenoic acid		Reference
	org	conv	org	conv	org	conv	org	conv	
Denmark in g/100g FA	2.2±0.1	2±0.1	0.82±0.05	0.68±0.4	0.94±0.02	0.46±0.02	-	-	(Slots et al., 2008)
Italy in g/100g FA	1.62	1.00	0.82	0.52	0.68	0.38	-	-	(Prandini et al., 2009)
Italy in g/100g milk fat	2.33±0.2	1.55±0.1	0.63±0.04	0.51±0.04	0.6±0.05	0.52±0.03	-	-	(Bergamo et al., 2003)
Switzerland in g/100g milk fat	2.98±0.16	2.75±0.1*	1.22±0.07	1.11±0.06	0.89 0.02	0.79±0.02	0.08±0.01	0.07±0.01	(Collomb et al., 2008)
UK in g/100g FA	1.62±0.06	1.15±0.06	0.74±0.02	0.56±0.02	0.69±0.02	0.44±0.02	0.07±0.00	0.06±0.00	(Butler et al., 2011)
USA in % of total FA	1.71±0.05	1.45±0.02	0.70±0.02	0.57±0.01	0.65±0.01	0.41±0.01	0.06±0.01	0.03±0.01	(O'Donnell et al., 2010)

*C18:1 t10 + C18:1 t11

Apart from variances in forage type, amount and quality, differences in breeds or strains in the studies mentioned above are potential contributors to the differences in milk FA profile reported between and within organic and conventional milk samples. It is impossible to determine the actual impact of the cow breed in the presented studies, but their effect cannot be excluded. Collomb et al. (2008) reported Brown Swiss, Swiss Fleckvieh, Simmental, and Red Holstein as the dominant breeds in the Swiss trial, while the study from Butler et al. (2011) in the UK excluded milk from minority breeds. Breed has not been mentioned by Slots et al. (2008) or O'Donnell et al. (2010). Therefore, one can only assume that the circumstances in which these studies were undertaken reflect conditions expected of these countries. Holstein is the dominant breed in the UK, USA, and Denmark, representing 95, 90, and 72% of all dairy cows, respectively (Nygaard, 2007, Department for Environment, Food and Rural Affairs, 2010), but there is a general trend towards non-Holstein breeds on organic dairy farms (Roderick and Burke, 2004, Benbrook et al., 2013, Sundberg et al., 2010, Benbrook et al., 2013). We can assume that the percentage of Holstein cows in the dairy herd varies between countries, as well as between organic and conventional farm systems; consequently, this will influence the FA composition in milk.

2.3.2 Milk from research units and dairy farms

Studies researching the difference between organic and conventional milk samples from a limited number of animals and dairy farms are constrained in their ability to conclude whether the two milk varieties differ in general. Depending on the number of farms and animals involved, these studies need to take more factors into account (e.g., animal genetic, individual management, specific herd characteristics, and microclimate), to avoid conclusions which are based on inter-herd or inter-animal variation (Hou, 2011) rather than the farming system (organic or conventional). The benefit of these studies lies in their ability to measure and control influence factors, and consequently characterise their effect. Adler et al. (2013), for example, compared milk samples from 28 organic and conventionally managed farms and was able to explain the differences between the two milk varieties, regarding pasture composition, FA profile in concentrate, and seasonal variations. Differences in FA profile between organic and conventional milk (amongst other) were found for C18:0, C18:1 c9, ALA, EPA and DHA. Concentration of C18:0 and C18:1 c9 were increased in concentrate feed for conventional cows, which was observed in conventional milk. The same was seen for ALA in organic concentrate feed and reflected in organic milk. A higher amount of EPA and DHA in organic milk has been explained by the fish oil supplementation for organic cows. Other studies are less conclusive in their reporting of the (possible) causes for their results. Butler et al. (2008) investigated 25 commercial farms during

the grazing period and classified them into high input (HI) conventional, LI organic, and LI conventional farms. Low input organic and conventional showed now significant differences for VA, ALA, total SFA, MUFA, PUFA and ω-6:ω-3 ratio. For conventional HI farms the total SFA content was increased while total MUFA, PUFA, VA, ALA and CLA was decreased in milk compared to LI farms. This difference in concentration was even more pronounced for conventional LI farms compared to organic LI farms. The differences between HI and LI farms in milk FA profile and the similarity between the two LI systems can be explained by the diet; with both LI systems have more than 80% of their diets as fresh forage, compared to less than 40% for the HI farm. In this study (Butler et al., 2008) LI organic cows have a larger part of their diet as conserved forages and concentrates compared to LI conventional, which might explain the significantly higher CLA concentration for LI conventional milk. A possible cause for the higher proportion of fresh forage in the LI conventional diet might be related to the application of phosphate and nitrogen fertilizer on the LI conventional farm which possibly resulted in higher dry matter yield compared to the same area at organic LI farms. Anacker (2007) studied one organic and two conventional farms, for consecutive years on a monthly basis, and reported higher amounts of C18:0, LA, CLA, ALA and ω-3 FA in organic milk, while no significant difference was observed for C18:1 c9 and SFA between organic and conventional milk. No dietary specifics were given to explain these results, but organic cows had access to green fodder and a maximum of 40% concentrate in their diet. Further, no chemical fertilizer or pesticides were used on the organic farm. The higher amount of ALA and PUFA in organic milk could be explained by the assumption of higher forage intake for organic compared to conventional cows. Higher values for ω-3 FA and CLA ($P = 0.067$) in organic bulk milk samples were also found by Bloksma et al. (2008), who reports a higher intake of pasture and clover silage for organic cows, while conventional cows consumed more concentrate and maize silage. Jahreis et al. (1997) observed significantly higher amounts of CLA in milk from organic and conventional farms with pasture access in summer and a silage rich diet in winter, compared to milk from conventional cows kept indoors feeding on cereal and maize silage. This was explained by the higher amount of PUFA in fresh pasture leading to an increase in VA and CLA. Higher amounts of CLA in milk from organic cows compared to forage (including pasture) fed conventional cows, were explained by the differences in silage used during the winter period. Organic cows consumed grass and clover silages which are rich in PUFA compared to maize silage. It was also suggested that organic pastures and silages contained a higher amount of crude fibre which could have influenced the rumen micro-biota composition (Jahreis et al., 1997). Ellis et al. (2006) observed no significant difference between the milk varieties for CLA overall. This was explained by the extended sample set of 36 farms, resulting in a wider variation of CLA concentrations within

farm systems than in other studies, with individual farm factors having significant influence on CLA concentration. Molkentin and Giesemann (2007) similarly reported a large deviation in ALA concentration in organic milk, caused by the larger variety of feed used, compared to conventional farm management. Pasture grazing lead to a significantly increased CLA concentration in milk compared to silage and total mixed ratio (TMR) feeding. Ellis et al. (2006) also demonstrated that pasture feeding, a preference for mixed breed herds, and herds with lower milk yields are factors which significantly increased ω-3 FA concentration in milk. Consequently, several influence factors and their interactions have to be considered before evaluating the difference between organic and conventional milk.

2.4 CONVENTIONAL VERSUS ORGANIC MILK – MINOR COMPONENTS

2.4.1 Vitamins and antioxidants

Milk contains water and fat soluble vitamins and a number of research studies have investigated whether the concentration of these essential nutrients was different between organic and conventionally produced milk. Several studies focussed on vitamin A, its precursor β-carotene, and α-tocopherol, a form of vitamin E. As antioxidants they are of interest to milk processors as they may prevent spontaneous oxidized flavour in milk. A higher amount of PUFA, as commonly associated with organic milk, and the resulting greater risk of oxidation makes it desirable to have a greater quantity of antioxidants present in organic milk. The content of α-tocopherol and β-carotene in milk depends on the content in the diet (Swensson and Lindmark-Mansson, 2007, Mogensen et al., 2012). The highest concentration of vitamins (α-tocopherol and β-carotene) can be found in fresh forage. The loss of vitamins occurs during wilting, ensiling and storage, affecting different crops (e.g., rye grass, clover, and maize) differently (Kalač, 2011, Blank et al., 2013). Conserved or dried forages and cereals are considered a poorer source of α-tocopherol and β-carotene compared to fresh forage (Kay et al., 2005). However, results on whether or not milk derived from a diet rich in fresh forages (commonly organic) contains more β-carotene and α-tocopherol than milk from animals consuming larger amounts of concentrates (commonly conventional) are inconsistent as concentrates can be supplemented with vitamins. Butler et al. (2008) reported higher amounts of α-tocopherol and β-carotene in bulk milk samples from organic and LI conventional farms compared to milk from HI conventional farms. Higher concentrations of α-tocopherol and β-carotene in organic milk were also reported by Bergamo et al. (2003) and Slots et al. (2008). Slots et al. (2008) observed that the overall difference in α-tocopherol concentration between the two milk varieties was less significant ($P < 0.023$) then that for individual stereoisomers. The natural stereoisomer RRR α-tocopherol was significantly higher in organic milk,

while the synthetic 2R stereoisomer of α-tocopherol was significantly higher in conventional milk ($P < 0.001$). Similar results were described by Butler et al. (2008) who reported significantly higher amounts of RRR α-tocopherol in LI organic and LI conventional milk compared to HI conventional milk, with no significant difference for the synthetic 2R stereoisomer observed between the three milk varieties. The study indicates that synthetic antioxidants can be present in organic milk and that conventional milk can have similar high concentrations of α-tocopherol, caused by the fortification of concentrates. Significant differences were also reported for the amount of carotenoids (including β-carotene), with the highest concentration and the lowest observed in LI conventional and HI conventional milk, respectively. The difference in antioxidant concentration between LI organic and LI conventional milk might be related to the difference in fresh forage intake (Butler et al., 2008). No significant difference in α-tocopherol and β-carotene levels in organic and conventional milk was found by Ellis et al. (2007b), while vitamin A was found to be higher in conventional milk. They observed that concentrate feeding was positively correlated with vitamin A, α-tocopherol, and β-carotene concentration in milk, with individual farm effects sampling month, and milk yield as additional influence factors. Similarly, no significant difference between organic and conventional milk for β-carotene and α-tocopherol was found by Fall and Emanuelson (2011), who compared organic and conventional dairy herds during winter. Lack of fresh pasture for organic cows and therefore a similarity in diet between the herds was given as explanation for these results. Zagorska and Ciprovica (2008) reported on the concentration of the water soluble vitamins thiamine and riboflavin (B1 and B2) in milk. Samples were taken from five organic and conventional farms in Latvia with significantly lower concentrations ($P < 0.05$) for both vitamins observed in organic milk samples. Both vitamins are found in cereals (Powers, 2003, Gołda et al., 2004) and an increased amount in conventional milk could be explained by a higher intake of grains in the diet of conventional dairy cows. All studies demonstrate that feed composition rather than farming system (organic vs conventional) influence concentration of vitamins (and their precursors) in milk.

2.4.2 Minerals

Several research studies compared the mineral content of organic and conventional milk. Individual minerals have to be considered separately as they are regarded either as beneficial for animals and humans, or are considered as contaminants. Mineral content in milk is, depending on the element, influenced by individual cow genetics (van Hulzen et al., 2009), farm management and surrounding environment (Gabryszuk et al., 2008). Factors which influence soil and pasture mineral composition are for example fertilizer application (McKenzie

and Jacobs, 2002), disposal of sewage sludge (Percival, 2003), soil type (Mut et al., 2009), and proximity to mining areas (Smith et al., 2009), industrial activities (Gabryszuk et al., 2008) or automotive emissions (Ward et al., 1977).

Calcium and Magnesium Concentrations of Ca and Mg in milk are highly heritable and only marginally influenced by diet (van Hulzen et al., 2009). Ca in milk is associated with casein, which remains relatively constant in milk during dietary changes of the animal (Haug et al., 2007). Higher concentrations of both elements, as well as P, can be found in breeds with higher casein and phospholipid concentration (e.g. Jersey compared to Holstein) (Hermansen et al., 2005). The concentrations of Ca and Mg increases with stage of lactation from increased degradation of alpha(s)-casein as a result of pH changes (Sapru et al., 1997, Coulon et al., 1998). Although, not discussed by Gabryszuk et al. (2008) stage of lactation might have contributed to higher concentration of Ca ($P < 0.01$) and Mg ($P < 0.05$) in milk from HI conventional (lactation average 162 d), and LI organic cows (lactation average 193 and 173 d), compared to LI conventional cows (lactation average 117 d). Čuboň et al. (2008) reported higher Ca levels in bulk organic milk, but found no difference in the total protein concentration between organic and conventional milk. The bulk milk samples in this study originated from one organic and one conventional herd of similar size and breed (Slovac Prinzgau), located in the same geographical area with morning and evening milk sampled over several months (May - February). No explanation for differences in Ca concentration was given, but different months were reported for minimum protein concentration in organic (August) and conventional milk (May). This might indicate differences in casein concentration and potential differences in stage of lactation between the farms. The use of Na fertilizer or Na supplements can also increase the Ca and Mg status in bovine milk, while decreasing the SCC (Phillips et al., 2000).

Iodine and Selenium I and Se content in organic and conventional milk has been extensively researched, with both elements essential for animal and human health. The concentration of both elements in milk greatly depends on dietary intake, and dairy cows have been supplemented with I for decades to prevent deficiencies (Bath et al., 2012). I is readily taken up from the diet and introduced into milk, with milk produced by concentrate fed cows showing higher I levels than milk from cows grazing pasture (Gabryszuk et al., 2008). In countries with winter housing, concentrations of I in milk are largely influenced by season and the subsequent change in diet, with levels decreasing in summer (Haug et al., 2007). A study of retail milk in the UK showed that while there were regional variations in I levels, conventional retail milk contained up to 42% more I than

organic milk (Bath et al., 2012). Similar results have been reported by studies from Germany (Johner et al., 2012), Norway (Dahl et al., 2003) and Spain (Rey-Crespo et al., 2013). For all studies, I concentration was significantly lower in organic milk, a difference which was even more pronounced during summer season when pasture feeding increases. Use of iodophor sanitizers for teats and equipment could additionally contribute to I levels in milk, and might explain the variability in I concentrations observed in conventional milk (Bath et al., 2012, Rey-Crespo et al., 2013). Selenium is also an essential mineral, and ruminants are susceptible to selenium deficiency caused by a lack of absorption from the diet (van Hulzen et al., 2009). This is especially prevalent in animals fed high amounts of pasture rather than silage or total mixed ratio (Gabryszuk et al., 2008). Pilarczyk et al. (2011) found that in areas of low soil selenium levels, conventional cows fed diets based on hay, cereals, and pasture had significantly lower selenium levels in milk than cows feeding on total mixed ratio. Selenium content in milk from organic cows, which consumed a diet high in hay and maize silage, was significantly higher ($P < 0.001$) than that of conventional cows with access to pasture (Pilarczyk et al., 2011). Fall and Emanuelson (2011) could not establish any differences between selenium levels in milk of organic and conventional cows in Sweden during winter, however, and explained these findings with the similarity in diets.

Heavy metals Concentrations of heavy metals in bovine milk has been a research objective in many countries (Licata et al., 2004, Qin et al., 2009, Abdulkhaliq et al., 2012), and is predominantly related to concerns for human health. Environment and diet are main influence factors on metal concentration in milk, with different breeds affected differently (Hermansen et al., 2005) and correlations between elements observed (Pilarczyk et al., 2013). Anacker (2007) reported that while no difference was observed between organic and conventional milk, the concentrations of As, Cd, Cu and Hg changed significantly between years. The main source for heavy metals (e.g., As, Cd, Hg, and Pb) in agricultural systems are fertilizers (Gray et al., 2003, Mirlean et al., 2008). Differences in fertilizer application and pasture growth rate might explain the variation in heavy metal concentrations for different years reported by Anacker (2007). No differences and generally very low concentrations for Cd and Pb were observed by Ghidini et al. (2005) who compared organic and conventionally produced milk and meat in Italy. Comparable results for Cd, Cu, Fe, and Zn concentrations in organic and conventional bulk milk were found by (Zagorska and Ciprovica, 2005) who analysed samples from different regions of Latvia. Hanus et al. (2008b) reported elevated Cu levels in conventional milk in a comparative study of organic and conventional farms in the Czech Republic. Similarly, Rey-Crespo et al. (2013) observed higher

concentration of Cu, Se and Zn in conventional milk on a farm and retail level compared to organic farm milk, which was explained with the high supplementation rate of these essential elements in concentrate feed.

2.4.3 Hormones

Milk and dairy products naturally contain estrogens (Malekinejad et al., 2006) and the possible impact on human health has been of research interest (Daxenberger et al., 2001). Estrone (E_1) and estradiol (αE_2 and βE_2) concentrations in bovine milk are positively correlated with stage of gestation in the animal. Estrogen concentrations in retail milk vary depending on the milk fat percentage, which can be explained by the lipophilic character of these hormones (Pape-Zambito et al., 2010). No significant difference in estrone concentration between organic and conventional milk was detected. The concentration of estradiol (βE_2) in organic milk increased at a greater rate with an increase in fat compared to conventional milk. Although these differences were significant, they were not considered to be biologically relevant. A higher fat percentage in organic milk than indicated on the label might have been the cause for the reported difference (Pape-Zambito et al., 2010). Vicini et al. (2008) analysed estradiol and progesterone concentrations in organic and conventional retail milk from 48 States within the USA collected within three weeks. They reported higher levels of both hormones in organic milk ($P < 0.05$) and explained these differences with potentially lower feed intake of organic cows, and differences in average gestation state between organic and conventional cows.

2.5 CONVENTIONAL VERSUS ORGANIC MILK – OTHER

2.5.1 Udder Health and Somatic Cell Count

Management issues such as milking hygiene and cow cleanliness (Ellis et al., 2007a) influence the incidence of udder infection, which can affect milk yield and composition. Milk protein and fat, yield and percentage have been shown to be negatively correlated with a high somatic cell count (SCC) (Juozaitiene et al., 2004, Ogola et al., 2007, Guo et al., 2010). Consequently, conclusions on compositional differences between organic and conventionally produced milk should be made after taking into account udder health. The SCC of organic and conventional milk has been compared in a range of published studies, most of which reported no significant difference between the milk types (Muller and Lehmann, 2007, Müller and Sauerwein, 2010, Mullen et al., 2013, Stiglbauer et al., 2013). Sundberg et al. (2009) studied records of 471 organic herds and almost 14,000 conventional herds during 1998 to 2005 in Sweden, and found no differences in SCC at a given production level. Others reported lower SCC in organic milk (Ellis et al., 2007a, Čuboň et al., 2008, Garmo et al., 2010). Roesch et al. (2007) found higher median SCC in organic milk 31 days postpartum and similar SCC for organic

and conventional herds at 102 days postpartum. Similarly, cases of subclinical and clinical mastitis were not different between organic and conventional cows (Sundberg et al., 2009). Vaarst and Bennedsgaard (2002) analysed incidences of mastitis treatments for 27 organic and 57 conventional herds in Denmark. The farming system (organic vs conventional) appeared to have less influence on udder health compared to management factors (e.g., routine teat dipping). Although Valle et al. (2007) reported that differences in health handling (e.g., seeking veterinary treatment) rather than differences in actual animal health between organic and conventional cows may influence mastitis statistics. No differences in animal health between farming types were found, with the exception of fewer incidences of clinical mastitis in organic farms. These were thought to be partly caused by lower milk production of organic cows (Valle et al., 2007). Richert et al. (2013) described that farming intensity rather than system (organic vs conventional), influenced the frequency of veterinary visits. Ahlman et al. (2011) reported a higher culling rate for organic cows due to poor udder health compared to conventional cows, when studying 402 organic and 5335 conventional herds between 1998 and 2003. Ahlman et al. (2011) discusses, similar to Valle et al. (2007), that culling reasons might not solely depend on udder health status, but on the priorities and tolerance levels of individual farmers. A generalisation on whether or not organic farmers have a lower tolerance for poor udder health is not possible as, ethical considerations may and regulations, regarding the use of antibiotics as treatment option for organic cows, do vary between countries (Mullen et al., 2013).

2.5.2 Flavour and Taste

Organic milk has not only been associated with the image of being safe and environmentally friendly, it is also regarded as more tasteful than conventional milk (Managi et al., 2008)(Liu et al., 2013). Flavour differences have been studied in milk from cows feeding different amounts of concentrate and pasture (Croissant et al., 2007, Bloksma et al., 2008, Bovolenta et al., 2009), with no difference in consumer acceptance reported. Similarly, no obvious difference in taste was established when comparing organic and conventional milk, but trials found organic milk to be creamier and with a greater intensity of grassy flavour (Bloksma et al., 2008). Temperature of the milk consumed (7 and 15ºC, respectively) affected the noticeability of specific flavours (Croissant et al., 2007), which can be explained by the increased volatility of flavour compounds by raising temperatures. Cmen et al. (2010) suggested that a lower concentration of fat in organic milk was related to the loss in flavour, while Coggins et al. (2008) reported that trained panellists were not able to differentiate between plain yoghurts of different fat content or milk varieties (organic vs conventional). Gallina Toschi et al. (2012)

similarly observed that consumers did not distinguish between odour and taste of yoghurt produced from organic and conventional milk, but that the most liked conventional yoghurt scored higher when it was labelled as organic.

2.5.3 Identification

Partly due to the demand of premium prices for organic milk in a growing retail market, researchers investigated factors to identify or distinguish organic from conventional milk. Several marker molecules have been considered in regard to their significantly different concentration in organic and conventional milk. The supposed difference in concentration relates back to a significant difference in diet between organic and conventional cows. So far all suggested markers have failed when organic and conventional diets were rather similar (e.g. in LI organic and LI conventional farms). Phytanic acid, for example, which is converted from phytol released from chlorophyll, can be used as an indicator for the amount of green fodder in the diet of a dairy cow (Vetter and Schröder, 2010, Schröder et al., 2011). Therefore, phytanic acid could be used to identify organic milk, with the limitation that comparisons could only be made between milk from conventional cows which have limited access to green fodder and organic cows mainly fed on forage. The same limitations are found for ALA as marker molecule (Molkentin, 2009). Organic milk generally contains increased amounts of ALA, caused by higher amounts of fresh forage in the diet, but Flowers et al. (2008) showed that supplementation with 5% linseed oil doubled the levels of ALA in conventional milk, thereby matching the values observed in organic milk. The method described by Molkentin, (2008) determines the carbon stable isotope ratio ($\delta^{13}C$) in milk. It is based on the fact that maize (which is commonly used in concentrate feed for conventional cows) is a C_4 plant (compared to other common feed plants) which uses a different biosynthetic pathway to fixate atmospheric CO_2. This leads as consequence to a stronger accumulation of the ^{13}C isotope in the plant which can be detected in milk. The method would therefore enable the determination of the amount of maize in the diet of the animal. The limitations of this method lay in the demand for a difference in maize concentration between organic and conventional diets, and in the inability to establish whether or not maize was produced organic or conventionally. A characterisation of organic and conventionally produced milk using metabolomics has been applied by Boudonck et al. (2009). Hippurate, proline, ribose 5-phosphate and carnitine were amongst the 14 identified metabolites significantly different between organic and conventional whole milk brands. Whether or not these differences are caused by differences in diet or metabolic pathways in the animals needs to be established. Hippuric acid has been considered as a marker molecule, but was found to be unsuitable as its content depends on the feeding regime

rather than the production system (Boundonck et al., 2009, Carpio et al., 2010). Capuano et al. (2014) described the feasibility to distinguish between milk samples from cows with or without pasture access via Fourier transformed infrared spectroscopy (FITR). However, similarly to other studies, the classification on whether or not the milk samples came from organic or conventionally reared cows had to be considered more cautiously and general conclusions could not be made. All current approaches described in the literature depend on a significant difference between diets, which either results in a measurable change in the amount of a certain marker molecule or in a characteristic alteration of an isotope ratio in milk. As such, these methods are not able to differentiate between intensive organic and extensive conventional farming systems.

2.6 CONCLUSION

The number of factors that influence milk composition are numerous, and knowledge in regard to their interactions is limited. The same can be said in regard to the large amount of studies comparing organic and conventional milk, and the limited number of generally accepted research conclusions considering the difference between organic and conventionally produced milk. This is caused by two facts. Firstly: a lack of comparable conditions within and between trials. In general, most researchers have not controlled enough variables to allow a valid comparison between organic and conventionally produced cow's milk and draw overall conclusions. Diet composition and breed of cow are the minimum factors which need to be considered and reported when aiming to compare milk samples. Secondly: the current regulations for organic milk production do not allow for a distinct separation from conventionally produced milk. In other words, there is no 'organic effect' which can be credited to a 'holistic' combination of factors affected by the organic system. If animal genetic, health, breed, diet, management or environment differ, then so will the composition of the milk produced.

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REFERENCES

Abdulkhaliq, A., K. M. Swaileh, R. M. Hussein, and M. Matani. 2012. Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. International Food Research Journal 19:1089-1094.

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91(5):2033-2045.

Adler, S. A., S. K. Jensen, E. Govasmark, and H. Steinshamn. 2013. Effect of short-term versus long-term grassland management and seasonal variation in organic and conventional dairy farming on the composition of bulk tank milk. Journal of Dairy Science 96:5793-5810.

Ahlman, T., B. Berglund, L. Rydhmer, and E. Strandberg. 2011. Culling reasons in organic and conventional dairy herds and genotype by environment interaction for longevity. Journal of Dairy Science 94:1568-1575. doi:10.3168/jds.2010-3483.

Anacker, G. Differences between composition of organic milk and conventional milk. 2007. DMZ, Lebensmittelindustrie und Milchwirtschaft. 128: 17, 20-25. AVA Agrar-Verlag Allgau GmbH.

Arnold, C., and G. Jahreis. 2011. Milk fat and health. Milchfett und Gesundheit 58:177-181.

AsureQuality. 2013. AsureQuality Organic Standard For Primary Producers - Version 5. Page 129. AsureQuality Ltd., New Zealand.

Auldist, M. J., K. A. Johnston, N. J. White, W. P. Fitzsimons, and M. J. Boland. 2004. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. Journal of Dairy Research 71:51-57. doi:10.1017/s0022029903006575.

Auldist, M. J., A. R. Napper, and E. S. Kolver. 2000. Contribution of nutrition to seasonal variation of milk composition in New Zealand Friesian and US Holstein dairy cows. Asian-Australasian Journal of Animal Sciences 13:513-515.

Baars, T., J. Wohlers, D. Kusche, and G. Jahreis. 2011. Experimental improvement of cow milk fatty acid composition in organic winter diets. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.5525.

Ballou, L. U., M. Pasquini, R. D. Bremel, T. Everson, and D. Sommer. 1995. Factors affecting herd milk composition and milk plasmin at four levels of somatic cell counts. Journal of Dairy Science 78:2186-2195.

Bartl, K., C. A. Gomez, M. García, T. Aufdermauer, M. Kreuzer, H. D. Hess, and H. R. Wettstein. 2008. Milk fatty acid profile of Peruvian Criollo and Brown Swiss cows in response to different diet qualities fed at

low and high altitude. Archives of Animal Nutrition 62:468-484. doi:10.1080/1745039080245345010.1016/j.livsci.2008.07.022

Bath, S. C., S. Button, and M. P. Rayman. 2012. Iodine concentration of organic and conventional milk: implications for iodine intake. British Journal of Nutrition 107: 37 ref. Cambridge University Press.

Benbrook, C. M., G. Butler, M. A. Latif, C. Leifert, and D. R. Davis. 2013. Organic production enhances milk nutritional quality by shifting fatty acid composition: A United States-wide, 18-month study. PLoS ONE 8. doi:10.1371/journal.pone.00824

2910.1001/jamapediatrics. 2013.2408.

Bergamo, P., E. Fedele, L. Iannibelli, and G. Marzillo. 2003. Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. Food Chemistry 82:625-631. doi:10.1016/s0308-8146(03)00036-0.

Bilik, K., and M. Lopuszanska-Rusek. 2010. Effect of organic and conventional feeding of Red-and-White cows on productivity and milk composition. Annals of Animal Science 10:441-458.

Blank, B., D. Schaub, H. M. Paulsen, and G. Rahmann. 2013. Comparison of performance and feeding parameters in organic and conventional dairy farms in Germany. Vergleich von Leistungs- und Fütterungsparametern in ökologischen und konventionellen Milchviehbetrieben in Deutschland 63:21-27. doi:10.3220/lbf-2013-21-28.

Bleck, G. T., M. B. Wheeler, L. B. Hansen, H. Chester-Jones, and D. J. Miller. 2009. Lactose synthase components in milk: Concentrations of α-lactalbumin and β1,4-galactosyltransferase in milk of cows from several breeds at various stages of lactation. Reproduction in Domestic Animals 44:241-247.

Bloksma, J., R. Adriaansen-Tennekes, M. Huber, L. P. L. van de Vijver, T. Baars, and J. de Wit. 2008. Comparison of organic and conventional raw milk quality in The Netherlands. Biological Agriculture and Horticulture 26:69-83.

Boudonck, K. J., M. W. Mitchell, J. Wulff, and J. A. Ryals. 2009. Characterization of the biochemical variability of bovine milk using metabolomics. Metabolomics 5:375-386. doi:10.1007/s11306-009-0160-810.1017/S0022029998002970

Bovolenta, S., M. Corazzin, E. Saccà, F. Gasperi, F. Biasioli, and W. Ventura. 2009. Performance and cheese quality of Brown cows grazing on mountain pasture fed two different levels of supplementation. Livestock Science 124:58-65. doi:10.1016/j.livsci.2008.12.009.

Butler, G., J. H. Nielsen, M. K. Larsen, B. Rehberger, S. Stergiadis, A. Canever, and C. Leifert. 2011. The effects of dairy management and processing on quality characteristics of milk and dairy products. NJAS - Wageningen Journal of Life Sciences 58:97-102. doi:10.1016/j.njas.2011.04.002.

Butler, G., J. H. Nielsen, T. Slots, C. Seal, M. D. Eyre, R. Sanderson, and C. Leifert. 2008. Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: Seasonal variation. Journal of the Science of Food and Agriculture 88:1431-1441. doi:10.1002/jsfa.3235.

Cabrita, A. R. J., R. J. B. Bessa, S. P. Alves, R. J. Dewhurst, and A. J. M. Fonseca. 2007. Effects of dietary protein and starch on intake, milk production, and milk fatty acid profiles of dairy cows fed corn silage-based diets. Journal of Dairy Science 90:1429-1439.

Canadian General Standards Board. 2011. Organic Production Systems General Principles and Management Standards. Page 39. Standards Council of Canada.

Capuano, E., J. Rademaker, H. van den Bijgaart, and S. M. van Ruth. 2014. Verification of fresh grass feeding, pasture grazing and organic farming by FTIR spectroscopy analysis of bovine milk. Food Research International doi:10.1016/j.foodres.2013.12.024.

Carpio, A., V. Rodríguez-Estévez, M. Sánchez-Rodríguez, L. Arce, and M. Valcárcel. 2010. Differentiation of organic goat's milk based on its hippuric acid content as determined by capillary electrophoresis. Electrophoresis 31:2211-2217.

Carroll, S. M., E. J. DePeters, S. J. Taylor, M. Rosenberg, H. Perez-Monti, and V. A. Capps. 2006. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Animal Feed Science & Technology 131:451-473.

Cmen, M., N. Yldrm, A. Dkc, O. Kaplan, and N. C. Yldrm. Seasonal variations of biochemical taste parameters in milks from conventional and environment - friendly organic farming. Page 13 ref. Bulgarian Journal of Agricultural Science; 2010. 16 Agricultural Academy - Bulgaria.

Coggins, P. C., M. W. Schilling, S. Kumari, and P. D. Gerrard. 2008. Development of a sensory lexicon for conventional milk yogurt in the United States. Journal of Sensory Studies 23:671-687. doi:10.1111/j.1745-459X.2008.00179.x.

Coleman, J., K. M. Pierce, D. P. Berry, A. Brennan, and B. Horan. 2010. Increasing milk solids production across lactation through genetic selection and intensive pasture-based feed system. Journal of Dairy Science 93:4302-4317. doi:10.3168/jds.2009-2591.

Collomb, M., W. Bisig, U. Buetikofer, R. Sieber, M. Bregy, and L. Etter. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. International Dairy Journal 18:976-982.

Coppa, M., A. Ferlay, C. Chassaing, C. Agabriel, F. Glasser, Y. Chilliard, G. Borreani, R. Barcarolo, T. Baars, D. Kusche, O. M. Harstad, J. Verbič, J. Golecký, and B. Martin. 2013. Prediction of bulk milk fatty acid composition based on farming practices collected through on-farm surveys. Journal of Dairy Science 96:4197-4211. doi:10.3168/jds.2012-6379.

Coulon, J. B., I. Verdier, P. Pradel, and M. Almena. 1998. Effect of lactation stage on the cheesemaking properties of milk and the quality of Saint-Nectaire-type cheese. Journal of Dairy Research 65:295-305.

Craninx, M., A. Steen, H. Van Laar, T. Van Nespen, J. Martin-Tereso, B. De Baets, and V. Fievez. 2008. Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. Journal of Dairy Science 91:2662-2677. doi:10.3168/jds.2007-0656.

Croissant, A. E., S. P. Washburn, L. L. Dean, and M. A. Drake. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. Journal of Dairy Science 90:4942-4953.

Čuboň, J., V. Foltys, P. Haščík, M. Kačániová, I. Ubrežiová, S. Kráčmar, and K. Vavrišínová. 2008. The raw milk quality from organic and conventional agriculture. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 56:25-30.

Dahl, L., J. A. Opsahl, H. M. Meltzer, and K. Julshamn. 2003. Iodine concentration in Norwegian milk and dairy products. British Journal of Nutrition 90:679-685. doi:10.1079/bjn2003921.

Dangour, A. D., K. Lock, A. Hayter, A. Aikenhead, E. Allen, and R. Uauy. 2010. Nutrition-related health effects of organic foods: A systematic review. American Journal of Clinical Nutrition 92:203-210. doi:10.3945/ajcn.2010.29269.

Daxenberger, A., D. Ibarreta, and H. H. D. Meyer. 2001. Possible health impact of animal oestrogens in food. Human Reproduction Update 7:340-355. doi:10.1093/humupd/7.3.340.

Department for Environment, Food and Rural Affairs. 2010. Guidance Document on European Union Organic Standards. Page 31. Welsh Assembly Government; Department of Agriculture and Rural Development; The Scottish Government.

Department for Environment, Food and Rural Affairs. 2011. The Cattle Book 2008. Descriptive Statistics of Cattle Numbers in Great Britain on June 2008. Defra, London, UK.

Dewhurst, R. J., W. J. Fisher, J. K. S. Tweed, and R. J. Wilkins. 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86:2598-2611.

Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Animal Feed Science and Technology 131:168-206.

Dunshea, F. R., G. P. Walker, E. Ostrowska, and P. T. Doyle. 2008. Seasonal variation in the concentrations of conjugated linoleic and trans fatty acids in milk fat from commercial dairy farms is associated with pasture and grazing management and supplementary feeding practices. Australian Journal of Experimental Agriculture 48:1062-1075. doi:10.1071/ea0728610.1071/EA99132

Elgersma, A., G. Ellen, H. Van Der Horst, H. Boer, P. R. Dekker, and S. Tamminga. 2004. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. Animal Feed Science and Technology 117:13-27. doi:10.1016/j.anifeedsci.2004.08.003.

Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. Animal Feed Science and Technology 131:207-225. doi:10.1016/j.anifeedsci.2006.06.012.

Ellis, K. A., G. Innocent, D. Grove-White, P. Cripps, W. G. McLean, C. V. Howard, and M. Mihm. 2006. Comparing the fatty acid composition of organic and conventional milk. Journal of Dairy Science 89:1938-1950.

Ellis, K. A., G. T. Innocent, M. Mihm, P. Cripps, W. G. McLean, C. V. Howard, and D. Grove-White. 2007a. Dairy cow cleanliness and milk quality on organic and conventional farms in the UK. Journal of Dairy Research 74:302-310. doi:10.1017/s002202990700249x.

Ellis, K. A., A. Monteiro, G. T. Innocent, D. Grove-White, P. Cripps, W. Graham McLean, C. V. Howard, and M. Mihm. 2007b. Investigation of the vitamins A and E and β-carotene content in milk from UK organic and conventional dairy farms. Journal of Dairy Research 74:484-491. doi:10.1017/s0022029907002816.

Emmett, P. M., and I. S. Rogers. 1997. Properties of human milk and their relationship with maternal nutrition. Early Human Development 49:S7-S28. doi:10.1016/s0378-3782(97)00051-0.

Fall, N., and U. Emanuelson. 2009. Milk yield, udder health and reproductive performance in Swedish organic and conventional dairy herds. Journal of Dairy Research 76:402-410. doi:10.1017/s0022029909990045.

Fall, N., and U. Emanuelson. 2011. Fatty acid content, vitamins and selenium in bulk tank milk from organic and conventional Swedish dairy herds during the indoor season. Journal of Dairy Research 78:287-292. doi:10.1017/s0022029911000392.

Ferlay, A., C. Agabriel, C. Sibra, C. Journal, B. Martin, and Y. Chilliard. 2008. Tanker milk variability in fatty acids according to farm feeding and husbandry practices in a French semi-mountain area. Dairy Science and Technology 88:193-215. doi:10.1051/dst:2007013.

Flowers, G., S. A. Ibrahim, and A. A. AbuGhazaleh. 2008. Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. Journal of Dairy Science 91:722-730. doi:10.3168/jds.2007-0410.

Forman, J., J. Silverstein, J. J. S. Bhatia, S. A. Abrams, M. R. Corkins, S. D. De Ferranti, N. H. Golden, J. A. Paulson, A. C. Brock-Utne, H. L. Brumberg, C. C. Campbell, B. P. Lanphear, K. C. Osterhoudt, M. T. Sandel, L. Trasande, and R. O. Wright. 2012. Organic foods: Health and environmental advantages and disadvantages. Pediatrics 130:e1406-e1415. doi:10.1542/peds.2012-2579.

Forsbäck, L., H. Lindmark-Månsson, A. Andrén, M. Åkerstedt, L. Andrée, and K. Svennersten-Sjaunja. 2010. Day-to-day variation in milk yield and milk composition at the udder-quarter level. Journal of Dairy Science 93:3569-3577.

Gabryszuk, M., K. Słoniewski, and T. Sakowski. 2008. Macro- and microelements in milk and hair of cows from conventional vs. organic farms. Animal Science Papers and Reports 26:199-209.

Gallina Toschi, T., A. Bendini, S. Barbieri, E. Valli, M. L. Cezanne, K. Buchecker, and M. Canavari. 2012. Organic and conventional nonflavored yogurts from the Italian market: Study on sensory profiles and consumer acceptability. Journal of the Science of Food and Agriculture 92:2788-2795. doi:10.1002/jsfa.5666.

Garmo, R. T., S. Waage, S. Sviland, B. I. F. Henriksen, O. Østerås, and O. Reksen. 2010. Reproductive performance, udder health, and antibiotic resistance in mastitis bacteria isolated from norwegian red cows in conventional and organic farming. Acta Veterinaria Scandinavica 52. doi:10.1186/1751-0147-52-11.

Ghidini, S., E. Zanardi, A. Battaglia, G. Varisco, E. Ferretti, G. Campanini, and R. Chizzolini. 2005. Comparison of contaminant and residue levels in organic and conventional milk and meat products from Northern Italy. Food Additives and Contaminants 22:9-14. doi:10.1080/02652030400027995.

Givens, D. I. 2005. The role of animal nutrition in improving the nutritive value of animal-derived foods in relation to chronic disease. Proceedings of the Nutrition Society 64:395-402. doi:10.1079/pns2005448.

Gołda, A., P. Szyniarowski, K. Ostrowska, A. Kozik, and M. Rapała-Kozik. 2004. Thiamine binding and metabolism in germinating seeds of selected cereals and legumes. Plant Physiology and Biochemistry 42:187-195.

Gray, C. W., R. G. McLaren, and A. H. C. Roberts. 2003. Cadmium leaching from some New Zealand pasture soils. European Journal of Soil Science 54:159-166. doi:10.1046/j.1365-2389.2003.00495.x.

Gross, J., H. A. Van Dorland, R. M. Bruckmaier, and F. J. Schwarz. 2011. Milk fatty acid profile related to energy balance in dairy cows. Journal of Dairy Research 78:479-488. doi:10.1017/s0022029911000550.

Gruber, L., R. Steinwender, T. Guggenberger, J. Häusler, and A. Schauer. 2001. Comparison of organic and conventional fanning on a grassland farm. 2nd Communication: Feed intake, milk yield, health and fertility parameters. Vergleich zwischen biologischer und konventioneller wirtschaftsweise im grünlandbetrieb. 2. Mitteilung: Futteraufnahme, milchleistung, gesundheit und fruchtbarkeit 52:55-70.

Guéguen, L., and G. Pascal. 2010. An update on the nutritional and health value of organic foods. Cahiers de Nutrition et de Dietetique 45:130-143. doi:10.1016/j.cnd.2010.02.002.

Guo, J. Z., X. L. Liu, A. J. Xu, and Z. Xia. 2010. Relationship of somatic cell count with milk yield and composition in Chinese Holstein population. Agricultural Sciences in China 9:1492-1496.

Hanus, O., Z. Vorlicek, K. Sojkova, R. Rozsypal, M. Vyletelova, P. Roubal, V. Gencurova, J. Pozdisek, and H. Landova. 2008a. A comparison of selected milk indicators in organic herds with conventional herd as reference. Page 19 ref. Folia Veterinaria; 52 University of Veterinary Medicine.

Hanus, O., J. Brychtova, V. Gencurova, J. Pesl, I. Hulova, M. Vyletelova, R. Jedelska, and J. Kopecky. 2008b. Effect of conversion from conventional to organic dairy farm on milk quality and health of dairy cows. Page 43 ref. Folia Veterinaria; 52.

Haug, A., A. T. Hostmark, and O. M. Harstad. 2007. Bovine milk in human nutrition - a review. Lipids in Health and Disease Vol. 6. doi:2510.1186/1476-511x-6-25.

Heck, J. M. L., H. J. F. van valenberg, J. Dijkstra, and A. C. M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. Journal of Dairy Science 92:4745-4755. doi:10.3168/jds.2009-2146.

Hermansen, J. E., J. H. Badsberg, T. Kristensen, and V. Gundersen. 2005. Major and trace elements in organically or conventionally produced milk. Journal of Dairy Research 72:362-368. doi:10.1017/s0022029905000968.

Hermansen, J. E., S. Ostersen, and O. Aaes. 1994. Effect of the levels of N fertilizer, grass and supplementary feeds on nitrogen composition and renneting properties of milk from cows at pasture. Journal of Dairy Research 61:179-189.

Honorato, L. A., L. C. P. Machado Filho, I. D. Barbosa Silveira, and M. J. Hötzel. 2014. Strategies used by dairy family farmers in the south of Brazil to comply with organic regulations. Journal of Dairy Science 97:1319-1327. doi:10.3168/jds.2012-6532.

Hou, J. 2011. Variations of trans octadecenoic acids, especially trans-10 C18:1 and trans-11 C18:1 in milk fat. Milchwissenschaft 66:189-192.

Jahreis, G., J. Pritsche, and H. Steinhart. 1997. Conjugated linoleic acid in milk fat: High variation depending on production system. Nutrition Research 17:1479-1484. doi:10.1016/s0271-5317(97)00138-3.

Jenkins, T. C., and M. A. McGuire. 2006. Major advances in nutrition: Impact on milk composition. Journal of Dairy Science 89:1302-1310.

Johner, S. A., K. v. Nida, G. Jahreis, and T. Remer. 2012. Time trends and seasonal variation of iodine content in German cows' milk - investigations from Northrhine-Westfalia. Berliner und Muenchener Tieraerztliche Wochenschrift 125:76-82.

Juozaitiene, V., A. Zakas, and A. Juozaitis. 2004. Relationship of somatic cell count with milk yield and composition in the herds of Black-and-White cattle. Medycyna Weterynaryjna 60:701-704.

Kalač, P. 2011. The effects of silage feeding on some sensory and health attributes of cow's milk: A review. Food Chemistry 125:307-317. doi:10.1016/j.foodchem.2010.08.077.

Kay, J. K., J. R. Roche, E. S. Kolver, N. A. Thomson, and L. H. Baumgard. 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. Journal of Dairy Research 72:322-332. doi:10.1017/s0022029905000944.

Khiaosa-Ard, R., F. Klevenhusen, C. R. Soliva, M. Kreuzer, and F. Leiber. 2010. Transfer of linoleic and linolenic acid from feed to milk in cows fed isoenergetic diets differing in proportion and origin of concentrates and roughages. Journal of Dairy Research 77:331-336. doi:10.1017/s0022029910000257.

Kliem, K. E., R. Morgan, D. J. Humphries, K. J. Shingfield, and D. I. Givens. 2008. Effect of replacing grass silage with maize silage in the diet on bovine milk fatty acid composition. Animal 2:1850-1858. doi:10.1017/s1751731108003078.

Kliem, K. E., K. J. Shingfield, K. M. Livingstone, and D. I. Givens. 2013. Seasonal variation in the fatty acid composition of milk available at retail in the United Kingdom and implications for dietary intake. Food Chemistry 141:274-281. doi:10.1016/j.foodche

m.2013.02.116.

Kuczyńska, B., K. Puppel, M. Gołębiewski, E. Metera, T. Sakowski, and K. Słoniewski. 2012. Differences in whey protein content between cow's milk collected in late pasture and early indoor feeding season from conventional and organic farms in Poland. Journal of the Science of Food and Agriculture. 49: 61-67 doi:10.1002/jsfa.56

63.

Kuczynska, B., K. Puppel, E. Metera, P. Klis, A. Grodzka, and T. Sakowski. 2011. Fatty acid composition of milk from Brown Swiss and Holstein-Friesian black and white cows kept in a certified organic farm. Annals of Warsaw University of Life Sciences SGGW, Animal Science 49:61-67.

Larsen, M. K., X. C. Fretté, T. Kristensen, J. Eriksen, K. Søegaard, and J. H. Nielsen. 2012. Fatty acid, tocopherol and carotenoid content in herbage and milk affected by sward composition and season of grazing. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.5620.

Leiber, F., M. Kreuzer, D. Nigg, H. R. Wettstein, and M. R. L. Scheeder. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids 40:191-202. doi:10.1007/s11745-005-1375-3.

Lemosquet, S., E. Delamaire, H. Lapierre, J. W. Blum, and J. L. Peyraud. 2009. Effects of glucose, propionic acid, and nonessential amino acids on glucose metabolism and milk yield in Holstein dairy cows. Journal of Dairy Science 92:3244-3257.

Licata, P., D. Trombetta, M. Cristani, F. Giofrè, D. Martino, M. Calò, and F. Naccari. 2004. Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environment International 30:1-6. doi:10.1016/s0160-4120(03)00139-9.

Lindmark Månsson, H. 2008. Fatty acids in bovine milk fat. Food and Nutrition Research 52. doi:10.3402/fnr.v52i0.1821.

Liu, Z., C. A. Kanter, K. D. Messer, and H. M. Kaiser. 2013. Identifying significant characteristics of organic milk consumers: A CART analysis of an artefactual field experiment. Applied Economics 45:3110-3121.

Lloyd, M. A., M. A. Drake, and P. D. Gerard. 2009. Flavor variability and flavor stability of U.S.-produced whole milk powder. Journal of Food Science 74:S334-S343. doi:10.1111/j.1750-3841.2009.01299.x.

Lock, A. L., C. L. Preseault, J. E. Rico, K. E. DeLand, and M. S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. Journal of Dairy Science 96:6650-6659. doi:10.3168/jds.2013-6892.

Loften, J. R., J. G. Linn, J. K. Drackley, T. C. Jenkins, C. G. Soderholm, and A. F. Kertz. 2014. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. Journal of Dairy Science 97:4661-4674. doi:10.3168/jds.2014-7919.

Lonnerdal, B., E. Forsum, M. Gebre Medhin, and L. Hambraeus. 1976. Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen, and protein contents. American Journal of Clinical Nutrition 29:1134-1141.

Lourenço, M., G. Van Ranst, B. Vlaeminck, S. De Smet, and V. Fievez. 2008. Influence of different dietary forages on the fatty acid composition of rumen digesta as well as ruminant meat and milk. Animal Feed Science and Technology 145:418-437. doi:10.1016/j.anifeedsci.2007.05.043.

Macdonald, K. A., G. A. Verkerk, B. S. Thorrold, J. E. Pryce, J. W. Penno, L. R. McNaughton, L. J. Burton, J. A. S. Lancaster, J. H. Williamson, and C. W. Holmes. 2008. A comparison of three strains of Holstein-Friesian grazed on pasture and managed under different feed allowances. Journal of Dairy Science 91:1693-1707. doi:10.3168/jds.2007-044110.3168/jds.2007-0630

Mackle, T. R., C. R. Parr, and A. M. Bryant. 1996. Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. New Zealand Journal of Agricultural Research 39:341-356.

Magkos, F., F. Arvaniti, and A. Zampelas. 2003. Organic food: Nutritious food or food for thought? A review of the evidence. International Journal of Food Sciences and Nutrition 54:357-371. doi:10.1080/09637480120092071.

Malekinejad, H., P. Scherpenisse, and A. A. Bergwerff. 2006. Naturally occurring estrogens in processed milk and in raw milk (from gestated cows). Journal of Agricultural and Food Chemistry 54:9785-9791. doi:10.1021/jf061972e.

Managi, S., Y. Yamamoto, H. Iwamoto, and K. Masuda. 2008. Valuing the influence of underlying attitudes and the demand for organic milk in Japan. Agricultural Economics 39:339-348. doi:10.1111/j.1574-0862.2008.00337.x.

Maréchal, C. L., R. Thiéry, E. Vautor, and Y. L. Loir. 2011. Mastitis impact on technological properties of milk and quality of milk products - A review. Dairy Science and Technology 91:247-282.

Maurice-Van Eijndhoven, M. H. T., S. J. Hiemstra, and M. P. L. Calus. 2011. Short communication: Milk fat composition of 4 cattle breeds in the Netherlands. Journal of Dairy Science 94:1021-1025. doi:10.3168/jds.2009-3018.

McKenzie, F. R., and J. L. Jacobs. 2002. Effects of application of nitrogen fertilizer on concentrations of P, K, S, Ca, Mg, Na, Cl, Mn, Fe, Cu and Zn in perennial ryegrass/white clover pastures in south-western Victoria, Australia. Grass and Forage Science 57:48-53.

Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): Milk production from late-lactation dairy cows. Grass and Forage Science 56:383-394. doi:10.1046/j.1365-2494.2001.00288.x.

Ministry of Agriculture, Forestry and Fisheries. 2012. Japanese Agricultural Standard for Organic Livestock Products. Notification No. 1608 of the Ministry of Agriculture, Forestry and Fisheries of October 27, 2005. Partial Revision 2012:17

Mirlean, N., P. Baisch, I. Machado, and E. Shumilin. 2008. Mercury contamination of soil as the result of long-term phosphate fertilizer production. Bulletin of Environmental Contamination and Toxicology 81:305-308. doi:10.1007/s00128-008-9480-z.

Mogensen, L., T. Kristensen, K. Søegaard, S. K. Jensen, and J. Sehested. 2012. Alfa-tocopherol and beta-carotene in roughages and milk in organic dairy herds. Livestock Science 145:44-54. doi:10.1016/j.livsci.2011.12.021.

Molkentin, J. 2008. Laboratory authentication of organic milk. Deutsche Milchwirtschaft 59:873-874.

Molkentin, J. 2009. Authentication of organic milk using ^{13}C and the alpha-linolenic acid content of milk fat. Journal of Agricultural and Food Chemistry 57:785-790.

Molkentin, J., and A. Giesemann. 2007. Differentiation of organically and conventionally produced milk by stable isotope and fatty acid analysis. Analytical and Bioanalytical Chemistry 388:297-305.

Moorby, J. M., M. R. F. Lee, D. R. Davies, E. J. Kim, G. R. Nute, N. M. Ellis, and N. D. Scollan. 2009. Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. Journal of Dairy Science 92:1148-1160. doi:10.3168/jds.2008-1771.

Mullen, K. A. E., L. G. Sparks, R. L. Lyman, S. P. Washburn, and K. L. Anderson. 2013. Comparisons of milk quality on North Carolina organic and conventional dairies. Journal of Dairy Science 96:6753-6762. doi:10.3168/jds.2012-6519.

Muller-Lindenlauf, M., C. Deittert, and U. Kopke. 2010. Assessment of environmental effects, animal welfare and milk quality among organic dairy farms. Livestock Science 128:140-148. doi:10.1016/j.livsci.2009.11.013.

Muller, U., and I. Lehmann. Factors influencing animal health and milk quality. Okologie & Landbau; 2007. 142: 2, 40-41. Stiftung Okologie & Landbau (SOL).

Müller, U., and H. Sauerwein. 2010. A comparison of somatic cell count between organic and conventional dairy cow herds in West Germany stressing dry period related changes. Livestock Science 127:30-37. doi:10.1016/j.livsci.2009.08.003.

Mut, H., I. Ayan, Z. Acar, U. Basaran, Ö. Töngel, and O. Asci. 2009. Relationship between soil structure and botanical composition of the flat pastures in costal region of Samsun province. Asian Journal of Chemistry 21:971-978.

Nagpal, R., H. Yadav, A. K. Puniya, K. Singh, S. Jain, and F. Marotta. 2007. Conjugated linoleic acid: Sources, synthesis and potential health benefits - An overview. Current Topics in Nutraceutical Research 5:55-65.

Nantapo, C. T. W., V. Muchenje, and A. Hugo. 2013. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian×Jersey cross cow milk under a pasture-based dairy system. Food Chemistry 146:127-133. doi:10.1016/j.foodchem.2013.09.009.

Nauta, W. J., T. Baars, H. Saatkamp, D. Weenink, and D. Roep. 2009. Farming strategies in organic dairy farming: Effects on breeding goal and choice of breed. An explorative study. Livestock Science 121:187-199. doi:10.1016/j.livsci.2008.06.011.

Nauta, W. J., R. F. Veerkamp, E. W. Brascamp, and H. Bovenhuis. 2006. Genotype by environment interaction for milk production traits between organic and conventional dairy cattle production in The Netherlands. Journal of Dairy Science 89:2729-2737.

Nygaard, H. 2007. Farming and milk production in Denmark. in 27th European Holstein and Red Holstein Conference, 2007 World Holstein Friesian Federation, Denmark.

O'Donnell, A. M., K. P. Spatny, J. L. Vicini, and D. E. Bauman. 2010. Survey of the fatty acid composition of retail milk differing in label claims based on production management practices. Journal of Dairy Science 93:1918-1925. doi:10.3168/jds.2009-279910.1016/j.plefa.2009.05.011

Ogola, H., A. Shitandi, and J. Nanua. 2007. Effect of mastitis on raw milk compositional quality. Journal of Veterinary Science 8:237-242.

Organic Industry Standards and Certification Committee. 2013. National Standard for Organic and Bio-Dynamic Produce—Edition 3.5. Page 74. Department of Agriculture, Australia.

Palladino, R. A., F. Buckley, R. Prendiville, J. J. Murphy, J. Callan, and D. A. Kenny. 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. Journal of Dairy Science 93:2176-2184.

Palupi, E., A. Jayanegara, A. Ploeger, and J. Kahl. 2012. Comparison of nutritional quality between conventional and organic dairy products: a meta-analysis. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.5639.

Pape-Zambito, D. A., R. F. Roberts, and R. S. Kensinger. Estrone and 17 beta -estradiol concentrations in pasteurized-homogenized milk and commercial dairy products. Journal of Dairy Science; 2010. 93: 6, 2533-2540.

Partida L, E., N. Perez Villalobos, and S. Astiz Blanco. 2007. Evaluation of the conversion of a dairy cattle farm with 200 Holstein cows from conventional to organic production, in the Southern Region of Madrid. Page 21 ref. Redvet; 8 Veterinaria Organizacion S.L.

Percival, H. J. 2003. Soil and soil solution chemistry of a New Zealand pasture soil amended with heavy metal-containing sewage sludge. Australian Journal of Soil Research 41:1-17. doi:10.1071/sr01061.

Phillips, C. J. C., P. C. Chiy, D. R. Arney, and O. Kärt. 2000. Effects of sodium fertilizers and supplements on milk production and mammary gland health. Journal of Dairy Research 67:1-12.

Piccand, V., E. Cutullic, S. Meier, F. Schori, P. L. Kunz, J. R. Roche, and P. Thomet. 2013. Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system. Journal of Dairy Science 96:5352-5363. doi:10.3168/jds.2012-6444.

Pilarczyk, B., R. Pilarczyk, A. Tomza-Marciniak, A. Kowieska, J. Wojcik, P. Sablik, A. Tylkowska, and D. Hendzel. 2011. Selenium concentrations in the serum and milk of cows from organic and conventional farms in West Pomerania. Page 25 ref. Tierarztliche Umschau; 66 Terra-Verlag GmbH.

Powers, H. J. 2003. Riboflavin (vitamin B-2) and health. American Journal of Clinical Nutrition 77:1352-1360.

Prandini, A., S. Sigolo, and G. Piva. 2009. Conjugated linoleic acid (CLA) and fatty acid composition of milk, curd and Grana Padano cheese in conventional and organic farming systems. Journal of Dairy Research 76:278-282. doi:10.1017/s0022029909004099.

Qin, L. Q., X. P. Wang, W. Li, X. Tong, and W. J. Tong. 2009. The minerals and heavy metals in cow's Milk from China and Japan. Journal of Health Science 55:300-305. doi:10.1248/jhs.55.300.

Ramalho, H. M., S. D. Campos, S. Casal, R. Alves, and M. B. P. Oliveira. 2012. Lipid fraction quality of milk produced by Minhota (Portuguese autochthonous breed) compared to Holstein Friesian cow's. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.5713.

Rego, O. A., H. J. D. Rosa, S. M. Regalo, S. P. Alves, C. M. M. Alfaia, J. A. M. Prates, C. M. Vouzela, and R. J. B. Bessa. 2008. Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores. Journal of the Science of Food and Agriculture 88:1855-1859. doi:10.1002/jsfa.3289.

Revello Chion, A., E. Tabacco, D. Giaccone, P. G. Peiretti, G. Battelli, and G. Borreani. 2010. Variation of fatty acid and terpene profiles in mountain milk and "Toma piemontese" cheese as affected by diet composition in different seasons. Food Chemistry 121:393-399. doi:10.1016/j.foodchem.2009.12.048.

Rey-Crespo, F., M. Miranda, and M. López-Alonso. 2013. Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. Food and Chemical Toxicology 55:513-518. doi:10.1016/j.fct.2013.01.040.

Richert, R. M., K. M. Cicconi, M. J. Gamroth, Y. H. Schukken, K. E. Stiglbauer, and P. L. Ruegg. 2013. Management factors associated with veterinary usage by organic and conventional dairy farms. Journal of the American Veterinary Medical Association 242:1732-1743. doi:10.2460/javma.242.12.1732.

Rius, A. G., J. A. D. R. N. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. L. McGilliard, B. J. Bequette, R. M. Akers, and M. D. Hanigan. 2010. Regulation of protein synthesis in mammary glands of

lactating dairy cows by starch and amino acids. Journal of Dairy Science 93:3114-3127. doi:10.3168/jds.2009-2743.

Roche, J. R., L. R. Turner, J. M. Lee, D. C. Edmeades, D. J. Donaghy, K. A. Macdonald, J. W. Penno, and D. P. Berry. 2009. Weather, herbage quality and milk production in pastoral systems. 4. Effects on dairy cattle production. Animal Production Science 49:222-232. doi:10.1071/ea0731010.1017/ S0022029906002160

Roderick, S., and J. Burke. 2004. Organic farming in Cornwall. Results of the 2002 Farmer Survey. Organic Studies Centre, Cornwall.

Roesch, M., M. G. Doherr, and J. W. Blum. 2005. Performance of dairy cows on Swiss farms with organic and integrated production. Journal of Dairy Science 88:2462-2475.

Roesch, M., M. G. Doherr, W. Schären, M. Schällibaum, and J. W. Blum. 2007. Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. Journal of Dairy Research 74:86-92. doi:10.1017/s002202990600210x.

Rosati, A., and A. Aumaitre. 2004. Organic dairy farming in Europe. Livestock Production Science 90:41-51. doi:10.1016/j.livprodsci.2004.07.005.

Sapru, A., D. M. Barbano, J. J. Yun, L. R. Klei, P. A. Oltenacu, and D. K. Bandler. 1997. Cheddar cheese: Influence of milking frequency and stage of lactation on composition and yield. Journal of Dairy Science 80:437-446.

Schönfeldt, H. C., N. G. Hall, and L. E. Smit. 2012. The need for country specific composition data on milk. Food Research International 47:207-209. doi:10.1016/j.foodres.2011.05.018.

Schröder, M., F. Yousefi, and W. Vetter. 2011. Investigating the day-to-day variations of potential marker fatty acids for organic milk in milk from conventionally and organically raised cows. European Food Research and Technology 232:167-174. doi:10.1007/s00217-010-1374-810. 1007/s11746-010-1682-y

Shahbazkia, H. R., M. Aminlari, A. Tavasoli, A. R. Mohamadnia, and A. Cravador. 2010. Associations among milk production traits and glycosylated haemoglobin in dairy cattle; Importance of lactose synthesis potential. Veterinary Research Communications 34:1-9. doi:10.1007/s11259-009-9324-2.

Simopoulos, A. P. 2003. Importance of the ratio of ω-6 /ω-3 essential fatty acids: evolutionary aspects. World Review of Nutrition and Dietetics 92:1-22.

Simopoulos, A. P. 2010. The ω-6 /ω-3 fatty acid ratio: Health implications. OCL - Oleagineux Corps Gras Lipides 17:267-275. doi:10.1684/ocl.2010.0325.

Slots, T., J. Sorensen, and J. H. Nielsen. 2008. Tocopherol, carotenoids and fatty acid composition in organic and conventional milk. Milchwissenschaft 63:352-355.

Slots, T., G. Butler, C. Leifert, T. Kristensen, L. H. Skibsted, and J. H. Nielsen. 2009. Potentials to differentiate milk composition by different feeding strategies. Journal of Dairy Science 92:2057-2066. doi:10.3168/jds.2008-1392.

Smith-Spangler, C., M. L. Brandeau, G. E. Hunter, J. Clay Bavinger, M. Pearson, P. J. Eschbach, V. Sundaram, H. Liu, P. Schirmer, C. Stave, I. Olkin, and D. M. Bravata. 2012. Are organic foods safer or healthier than conventional alternatives?: A systematic review. Annals of Internal Medicine 157:348-366.

Smith, K. M., P. W. Abrahams, M. P. Dagleish, and J. Steigmajer. 2009. The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil-metal partitioning and potential availability to pasture herbage and livestock. Science of the Total Environment 407:3731-3739. doi:10.1016/j.scitotenv.2009.02.032.

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. Journal of Dairy Science 91:3611-3626. doi:10.3168/jds.2007-0971.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2006. Variation in fatty acid contents of milk and milk fat within and across breeds. Journal of Dairy Science 89:4858-4865.

Soyeurt, H., A. Gillon, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2007. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. Journal of Dairy Science 90:4435-4442. doi:10.3168/jds.2007-0054.

Steinshamn, H., and E. Thuen. 2008. White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livestock Science 119:202-215. doi:10.1016/j.livsci.2008.04.004.

Stergiadis, S., C. J. Seal, C. Leifert, M. D. Eyre, M. K. Larsen, and G. Butler. 2013. Variation in nutritionally relevant components in retail Jersey and Guernsey whole milk. Food Chemistry 139:540-548. doi:10.1016/j.foodchem.2013.01.078.

Stiglbauer, K. E., K. M. Cicconi-Hogan, R. Richert, Y. H. Schukken, P. L. Ruegg, and M. Gamroth. 2013. Assessment of herd management on organic and conventional dairy farms in the United States. Journal of Dairy Science 96:1290-1300. doi:10.3168/jds.2012-5845.

Stoop, W. M., H. Bovenhuis, J. M. L. Heck, and J. A. M. van Arendonk. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. Journal of Dairy Science 92:1469-1478. doi:10.3168/jds.2008-1468.

Sundberg, T., B. Berglund, L. Rydhmer, and E. Strandberg. 2009. Fertility, somatic cell count and milk production in Swedish organic and conventional dairy herds. Livestock Science 126:176-182. doi:10.1016/j.livsci.2009.06.022.

Sundberg, T., L. Rydhmer, W. F. Fikse, B. Berglund, and E. Strandberg. 2010. Genotype by environment interaction of Swedish dairy cows in organic and conventional production systems. Acta Agriculturae Scandinavica A: Animal Sciences 60:65-73. doi:10.1080/09064702.2010.496003.

Swensson, C., and H. Lindmark-Mansson. 2007. The prospect of obtaining beneficial mineral and vitamin contents in cow's milk through feed. Journal of Animal and Feed Sciences 16:21-41.

Toledo, P., A. Andrén, and L. Björck. 2002. Composition of raw milk from sustainable production systems. International Dairy Journal 12:75-80. doi:10.1016/s0958-6946(01)00148-0.

Trachsel, P., A. Busato, and J. W. Blum. 2000. Body conditions scores of dairy cattle in organic farms. Journal of Animal Physiology and Animal Nutrition 84:112-124.

Tu, W. C., R. J. Cook-Johnson, M. J. James, B. S. Mühlhäusler, and R. A. Gibson. 2010. Ω-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression. Prostaglandins Leukotrienes and Essential Fatty Acids 83:61-68. doi:10.1016/j.plefa.2010.04.001.

US Government Printing Office. 2014. Electronic Code of Federal Regulations: Regulations of the Department of Agriculture: Subchapter M–Organic Foods Production Act Provisions. Vol. 2014.

Vaarst, M., and T. W. Bennedsgaard. 2002. Reduced medication in organic farming with emphasis on organic dairy production. Acta veterinaria Scandinavica. Supplementum 43 51-57. doi:10.1186/1751-0147-43-S1-S51.

Valle, P. S., G. Lien, O. Flaten, M. Koesling, and M. Ebbesvik. 2007. Herd health and health management in organic versus conventional dairy herds in Norway. Livestock Science 112:123-132. doi:10.1016/j.livsci.2007.02.005.

van Hulzen, K. J. E., R. C. Sprong, R. van der Meer, and J. A. M. van Arendonk. 2009. Genetic and nongenetic variation in concentration of selenium, calcium, potassium, zinc, magnesium, and phosphorus in milk of Dutch Holstein-Friesian cows. Journal of Dairy Science 92:5754-5759. doi:10.3168/jds.2009-2406.

Vanhatalo, A., T. Gäddnäs, and T. Heikkilä. 2006. Microbial protein synthesis, digestion and lactation responses of cows to grass or grass-red clover silage diet supplemented with barley or oats. Agricultural and Food Science 15:252-267. doi:10.2137/14596060677

9216236.

Vetter, W., and M. Schröder. 2010. Concentrations of phytanic acid and pristanic acid are higher in organic than in conventional dairy products from the German market. Food Chemistry 119:746-752. doi:10.1016/j.foodchem.2009.07.027.

Vicini, J., T. Etherton, P. Kris-Etherton, J. Ballam, S. Denham, R. Staub, D. Goldstein, R. Cady, M. McGrath, and M. Lucy. 2008. Survey of retail milk composition as affected by label claims regarding farm-management practices. Page 30 ref. Journal of the American Dietetic Association; 108.

Villeneuve, M. P., Y. Lebeuf, R. Gervais, G. F. Tremblay, J. C. Vuillemard, J. Fortin, and P. Y. Chouinard. 2013. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. Journal of Dairy Science 96:7181-7194. doi:10.3168/jds.2013-6785.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131:389-417. doi:10.1016/j.anifeedsci.2006.06.017.

Vyas, D., B. B. Teter, and R. A. Erdman. 2012. Milk fat responses to dietary supplementation of short- and medium-chain fatty acids in lactating dairy cows. Journal of Dairy Science 95:5194-5202. doi:10.3168/jds.2011-5277.

Waiblinger, S., C. Menke, and G. Coleman. 2002. The relationship between attitudes, personal characteristics and behaviour of stockpeople and subsequent behaviour and production of dairy cows. Applied Animal Behaviour Science 79:195-219. doi:10.1016/s0168-1591(02)00155-7.

Walker, G. P., F. R. Dunshea, and P. T. Doyle. 2004. Effects of nutrition and management on the production and composition of milk fat and protein: A review. Australian Journal of Agricultural Research 55:1009-1028.

Wang, C., J. X. Liu, Z. P. Yuan, Y. M. Wu, S. W. Zhai, and H. W. Ye. 2007. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. Journal of Dairy Science 90:2960-2965.

Ward, N. I., R. R. Brooks, E. Roberts, and C. R. Boswell. 1977. Heavy-metal pollution from automotive emissions and its effect on roadside soils and pasture species in New Zealand. Environmental Science and Technology 11:917-920.

Wijesundera, C., Z. Shen, W. J. Wales, and D. E. Dalley. 2003. Effect of cereal grain and fibre supplements on the fatty acid composition of milk fat of grazing dairy cows in early lactation. Journal of Dairy Research 70:257-265. doi:10.1017/s002202990300 6241.

Wiking, L., P. K. Theil, J. H. Nielsen, and M. T. Sørensen. 2010. Effect of grazing fresh legumes or feeding silage on fatty acids and enzymes involved in the synthesis of milk fat in dairy cows. Journal of Dairy Research 77:337-342. doi:10.1017/s002202991000021x.

Zagorska, J., and I. Ciprovica. 2008. The chemical composition of organic and conventional milk in Latvia. Proceedings of the 3rd Baltic Conference on Food Science and Technology. FOODBALT-2008. 10-14. 15

Zagorska, J., and I. Ciprovica. 2005. The comparison of chemical pollution between organic and conventional milk. Research for rural development Latvia University of Agriculture 195.196-198.

Załecka, A., S. Bügel, F. Paoletti, J. Kahl, A. Bonanno, A. Dostalova, and G. Rahmann. 2014. The influence of organic production on food quality - research findings, gaps and future challenges. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.6578.

Chapter 3

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Fatty acid profile differs between organic and conventionally produced cow's milk independently of season or milking time

Summary

Variations in fatty acid profile between organic and conventionally produced cow's milk have been widely reported, and can generally be explained by differences in diet between the two farming systems. The objective of our study was to determine whether there is a difference in milk fatty acid composition when both organic and conventional farmed cows are kept in similar year round pasture grazing systems. We also investigated the effect of sampling time (throughout the year and day) on milk fatty acid composition, as well as interactions between all three factors (system, season, and time of day).

ABSTRACT

Differing amounts of fresh forage and concentrates fed, and level of input contributes to the differences reported in fatty acid (**FA**) composition of organic and conventionally-produced cow's milk. In many previous studies designed to investigate this phenomenon, comparisons were made between grazed organic cows and housed conventional cows. In the present study, we have investigated differences between organic and conventional milk produced using year-round pasture grazing, as practiced in New Zealand. FA composition was determined in milk sampled at morning and evening milking in both spring and autumn. Samples were taken from 45 cows from the Massey University organic herd and compared to 50 cows from the corresponding conventional herd grazed and managed similarly at the same location. Forty-three out of 51 analysed FA were influenced by season ($P < 0.001$), while 28 were different between production systems ($P < 0.001$). In addition, one-half were also different ($P < 0.001$) due to time of milking. Levels of linoleic acid (**LA**) and α-linolenic acid

(**ALA**) were higher in organic milk whereas conjugated linoleic acid (**CLA**) and vaccenic acid (**VA**) were higher in conventional milk ($P < 0.001$). The first three FA (LA, ALA, and CLA) were more abundant in milk harvested during autumn, and the CLA concentration was also significantly influenced by time of milking. Our results confirm reports that the FA profile is affected by season and time of milking, and we also showed an effect due to the production system, when both sets of cows were kept continuously on pasture, even after taking milking time and seasonal effect into account.

3.1 INTRODUCTION

Milk contains approximately 400 different fatty acids (FA) which makes it the most complex natural fat system (Lindmark Månsson, 2008). The FA profile in cow's milk is influenced by diet, with variations predominantly caused by differing amounts of fresh forage and concentrates eaten (Croissant et al., 2007, Coppa et al., 2013). Other factors reported to influence the FA profile of milk include differences within and between breed (Soyeurt et al., 2008, Maurice-Van Eijndhoven, 2011), season (Heck et al., 2009), climate (Kamleh et al., 2010), stage of lactation (Craninx et al., 2008) and management (Fall et al., 2008). Any of those factors, as well as the interactions between them, might contribute to the concentration of individual FA in milk, with many of the mechanisms behind those effects not fully understood. Consequently, when attempting to study the effect of one specific factor (e.g., diet) on milk FA profile, it is necessary to eliminate or to account for and control, other potential influences. Estimations of the differences between the FA composition of milk from organic and conventional farming systems are compromised in that many studies investigating the compositional disparities between organic and conventionally produced milk have not considered or been able to control factors which could have resulted in, or contributed to, such differences (e.g., diet, breed, etc.). Consequently, differences were attributed solely to the effect of the farming system. Many studies to date reporting differences between organic and conventionally produced milk have not utilized similar diets. It has to be acknowledged that this factor is most likely to differ between these systems. It demonstrates the difficulty when comparing data from organic and conventional farm data, as the factors which constitute the differences between the systems are, in most countries, irrevocable components of the systems. On the other hand, studies investigating the effect of diet did not consider the possible impact of the farming system (organic vs. conventional). Additionally, comparisons among studies are problematic as it is difficult to account for any number of variables, including sampling conditions, inherent differences in farming systems among regions, levels of inputs, and even regulatory differences in conventional and organic production among countries. This may explain the differences in quantity

for individual FA in and between systems as reported in different studies comparing organic and conventionally produced milk (Ellis et al., 2006, Collomb et al., 2008, Slots et al., 2009). A study with multiple side-by-side organic vs. conventionally managed pasture-based herds observed over several years would be desirable to account variation within each system at different locations and climatic conditions. In practice, however, it has not been possible to identify several farms which would be able to exclude the variety of influence factors we were able to exclude in our study.

The aim of the present study was to determine whether there are differences in FA composition between organic and conventional cow's milk produced in an all year round pasture grazing system as commonly used in New Zealand.

3.2 MATERIAL AND METHODS

3.2.1 Farm and Herd Data

During the 2010-2011 milking season, milk samples were collected from individual cows of one organic (45 cows) and one conventional herd (50 cows) at Massey University, Palmerston North, New Zealand (Kelly et al., 2005). Both herds were derived from a single herd which was divided in 2001 after taking breeding value, production value, somatic cell count, age and parity of each individual animal into account in order to create two matching herds. Characteristics of both farms and herds during the 2010-2011 milking season are averaged over the milking season and listed in Table 3.1, with animal data originating from monthly herd testing. Milking was conducted at 0600 h in the morning and 1400 h in the afternoon, with cows given access to new pasture after each milking event. Daylight hours were from 0544 to 2033 h, and from 0714 to 1947 h in spring and autumn, respectively. The amount of pasture available for each cow before milk sample collection in spring was 9.0 and 9.3 kgDM for conventional and 11.8 and 11.8 kgDM for organic cows, in the morning and afternoon, respectively. In autumn the amount of pasture available was 8.1 and 8.1 kgDM for conventional and 11.4 and 8.7 kgDM for organic cows, in the morning and afternoon, respectively.

A cider vinegar-garlic mixture (Dairy-Mate Direct Health Products Ltd, NZ), was added daily to the water trough of organic cows, as a food supplement and natural antibiotic (Ozturk et al., 2009). This resulted in an estimated consumption of 10 mg garlic oil per cow per day. Additionally, during late spring and early summer (October-December), organic cows were drenched daily with approximately 18 g (20 ml) of fish oil (BioSea Ltd, NZ), to influence the oestrous cycle and as a bloat preventative agent. Conventional cows were treated with antibiotics and oxytocin when necessary, which are not approved by the International Foundation for Organic

Agriculture (IFOAM). Both herds were grazed and managed similarly on different paddocks at adjacent locations under the same management, which was representative of organic and conventional dairy herds for this geographical area in New Zealand. No supplemental feed was provided to either herd in the six weeks leading up to and on the day of sampling, with pasture growth being sufficient to feed the animals.

Botanical composition of pasture from both farms was analysed twice throughout the 2010-2011 milking season (Figure 3.1). For this purpose, pasture samples were taken from 10 paddocks of each farm. Chemical composition was analysed from pasture samples from six paddocks of each farm, seven times between August 2010 and May 2011 (Table 3.2).

3.2.2 Sampling and Sample Treatment

Four milk samples each were taken from 45 organic and 50 conventional cows. One sample each from morning and afternoon milking were collected during one day in New Zealand spring (November 2010) and one day in New Zealand autumn (March 2011). Samples were taken from the milking line during the routine milking process in 100 ml plastic screw top containers and stored at -20 ºC until analysis.

Table 0.1 Farm and animal characteristics averaged over lactation period for organic and conventional herds.

	Organic	Conventional
Farm Factors		
Number of cows	45	50
Stocking rate cow/ha	2.2	2.4
N Fertilizer application in kg/ha	14.7	123.0
	Organic fertilizer[1]	urea, ammonium sulphate
Herbage yield in t DM/ha	10.4	11.4
Animal factors		
Breed[2]	F[3] 56.1 %	F 77.7 %
	J[4] 40.2 %	J 21.0 %
	A[5] 1.1 %	
Mean Days in Lactation at Sampling in		
Spring / Autumn	90 / 202	100 / 212
Mean Breeding worth[4]	79	94
Mean Production value	95	112
Mean Age, years	3.7	4.0
Mean Milk volume, l/cow day	17.2	17.8
Mean Milk protein, %	3.67	3.60
Mean Milk fat, %	5.53	5.00
Mean SCC (x 1000 cells/ml)	163.1	151.6

[1]Osflo Fertilizer Ltd, NZ

[2]Average blood blend of the herd

[3]Friesian[4]Jersey [5]Ayrshire

[4] New Zealand ranks dairy cows by their expected ability to breed high merit replacements, described as Breeding Worth (BW)

3.2.3 Fatty Acid Analyses

The extraction and methylation process described by Toledo et al. (2002) was adjusted to a smaller sample amount of 1 ml. Samples were extracted with 2 ml 2-propanol and 1.5 ml hexane which contained [1,1,1-^{13}C] Trioctanoin (Larodan Fine Chemicals, Sweden) as an internal standard. The hexane layer was dried under N_2 and the milk fat was dissolved in 1 ml hexane before the addition of 2 ml of 0.1 M KOH in methanol. Tubes were sealed and heated for 1 h at 50 ºC, to facilitate the trans-esterification process of glycerides into the corresponding FA methyl esters (**FAME**). The resulting alkali mixture was neutralised with 0.1 M HCl. The organic solvent layer containing FAMEs was diluted 1:1 (vol:vol) with hexane and then injected into a gas chromatograph-mass spectrometer (GCMS; Shimadzu *GC-17A QP5050A, Shimadzu, Japan*).

FAMEs were separated on a 60 m SGE BPX70 column (ID 0.25 mm; film thickness 0.25 μm) with a 60 min run time. Injection port and interface temperatures were maintained at 240 °C. Column temperature profile was as follows: held at 50 ºC for 5 min, increased at 12.5 ºC/min to 170 ºC, increased at 1.0 ºC/min to 193 ºC, increased at 4.0 ºC/min to 240 ºC, and held for 8 min at 240 ºC. An Inlet pressure of 180 kPa resulted in a column flow of 1.7 ml/min, with helium used as carrier gas. The mass spectrometer was used in selected ion mode and acquired data for masses 55, 67, 74, 75 and 79 m/z.

Fifty-one FAME were identified through retention time, external standard (Supelco® FAME Mix C4-C24; Sigma-Aldrich, USA), and intensity ratios of acquired ions to the base ion. The latter confirmed the degree of saturation of each individual FA (Härtig, 2008). Internal standard was identified using the base ion m/z 75. Results were calculated via cross-multiplication taking into account the peak areas of the external standard and sample FA, and the mass of the external standard for the FA concerned. FA not included in the external standard mixture were calculated using standard FA with identical chain length (e.g. C18:1 t11 was calculated using area and mass of C18:1 t9). All FA are expressed as g FA per 100 g FA.

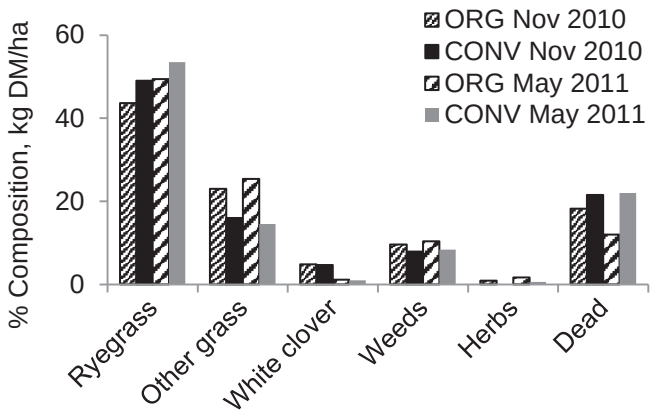


Figure 0.1 Botanical composition of organic (ORG) and conventional (CONV) pasture in spring (Nov) and autumn (May)

Table 0.2 Measured chemical composition of organic and conventional pasture

Item	Organic	Conventional	SED[1]	*P*-Value
	(*n* = 7)	(*n* = 7)		
Dry matter (DM), %	21.6	19.3	2.7	NS
Crude Protein, % DM	20.8	22.2	1.8	NS
Lipid, % DM	3.3	3.7	0.3	NS
Ash, % DM	9.4	9.5	0.7	NS
Neutral Detergent Fiber , % DM	47.1	46.4	1.4	NS
Soluble sugars and starches, % DM	11.3	12.1	1.8	NS
Metabolisable Energy (MJ/kg DM)	11.8	12.0	0.3	NS

NS $P > 0.1$

[1]Standard error of the difference

Analysis was undertaken by: feedTECH AgResearch, Palmerston North, New Zealand via NIRS, with samples (n = 7) collected between July 2010 and March 2011).

3.2.4 Statistical Analyses

Data on the composition of pasture were analysed by use of analysis of variance using a general linear model in SAS (9.3) with system as fixed and date as random effects. Data for FA were tested for normality and outliers, and statistically explored, to test for a difference between the group means, through analysis of variance using a mixed model. It included fixed effects of system (organic versus conventional), sampling date (spring versus autumn) and sampling time (morning (AM) versus afternoon (PM)), as well as their interactions, while cow within system was a random effect. An F-test was used to ascertain the degree of differences, and a multiple range test to compare the interaction combinations. The data set was further explored with principal component analysis (PCA) and discriminant function analysis (DFA). DFA is used to predict group membership of a sample into one of several naturally occurring groups. The prediction is based on linear combination of variables which discriminate between groups. In our trial, 17 FA (variables) were selected by having been identified as the best predictors of whether or not a milk sample belonged to a specific milking event, depending on system, season and time of milking.

3.3 RESULTS AND DISCUSSION

The results from the ANOVA showed a variety of differences between milk samples. These were possibly caused by a number of factors, which are discussed below.

3.3.1 System effect

Twenty-eight of the 51 FA analysed showed different concentrations ($P < 0.001$) between organic and conventional milk samples (Table 3.3). Butyric acid (C4:0), stearic acid (C18:0, $P < 0.01$), linoleic acid (C18:2 c9,12; **LA**), and α-linolenic acid (C18:3 c9,12,15; **ALA**) were higher in organic milk, whereas conventional milk contained a greater amount of odd- and branched-chain FA (**OBCFA**), as well as vaccenic acid (C18:1 t11; **VA**) and conjugated linoleic acid (C18:2 c9t11; **CLA**). These differences between organic and conventionally produced milk were observed even though cows from both herds were fed solely on pasture of similar species diversity, and similar botanical (Figure 3.1) and chemical composition (Table 3.2). Paddocks for the conventional herd had a larger proportion of ryegrass and dead material whereas those for the organic herd contained a higher amount of other grasses, weeds and herbs.

Table 0.3 Effect of system, season, and time of milking on fatty acids (FA).

	System		SED[1]	Season		SED[1]	Time of milking		SED[1]	*P*-Value						
FA (g/100g FA)	Conv[2]	Organic		Spring	Autumn		AM	PM		System	Season	Time	T*S[3]	Sys*S[4]	Sys*T[5]	Sys*T*S[6]
Even-chain saturated FA																
C4:0	2.11	2.30	0.040	2.07	2.34	0.034	2.29	2.11	0.034	***	***	***	NS	***	NS	NS
C6:0	1.69	1.78	0.027	1.74	1.74	0.020	1.79	1.68	0.020	**	NS	***	***	***	NS	†
C8:0	1.18	1.18	0.019	1.24	1.13	0.012	1.22	1.14	0.012	NS	***	***	***	***	NS	NS
C10:0	2.67	2.69	0.063	2.93	2.43	0.029	2.74	2.61	0.029	NS	***	***	***	***	**	NS
C12:0	3.22	3.20	0.084	3.49	2.92	0.036	3.27	3.15	0.036	NS	***	**	***	***	*	NS
C14:0	11.32	11.29	0.177	11.87	10.73	0.097	11.42	11.19	0.096	NS	***	*	***	*	***	NS
C16:0	31.53	32.79	0.527	31.54	32.79	0.261	32.80	31.53	0.260	*	***	***	***	NS	NS	***
C18:0	9.56	10.47	0.302	10.82	9.22	0.150	9.78	10.26	0.150	**	***	**	NS	***	†	NS
C20:0	0.091	0.104	0.0036	0.091	0.105	0.0025	0.099	0.097	0.0026	***	***	NS	***	***	***	†
C22:0	0.031	0.035	0.0015	0.030	0.037	0.0010	0.033	0.033	0.0010	**	***	NS	***	†	***	NS
C24:0	0.020	0.022	0.0009	0.020	0.022	0.0006	0.020	0.021	0.0007	†	***	NS	***	NS	***	***
Odd-chain saturated FA																
C7:0	0.021	0.016	0.0008	0.019	0.018	0.0005	0.020	0.017	0.0005	***	**	***	NS	***	*	***
C9:0	0.023	0.019	0.0010	0.022	0.020	0.0005	0.022	0.021	0.0005	***	***	*	*	**	*	NS
C11:0	0.041	0.034	0.0020	0.042	0.033	0.0009	0.038	0.037	0.0009	***	***	NS	**	**	*	**
C13:0	0.077	0.062	0.0023	0.073	0.066	0.0012	0.069	0.070	0.0012	***	***	NS	**	†	***	NS
C15:0	1.319	1.138	0.0213	1.189	1.268	0.0114	1.190	1.267	0.0114	***	***	***	***	*	***	NS
C17:0	0.578	0.566	0.0092	0.615	0.519	0.0058	0.551	0.593	0.0058	NS	***	***	**	***	***	NS
C21:0	0.017	0.021	0.0009	0.018	0.020	0.0005	0.018	0.020	0.0005	***	***	***	**	*	***	*

C23:0	0.0014	0.0018	0.00008	0.0017	0.0016	0.0001	0.0016	0.0018	0.00007	***	NS	**	***	***	***	**
C25:0	0.016	0.016	0.0007	0.016	0.016	0.0003	0.015	0.017	0.0003	NS	NS	***	***	NS	**	***
Branched-chain FA																
C13:0 iso	0.032	0.028	0.0007	0.027	0.034	0.0005	0.030	0.031	0.0005	***	***	*	NS	***	***	**
C14:0 iso	0.103	0.081	0.0021	0.090	0.095	0.0010	0.088	0.097	0.0010	***	***	***	*	***	***	**
C15:0 iso	0.409	0.317	0.0107	0.386	0.341	0.0046	0.339	0.388	0.0046	***	***	***	***	†	***	NS
C15:0 anteiso	0.478	0.381	0.0152	0.483	0.375	0.0094	0.468	0.390	0.0094	***	***	***	***	NS	NS	NS
C16:0 iso	0.184	0.156	0.0034	0.177	0.162	0.0022	0.161	0.178	0.0021	***	***	***	NS	NS	NS	†
C17:0 iso	0.331	0.288	0.0073	0.268	0.351	0.0043	0.284	0.334	0.0043	***	***	***	***	NS	***	NS
C17:0 anteiso	0.355	0.336	0.0057	0.365	0.326	0.0036	0.327	0.365	0.0036	**	***	***	***	NS	NS	***
C18:0 iso	0.030	0.028	0.0008	0.031	0.027	0.0006	0.028	0.030	0.0006	*	***	**	*	***	***	NS
Monounsaturated FA																
C10:1	0.22	0.20	0.006	0.20	0.22	0.003	0.21	0.21	0.003	***	***	†	***	***	NS	NS
C14:1 c9	1.15	0.99	0.042	0.95	1.19	0.015	1.05	1.09	0.015	***	***	*	**	NS	†	*
C16:1 c9	1.47	1.41	0.053	1.35	1.54	0.022	1.37	1.51	0.021	NS	***	***	***	***	†	NS
C16:1 t9	0.17	0.18	0.007	0.20	0.16	0.006	0.15	0.21	0.006	†	***	***	***	***	†	***
C17:1 c9	0.20	0.18	0.005	0.18	0.19	0.003	0.18	0.20	0.003	***	***	***	NS	***	**	†
C18:1 c9	16.09	15.79	0.365	15.79	16.09	0.201	15.05	16.82	0.202	NS	NS	***	***	***	NS	NS
C18:1 t9	0.21	0.18	0.007	0.18	0.22	0.005	0.20	0.20	0.005	***	***	NS	NS	†	***	NS
C18:1 c11	0.30	0.26	0.010	0.28	0.29	0.005	0.28	0.29	0.005	***	**	NS	*	***	***	NS
C18:1 t11 VA	4.26	2.59	0.195	3.50	3.34	0.066	3.28	3.57	0.066	***	*	***	***	NS	NS	NS
C20:1	0.06	0.04	0.002	0.04	0.06	0.002	0.05	0.05	0.002	***	***	NS	*	***	NS	NS
Polyunsaturated FA																

C18:2 t9,12	0.688	0.747	0.0210	0.681	0.753	0.0155	0.685	0.750	0.0158	***	***	***	*	***	*	NS
C18:2 c9,12 LA	0.814	0.909	0.0221	0.744	0.980	0.0177	0.863	0.861	0.0180	***	***	NS	***	***	***	NS
C18:3 c9,12,15 ALA	0.902	1.093	0.0339	0.927	1.068	0.0246	0.987	1.009	0.0251	***	***	NS	†	***	***	**
C18:2 c9t11 CLA	1.582	0.929	0.1074	0.998	1.512	0.0760	1.173	1.338	0.0771	**	***	**	NS	***	NS	*
C18:2 t10t12 CLA	0.053	0.046	0.0024	0.039	0.060	0.0017	0.050	0.050	0.0017	***	***	NS	***	***	**	***
C20:2 c,c (n-6)	0.031	0.036	0.0015	0.029	0.038	0.0016	0.036	0.032	0.0016	***	***	**	***	***	*	NS
C20:3 (n-6)	0.026	0.029	0.0009	0.022	0.033	0.0008	0.027	0.028	0.0008	***	***	†	***	NS	**	**
C20:3 (n-3)	0.038	0.040	0.0013	0.033	0.045	0.0012	0.038	0.040	0.0012	NS	***	*	***	***	***	†
C20:4 (n-6)	0.0100	0.0105	0.0003	0.0103	0.0102	0.0003	0.0104	0.0100	0.0003	*	NS	NS	NS	NS	NS	NS
C20:5 (n-3)	0.011	0.011	0.0005	0.010	0.012	0.0004	0.010	0.011	0.0004	NS	***	***	**	†	NS	*
C22:3 (n-3)	0.079	0.081	0.0026	0.076	0.084	0.0025	0.075	0.084	0.0025	NS	***	***	**	***	***	***
C22:5 (n-3)	0.141	0.141	0.0049	0.123	0.158	0.0036	0.136	0.145	0.0037	NS	***	***	***	***	***	***
C22:6 (n-3)	0.010	0.010	0.0003	0.011	0.009	0.0003	0.010	0.011	0.0003	†	***	***	NS	*	**	***

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, † $0.05 < P < 0.1$, NS $P > 0.1$

[1] Standard error of the difference

[2] Conventional

[3] Interaction between milking time and season

[4] Interaction between system and season

[5] Interaction between system and milking time

[6] Interaction between system, milking time, and season

The clover distribution in the paddocks was similarly low for both treatments with a seasonal variation resulting in 4.8 and 4.7% DM of pasture cover in November 2010 and 1.1 and 1.0% in May 2011 for organic and conventional pasture, respectively. Organic pasture contained a higher amount of herbs, including plantain, and other grasses (1.3 and 23.8% DM) compared to conventional pastures (0.3 and 15.2% DM). Although the herb content differed between the pastures, it was very low for both. The organic herd also had a larger amount of total pasture available per cow, 20.8 kg DM/d compared to 16.4 kg DM/d for conventional cows. None of the chemical compounds measured showed a significant difference between organic and conventional pastures. Conventional pastures produced about 10% more DM per hectare than organic pastures while receiving an approximately nine times higher application of nitrogen fertilizer. The limited number of studies conducted in a setting comparable to our trial limits possible comparisons and subsequent conclusions about the causes for the differences between the FA profile of organic and conventional milk in a low input (**LI**) farming system.

Differences in milk FA composition have been reported by Butler et al. (2009) when comparing milk from LI organic versus LI conventional dairy farms. The farming system described by Butler et al. (2009) was comparable to the present study, with the two sets of farms practicing spring block calving and an average of over 90% of the diet DM coming from grazing. Milk from LI conventional farms had, similar to our results, higher amounts of VA and CLA, whereas no difference between milk varieties was reported for LA and ALA. Butler et al. (2009) assumed that those differences were related to higher dietary intake of LA by LI conventional cows caused by differences in sward composition. Collomb et al. (2008) studied milk fat composition from organic and integrated dairy farms and found, contrary to Butler et al. (2009), higher amounts of VA, ALA, CLA and branched chain FA in organic milk. These differences were attributed to the higher amounts of grasses (87 and 83% of total DM intake for organic and conventional, respectively) and lower amounts of concentrate in the diet of organic cows. Collomb et al. (2008) suggested organic cows developed specific rumen ecology as a consequence of the higher grass diet. Kusche et al. (2010), who compared LI biodynamic and LI conventional milk, reported no significant difference in CLA levels and higher amounts of n-3 FA in LI biodynamic milk. The latter was, similarly to Collomb et al. (2008), attributed to a higher amount of fresh grass in the diet (81% biodynamic LI versus 58% conventional LI). Organic and conventional cows in our study had the same amount of pasture in their diets (100%), which were similar in chemical and botanical composition, and differences in FA profile between the two milk types must, therefore, relate to causes other than fresh forage intake.

Although not usually mentioned, differences in fertilizer application generally can be assumed for most studies comparing organic and conventional milk from pasture grazed cows. In our study, we did not observe a difference in the proximate chemical and botanical composition of pastures, despite differences in fertilizer application rates. Consequently, the impact of fertilizer in regard to those two factors appears to be minimal in our study. Similar to our results, Mackle et al. (1996) reported only minimal differences in chemical composition when comparing pastures that had high (100-150 kg/Ha) and low (20 kg/Ha) rates of N fertilizer applied. Despite similarity between the chemical compositions of pastures, Mackle et al. (1996) reported higher rumen pH in cows fed on high N-pasture, which could affect rumen ecology, and consequently could alter the rate of biohydrogenation of unsaturated FA.

Similarity in proximate chemical composition of pastures as seen in our trial should not lead to the assumption that there is no other differences between chemical compositions of the pastures. Several studies (Boufaïed et al., 2003, Elgersma et al., 2005, Arvidsson et al., 2012, and Glasser et al., 2013) reported higher content of total FA and ALA after application of various N fertilizer levels (30-120 N kg/ha) on the same forage. ALA is the most abundant FA in most common pasture grasses (Dewhurst et al., 2001, Elgersma et al., 2005), representing between 60 and 70% of all FA. Elgersma et al. (2005) reported an approximate increase of 3g ALA kg/DM in ryegrass pasture per 50 kg N/ha. This would result in a significant difference in total amount of ALA taken up by cows feeding solely on fertilized pasture compared to cows feeding on pasture with lower N fertilizer treatment.

Up to 99% of ALA and LA consumed by cows is biohydrogenated in the rumen, with VA being a main derivative (Lee and Jenkins, 2011). VA is then partly desaturated to CLA in the mammary gland, explaining the elevated content of VA and CLA in milk from predominantly grass-fed cows (Destaillats et al., 2005, Leiber et al., 2005). A higher dietary intake of ALA should consequently lead to a higher amount of VA and CLA in milk. This is supported by Leiber et al. (2005) who reported that lowland pasture containing nearly twice as much ALA resulted in 25% more CLA in milk than cows grazing alpine pasture. It can only be speculated if differences in FA composition existed between the two pastures in our trial, but other grazing studies observed similar results where VA and CLA were increased and ALA decreased in milk from cows grazing on lowland and rotational grazed pasture, respectively (Leiber et al., 2005, Coppa et al., 2011).

None of these studies mentioned fertilizer application, but this may be deduced by examining differences in plant diversity among pastures, as shown below. Leiber et al. (2005) compared the effect of

lowland and alpine pasture on milk FA composition, whereas Coppa et al. (2011) studied the differences of rotational and permanent grazing on milk FA composition. Alpine pasture and permanently grazed pasture reportedly provided a higher variety in plant species compared to lowland and rotationally grazed pasture. Although this can only be speculated, alpine pasture and permanently grazed pasture might have been treated with less fertilizer, then lowland and rotationally grazed pasture, respectively, with a more diverse botanical composition as an indicator for a lesser amount of fertilizer.

Fertilizer application and its impact on the botanical composition of pastures have been studied, with results depending on the amount and type of fertilizer applied. Mackle et al. (1996) and McKenzie et al. (1999) found that clover content in pasture was not affected by N application (45-150 N kg/ha), whereas Bolland and Guthridge, (2007) and Bochi-Brum et al. (2011) reported a continuous increase in grass content with greater N fertilizer application (60-320 N kg/ha and 60-180 N kg/ha, respectively). Lambert et al. (1986) reported a change in botanical composition over a nine year period with ryegrass continuously increasing with application of P fertilizer. Differences in composition in regard to clover and grass mixtures have been reported to affect FA in milk, with clover reportedly increasing the concentration of ALA in milk (Lourenço et al., 2007, Vanhatalo et al., 2007, Moorby et al., 2009).

A number of studies which compared pasture based diets (Wiking et al., 2010, Larsen et al., 2012) found that variation in botanical composition between pastures are reflected in the differences in chemical composition of the pasture which can then affect the milk FA composition (Falchero et al., 2010, Coppa et al., 2011, Gorlier et al., 2013). Baars et al. (2011) reported greater amounts of all branched-chain FA in milk when cows were fed hay which contained a higher amount of herbaceous plant material. Herbs, like chicory (Molan et al., 2003) and plantain (Jarzomski et al., 2000), contain condensed tannins and secondary plant metabolites which are known to influence the bio-hydrogenation of FA in the rumen (Patra and Saxena, 2011, Petersen et al., 2011). For our study, the differences in clover and herb content between farms were considered minor and could not be statistically explored due to the lack of repetitive pasture composition measurements. Consequently, we are unable to completely exclude the possibility that slight differences in botanical composition had an effect on milk FA composition.

With only a limited number of studies focussing on intensity of the farming system (high or low input) (Coppa et al., 2013), rather than the farming style (conventional or organic), and inconsistent results for

individual FA of these studies, comparing milk from LI organic and conventional dairy farms makes an explanation for our findings challenging.

Another possible cause for the differences in FA profiles in our study, as suggested by Collomb et al. (2008), may have been differences in the rumen ecology between the two herds. Our results show higher amounts ($P < 0.001$) for 11 OBCFA, including the most abundant ones (C15:0, C15:0 iso and anteiso) in conventionally produced milk. Changes in the OBCFA profile leaving the rumen, which are subsequently reflected in milk FA profile, are largely caused by alterations in the relative abundance of specific bacterial populations rather than by the availability of precursors for OBCFA (Vlaeminck et al., 2006, French et al., 2012). No samples of ruminal contents were taken in this trial and it was therefore not possible to assess if there were differences in the rumen microbiota between organic and conventional farmed cows.

The drenching of the organic herd in early lactation with fish oil and the continuous supplementation with cider-vinegar garlic was not considered to have an effect on milk FA composition in our trial. This assumption is supported by the fact that all n-3 PUFA were significantly higher in autumn milk when no fish oil supplementation occurred. Studies which reported changes in milk FA profile when using fish oil generally involved administration of the oil at 1-3% of DM intake (Donovan et al., 2000, Osborne et al., 2008, Huws et al., 2010). Fish oil contains large amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as lower levels of docosapentaenoic acid (DPA), FA which can be found at higher levels in milk from supplemented cows (Bharathan et al., 2008). Moate et al. (2013) reported a linear response between increasing DPA and DHA levels in the diet and the transfer into milk, with DPA having a greater transfer coefficient. Organic cows had been drenched continuously for 50 days before the collection of the first milk sample set in November (Spring). The amount of fish oil supplemented represented 0.2% of the DM intake. Consequently, the fish oil amount administered to organic cows in the current trial was not considered to be sufficient to increase levels of EPA, DPA, and DHA in milk.

The lack of research on the long-term effects of chronic supplementation with small doses of garlic makes it difficult to determine the impact regular supplementation of cows with cider-vinegar garlic mix could have had in our study. Garlic is known for its antimicrobial properties (Feldberg et al., 1988) and has been reported to influence ruminal volatile FA composition (Calsamiglia et al., 2007). Recent research measuring the *in vitro* effect of garlic on methanogens from the rumen observed a change in the ratio of acetate, propionate, and butyrate when 30 or 300 mg/L garlic oil was added to buffered rumen fluid (Busquet et al., 2005). The

amount of garlic oil administered to the organic cows in our trial was approximately 10 mg/d and therefore far lower, given that those were added to approximately 60 L of rumen fluid with an approximately 8 h turnover rate (Woodford and Murphy, 1988). Additionally, Cardozo et al. (2004) reported that while plant extracts modified ruminal fermentation, microbes adapted to some extracts after only six days. It is therefore questionable if the supplementation with a vinegar-garlic mix contributed to the differences in FA profiles seen between the two milk varieties.

The effect of the breed in our study was also considered as a possible influence on the FA profile in organic and conventional milk. Palladino et al. (2010) reported differences in FA profile between Holstein Friesian (HF) and Jersey cows, with higher amounts of CLA and C15:0 in milk from HF cows. In our trial, despite originating from the same herd ten years previously, herds genetically diverged, as reflected in the differences in breed composition, LW, breed and production value. While the number of Jersey and Friesian cows significantly varied between the herds ($P < 0.05$), the effect of system (organic or conventional) on FA concentration was still apparent, even when the percentage of Jersey or Friesian, was used as a covariate in the analysis. In our study, while the genomic makeup of the organic herd contained 19% more Jersey and the conventional herd had 21% more Friesian, the majority of both herds was comprised of crossbreed cows. Differences in milk FA profile has been reported between purebred Jersey and Friesen herds, however, any differences rapidly disappear in crossbreeds. Palladino et al. (2010), investigated the differences in milk FA profile between Holstein Friesian and Jersey cows, and their F1 hybrid (Jersey x Holstein). Five of the 16 FA reported were significantly different between the breeds. Milk FA profile of crossbreed and purebred cows showed even greater similarity, with only one FA (C15:0) different between Jersey and F1 hybrid, and two FA (C16:0 and LA) different between Friesian and F1 hybrid (Palladino et al., 2010). Nantapo et al. (2014) investigated milk FA profiles from Friesian, Jersey and Friesian × Jersey cross cows and reported similar results to Palladino et al. (2010) with five FA significantly different between the two purebred lines. Only one milk FA differed between each of the purebreds and the crossbreed cows. The amount of LA and palmitoleic acid (C16:1 c9) varied between Friesian and crossbreed, and Jersey and crossbreed, respectively. The reported results for the amounts of C16:0, C16:1 c9 and LA in milk of crossbreed cows relative to purebred Jersey or Friesian cows, however, did not agree between the two studies, which indicates that factors other than breed (e.g., differences in concentrate feeding) may had an influence. Both studies (Palladino et al., 2010, Nantapo et al., 2014) demonstrated that milk FA composition in purebreds differs from each other, but previous differences

disappeared upon crossbreeding. We can infer that the difference in breed percentage between the herds in our study was not large enough to statistically affect milk FA profile. Effect of season and stage of lactation

Of the 51 FA analysed, 43 were affected by the sampling date ($P < 0.001$). The New Zealand seasonal management system uses synchronous calving tied to season and pasture growth, so the stage of lactation was similar in both herds. Consequently, it is difficult to determine to what degree the differences in FA profile seen between the two sampling dates (spring and autumn) are due to changes in season, the progression of lactation, or the interaction of both factors. FA affected by different sampling dates were: branched-chain FA, all even-chain saturated FA except for hexanoic acid (C6:0), and all polyunsaturated FA except for arachidonic acid (C20:4 n6). The levels of the majority of *de novo* FA were higher in spring, except for butanoic acid (C4:0) and palmitic acid (16:0) while the levels of most polyunsaturated FA (**PUFA**), including LA, CLA, ALA, were higher in milk harvested in autumn.

As all cows were feeding solely on pasture when milk samples were taken, changes in chemical and botanical pasture composition have to be considered when trying to explain differences between the two sampling dates. Pasture quality, though, was similar for both sampling periods and average body condition scores (BSC) changed only minimally between the two sampling dates, being 3.7 and for 3.9 in spring and 3.6 and 3.8 in autumn for organic and conventional cows, respectively.

No differences in chemical composition between the pastures have been found, but no detailed analysis of individual plant compounds (e.g., FA, secondary plant metabolites) has been reported. Pasture composition changes over the season (Walker et al., 2004) and the botanical composition of pastures with mixed swards is affected by varying responses of different species to grazing intervals, nutrient input and herbage re-growth (Nie et al., 1997, Belesky et al., 1999). Differences in milk FA composition between seasons have been reported in various studies (Dunshea et al., 2008, Heck et al., 2009, Rutten et al., 2009). Larsen et al. (2012) reported a decline in ALA, LA and in C16:0 pasture during the milking season but found an increase for oleic acid (C18:1 c9). Meľuchová et al. (2008) found ALA decreased during summer, but increased in autumn, whereas C16:0, oleic acid and LA reached their maximum concentration in summer. The differences among the studies may be due to variations in botanical composition. The concentration of long-chain unsaturated FA in milk is influenced by dietary intake of those FA by the animal. ALA and LA undergo hydrogenation in the rumen to yield VA, which then acts as a precursor for CLA in the mammary gland (Walker et al., 2004). A change in ALA

concentration in pasture will, therefore, be reflected in CLA in milk (Meľuchová et al., 2008). In the present study, a higher concentration for ALA and CLA in milk was found in autumn, which supports these findings.

The stage of lactation also influences the FA profile and might therefore cause some of the changes seen. Craninx et al. (2008) observed decreased FA concentration during the first 18 weeks of lactation for C17:0, while C15:0 and C15:0 anteiso increased during this period. Kgwatalala et al. (2009) found higher amounts of C6:0, C10:0, and C12:0 and SFA in milk samples during mid-lactation (100-200 d in lactation), compared to milk samples from late lactation (>200 d in lactation). Oleic acid and MUFA were significantly lower in mid-lactation compared to early and late lactation, whereas no significant difference was observed for the concentration of VA, LA, ALA and CLA between mid and late lactations (Kgwatalala et al., 2009). Similar trends were observed for the first 21 weeks of lactation by Gross et al. (2011) with concentrations of C8:0, C10:0, C12:0, and C16:0 lower during the first four weeks of lactation, but increasing in mid lactation (week 17-21), while the reverse was seen for oleic acid (C18:1 c9). Stoop et al. (2009) detected the same trend for saturated and unsaturated FA and reported no change for levels of odd-chain FA (C5:0 - C15:0), but saw a strong increase in C16:0 and a decrease in C18:0 during mid-lactation. Our results agree with findings by Kgwatalala et al. (2009), Stoop et al. (2009) and Gross et al. (2011) for *de novo* synthesised FA, which may indicate that those FA are influenced predominantly by stage of lactation rather than season. Changes in diet throughout the milking season as reported by Gross et al. (2011) have to be considered when reporting changes in concentration of *de novo* FA in milk. Differences in forage to concentrate ratios will change the ratio of acetate and butyrate versus propionate coming from the rumen, affecting the amount of de novo FA and protein in the milk. The pronounced increase in oleic acid levels during the first weeks of lactation, as described by Kgwatalala et al. (2009) and Gross et al. (2011), indicated a negative energy balance and lipid mobilization from adipose tissue in high yielding animals. In our study, no difference in the concentration of oleic acid was found during different stages of lactation, suggesting that in both herds cows were able to sufficiently support their energy demands by feed intake. Dunshea et al. (2008) found no correlation for variations in concentration of VA and CLA and calving time during the year. This suggests that these FA are influenced by season rather than stage of lactation.

3.3.2 Time of the day effect

Levels of twenty-seven of the 51 FA we quantified differed between morning and afternoon milk samples (Table 3.4). Even-chain saturated FA (C4:0 - C16:0) were increased in morning milk, whereas higher amounts of OBCFA and VA, oleic acid and CLA were found in milk samples collected in the afternoon. Due to

differences in experimental protocols (e.g., restricted pasture access, and once a day pasture allocation) the comparability of our results with those from other trials (Loor et al., 2003, Sun and Gibbs, 2012) is limited.

Effects of diurnal variation and time since last milking on milk composition are not unprecedented. Some of these effects are because chemical composition of pasture undergoes diurnal changes, and herbage from temperate pastures often increases in nutritional value throughout the day (Vibart et al., 2012). Dry matter and water soluble carbohydrates (WSC) become more concentrated and accumulate over the day (Fulkerson and Donaghy, 2001, Orr et al., 2001) due to evaporation and photosynthesis (Griggs et al., 2005). Water soluble carbohydrate content positively influences palatability (Horadagoda et al., 2009) and affects grazing behavior and rumination frequency as a consequence (Orr et al., 2001, Sun and Gibbs, 2012). In a study by Orr et al., 2001, they reported a longer (> 4 h), more continuous grazing period after afternoon milking and before sunset when dairy cows were offered a new pasture allocation compared to a shorter (2 to 3 h) and more intermittent grazing period when fresh pasture was offered after morning milking. Bite mass, bite frequency and time spent ruminating were also affected by time of pasture allocation (Orr et al., 2001), with ruminating time for cows given afternoon pasture allocation shorter than for cows which received fresh pasture after morning milking.

In our study, cows had access to fresh pasture after each milking, but differences in grazing pattern caused by diurnal changes in WSC content in pasture cannot be excluded. Differences in grazing behaviour and time spent ruminating add physical influence factors such as rumen fill and rumen passage rate to the already mentioned changes in chemical composition throughout the day. Findings from Sun and Gibbs (2012) correspond readily to changes in grazing behaviour reported by Orr et al. (2001). In their trial, cows which received once daily pasture allocation after afternoon milking showed a significant reduction in ruminl pH and OBCFA, and plant derived PUFA in the rumen significantly increased 2 h after pasture allocation (Sun and Gibbs, 2012). Reduction in pH was explained by the fast intake and rapid fermentation of a large amount of DM which overwhelmed the buffering system in the rumen as a consequence. In relation to milk composition, Loor et al. (2003) studied differences in milk FA profile for cows having limited access to pasture in the morning or afternoon while being fed TMR outside of grazing hours. Cows with access to afternoon grazing derived a larger amount of their DMI from pasture than cows which grazed in the morning, which might be a result of previously mentioned changes in WSC concentration, palatability and consequent grazing behaviour. When morning and afternoon milking were exactly 12 h apart, however, little variability in milk FA composition was reported (Loor et al., 2003). The only FA affected by time of milking were VA and CLA (C18:2, c9t11), both were increased

($P < 0.05$) in milk from cows with access to pasture in the afternoon, which indicates a difference in grazing behaviour depending on time of pasture allocation (Sun and Gibbs, 2012).

Besides changes in grazing behaviour and WSC content, a larger impact on milk FA profile may result from differences in time since last milking, with 16 and 8 h since last milking for morning and afternoon milking, respectively. Despite mentioned differences in WSC content, access to fresh pasture after morning milking leads to an extended grazing period (> 2 h), which results in a reduction in ruminal pH and increase in ruminal VFA (Sun and Gibbs, 2012). In addition to diurnal changes in ruminal VFA, a similar diurnal response has been reported for urea in rumen fluid, plasma, and milk (Gustafsson and Palmquist, 1993) and for the concentration of indole and skatole in milk (Lane, 2008). Gustafsson and Palmquist (1993) reported a time lag of 1.5 – 2.0 h between rumen ammonia peak and urea peak in serum, with an additional further 1 - 2 h between serum peak and urea peak in milk Lane et al. (2008) reported two-fold higher amounts of indole and skatole in milk from afternoon milking compared to morning milking in cows which had been grazed continuously. As indole and skatole are formed in the rumen as products from tryptophan degradation (originating from forage protein), similar diurnal changes resulting from time since last milking would not be unexpected for long chain PUFA derived from feed and could explain differences we observed in our trial.

3.3.3 Interactions

In our study, only three out of 51 FA showed no significant interactions (P <0 .001) between at least two of the three factors considered (time of the day, season, and system). Depending on the FA, effect of system varied for different seasons and time of the day. Similarly, sampling time throughout the day had a greater or a lesser effect for individual FA during different seasons. Interactions between all three factors were observed for ten FA ($P < 0.001$), including palmitic acid (C16:0), DPA and DHA.

A PCA was conducted to achieve an overall view of the difference between the groups, considering multiple FA simultaneously. It showed that all cows within one farming system are similarly affected by season and sampling time throughout the day. Seventeen FA (C4:0, C10:0, C11:0, C12:0, C13:0, C13 iso, C14:0 iso, C15:0 iso, C15:0 anteiso, C17:0 iso, C18:0 iso, C17:1 c9, C18:1 t9, VA, LA, C18:2 t10t12, and C22:6n3) were selected considering their loadings, and the PCA analysis was performed on a matrix of 17 analytical parameters, for 354 samples. The first eight principal components explain 89% of the total variance whereas PC1 and PC2 describe 53% of the total variance. The FA accounting for most variation in the PCA were C10:0 and C14:0 iso (positive loading) and C13:0 and LA (negative loading) for PC1 and PC2, respectively. For better visibility the

PCA results were divided into four individual plots: Spring AM, Spring PM, Autumn AM, and Autumn PM (Figure 3.2).

Discriminant function analysis (DFA) was performed using eight classes of data grouped by system x season x time to define a set of discriminant functions (DFs). The data was then re-evaluated using the DFs to assign them the closest group and the proportion of correctly and mis-assigned data determined (Table 3.4), with the same 17 FA chosen as predictors. The same predictors were also used to classify the samples into two groups with 95% correctly assigned into organic or conventional milk, and 96% correctly assigned into spring or autumn, respectively. The FA accounting for the most variation between the groups were C13:0 iso and C18:0 iso (positive loading) and C11:0 and C14:0 iso (negative loading) for the first and the second variate, respectively.

In our trial it is, therefore, possible to discriminate between organic and conventionally produced milk. Further research is necessary to determine whether the same DFs can be applied to other sets of organic and conventionally produced milk.

Discussion on interactions among factors (system, season, and time of milking) is purely speculative as not enough is known on the effect each individual factor alone. Our study excluded a large number of known influence factors, but still the drivers of reported differences in milk FA for each remaining factor is not known with certainty. It cannot be assumed either that the effect of two or more factors (system, season, and time of milking) is equal to the sum of each individual effect. No pattern or trend could be identified in our study, and interpretation of the multitude of different interactions is complicated by the lack of understanding of the causes. The effect of interactions has to be explained for each individual FA to be put into wider context of changes in milk FA profile. Taking ALA, VA and CLA, as examples (Figure 3.3), only ALA and CLA show a three-way interaction between system, season and time of milking. ALA is higher in organic milk throughout the first three time points (Spr × AM, Spr × PM, Aut × AM), while in conventional milk ALA is significantly increased in Aut × AM and is even greater in Aut x PM, compared to spring. VA is significantly higher in conventional milk throughout all sampling points with concentrations showing the same trend for organic and conventional milk, with an increase in Spr × PM. The concentration of CLA in conventional milk is, similar to VA, higher than organic milk through all sampling points, with a marked increase in Aut × AM and a further rise in Aut × PM.

Table 0.4 Proportion of correctly grouped data assigned by Discriminant Function Analysis.

Group	Spr[1]×AM[3]×Conv[5]	Spr×AM×Org[6]	Spr×PM[4]×Conv	Spr×PM×Org	Aut2×AM×Conv	Aut×AM×Org	Aut×PM×Conv	Aut×PM×Org
Proportion	0.936	0.956	0.900	0.929	0.891	0.791	0.870	0.800

[1] Spring [2] Autumn [3] Morning [4] Afternoon [5] Conventional [6] Organic

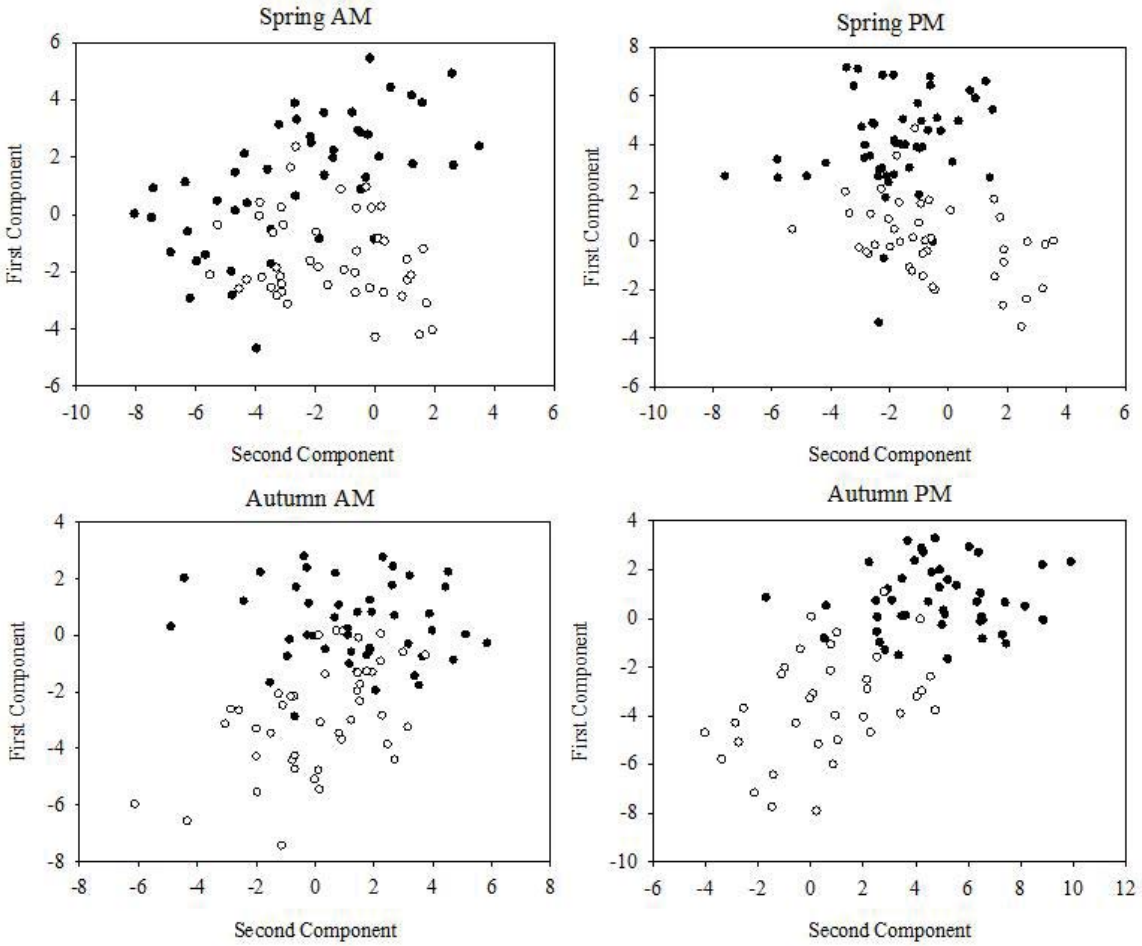
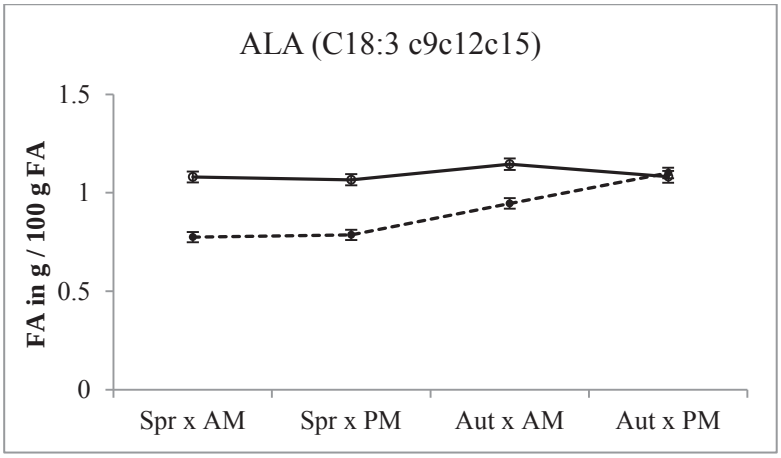


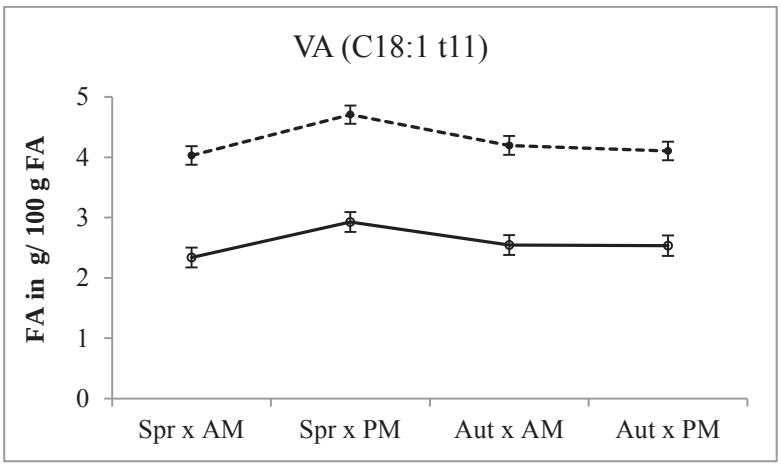
Figure 0.2 Principal component analysis of eight milk sets collected, separated by season and sampling time of the day. Organic milk is represented by (○) and conventional milk by (●).

In organic milk CLA levels are relatively constant, except for a lower concentration in Spr × AM. There is a noticeable similarity in concentration changes between ALA and CLA over the four sampling points within each system.

Although we can only speculate, significantly higher levels of VA and CLA in conventional milk may be related to greater amounts of N fertilizer applied to conventional pastures. Presumably, this could have resulted in higher amounts of ALA in the conventional pasture (as discussed above under 'system effect'). The fact that VA showed the same trend in organic and conventional milk leads us to speculate that in our study, apart from dietary intake of ALA, there are no other major influence factors on VA in milk. In addition, as VA is the major precursor of CLA in the mammary gland, an overall greater amount of CLA in conventional milk is equally linked to a higher amount N fertilizer. CLA and ALA showed an increase in autumn compared to spring sampling, which was more marked in conventional milk as well.

Differences in concentration between sampling months can be related to a combination of interdependent factors, e.g. changes in stage of lactation, Δ9-desaturase activity (Heck, 2009), and FA composition in feed (Khiaosa-Ard, 2010). Further investigation would be necessary to quantify the specific impact of each of these factors on the FA profile.





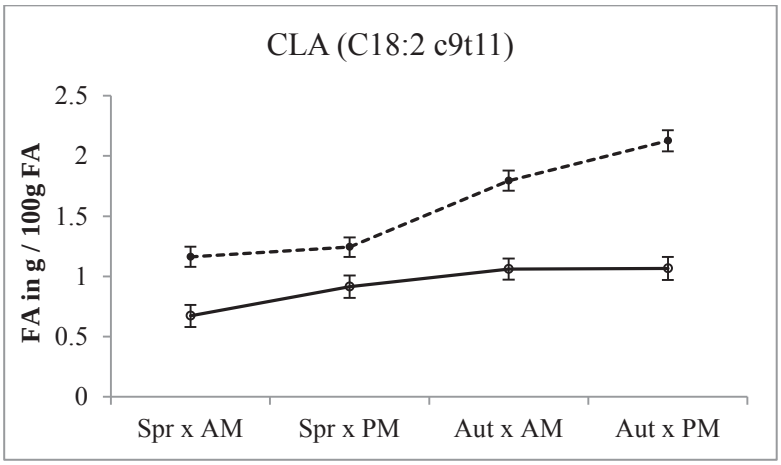


Figure 0.3 Interactions of α-linolenic acid (ALA), vaccenic acid (VA), and conjugated linoleic acid (CLA) concentration on sampling time for each system. Organic milk is represented by (–○–) and conventional milk by (- -●- -)

3.4 CONCLUSIONS

The present study indicated that in a system where many of the factors known to influence milk FA composition have been controlled, differences between organic and conventional milk samples can still be found. Several possible causes for variation in FA profile were discussed, and some were excluded (e.g., fish oil supplementation of the organic herd, and differences in botanical composition). The influence of several other factors, among them application rate of N fertilizer on pasture, time between milking, and time between main feeding time and milking, requires further investigation to determine their impacts on milk FA profile. Future studies on milk FA profile will also benefit from consideration of the FA composition of pasture and ruminal fluid. In addition, our results showed how much variation in FA profile in each milk sample can be accounted for by sampling time throughout the day and throughout the year. This will help to understand some of the variation across results presented in different studies comparing organic and conventional milk FA.

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REFERENCES

Baars, T., J. Wohlers, D. Kusche, and G. Jahreis. 2011. Experimental improvement of cow milk fatty acid composition in organic winter diets. Journal of the Science of Food and Agriculture. doi:10.1002/jsfa.5525.

Belesky, D. P., J. M. Fedders, K. E. Turner, and J. M. Ruckle. 1999. Productivity, botanical composition, and nutritive value of swards including forage chicory. Agronomy Journal 91:450-456.

Bharathan, M., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, M. L. Gibson, and K. Karges. 2008. Conjugated linoleic acid increases in milk from cows fed condensed corn distillers solubles and fish oil. Journal of Dairy Science 91:2796-2807. doi:10.3168/jds.2007-0938.

Bochi-Brum, O., R. García, R. Bodas, A. Calleja, S. Andrés, and S. López. 2011. Nutritive value of herbage from mountain hay meadow managed under traditional and intensive harvest systems as affected by nitrogen fertilisation and time of cutting. Animal Production Science 51:549-556. doi:10.1071/an10182Bolland, M. D. A., and I. F. Guthridge. 2007. Responses of intensively grazed dairy pastures to applications of fertiliser

nitrogen in south-western Australia. Australian Journal of Experimental Agriculture 47:927-941. doi:10.1071/ea06014

Bolland, M. D. A., and I. F. Guthridge. 2007. Responses of intensively grazed dairy pastures to applications of fertiliser nitrogen in south-western Australia. Australian Journal of Experimental Agriculture 47:927-941. doi:10.1071/ea0601410.1016/0377-8401(88)90044-2

Boufaïed, H., P. Y. Chouinard, G. F. Tremblay, H. V. Petit, R. Michaud, and G. Bélanger. 2003. Fatty acids in forages. I. Factors affecting concentrations. Canadian Journal of Animal Science 83:501-511.

Busquet, M., S. Calsamiglia, A. Ferret, P. W. Cardozo, and C. Kamel. 2005. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. Journal of Dairy Science 88:2508-2516.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science 90:2580-2595. doi:10.3168/jds.2006-644.

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2004. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. Journal of Animal Science 82:3230-3236.

Collomb, M., W. Bisig, U. Buetikofer, R. Sieber, M. Bregy, and L. Etter. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. International Dairy Journal 18:976-982.

Coppa, M., A. Ferlay, F. Monsallier, I. Verdier-Metz, P. Pradel, R. Didienne, A. Farruggia, M. C. Montel, and B. Martin. 2011. Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. Journal of Dairy Science 94:1132-1145. doi:10.3168/jds.2010-3510.

Coppa, M., A. Ferlay, C. Chassaing, C. Agabriel, F. Glasser, Y. Chilliard, G. Borreani, R. Barcarolo, T. Baars, D. Kusche, O. M. Harstad, J. Verbič, J. Golecký, and B. Martin. 2013. Prediction of bulk milk fatty acid composition based on farming practices collected through on-farm surveys. Journal of Dairy Science 96:4197-4211. doi:10.3168/jds.2012-6379.

Craninx, M., A. Steen, H. Van Laar, T. Van Nespen, J. Martin-Tereso, B. De Baets, and V. Fievez. 2008. Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. Journal of Dairy Science 91:2662-2677. doi:10.3168/jds.2007-0656.

Croissant, A. E., S. P. Washburn, L. L. Dean, and M. A. Drake. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. Journal of Dairy Science 90:4942-4953.

Destaillats, F., M. de Wispelaere, F. Joffre, P. A. Golay, B. Hug, F. Giuffrida, L. Fauconnot, and F. Dionisi. 2006. Authenticity of milk fat by fast analysis of triacylglycerols. Application to the detection of partially hydrogenated vegetable oils. Journal of Chromatography A 1131:227-234. doi:10.1016/j.chroma.2006.07.040.

Dewhurst, R. J., N. D. Scollan, S. J. Youell, J. K. S. Tweed, and M. O. Humphreys. 2001. Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. Grass and Forage Science 56:68-74.

Donovan, D. C., D. J. Schingoethe, R. J. Baer, J. Ryali, A. R. Hippen, and S. T. Franklin. 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. Journal of Dairy Science 83:2620-2628.

Dunshea, F. R., G. P. Walker, E. Ostrowska, and P. T. Doyle. 2008. Seasonal variation in the concentrations of conjugated linoleic and trans fatty acids in milk fat from commercial dairy farms is associated with pasture and grazing management and supplementary feeding practices. Australian Journal of Experimental Agriculture 48:1062-1075. doi:10.1071/ea07286

Elgersma, A., P. Maudet, I. M. Witkowska, and A. C. Wever. 2005. Effects of Nitrogen fertilisation and regrowth period on fatty acid concentrations in perennial ryegrass (Lolium perenne L.). Annals of Applied Biology 147:145-152.

Ellis, K. A., G. Innocent, D. Grove-White, P. Cripps, W. G. McLean, C. V. Howard, and M. Mihm. 2006. Comparing the fatty acid composition of organic and conventional milk. Journal of Dairy Science 89:1938-1950.

Falchero, L., G. Lombardi, A. Gorlier, M. Lonati, M. Odoardi, and A. Cavallero. 2010. Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. Dairy Science and Technology 90:657-672. doi:10.1051/dst/2010035.

Fall, N., K. Forslund, and U. Emanuelson. 2008. Reproductive performance, general health, and longevity of dairy cows at a Swedish research farm with both organic and conventional production. Livestock Science 118:11-19. doi:10.1016/j.livsci.2008.01.017.

Feldberg, R. S., S. C. Chang, A. N. Kotik, M. Nadler, Z. Neuwirth, D. C. Sundstrom, and N. H. Thompson. 1988. In vitro mechanism of inhibition of bacterial cell growth by allicin. Antimicrobial Agents and Chemotherapy 32:1763-1768.

French, E. A., S. J. Bertics, and L. E. Armentano. 2012. Rumen and milk odd- and branched-chain fatty acid proportions are minimally influenced by ruminal volatile fatty acid infusions. Journal of Dairy Science 95:2015-2026. doi:10.3168/jds.2011-4827.

Fulkerson, W. J., and D. J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Australian Journal of Experimental Agriculture 41:261-275. doi:10.1071/ea00062.

Glasser, F., M. Doreau, G. Maxin, and R. Baumont. 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Animal Feed Science and Technology 185:19-34.

Gorlier, A., M. Lonati, M. Renna, C. Lussiana, G. Lombardi, and L. M. Battaglini. 2013. Changes in pasture and cow milk compositions during a summer transhumance in the western Italian Alps. Journal of Applied Botany and Food Quality 85:216-223.

Griggs, T. C., J. W. MacAdam, H. F. Mayland, and J. C. Burns. 2005. Nonstructural carbohydrate and digestibility patterns in orchardgrass swards during daily defoliation sequences initiated in evening and morning. Crop Science 45:1295-1304. doi:10.2135/cropsci2003.0613.

Gross, J., H. A. Van Dorland, R. M. Bruckmaier, and F. J. Schwarz. 2011. Milk fatty acid profile related to energy balance in dairy cows. Journal of Dairy Research 78:479-488. doi:10.1017/s0022029911000550.

Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76:475-484.

Härtig, C. 2008. Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography-mass spectrometry database. Journal of Chromatography A 1177:159-169. doi:10.1016/j.chroma.2007.10.089.

Heck, J. M. L., H. J. F. van valenberg, J. Dijkstra, and A. C. M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. Journal of Dairy Science 92:4745-4755. doi:10.3168/jds.2009-2146.

Horadagoda, A., W. J. Fulkerson, K. S. Nandra, and I. M. Barchia. 2009. Grazing preferences by dairy cows for 14 forage species. Animal Production Science 49:586-594. doi:10.1017/S002185969800584.

Huws, S. A., M. R. F. Lee, S. M. Muetzel, M. B. Scott, R. J. Wallace, and N. D. Scollan. 2010. Forage type and fish oil cause shifts in rumen bacterial diversity. FEMS Microbiology Ecology 73:396-407. doi:10.1111/j.1574-6941.2010.00892.x.

Jarzomski, C. M., N. E. Stamp, and M. D. Bowers. 2000. Effects of plant phenology, nutrients and herbivory on growth and defensive chemistry of plantain, Plantago lanceolata. Oikos 88:371-379.

Kamleh, R., J. Fanni, and E. El Mayda. 2010. The characteristics of cow's milkfat produced in four different dairy regions in Bekaa Valley (Lebanon). Journal of Food, Agriculture and Environment 8:182-185.

Kelly, T., N. Butcher, K. Harrington, C. Holmes, D. Horne, P. Kemp, A. Palmer, A. Quinn, N. Shadbolt, and A. Thatcher. 2005. Organic-conventional dairy systems trial in New Zealand: four years' results. Pages 268-271 in Researching sustainable systems. Proceedings of the First Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR), held in Cooperation with the International Federation of Organic Agriculture Movements (IFOAM) and the National Association for Sustainable Agriculture, Australia (NASAA), Adelaide Convention Centre, Adelaide, South Australia, 21-23 September, 2005. U. Kopke, U. Niggli, D. Neuhoff, P. Cornish, W. Lockeretz, and H. Willer, ed. International Society of Organic Agricultural Research (ISOFAR), Bonn.

Kgwatalala, P. M., E. M. Ibeagha-Awemu, A. F. Mustafa, and X. Zhao. 2009. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. Animal Genetics 40:609-615. doi:10.1111/j.1365-2052.2009.01887.x.

Khiaosa-Ard, R., F. Klevenhusen, C. R. Soliva, M. Kreuzer, and F. Leiber. 2010. Transfer of linoleic and linolenic acid from feed to milk in cows fed isoenergetic diets differing in proportion and origin of concentrates and roughages. Journal of Dairy Research 77:331-336. doi:10.1017/s0022029910000257.

Kusche, D., K. Ruebesam, and T. Baars. 2010. Fatty acids and antioxidant profiles in summer milk from different biodynamic and conventional systems in Southern Germany. (Grassland Science in Europe, Volume 15). H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage, and A. Hopkins, ed. Mecke Druck und Verlag, Duderstadt.

Lambert, M. G., D. A. Clark, D. A. Grant, D. A. Costall, and Y. S. Gray. 1986. Infuence of fertiliser and grazing management on North Island moist hill country. 4. Pasture species abundance. New Zealand Journal of Agricultural Research 29:23-31.

Lane, G.A., K. Fraser, M. Yu, D. Pacheco, D.E. Dalley and L.A. Hofmann. 2008. BRIEF COMMUNICATION: Effect of once daily milking on milk flavour chemistry. Proceedings of the New Zealand Society of Animal Production. Vol 68: 162-163

Larsen, M. K., X. C. Fretté, T. Kristensen, J. Eriksen, K. Søegaard, and J. H. Nielsen. 2012. Fatty acid, tocopherol and carotenoid content in herbage and milk affected by sward composition and season of grazing. Journal of the Science of Food and Agriculture 92:2891-2898. doi:10.1002/jsfa.5620.

Lee, Y. J. and T. C. Jenkins. 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. Journal of Nutrition 141(8):1445-1450.

Leiber, F., M. Kreuzer, D. Nigg, H. R. Wettstein, and M. R. L. Scheeder. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids 40:191-202. doi:10.1007/s11745-005-1375-3.

Lindmark Månsson, H. 2008. Fatty acids in bovine milk fat. Food and Nutrition Research 52. doi:10.3402/fnr.v52i0.1821.

Loor, J. J., F. D. Soriano, X. Lin, J. H. Herbein, and C. E. Polan. 2003. Grazing allowance after the morning or afternoon milking for lactating cows fed a total mixed ration (TMR) enhances trans 11-18:1 and cis9,trans 11-18:2 (rumenic acid) in milk fat to different extents. Animal Feed Science and Technology 109:105-119. doi:10.1016/s0377-8401(03)00175-5.

Lourenço, M., G. Van Ranst, B. Vlaeminck, S. De Smet, and V. Fievez. 2008. Influence of different dietary forages on the fatty acid composition of rumen digesta as well as ruminant meat and milk. Animal Feed Science and Technology 145:418-437. doi:10.1016/j.anifeedsci.2007.05.043.

Mackle, T. R., C. R. Parr, and A. M. Bryant. 1996. Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. New Zealand Journal of Agricultural Research 39:341-356.

Maurice-Van Eijndhoven, M. H. T., S. J. Hiemstra, and M. P. L. Calus. 2011. Short communication: Milk fat composition of 4 cattle breeds in the Netherlands. Journal of Dairy Science 94:1021-1025. doi:10.3168/jds.2009-3018.

McKenzie, F. R., J. L. Jacobs, M. J. Ryan, and G. Kearney. 1999. Effect of rate and time of nitrogen application from autumn to midwinter on perennial ryegrass-white clover dairy pastures in western Victoria. 2. Pasture nutritive value. Australian Journal of Agricultural Research 50:1067-1072. doi:10.1071/ar98197.

Meľuchová, B., J. Blaško, R. Kubinec, R. Górová, J. Dubravská, M. Margetín, and L. Soják. 2008. Seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. Small Ruminant Research 78:56-65.

Moate, P. J., S. R. O. Williams, M. C. Hannah, R. J. Eckard, M. J. Auldist, B. E. Ribaux, J. L. Jacobs, and W. J. Wales. 2013. Effects of feeding algal meal high in docosahexaenoic acid on feed intake, milk production, and methane emissions in dairy cows. Journal of Dairy Science 96:3177-3188.

Molan, A. L., A. J. Duncan, T. N. Barry, and W. C. McNabb. 2003. Effects of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworm and gastrointestinal nematodes. Parasitology International 52:209-218.

Moorby, J. M., M. R. F. Lee, D. R. Davies, E. J. Kim, G. R. Nute, N. M. Ellis, and N. D. Scollan. 2009. Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. Journal of Dairy Science 92:1148-1160. doi:10.3168/jds.2008-1771.

Nantapo, C. T. W., V. Muchenje, and A. Hugo. 2014. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian × Jersey cross cow milk under a pasture-based dairy system. Food Chemistry 146:127-133.

Nie, Z. N., A. D. Mackay, D. J. Barker, I. Valentine, and J. Hodgson. 1997. Changes in plant population density, composition and sward structure of a hill pasture during a pastoral fallow. Grass and Forage Science 52:190-198.

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56:352-361.

Osborne, V. R., S. Radhakrishnan, N. E. Odongo, A. R. Hill, and B. W. McBride. 2008. Effects of supplementing fish oil in the drinking water of dairy cows on production performance and milk fatty acid composition. Journal of Animal Science 86:720-729. doi:10.2527/jas.2007-0342.

Ozturk, A., Y. Ozdemir, and Z. Goksel. 2009. Apple vinegar and its therapeutic effects. TABAD, Tarim Bilimleri Arastirma Dergisi 2:155-158.

Palladino, R. A., F. Buckley, R. Prendiville, J. J. Murphy, J. Callan, and D. A. Kenny. 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. Journal of Dairy Science 93:2176-2184.

Patra, A. K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture 91:24-37. doi:10.1002/jsfa.4152.

Petersen, M. B., K. Søegaard, and S. K. Jensen. 2011. Herb feeding increases n-3 and n-6 fatty acids in cow milk. Livestock Science 141:90-94. doi:10.1016/j.livsci.2011.05.004.

Rutten, M. J. M., H. Bovenhuis, K. A. Hettinga, H. J. F. van Valenberg, and J. A. M. van Arendonk. 2009. Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. Journal of Dairy Science 92:6202-6209. doi:10.3168/jds.2009-2456.

Slots, T., G. Butler, C. Leifert, T. Kristensen, L. H. Skibsted, and J. H. Nielsen. 2009. Potentials to differentiate milk composition by different feeding strategies. Journal of Dairy Science 92:2057-2066. doi:10.3168/jds.2008-1392.

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. Journal of Dairy Science 91:3611-3626. doi:10.3168/jds.2007-0971.

Stoop, W. M., H. Bovenhuis, J. M. L. Heck, and J. A. M. van Arendonk. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. Journal of Dairy Science 92:1469-1478. doi:10.3168/jds.2008-1468.

Toledo, P., A. Andrén, and L. Björck. 2002. Composition of raw milk from sustainable production systems. International Dairy Journal 12:75-80. doi:10.1016/s0958-6946(01)00148-0.

Vanhatalo, A., K. Kuoppala, V. Toivonen, and K. J. Shingfield. 2007. Effects of forage species and stage of maturity on bovine milk fatty acid composition. European Journal of Lipid Science and Technology 109:856-867.

Vibart, R. E., D. Pacheco, K. Lowe, B. A. Barrett. 2012. The effects of time of allocation of a ryegrass-based pasture on animal performance, nitrogen utilization and grazing behavior from late-lactation dairy cows. Journal of Dairy Science 95(2):727.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131:389-417. doi:10.1016/j.anifeedsci.2006.06.017.

Walker, G. P., F. R. Dunshea, and P. T. Doyle. 2004. Effects of nutrition and management on the production and composition of milk fat and protein: A review. Australian Journal of Agricultural Research 55:1009-1028.

Wiking, L., P. K. Theil, J. H. Nielsen, and M. T. Sørensen. 2010. Effect of grazing fresh legumes or feeding silage on fatty acids and enzymes involved in the synthesis of milk fat in dairy cows. Journal of Dairy Research 77:337-342. doi:10.1017/s002202991000021x.

Woodford, S. T., and M. R. Murphy. 1988. Effect of forage physical form on chewing activity, dry matter intake, and rumen function of dairy cows in early lactation. Journal of Dairy Science 71:674-686.

Chapter 4

This chapter is published as presented:

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Pasture feeding conventional cows removes differences between organic and conventionally produced milk

Highlights

- CLA and VA are increased in conventional milk from cows fed pasture
- Oligosaccharide composition is variable between organic and conventionally produced milk
- Protein and milk volatile composition are independent from farming system

ABSTRACT

Perceptions of production methods for organic and conventional milk are changing, with consumers prepared to pay premium prices for milk from either certified organic or conventional grass-fed cows. Our study investigated whether chemical composition differed between milk produced by these two farming systems. Sampling was conducted on two farms sets, each comprised of one organic and one conventional farm. All farms applied year-round pasture grazing. Milk samples were collected throughout the milking season and analysed for free oligosaccharides, fatty acids, major casein and whey proteins, and milk fat volatiles. Fatty acids were influenced by breed and fertilizer application. Oligosaccharides differed between farming systems, with causes presently unknown, while farm set was the dominant influence factor on protein composition. Factors identified in this study influencing milk composition are not exclusive to either farming system, and pasture feeding conventional cows will remove differences previously reported for organic and conventionally produced milk.

4.1 INTRODUCTION

Organic food products are commonly perceived by consumers to be healthier. That is a key reason for their purchase and the acceptance of premium prices (Lee & Yun, 2015). Previous research investigating differences between organic and conventionally produced milk and dairy products has focussed predominantly on the fatty acid (FA) profile. The majority of these studies reported higher amounts of beneficial poly-unsaturated FA, including conjugated linoleic acid (CLA, C18:2 c9t11), in organic milk (Tunick, Van Hekken, Paul, Ingham, & Karreman, 2016). The grass- or pasture-based diets fed to organic cows have been identified as the main cause for differences in FA composition between organic and conventional milk (Jahreis, Pritsche, & Steinhart, 1997). Although essential to produce "certified organic" dairy products, grazing on pasture is not exclusive to organic dairy systems, with consumers willing to pay premium prices for milk from conventional, pasture-fed cows (Elgersma, Tamminga, & Ellen, 2006). A similar diet for organic and conventional pasture-fed cows creates a major obstacle for testing the authenticity of organic milk as all suggested organic markers are based predominantly on the amount of fresh pasture a cow consumes. Presently it is questionable whether the FA profile of organic milk differs from that of pasture based conventional milk if animals are provided with comparable pasture access and pasture composition.

Few studies have investigated compounds other than FA when looking for differences between organic and conventionally produced milk (Payling, Juniper, Drake, Rymer, & Givens, 2015), with most of the differences reported resulting from diet differences between the organic and conventional cows.

In the present study, we constructed a more complete image of organic and conventional milk by investigating the composition of the proteins, oligosaccharides (OS) and volatile compounds in addition to FA. Protein composition, which is less susceptible than FA to diet, has received little attention in relation to organic and conventional milk production (Kuczyńska, Puppel, Gołębiewski, Metera, Sakowski, & Słoniewski, 2012). Current interest in bovine milk OS is dominated by the desire to create a bovine-based infant formula that mimics human milk in OS composition and concentration (Lee, MeloSilva, Liu, & Barile, 2015). Individual animal genetics, breed, and stage of lactation are known to influence milk OS composition (McJarrow & Van Amelsfort-Schoonbeek, 2004; Tao, DePeters, German, Grimm, & Lebrilla, 2009). Furthermore, bovine diet may influence OS composition in milk (Asakuma, Ueda, Akiyama, Uemura, Miyaji, Nakamura, et al., 2010; Liu, Moate, Cocks, & Rochfort, 2014), with greater total sialic acid concentration observed in milk from grazed cows, although research is limited.

Volatile compounds in milk fat have been investigated primarily in relation to flavour components derived from different diets and the development of off-flavours during heat treatment or storage (Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011). Also volatile secondary plant metabolites taken up in the diet might influence rumen microbiota (Collomb, Bisig, Buetikofer, Sieber, Bregy, & Etter, 2008), which in turn can affect the FA composition in milk. We analysed the composition of volatile compounds as it can provide information to observed differences in other compound classes.

The aim of our study was to investigate differences between organic and conventional milk from pasture-based systems. Comparisons of milk composition are commonly conducted between pasture-fed organic cows and conventional cows that are housed indoors and provided harvested feed. Reported differences in composition between organic and conventional cows are, therefore, the result of differences in diet and do not reflect the organic or conventional status of the animal. Knowledge about the chemical composition of milk from pasture based conventional and organically raised cows will provide consumers with greater confidence when choosing either certified organic or grass-fed dairy products, based on presumed health benefits.

4.2 MATERIAL AND METHODS

4.2.1 Farm and animal data

For this study, two sets of dairy farms in New Zealand were selected where a certified organic farm was directly adjacent to one that was managed conventionally, with farms in each set operated similarly under the same management. Both organic farms were managed according to Organic Foods Production Act Provisions 2014 (US Government Printing Office, 2014). Farm Set 1 belonged to Massey University, Palmerston North (38.23° S, 175.86° E), while approximately 320 km north near Tokoroa, Farm Set 2 was privately owned (40.38° S, 175.61° E). In both sets, both organic and conventional herds were on an all year round pasture grazing system as is commonly practiced in New Zealand. The cows in each respective herd in Farm Set 1 originated from one single herd that was split in 2001 and two matching herds were achieved by taking into account breed value, production value, somatic cell count, age, and parity of each individual cow (Schwendel, Morel, Wester, Tavendale, Deadman, Fong, et al., 2015). The organic and conventional herds in Farm Set 2 were originally from different farms, with both farms coming under the same management in 2007. Descriptions, and supplemental feeding of both farm sets, are shown in Table 4.1 and *Supplementary data, Table 4.2*.

Massey University Animal Ethics Committee approval was not required as no additional animal manipulations were undertaken to collect samples and farms adhered to all relevant laws pertaining to production animals in New Zealand.

4.2.2 Sample collection

Bulk milk samples were collected approximately twice per week from Farm Set 1 between August and May during the 2010-2011 milking season (n = 120 samples). Weekly bulk milk samples from Farm Set 2 were collected between October and March during the 2012-2013 milking season (n = 40 samples). All milk samples were collected in 200 ml polyethylene screw-top containers, subsampled and stored at -20 °C until analysis.

4.2.3 Oligosaccharide analysis

The method was adapted from (Liu, Moate, Cocks, & Rochfort, 2014) for detection of free oligosaccharides. Protein-bound oligosaccharides were not investigated. Milk samples were defatted by centrifugation at 4 °C (30 min, 12,400 × g). Skim milk samples were filtered through a 10 kDa filter (Vivaspin 500, GE Healthcare) to remove proteins. The filtrate (2 µl) was injected into a UHPLC system (Accela 1250, Thermo Scientific, Waltham, MA) using a Hypercarb column (100 mm × 2.1 mm, 5 µm particle size, Thermo Fisher Scientific, Auckland, NZ) combined with high resolution mass spectrometry (Q-Exactive, Thermo Scientific, Waltham, MA) in negative ionization mode. A gradient elution was conducted starting with 100% solvent A (0.1% formic acid in water), increasing to 35% and 95% solvent B (0.1% formic acid in acetonitrile), at 15 and 18 min respectively, applying a flow rate of 300 µl/min, before returning back to 100% solvent A after 21 min. Full scan data were collected in profile data acquisition mode over the mass range from 300 to 2000 mass over charge (m/z) and processed using the Xcalibur software package. Chromatographic features were tentatively assigned as oligosaccharides based on their accurate mass, considering adducts and source induced fragments, using The Human Metabolome Database as reference database (Wishart, Jewison, Guo, Wilson, Knox, Liu, et al., 2013). With the exception of m/z 632.2038, which represented 3'sialyllactose (3-SL) and 6'sialyllactose (6-SL) and was identified using external standards (prepared in-house from goat milk), overlapping isomers were not analysed separately. Results were presented as peak areas.

Table 0.1 Farm and animal factors of conventional and organic dairy farms from Farm Sets 1 and 2.

	Farm Set 1		**Farm Set 2**	
Farm characteristics	Conventional	Organic	Conventional	Organic
Number of cows	50	45	158	150
Stocking rate, cow/ha	2.4	2.2	2.2	2.2
Soil type	Silt loam	Silt loam	Pumice	Pumice
White clover in pasture, %	0.9 - 1.4	1.0 - 1.4	40	50 - 60
N Fertilizer	123.0	14.7	141.8	6.6
in kg/ha / Product	Urea, NH_4SO_4	organic fertilzer[1]	fertilzer[2]	organic fertilzer[1]
Elevation, m	40	40	326	326
Animal factors				
Herd composition				
Holstein-Friesian, %	77.7	56.1	92.6	62.5
Jersey, %	21.0	40.2	7.3	35.5
Ayrshire, %	--	3.7	--	2.1
Breed worth[3]	94	79	121/51	111/50
Production worth[4]	112	95	127/69	148/68
Cow age, y	4.0	3.7	4.5	4.7
Average calving date ± Stdev, d	21/08/2010 ± 18	26/08/2010 ±15	17/08/2012 ± 19	25/08/2012 ± 21
Milk volume per cow, l/d	17.9	16.4	25.1	22.2
Milk solids per cow, kg/d	1.5	1.5	2.1	2.0
Milk fat, %	4.8	5.3	4.9	5.1

[1] Osflo Fertilizer Ltd., New Plymouth, New Zealand.

[2] n-rich Multi, Ballance Agri-Nutrients Ltd., Tauranga, New Zealand

[3] Breed worth ranks male and female animals for their genetic merit for individual traits

[4] Production worth ranks female animals for their lifetime production ability

Table 0.2 ***Supplementary data.*** **Feed supplementation throughout the milking period for Farm sets 1 and 2.**

Farm	Supplement, in kg DM/cow	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Set 1											
Conventional	Hay	24	2	4							
	Maize silage	152	96								
	Grass silage		29	10		37					
	Baleage[1]					29	56	9	48		22
	PKE[2]	6									
	Turnips						52	112	20		
Organic	Hay	68	10	7							
	Baleage	133	69			22	73	36	20	139	34
	Turnips						48	16			
Set 2											
Conventional	Grass silage			21				102	212		
	PKE			12			51	186	92		
Organic	Grass silage					3		7			

[1] PKE – Palm kernel expeller
[2] Grass baled and wrapped immediately after cutting

4.2.4 Fatty acids

Samples were analysed for FA, with fatty acid methyl esters (FAME) seperated by a BPX70 column (ID 0.25 mm; film thickness 0.25 µm; 60 m; SGE, Trajan Scientific Australia Pty Ltd) with a Shimadzu GC-17A (Auckland, NZ) system and a Shimadzu QP5050A mass spectrometer as detector. Extraction and methylation have been conducted using the method described by (Schwendel, et al., 2015). Forty-eight FAME were identified using retention time, external standards (Supelco® F.A.M.E. Mix C4-C24; Sigma-Aldrich, Auckland, NZ), and the intensity ratios of the acquired ions to the base ion. The latter was used to confirm the degree of saturation of each individual FA, as described by (Härtig, 2008). Results were expressed as g FA per 100 g fat.

4.2.5 Protein analysis

Milk samples were analysed for protein according to the method of (Day, Williams, Otter, & Augustin, 2015). Briefly, 200 µl of milk were added to 600 µl of buffer (6 M guanidine hydrochloride, 0.1 M Bis-Tris, and 5.37 mM sodium citrate, pH 7). Samples were centrifuged and 500 µl of the supernatant was added to 490 µl of 4.5 M guanidine hydrochloride and 10 µl of 2-mercaptoethanol. Sample analysis was performed using a Shimadzu LC10ADvp HPLC system equipped with a UV-VIS detector (SPD 10A vp, Shimadzu). Samples were injected onto a Hi-Pore RP-318 column (250 mm × 4.80 mm, 5 µm particle size, Bio-Rad, NZ) and gradient elution was performed with a flow rate of 1 ml/min; solvent A (0.1% trifluoroacetic acid in water), solvent B (0.1% trifluoroacetic acid in 90% acetonitrile, Optima LC/MS, Fisher Chemical, New Zealand). Major milk proteins were identified using external standards (α-, β-, and κ-casein, and α-lactalbumin, β-lactoglobulin, Sigma-Aldrich, Auckland NZ) and comparison with HPLC traces reported by (Bordin, Cordeiro Raposo, De La Calle, & Rodriguez, 2001). Percentages of relative abundance of individual proteins were calculated based on peak area ratios from sample peaks and external standards.

4.2.6 Milk fat volatile compounds analysis

Milk samples were centrifuged at 4 °C (30 min, 1,000 × g). The resulting cream layer (5-10 g) was diluted with saturated NaCl and extracted by steam distillation with *t*-butyl methyl ether for 90 min using a Likens-Nickerson apparatus. Para-cresol and sodium butyrate *(prepared in-house)* were added as internal standards. Samples were analysed using a Shimadzu GC-17A gas chromatograph with a QP-5050A mass spectrometer as detector. Chromatographic separation was conducted with a BPX70 column (ID 0.25 mm; film thickness 0.25 µm; 60 m; SGE, Trajan Scientific Australia Pty Ltd 2015). Helium was used as the carrier gas

with a total flow rate of 126.1 ml/min and the sample was applied in split-less mode. The interface temperature was kept at 250 °C. After sample injection, the injector, interface, and ion source temperatures were held at 250 °C, while the oven temperature was held at 35 °C for 10 min, and then increased at 4 °C per min to 250 °C. Mass detection started after 4 min, with each sample analysed twice in SCAN (29 to 500 m/z) and selected ion mode. Selected ions monitored represent compounds of interest: indole 117/90 *m/z*, skatole 130/131 *m/z*, lactone derivate 99/ 71/ 55 *m/z*, garlic extract compounds 41/114 *m/z*, and cyclic terpenes 93 *m/z*. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST14 Mass Spectral Database.

4.2.7 Statistical analysis

Data from each farm set was tested for normality and outliers, and statistically explored to test for a difference between the group means using a general linear model that included the random effect of sample collection date throughout the milking period, and the fixed effect of farming system (organic or conventional). Each farm set was analysed separately. Analysis was carried out with SAS version 9.4 (Cary, NC, USA). The data set was further explored with principal component analysis (PCA). Heatmaps were prepared using Metaboanalyst 3.0 (Xia, Sinelnikov, Han, & Wishart, 2015)

4.3 RESULTS

An increase in SFA and a decrease in PUFA in the milk of the conventional herd in Farm Set 2 corresponded to the supplementary feeding of palm kernel expeller, which contained predominantly C12:0, C14:0 and C18:1 c9 (Figure 4.1 Supplementary data). As a consequence, the last five sampling points from Farm Set 2 were excluded from the analysis presented in Table 4.4. We did not observe effects of supplemental feeding on other milk components in samples taken when palm kernel expeller was fed and thus no data were excluded from those analyses.

4.3.1 Oligosaccharide

Eleven chromatographic features of the correct calculated m/z values, observed in all milk samples independent of farm set and sampling date, were putatively assigned as the corresponding bovine milk oligosaccharides (Table 4.3). Both farm sets showed similarity in both the absolute peak areas of each OS and the variability between farm systems. All OS were affected by sampling date ($P<0.001$, Farm Set 1; $P\leq0.05$, Farm Set 2), while approximately one-third were affected ($P\leq0.01$) by farming system independent of farm set. Two OS levels were affected by farm system depending on farm set, and four OS were unaffected by either

farming system or farm set. Four OS concentrations were increased *(P<0.05*) in organic milk (3 Hex; 3 Hex, 1 NeuAc; 4 Hex, 1 HexNAc; 3 Hex, 2 HexNAc) independent of the farm set.

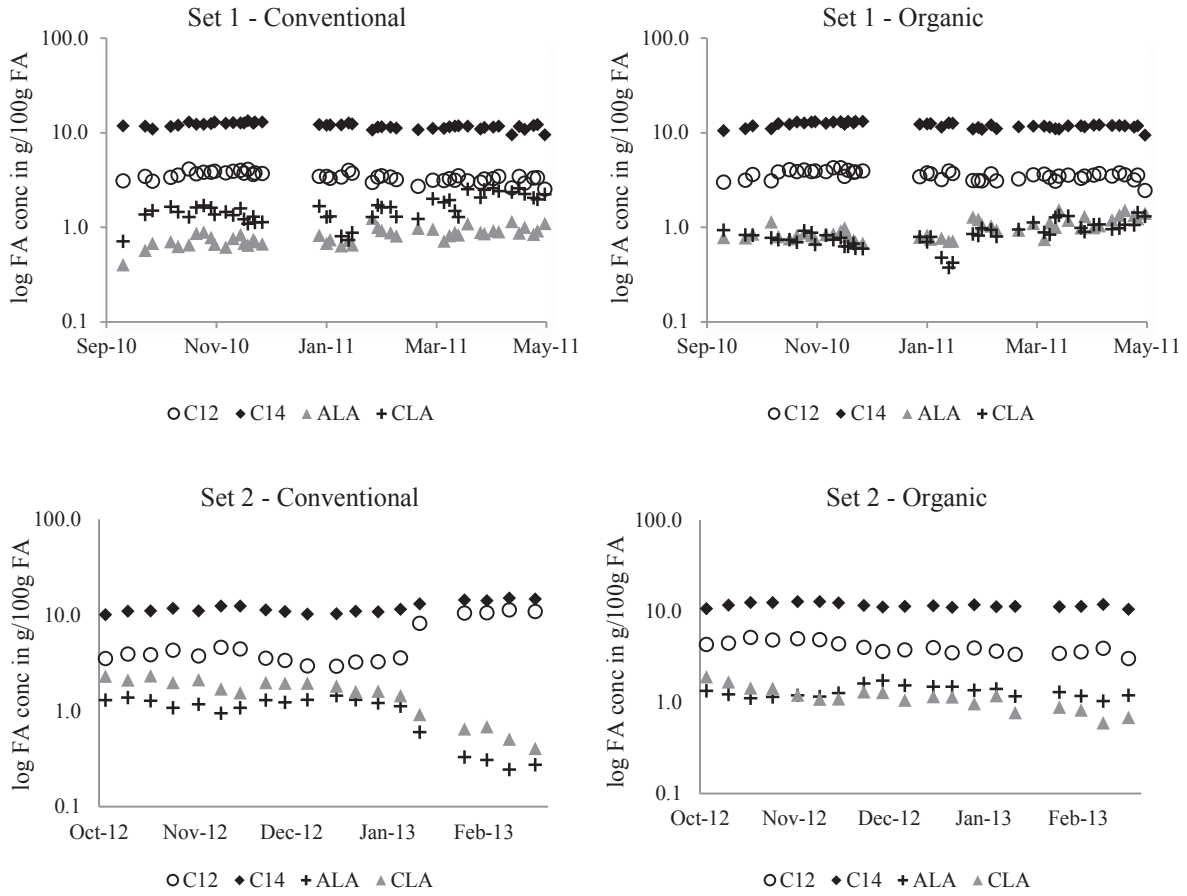

Figure 0.1 Concentration of selected fatty acids (FA) (○ C12:0; ◆ C14:0; ▲ ALA, C18:3 c9c12c15; + CLA, C18:2 c9t11) throughout the sampling period in organic and conventional milk from Farm Sets 1 and 2.

Table 0.3 Oligosaccharide in bulk milk samples from conventional (Con) and organic (Org) farms[1].

					Farm Set 1 (n=104)			**Farm Set 2** (n=40)			**Set 1**	**Set 2**
					Con	**Org**		**Con**	**Org**		**System**	
Oligosaccharide composition		m/z (exp)	Δ ppm	rt (min)	**Mean (Area, E+06)**		**SEM**	**Mean (Area, E+06)**		**SEM**	***P-value***	
3 Hex	Trisa	503.1618	1.1	5.19	55.86	60.82	1.15	51.37	61.11	2.10	0.004	0.004
2 Hex, 1 HexNAc	GLN	544.1876	0.5	5.04	123.51	105.40	2.84	105.45	98.22	3.77	<.0001	0.192
2 Hex, 1 NeuAc	6'SL	632.2044	0.9	8.27	81.03	86.48	1.96	83.97	90.94	3.84	0.060	0.216
2 Hex, 1 NeuAc	3'SL	632.205	1.9	13.40	615.09	675.22	25.24	554.43	648.41	22.80	0.105	0.009
2 Hex, 1 NeuGc		648.2002	2.2	12.77	3.69	3.72	0.08	3.50	3.71	0.14	0.773	0.313
1 Hex, 1 HexNAc, 1 NeuAc	6'SLN	673.2322	2.6	8.70	2.14	1.99	0.06	1.84	1.89	0.08	0.106	0.675
3 Hex, 1 HexNAc		706.2416	1.3	6.02	3.03	3.13	0.05	2.79	2.99	0.09	0.168	0.147
3 Hex, 1 NeuAc		794.2587	2.6	14.14	12.42	14.60	0.28	9.24	11.83	0.47	<.0001	0.001
2 Hex, 1 HexNAc, 1 NeuAc		835.2859	3.2	8.08	0.56	0.67	0.03	0.58	0.80	0.07	0.029	0.039
4 Hex, 1 HexNAc		868.2963	3.3	6.91	0.69	0.93	0.02	0.63	0.87	0.04	<.0001	0.000
3 Hex, 2 HexNAc		909.3253	5.8	6.11	0.25	0.31	0.01	0.25	0.33	0.02	0.000	0.014

[1]exp and Δ ppm, experimental m/z values and residual error in ppm, for deprotonated molecular ions (detected in negative mode); Trisa, Trisaccharides; Hex, glucose or galactose; HexNAc, N-acetylglucosamine or N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid (sialic acid); and NeuGc, N-glycolylneuraminic acid.

4.3.2 Fatty acid

Farm set, farm system (organic vs conventional), and sampling date significantly influenced milk FA composition. Of the 48 FA analysed, 22 where affected (*P≤0.001*) by the farming system in Farm Set 1, compare to 9 FA in Farm Set 2 (Table 4.4). Sampling date affected all reported FA (*P<0.01*) in Farm Set 1, compared to 13 FA in Farm Set 2, respectively. When considering individual FA classes, 7 of 11 saturated FA (SFA) were either similar for milk produced across both farming systems or greater (*P<0.01*) in organic milk for both farm sets. For the eight reported odd-chain FA (OFA), no similarities between farm sets could be observed, with four OFA greater (*P≤00.01*) in organic milk from Farm Set 2, while three OFA were increased in conventional milk from Farm Set 1. All branched-chain FA (BFA), except C13:0 *iso*, were greater (*P≤0.005*) in conventional milk from Farm Set 1, while Farm Set 2 showed fewer differences between milk produced in the two farming systems, with only C17:0 *iso* and C17:0 *anteiso* different between systems *(P<0.001)*. All mono-unsaturated FA were greater *(P<0.01)* in conventional milk at Farm Set 1, while four mono-unsaturated FA were similarly abundant between the two farming systems inFarm Set 2, and only vaccenic acid (VA) was greater *(P<0.001)* in conventional milk. Poly-unsaturated FA (PUFA) were similar between both farm sets, with conjugated linoleic acid (C18:2 c9t11, CLA) more abundant in conventional milk (*P<0.001)* and both linoleic acid (C18:2 9c12c, LA) and α-linolenic acid (C18:3 c9c12c15, ALA) more prevalent in organic milk (*P<0.05*). Of the 48 reported FA, 45 were significantly influenced by the sampling date (*P≤0.001*) on Farm Set 1, while only one-third were affected on Farm Set 2 (*P ≤ 0.001*), with PUFA the FA class least affected by sampling date, independent of farm set and system.

4.3.3 Protein

Total milk casein and whey concentrations did not vary between farm systems independent of the sampling date (Table 4.5), but the farm set had an effect on overall protein composition (*Supplementary data, Figure 4.2*) with Farm Set 2 showing a higher amount of casein per 100 g protein. Relative abundance of individual proteins varied between farm sets, especially with regards to whey protein composition. Milk from both farms in Set 2 contained less than half the amount of β-lactoglobulin B than milk from farms of Set 1.

Table 0.4 Fatty acids in bulk milk samples from conventional (Con) and organic (Org) farms.

Fatty acid (FA)	Farm Set 1 (n=104)			Farm Set 2 (n=30)			Set 1	Set 2	Set 1	Set 2
	Con	Org		Con	Org		System		Date	
(in g per 100 g fat)	Mean		SEM	Mean		SEM	*P-value*			
Saturated FA										
C4:0	2.82	2.95	0.03	2.78	2.82	0.03	<.0001	0.554	0.001	0.333
C6:0	1.90	2.00	0.02	2.26	2.40	0.03	<.0001	0.002	<.0001	0.013
C8:0	1.32	1.37	0.01	1.45	1.61	0.02	0.002	<.0001	<.0001	0.001
C10:0	2.93	3.07	0.02	3.20	3.66	0.04	<.0001	<.0001	<.0001	<.0001
C12:0	3.40	3.51	0.03	3.98	4.23	0.25	0.011	0.496	<.0001	0.453
C14:0	11.80	11.85	0.07	11.31	11.65	0.14	0.611	0.102	<.0001	0.014
C16:0	30.12	31.47	0.29	25.65	26.43	0.19	0.002	0.013	0.001	<.0001
C18:0	9.82	10.95	0.15	10.91	11.24	0.07	<.0001	0.006	<.0001	0.002
C20:0	0.058	0.066	0.001	0.087	0.096	0.002	<.0001	0.008	<.0001	0.050
C22:0	0.019	0.022	0.001	0.030	0.038	0.002	0.001	0.034	<.0001	0.891
C24:0	0.015	0.016	0.001	0.030	0.038	0.003	0.256	0.068	<.0001	0.784
Odd-chain FA										
C7:0	0.021	0.021	0.001	0.029	0.034	0.001	1.000	0.001	0.003	<.0001
C9:0	0.024	0.024	0.001	0.032	0.039	0.001	0.642	<0.001	0.001	<.0001

C11:0	0.043	0.041	0.001	0.052	0.067	0.002	0.115	<.0001	<0.001	<.0001
C13:0	0.072	0.066	0.001	0.086	0.100	0.002	<.0001	<.0001	<0.001	<.0001
C15:0	1.25	1.15	0.01	1.18	1.21	0.01	<.0001	0.025	<.0001	<.0001
C17:0	0.528	0.491	0.008	0.566	0.567	0.009	0.003	0.916	<.0001	0.302
C21:0	0.013	0.015	0.000	0.016	0.022	0.002	0.005	0.023	<.0001	0.960
C23:0	0.001	0.001	0.000	0.001	0.002	0.000	<.0001	0.014	<.0001	0.819
C25:0	0.013	0.014	0.001	0.023	0.028	0.003	0.168	0.290	<.0001	0.306
Branched-chain FA										
C13:0 iso	0.029	0.028	0.000	0.032	0.037	0.001	0.010	0.015	<0.001	0.580
C14:0 iso	0.088	0.075	0.001	0.082	0.082	0.002	<.0001	1.000	<.0001	0.001
C15:0 iso	0.245	0.228	0.002	0.428	0.425	0.004	<.0001	0.610	<.0001	<.0001
C15:0 anteiso	0.421	0.357	0.003	0.371	0.359	0.004	<.0001	0.079	<.0001	<.0001
C16:0 iso	0.560	0.492	0.006	0.172	0.167	0.003	<.0001	0.250	<.0001	0.004
C17:0 iso	0.336	0.298	0.003	0.307	0.279	0.003	<.0001	<.0001	<.0001	<.0001
C17:0 anteiso	0.361	0.339	0.003	0.387	0.350	0.003	<.0001	<.0001	<.0001	0.023
C18:0 iso	0.027	0.024	0.001	0.025	0.027	0.001	0.002	0.213	<.0001	0.810
Mono-unsaturated FA										
C10:1	0.22	0.21	0.00	0.24	0.25	0.00	0.001	0.178	<.0001	0.086

C14:1 c9	1.02	0.91	0.01	1.04	0.99	0.01	<.0001	0.020	<.0001	0.006
C16:1 c9	1.60	1.53	0.02	1.29	1.27	0.01	0.009	0.231	<.0001	0.001
C17:1 c9	0.22	0.19	0.00	0.19	0.18	0.00	<.0001	0.202	<.0001	0.109
C18:1 t9	0.21	0.18	0.01	0.20	0.18	0.01	<0.001	0.206	<.0001	0.334
C18:1 t11 (VA)	3.53	2.28	0.07	5.31	3.75	0.13	<.0001	<.0001	0.001	<.0001
C18:1 c9	18.57	17.84	0.19	18.07	17.47	0.16	0.011	0.018	<.0001	<.0001
C18:1 c11	0.29	0.25	0.00	0.28	0.30	0.01	<.0001	0.084	<.0001	0.372
Poly-unsaturated FA										
C18:2 c9,12 (LA)	0.708	0.816	0.012	1.06	1.12	0.02	<.0001	0.041	<.0001	<.0001
C18:3 c9,12,15 (ALA)	0.802	0.971	0.017	1.19	1.37	0.04	<.0001	0.008	<.0001	0.112
C18:2 c9t11 (CLA)	1.58	0.86	0.04	1.83	1.33	0.05	<.0001	<.0001	<.0001	0.002
C20:2 t (n-6)	0.048	0.045	0.002	0.053	0.052	0.003	0.123	0.869	<.0001	0.777
C20:2 c (n-6)	0.044	0.038	0.001	0.038	0.045	0.002	0.003	0.067	<.0001	0.278
C20:3 (n-6)	0.023	0.026	0.001	0.031	0.034	0.002	0.077	0.292	0.003	0.631
C20:3 (n-3)	0.036	0.037	0.001	0.038	0.045	0.003	0.617	0.118	0.006	0.510
C20:4 (n-6)	0.011	0.012	0.000	0.010	0.013	0.001	0.371	0.054	0.001	0.443
C22:3 (n-3)	0.067	0.072	0.001	0.097	0.111	0.005	0.004	0.061	<.0001	0.428
C20:5 (n-3) (EPA)	0.026	0.025	0.001	0.021	0.019	0.001	0.822	0.320	<.0001	0.007

C22:5 (n-3) (DPA)	0.132	0.137	0.003	0.145	0.149	0.009	0.355	0.740	<.0001	0.519
C22:6 (n-3) (DHA)	0.014	0.015	0.001	0.010	0.008	0.001	0.146	0.029	<.0001	0.320
Sum of FA	97.23	97.26	0.06	96.41	96.51	0.06	0.689	0.267	<.0001	<.0001
Saturated FA	64.17	67.25	0.29	61.50	64.02	0.42	<.0001	0.001	<.0001	0.003
Odd & branched chain FA	3.99	3.63	0.02	3.77	3.78	0.03	<.0001	0.885	<.0001	<.0001
Monounsaturated FA	25.57	23.33	0.22	26.61	24.39	0.28	<.0001	<.0001	<.0001	0.001
Polyunsaturated FA	3.48	3.04	0.05	4.51	4.30	0.12	<.0001	0.227	<.0001	0.095

Table 0.5 Casein and whey proteins in bulk milk samples from conventional (Con) and organic (Org) farms.

Protein	**Farm Set 1** (n=104)			**Farm Set 2** (n=40)			**Set 1**	**Set 2**	**Set 1**	**Set 2**
	Con	**Org**		**Con**	**Org**		**System**		**Date**	
(in g per 100 g protein)	**Mean**		**SEM**	**Mean**		**SEM**	***P-value***			
Casein										
α_{s1}- casein	28.22	29.07	0.15	27.34	27.12	0.10	<0.001	0.132	<0.001	0.561
α_{s2}-casein	12.55	12.15	0.17	14.79	14.79	0.15	0.094	0.998	<0.001	0.291
β-casein	26.84	26.20	0.10	28.50	28.37	0.12	<0.001	0.447	<0.001	0.109
κ-casein	13.15	13.79	0.11	14.32	14.50	0.15	<0.001	0.424	<0.001	0.582
Sum casein	81.06	81.00	0.15	85.01	84.71	0.18	0.781	0.239	0.260	0.600
Whey protein										
α-lactalbumin	1.47	1.57	0.03	4.64	4.62	0.07	0.017	0.847	<0.001	0.069
β-lactoglobulin A	6.58	6.18	0.07	5.73	5.88	0.11	<0.001	0.345	0.040	0.550
β-lactoglobulin B	11.00	11.22	0.10	4.89	4.80	0.07	0.143	0.415	<0.001	0.842
Sum of whey proteins	18.94	19.07	0.15	14.99	15.29	0.18	0.547	0.239	0.246	0.600

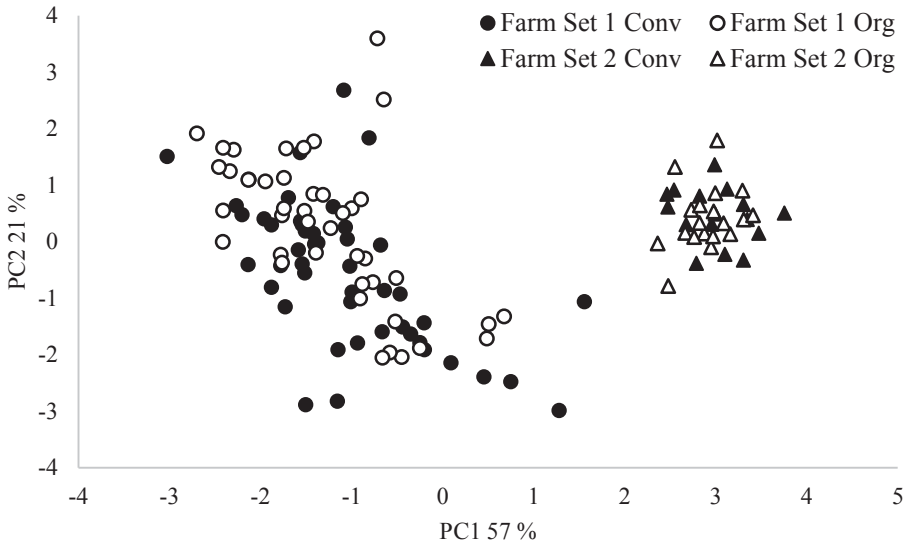

Figure 0.2 Supplementary Data. Principal component analysis of all major milk proteins in milk from (○) organic and (●) conventional Farm Set 1, and (△) organic and (▲) conventional Farm Set 2

Composition of individual casein and whey proteins in milk from Farm Set 1 varied between organic and conventional milk, with α_{s1}- casein and κ-casein significantly greater in organic milk, while β-casein and β-lactoglobulin A were greater in conventional milk. Furthermore, protein composition of Farm Set 1 was significantly *($P<0.05$)* affected by sampling date (*Supplementary data, Figure 4.3*) with κ-casein increased in late lactation, while β-lactoglobulin B was reduced throughout the lactation period. No compositional changes were observed in Farm Set 2 between farm systems and throughout the sampling period.

4.3.4 Milk fat volatile compounds

We putatively identified 33 volatile compounds in the milk samples (Figure 4.5). Twenty-one of the 33 compounds identified have been reported previously in other studies investigating milk volatiles *(Supplementary data, Table 4.6)*. Farm set, farm system, and sampling date did not affect volatile compound composition and concentration in any of the milk collected

4.4 DISCUSSION

This study set out to investigate whether there are unique features in milk produced from pasture-fed cows in either organic or conventional production systems. To answer this question, we analysed various compounds while taking into account factors known to influence milk composition. We were able to explain many of the differences observed between the organic and conventionally produced milk in our study, as well as the impacts of individual conditions for each farm set.

Above all, this study does not examine whether the statistical differences reported between the two milk varieties are biologically significant, and recent reviews commenting, that biological significance is minimal (Givens and Lovegrove, 2016).

4.4.1 Oligosaccharides

Presently, comparisons between OS research studies are difficult. Abundance of individual OS can vary between one and three orders of magnitude (Fong, Ma, & McJarrow, 2011). Differences in methodology applied to analyse OS, lack of consistent quantitative methods, and differences in reporting OS species complicate comparisons. As a consequence, a significant utility of our study is derived in providing foundation data to a developing research area. To our knowledge, no previous study has investigated effects of different farming systems and farms on OS composition and concentration while reporting in detail on animal and farm factors.

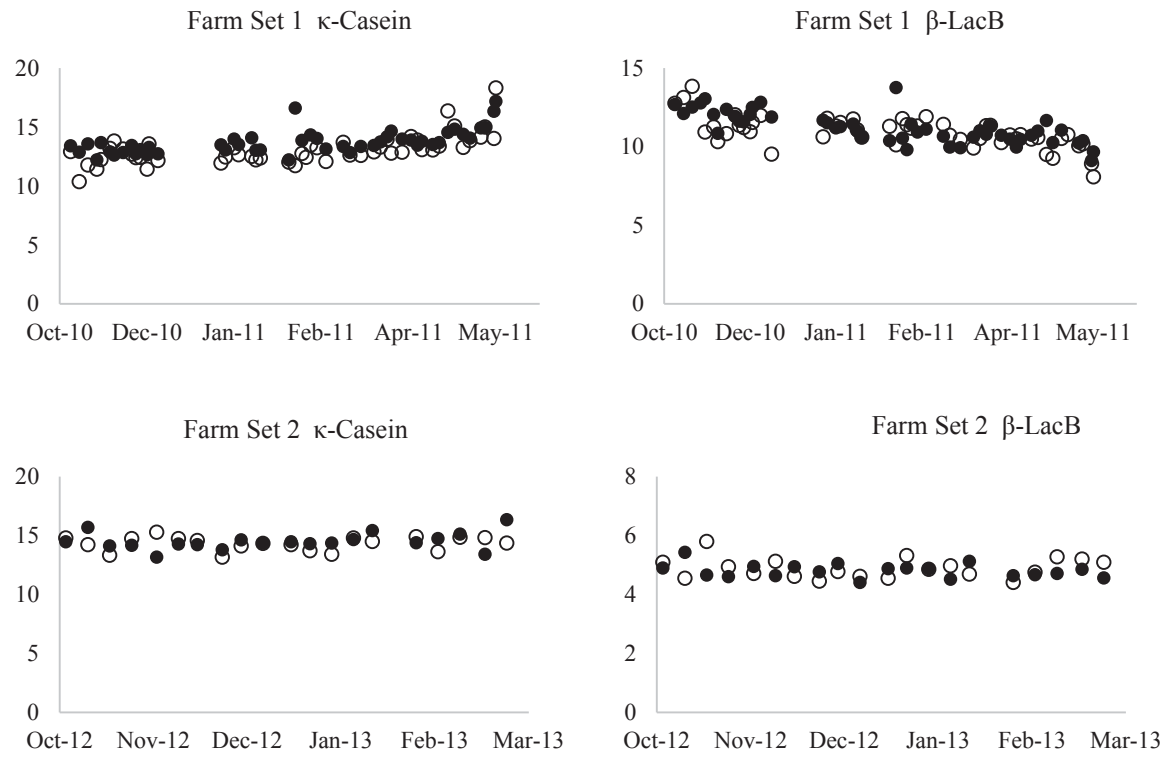

Figure 0.3. Concentration (in g per 100 g milk protein) of κ-Casein and β-Lactoglobulin B throughout the milking season from Farm Set 1 and 2 for organic (●) and conventionally (○) produced milk

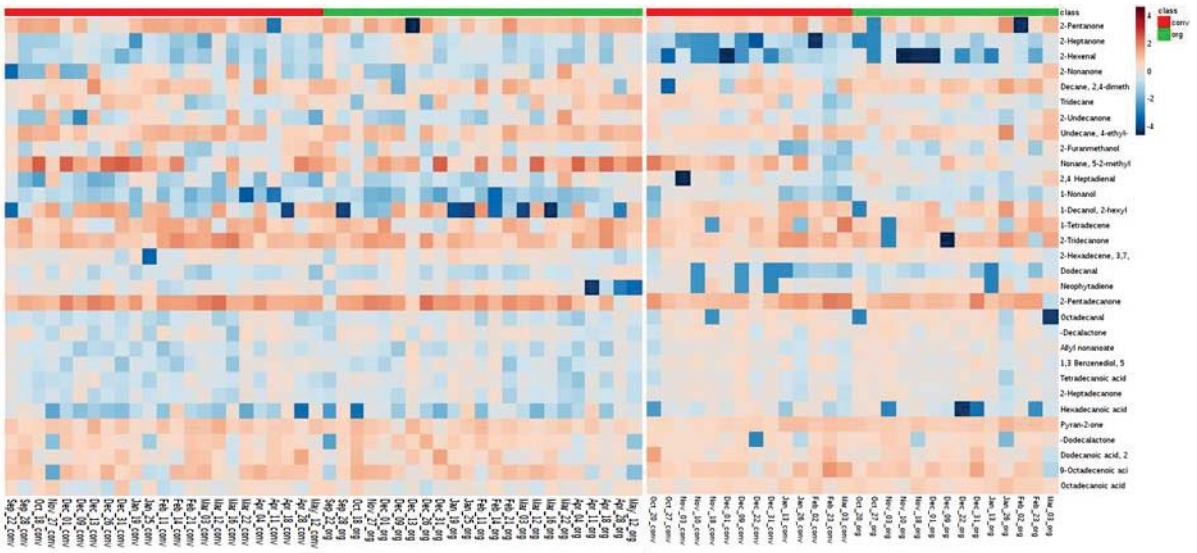

Figure 0.4 Heat maps of volatile compounds in milk fat measured throughout milking season. Farm set 1 (left) and Farm set 2 (right).

org – Organic, conv - Conventional

Table 0.6 Supplementary Data. Volatile compounds observed by this study in bulk milk samples from organic and conventional farms

	Compound	Rt in min		Reference
1	Tetradecanoic acid	38.1	Acid	(Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011; Croissant, Washburn, Dean, & Drake, 2007; Vagenas & Roussis, 2012; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001)
2	Hexadecanoic acid	40.6		(Croissant, Washburn, Dean, & Drake, 2007; Vagenas & Roussis, 2012)
3	Octadecanoic acid	42.4		(Croissant, Washburn, Dean, & Drake, 2007; Vagenas & Roussis, 2012)
4	9-Octadecenoic acid	45.3		(Croissant, Washburn, Dean, & Drake, 2007; Vagenas & Roussis, 2012)
5	Tridecane	17.7	Alkane	(Iličić, Milanović, Carić, Kanurić, Vukić, Hrnjez, et al., 2012)
6	1-Tetradecene	27.2	Alkene	
7	1-Nonanol	26.7	Alcohol	
8	2-Hexenal	13.3	Aldehyde	(Bendall, 2001; Hausner, Philipsen, Skov, Petersen, & Bredie, 2009)
9	2-Nonenal	18.8		(Hausner, Philipsen, Skov, Petersen, & Bredie, 2009)
10	Dodecanal	30.4		(Vagenas & Roussis, 2012)
11	Octadecanal	36		
12	2-Furanmethanol	22.8	Aromatic	(Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011; Croissant, Washburn, Dean, &

				Drake, 2007; Pionnier & Hugelshofer, 2006)
13	2,4 Heptadienal	24.3		(Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011; Hausner, Philipsen, Skov, Petersen, & Bredie, 2009)
14	1,3 Benzenediol, 5pentyl	37.9		
15	Decane, 2,4-dimethyl	16.3	Hydrocarbons	
16	Undecane, 4-ethyl-	22.1		
17	Nonane, 5-(2-methylpropyl)-	24		
18	1-Decanol, 2-hexyl-	26.9		
19	2-Hexadecene, 3,7,11,15-tetramethyl-	29.2		
20	2-Furfural	16.5	Heterocyclic aldehyde	(Hausner, Philipsen, Skov, Petersen, & Bredie, 2009; Pionnier & Hugelshofer, 2006; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001)
21	2-Pentanone	8.4	Ketone	(Contarini & Povolo, 2002; Croissant, Washburn, Dean, & Drake, 2007; Hausner, Philipsen, Skov, Petersen, & Bredie, 2009; Pionnier & Hugelshofer; Toso, Procida, & Stefanon, 2002; Vagenas & Roussis; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001)
22	2-Heptanone	11		(Contarini & Povolo, 2002; Croissant, Washburn, Dean, & Drake, 2007; Hausner, Philipsen, Skov, Petersen, & Bredie, 2009; Havemose, Justesen, Bredie, & Nielsen, 2007; Iličić, et al., 2012;

				Pionnier & Hugelshofer; Toso, Procida, & Stefanon, 2002; Vagenas & Roussis; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001)
23	2-Nonanone	14.3		(Contarini & Povolo, 2002; Croissant, Washburn, Dean, & Drake, 2007; Havemose, Justesen, Bredie, & Nielsen, 2007; Iličić, et al., 2012; Vagenas & Roussis, 2012)
24	2-Undecanone	20.9		(Contarini & Povolo, 2002; Croissant, Washburn, Dean, & Drake, 2007; Havemose, Justesen, Bredie, & Nielsen, 2007; Iličić, et al., 2012; Pionnier & Hugelshofer)
25	2-Tridecanone	27.3		{Pionnier 2006; Havemose, Justesen, Bredie, & Nielsen, 2007;Iličić2012)
26	2-Pentadecanone	33		(Iličić, et al., 2012)
27	2-Heptadecanone	38.4		(Iličić, et al., 2012)
28	δ-Decalactone	37.1	Lactone	(Bendall, 2001; Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011; Croissant, Washburn, Dean, & Drake, 2007; Havemose, Justesen, Bredie, & Nielsen, 2007; Pionnier & Hugelshofer; Vagenas & Roussis, 2012)
29	Pyran-2-one	42.3		(Bendall, 2001)
30	δ-Dodecalactone	42.7		(Bendall, 2001; Coppa, Martin, Pradel, Leotta, Priolo, & Vasta, 2011; Croissant, Washburn, Dean, & Drake, 2007; Havemose, Justesen, Bredie, &

				Nielsen, 2007; Pionnier & Hugelshofer, 2006; Vagenas & Roussis, 2012)
31	Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	43		
32	Neophytadiene	30.6	Diterpenoid	(Bendall, 2001)
	Allyl nonanoate			

We found that, similar to other studies (McJarrow & Van Amelsfort-Schoonbeek, 2004; Tao, DePeters, German, Grimm, & Lebrilla, 2009), bovine milk OS is predominantly acidic OS - 3'SL. This is different than in human milk, which is dominated by neutral OS. Other abundant OS in our study were trisaccharides, GLN, 6'SL, and 3 Hex 1 NeuAc. Disialyllactose was found in traces, which is in agreement with other studies using raw milk (Nakamura & Urashima, 2004; Tao, DePeters, German, Grimm, & Lebrilla, 2009). Studies analysing homogenised milk (Martín-Sosa, Alonso, Sánchez-Juanes, Zancada, García-Pardo, & Hueso, 2009; Fong, Ma, & McJarrow, 2011) observed a higher amount of disialyllactose, which indicates that destruction of fat micelles might positively influence disialyllactose recovery.

In our study, OS showed similarity in abundance independent of farm set or farming system, considering the variability in abundance for individual OS between studies. Farm system appears to have an effect on some OS, although the causes are presently unknown. Except for GLN and 3'SL, the farm set appears to be a less influential factor. This indicates that differences between farm sets (e.g., soil type, pasture composition, milk volume per cow/day, and sampling year) have little or no impact on OS produced in the mammary gland. Five of the reported OS are either unaffected by farming system and farm set (2 Hex, 1 NeuAc; 2 Hex, 1 NeuGc; 1 Hex, 1 HexNAc, 1 NeuAc; 3 Hex, 1 HexNAc) or show only a trend for a farm system effect (1 HexNAc, 1 NeuAc). We conclude that differences between organic and conventional pasture based farms (e.g., rate of fertilizer application) did not affect these OS levels, or that they were not significant enough (e.g., breed composition) to affect OS composition.

4.4.2 Fatty acids

Factors that affect the FA profile in milk have been extensively described previously (Schwendel, Wester, Morel, Tavendale, Deadman, Shadbolt, et al., 2014). We found that much of the variation observed between organic and conventional farms in our study can be attributed to breed composition of herds and fertilizer application rate to grazed pastures. We observed a higher amount of SFA in milk from both organic farms, compared to their aligned conventional farms. Holstein-Friesian cows are known to produce milk with a lower amount of SFA compared to Jersey cows in pasture-based systems (Palladino, Buckley, Prendiville, Murphy, Callan, & Kenny, 2010), and in our study, both organic farms had a lower percentage of Holstein-Friesian cows than the conventional farms. The differences in SFA content in milk can be related to the variation in breed composition between the herds. The lowest amount of SFA (61.5 g/100g) was observed for the conventional herd from Farm Set 2 (92.6% Holstein-Friesian), while the highest amount 67.25 g/100g) found for organic milk from Farm Set 1 where the herd was comprised of 56.1% Holstein Friesian cows.

In contrast to SFA, milk PUFA composition and concentration is predominantly influenced by intake of long chain PUFA. Cows eating fresh forage have greater PUFA composition in their milk compared to cows fed concentrate (grain) diets due to the greater concentrations of LA and ALA in forage plants (Lock & Bauman, 2004).

We postulated previously that greater VA and CLA content in conventional milk was due to a higher application rate of nitrogen fertilizer on the conventional farm of Farm Set 1 at Massey University (Schwendel, et al., 2015). Nitrogen fertilizer application was reported to increase ALA concentration in pasture (Mackle, Parr, & Bryant, 1996). Ruminal bio-hydrogenation of LA and ALA leads to formation of VA, which is a precursor for CLA in the mammary gland (Destaillats, Trottier, Galvez, & Angers, 2005). We observed that LA and ALA were greater in organic milk, and VA and CLA were greater in conventional milk. In this study we additionally observed greater VA and CLA in milk from the conventional farm of Farm Set 2 (*Figure 4.5, Supplementary data*) which, in a similar fashion to the conventional farm of Farm Set 1, applied a significantly higher amount of nitrogen fertilizer compared to the organic farms (Table 1). To our knowledge, there are no regulations limiting the amount (kg/ha) of nitrogen fertilizer applied on organic dairy farms. Consequently, the significantly lower amount of fertilizer used on both organic farms reflects the financial costs and difficulty sourcing certified organic fertilizer in New Zealand.

The overall higher amount of PUFA in Farm Set 2 is a reflection of the higher amount of white clover in Farm Set 2. White clover pasture contains higher FA concentrations than fescue or ryegrass pasture (Glasser, Doreau, Maxin, & Baumont, 2013), and a lower percentage of bio-hydrogenation in the rumen was reported for PUFA originating from white clover compared to pasture grasses (Lee, Harris, Dewhurst, Merry, & Scollan, 2003).

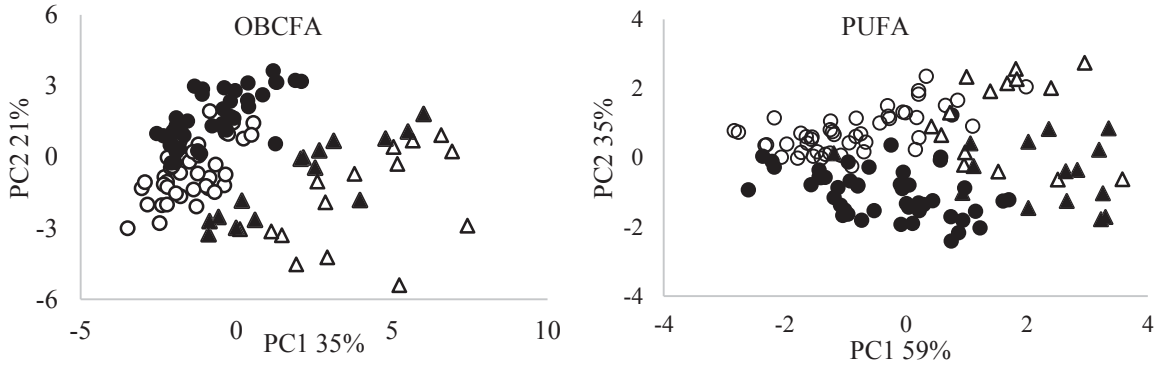

Figure 0.5 Principal component analysis of odd and branched chain fatty acid (OBCFA) and poly unsaturated fatty acids (PUFA; vaccenic acid, α-linolenic acid, linoleic acid, conjugated linoleic acid) in milk from (○) organic and (●) conventional Farm Set 1, and (△) organic and (▲)conventional Farm Set 2

The significant differences we observed between organic and conventional milk for BFA is probably due to small differences in diet composition resulting in changes in ruminal microbiota. Synthesis of BFA by ruminal microbiota is influenced by the ruminal ecosystem, which in turn is influenced by diet (Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006). Diets in our current study were pasture-based on all four farms, but small differences in secondary plant metabolites, as well as the influence of daily garlic, cider-vinegar drench in the organic herd on Farm Set 1, may explain differences observed in BFA composition. Garlic supplementation (Ramos-Morales, Martínez-Fernández, Abecia, Martin-García, Molina-Alcaide, & Yáñez-Ruiz, 2013) and differences in secondary plant metabolite composition (Falchero, Lombardi, Gorlier, Lonati, Odoardi, & Cavallero, 2010) have been reported to affect ruminal microbiota and consequently milk FA composition. Variation in pasture composition was larger between farm sets than within. Hence, differences in secondary plant metabolites are less likely to have caused the differences observed for BFA between farms in Farm Set 1. We previously questioned whether prolonged administration of garlic, cider-vinegar drench altered ruminal microbiota in organic cows from Farm Set 1 (Schwendel, et al., 2015) to explain the differences in BFA observed between organic and conventional milk. Composition of BFA showed greater similarity between organic and conventional milk from Farm Set 2, with no garlic drenching used on either herd.

Observations for OFA do not show the same clear trend as BFA, despite OFA also originate in the rumen. While the three most abundant OFA increased in the conventional milk of Farm Set 1, only minor OFA and C13:0 varied between farms on Farm Set 2, and all of them increased in organic milk. This further suggests variations in the availability of precursors for OFA caused by differences in rumen microbiota between farm sets and systems, with the specific influence factors presently unknown.

4.4.3 Protein

A higher amount of daily milk yield and milk solids could be observed in Farm Set 2 compared to Farm Set 1, with pasture composition significantly different between both farms sets. Pasture from Farm Set 2 contained 50% white clover compared to less than 5% for Farm Set 1. Clover content in bovine diet has been associated with higher milk volume and protein yield (Thomson, Beever, Haines, Cammell, Evans, Dhanoa, et al., 1985) compared to ryegrass based diets, which reflects our observations. Concentrations of individual milk proteins varied. Differences in the total casein percentage for both farm sets are presumably related to the higher clover content in pastures in Farm Set 2, with previous studies reporting higher contents of α- and β-casein in milk from cows grazing clover instead of ryegrass (Grandison, Manning, & Erson, 1985). This increase in casein

relative to whey was explained to result from a higher feed intake when grazing clover (Thomson, Beever, Haines, Cammell, Evans, Dhanoa, et al., 1985). Breed composition varied between our farm sets and systems, but for Farm Set 2 with a larger breed disparity between the organic and conventional herds, no system effect for any major protein was observed. We believe that breed can, therefore, be excluded as a cause differences in protein content. We observed changes in concentration of κ-casein and β-lactoglobulin in Farm Set 1 throughout the lactation period (*Supplementary data, Figure 4.3*) with κ-casein increased, and β-lactoglobulin decreased at the end of the lactation season. The higher proportion of κ-casein compared to α- and β-casein at the latter stage of lactation has been explained as being due to a greater resistance to proteolysis (Ostersen, Foldager, & Hermansen, 1997). Sampling over a shorter period effectively removed any effect due to sampling date on Farm Set 2 as the times corresponding to periods of slow pasture growth in Farm Set 1 were not included.

4.4.4 Milk fat volatile compounds

We observed similar volatile compounds in milk fat in our study to what others have reported (Croissant, Washburn, Dean, & Drake, 2007), except that we did not detect terpenes. The method we used was optimised to quantitate longer chain compounds (> C4:0), such as, alkanes, alcohols, ketones and FA. It employed a combined extraction and distillation procedure on milk fat followed by a stationary phase chromatographic separation and as a consequence, we did not observe the same quantity and variety of aromatic compounds (e.g., terpenes). We saw the greatest amount of overlap with what others have reported with ketones, where agreement between studies for individual compounds were independent of sample type, preparation, and analysis *(Supplementary data, Table 2)*. Hydrocarbons, which made up the second largest group of volatile compounds in our study, have been reported by others (Toso, Procida, & Stefanon, 2002). However, individual hydrocarbons vary between studies and, by comparison, we observed more long chain compounds, which may reflect our choice of column. Other classes of compounds we observed were aldehydes, non-esterified FA, and lactones, all of which have been reported by other researchers (Vagenas & Roussis, 2012).

None of the individual compounds or classes of compounds showed trends for farm set, system, or sampling date. This indicates that factors other than pasture composition and stage of lactation influence milk fat volatile composition. Similar to our findings, others have reported that most volatile compound classes (e.g., alcohols, aldehyde, hydrocarbons) are largely unaffected by dietary differences (Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001). Milk volatile compounds may arise from microorganisms in milk as there is no

correlation between volatiles in pasture and those found in in milk (Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001).

4.5 CONCLUSION

Most studies that purport to compare organic and conventionally produced milk do not take into account the major factors which affect milk composition (e.g., diet and breed). Our study was designed intentionally to minimize these differences. By using two farm sets with similarly managed, pasture-based systems on adjacent properties, we were able to make a true comparison of the impact of organic versus conventional dairy farming on milk composition, and not just a comparison of pasture versus concentrate feeding. We attempted to explain many of the differences observed. Our study investigated four different groups of compounds found in organic and conventional bovine milk produced in a pasture based system. We accounted for known factors that influence milk composition and selected farms that were >95% forage based and utilized Jersey-Holstein-Friesian crossbreed cows. We demonstrated that VA and CLA are increased in conventional milk, while LA and ALA, show greater abundance in organic milk independent of the Farm Set. Similar to FA, system effects have been observed for half of the reported OS, while three OS were not influenced by the farm or system. Presently, influence factors on reported OS in bovine milk are unknown and further research is required. Increased clover consumption is related to greater milk production, while affecting the ratio of casein and whey protein.

None of the factors identified by this study that potentially alter milk composition were unique to either farming system. Nitrogen fertilizer application, clover content in pasture, and garlic drenching are not unique to organic or conventional farming systems. Many conventionally produced dairy products might differ in their chemical composition from organically produced ones as a result of differences in diet, breed, and farm management between the two systems. However, increasing availability of milk from pasture fed conventional cows will reduce and potentially eliminate any overall differences previously reported between organic and conventionally produced milk.

Conflict of interest

Brigitte H. Schwendel, Timothy J. Wester, Patrick C.H. Morel, Bertram Fong, Michael H. Tavendale, Craig Deadman, Nicola M. Shadbolt, and Don E. Otter individually declare, that they have no conflict of interest.

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REFERENCES

Asakuma, S., Ueda, Y., Akiyama, F., Uemura, Y., Miyaji, M., Nakamura, M., Murai, M., & Urashima, T. (2010). Short communication: Effect of grazing on the concentrations of total sialic acid and hexose in bovine milk. *Journal of Dairy Science, 93*(10), 4850-4854.

Bendall, J. G. (2001). Aroma compounds of fresh milk from New Zealand cows fed different diets. Journal of Agricultural and Food Chemistry, 49(10), 4825-4832.

Bordin, G., Cordeiro Raposo, F., De La Calle, B., & Rodriguez, A. R. (2001). Identification and quantification of major bovine milk proteins by liquid chromatography. *Journal of Chromatography A, 928*(1), 63-76.

Bugaud, C., Buchin, S., Coulon, J. B., Hauwuy, A., & Dupont, D. (2001). Influence of the nature of alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk. *Lait, 81*(3), 401-414.

Collomb, M., Bisig, W., Buetikofer, U., Sieber, R., Bregy, M., & Etter, L. (2008). Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. *International Dairy Journal, 18*(10--11), 976-982.

Contarini, G., & Povolo, M. (2002). Volatile fraction of milk: Comparison between, purge and trap and solid phase microextraction techniques. Journal of Agricultural and Food Chemistry, 50(25), 7350-7355.

Coppa, M., Martin, B., Pradel, P., Leotta, B., Priolo, A., & Vasta, V. (2011). Effect of a hay-based diet or different upland grazing systems on milk volatile compounds. *Journal of Agricultural and Food Chemistry, 59*(9), 4947-4954.

Croissant, A. E., Washburn, S. P., Dean, L. L., & Drake, M. A. (2007). Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. *Journal of Dairy Science, 90*(11), 4942-4953.

Day, L., Williams, R. P. W., Otter, D., & Augustin, M. A. (2015). Casein polymorphism heterogeneity influences casein micelle size in milk of individual cows. *Journal of Dairy Science, 98*(6), 3633-3644.

Destaillats, F., Trottier, J. P., Galvez, J. M. G., & Angers, P. (2005). Analysis of α-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acids. *Journal of Dairy Science, 88*(9), 3231-3239.

Elgersma, A., Tamminga, S., & Ellen, G. (2006). Modifying milk composition through forage. *Animal Feed Science and Technology, 131*(3-4), 207-225.

Falchero, L., Lombardi, G., Gorlier, A., Lonati, M., Odoardi, M., & Cavallero, A. (2010). Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. *Dairy Science and Technology, 90*(6), 657-672.

Fong, B., Ma, K., & McJarrow, P. (2011). Quantification of bovine milk oligosaccharides using liquid chromatography-selected reaction monitoring-mass spectrometry. *Journal of Agricultural and Food Chemistry, 59*(18), 9788-9795.

Givens, D. I., and J. A. Lovegrove. 2016. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: A systematic literature review and meta- and redundancy analyses. *Br. J. Nutr. 116:1-2*. http://dx.doi.org/10.1017/s0007114516001604.

Glasser, F., Doreau, M., Maxin, G., & Baumont, R. (2013). Fat and fatty acid content and composition of forages: A meta-analysis. *Animal Feed Science and Technology, 185*(1-2), 19-34.

Grandison, A. S., Manning, D. J., & Erson, M. (1985). Chemical Composition, Rennet Coagulation Properties and Flavour of Milks From Cows Grazing Ryegrass or White Clover. *Journal of Dairy Research, 52*(1), 33-39.

Härtig, C. (2008). Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography-mass spectrometry database. *Journal of Chromatography A, 1177*(1), 159-169.

Hausner, H., Philipsen, M., Skov, T. H., Petersen, M. A., & Bredie, W. L. P. (2009). Characterization of the volatile composition and variations between infant formulas and mother's milk. Chemosensory Perception, 2(2), 79-93.

Havemose, M. S., Justesen, P., Bredie, W. L. P., & Nielsen, J. H. (2007). Measurement of volatile oxidation products from milk using solvent-assisted flavour evaporation and solid phase microextraction. International Dairy Journal, 17(7), 746-752.

Iličić, M. D., Milanović, S. D., Carić, M. D., Kanurić, K. G., Vukić, V. R., Hrnjez, D. V., & Ranogajec, M. I. (2012). Volatile compounds of functional dairy products. Acta Periodica Technologica, 43, 11-19.

Jahreis, G., Pritsche, J., & Steinhart, H. (1997). Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutrition Research, 17*(9), 1479-1484.

Kuczyńska, B., Puppel, K., Gołębiewski, M., Metera, E., Sakowski, T., & Słoniewski, K. (2012). Differences in whey protein content between cow's milk collected in late pasture and early indoor feeding season from conventional and organic farms in Poland. *Journal of the Science of Food and Agriculture*.

Lee, H., MeloSilva, V. L. D., Liu, Y., & Barile, D. (2015). Short communication: Quantification of carbohydrates in whey permeate products using high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Dairy Science, 98*(11), 7644-7649.

Lee, H. J., & Yun, Z. S. (2015). Consumers' perceptions of organic food attributes and cognitive and affective attitudes as determinants of their purchase intentions toward organic food. *Food Quality and Preference, 39*, 259-267.

Lee, M. R. F., Harris, L. J., Dewhurst, R. J., Merry, R. J., & Scollan, N. D. (2003). The effect of clover silages on long chain fatty acid rumen transformations and digestion in beef steers. *Animal Science, 76*(3), 491-501.

Liu, Z., Moate, P., Cocks, B., & Rochfort, S. (2014). Simple liquid chromatography-mass spectrometry method for quantification of major free oligosaccharides in bovine milk. *Journal of Agricultural and Food Chemistry, 62*(47), 11568-11574.

Lock, A. L., & Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids, 39*(12), 1197-1206.

Mackle, T. R., Parr, C. R., & Bryant, A. M. (1996). Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. *New Zealand Journal of Agricultural Research, 39*(3), 341-356.

McJarrow, P., & Van Amelsfort-Schoonbeek, J. (2004). Bovine sialyl oligosaccharides: Seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. *International Dairy Journal, 14*(7), 571-579.

Nakamura, T., & Urashima, T. (2004). The milk oligosaccharides of domestic farm animals. *Trends in Glycoscience and Glycotechnology, 16*(88), 135-142.

Ostersen, S., Foldager, J., & Hermansen, J. E. (1997). Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *Journal of Dairy Research, 64*(2), 207-219.

Palladino, R. A., Buckley, F., Prendiville, R., Murphy, J. J., Callan, J., & Kenny, D. A. (2010). A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. *Journal of Dairy Science, 93*(5), 2176-2184.

Payling, L. M., Juniper, D. T., Drake, C., Rymer, C., & Givens, D. I. (2015). Effect of milk type and processing on iodine concentration of organic and conventional winter milk at retail: Implications for nutrition. *Food Chemistry, 178*, 327-330.

Pionnier, E., & Hugelshofer, D. (2006). Characterisation of key odorant compounds in creams from different origins with distinct flavours. In Developments in Food Science, vol. 43 (pp. 233-236).

Ramos-Morales, E., Martínez-Fernández, G., Abecia, L., Martin-García, A. I., Molina-Alcaide, E., & Yáñez-Ruiz, D. R. (2013). Garlic derived compounds modify ruminal fatty acid biohydrogenation and induce shifts in the butyrivibrio community in continuous-culture fermenters. *Animal Feed Science and Technology, 184*(1-4), 38-48.

Schwendel, B. H., Morel, P. C. H., Wester, T. J., Tavendale, M. H., Deadman, C., Fong, B., Shadbolt, N. M., Thatcher, A., & Otter, D. E. (2015). Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. *Journal of Dairy Science, 98*(3), 1411-1425.

Schwendel, B. H., Wester, T. J., Morel, P. C. H., Tavendale, M. H., Deadman, C., Shadbolt, N. M., & Otter, D. E. (2014). Invited review: Organic and conventionally produced milk-An evaluation of influence factors on milk composition. *Journal of Dairy Science*.

Tao, N., DePeters, E. J., German, J. B., Grimm, R., & Lebrilla, C. B. (2009). Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. *Journal of Dairy Science, 92*(7), 2991-3001.

Thomson, D. J., Beever, D. E., Haines, M. J., Cammell, S. B., Evans, R. T., Dhanoa, M. S., & Austin, A. R. (1985). Yield and Composition of Milk From Friesian Cows Grazing Either Perennial Ryegrass Or White Clover In Early Lactation. *Journal of Dairy Research, 52*(1), 17-31.

Toso, B., Procida, G., & Stefanon, B. (2002). Determination of volatile compounds in cows' milk using headspace GC-MS. *Journal of Dairy Research, 69*(4), 569-577.

Tunick, M. H., Van Hekken, D. L., Paul, M., Ingham, E. R., & Karreman, H. J. (2016). Case study: Comparison of milk composition from adjacent organic and conventional farms in the USA. *International Journal of Dairy Technology, 69*(1), 137-142.

US Government Printing Office. 2014. Electronic Code of Federal Regulations: Regulations of the Department of Agriculture: Subchapter M–Organic Foods Production Act Provisions. Vol. 2014.

Vagenas, G., & Roussis, I. G. (2012). Fat-derived volatiles of various products of cows', ewes', and goats' milk. *International Journal of Food Properties, 15*(3), 665-682.

Valero, E., Villamiel, M., Miralles, B., Sanz, J., & Martínez-Castro, I. (2001). Changes in flavour and volatile components during storage of whole and skimmed UHT milk. Food Chemistry, 72(1), 51-58.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M., & Dewhurst, R. J. (2006). Factors affecting odd- and branched-chain fatty acids in milk: A review. *Animal Feed Science and Technology, 131*(3-4), 389-417.

Wishart, D. S., Jewison, T., Guo, A. C., Wilson, M., Knox, C., Liu, Y., Djoumbou, Y., Mandal, R., Aziat, F., Dong, E., Bouatra, S., Sinelnikov, I., Arndt, D., Xia, J., Liu, P., Yallou, F., Bjorndahl, T., Perez-Pineiro, R., Eisner, R., Allen, F., Neveu, V., Greiner, R., & Scalbert, A. (2013). HMDB 3.0-The Human Metabolome Database in 2013. *Nucleic Acids Research, 41*(D1), D801-D807.

Xia, J., Sinelnikov, I. V., Han, B., & Wishart, D. S. (2015). MetaboAnalyst 3.0--making metabolomics more meaningful. *Nucleic Acids Research, 43*(W1), W251-W257.

Chapter 5

Non-targeted approach does not reveal differences between organic and conventionally produced milk

ABSTRACT

Organic and conventionally produced milk has predominantly been compared in regards to milk fatty acid (FA) composition, which is easily influenced by a variety of parameters, predominantly the diet of the animal. Exploring overall milk composition with untargeted methods provides the opportunity to investigate potential markers from a variety of biological pathways. Nuclear magnetic resonance spectroscopy is an analytical platform used to investigate complex biological samples. For this study, two sets of dairy farms were selected where each set consisted of a certified organic farm adjacent to a conventional farm, with samples collected throughout the milking season. Additional milk was collected four times from individual cows. No differences between organic and conventionally produced milk for individual samples were observed, with the exception of β-hydroxybutyrate. For bulk milk samples, differences between the two systems were only observed in Farm Set 1 where sampling was conducted throughout the whole lactation period. No individual milk metabolite could be identified to be consistently different between both milk types across all data sets. Changes in metabolite concentration in Farm Set 1 have been attributed to differences in stage of lactation. Sampling date and time of milking had only minor effects on metabolite concentration. This leads to the overall conclusion that the metabolomic profile in milk of healthy animals that are fed to their requirements is not affected by other farming factors.

5.1 INTRODUCTION

Consumer demand for dairy products produced in accordance with organic regulations is still growing. Customers associate the term ‘organic’ with increased health benefits for consumers, reduced environmental impact, and improved animal welfare. However, these assumptions are based on the expectation that organic cows are raised on pasture, while conventional cows are housed and fed concentrates. Regulations for organic dairy production commonly mandate access to pasture, but vary in the number of days grazing required per year, and percentage of dry matter consumed as pasture or forage (Schwendel et al., 2014). Consequently, grazed fresh pasture may contribute as little as 10% to the overall diet of organic dairy cows (Organic Foods Production Act Provisions 2014, US Government Printing Office, 2014). More recently, milk produced by exclusively pasture

fed conventional cows has entered the market, with consumers prepared to pay a premium for 'pasture milk' similar to that paid for organic milk. Premium prices create a strong incentive to develop analytical methods that enable the authentication of organic dairy products. However, methodologies and markers presently selected to differentiate between organic and conventionally produced milk are related to the amount of pasture consumed by the animal and, therefore, fail to set apart milk from pasture fed conventional and pasture fed organic cows.

In the past, organic and conventionally produced milk has predominantly been compared in regard to milk fatty acid (FA) composition, which is easily influenced by a variety of parameters, predominantly the diet of the animal. Investigating overall milk composition with untargeted methods, rather than concentrating on specific compound groups, provides the opportunity to investigate potential markers derived from a variety of biological pathways. Nuclear magnetic resonance (NMR) spectroscopy is, after mass spectrometry (MS), the analytical platform used to investigate the metabolomic composition of complex biological samples. NMR, although significantly limited by the number of compounds which can be identified per sample, provides a standardised methodology with minimal changes in chemical shift and signal intensity between samples, independent of instrumentation or sample type. It, therefore, allows the analysis of a multitude of samples without run order or batch effects, the direct comparison between different studies, and the creation of extended spectral libraries. Consequently, the ratio of compounds detected vs compounds identified is presently significantly greater compared to MS analysis.

Our study investigated if NMR metabolomics can identify markers to differentiate between organic and conventionally produced milk from all-year-round-pasture fed cows.

5.2 MATERIAL AND METHODS

5.2.1 Farm Data and Sampling

For this study, two sets of dairy farms were selected where each set consisted of a certified organic farm adjacent to a conventional farm as described in {Schwendel, 2016}. Farm Set 1, was based at Massey University, Palmerston, NZ with samples collected twice a week (n = 120) between August and May during the 2010-2011 milking season. Farm Set 2 was privately owned and located at Tokoroa, NZ, with samples collected once a week (n = 40) between October and March during the 2012-2013 milking season. Additional milk was collected four times from individual cows from Farm Set 1 (n = 45 organic, n = 50 conventional) throughout the milking season (Schwendel et al., 2015), with one sample each from morning and afternoon milking collected during one day in New Zealand spring (November 2010) and one day in New Zealand autumn (March 2011). Bulk milk samples were collected from the vat, while individual samples were taken from the milking line during the

routine milking process. All samples were collected in 100 or 200 mL plastic screw-top containers and stored at −20 °C until analysis.

5.2.2 Sample preparation and NMR analysis

Milk samples where defatted by centrifugation at 4 °C, and skim milk samples were filtered through a 10 kDA filter (Vivaspin 500, GE Healthcare) to remove protein. The supernatant was combined in equal parts with phosphate buffer containing 0.05 g trimethylsilylpropanoic acid, vortexed and 600µl transferred into a NMR tube for analysis.

NMR data were recorded using a Bruker Avance 700 NMR spectrometer operating at 700.13 MHz and equipped with a 5 mm TCI cryoprobe. 1D 1H spectra were recorded using the standard "noesygppr1d" pulse sequence with a spectral width of 8.33 kHz and an acquisition time of 2.12 s. The mixing time was 10 ms and the solvent presaturation field strength was 50 Hz. The signal was averaged for 160 scans using a recycle delay of 2 s at the end of each acquisition. All data was collected at 300K. Samples were run random order.

Data was processed using Bruker's Topspin software (v. 2.1.6). Spectra were apodized using an exponential window function with 1 Hz line broadening and zero-filled to 130k points before Fourier transformation. The transformed spectra were then phased, referenced to internal trimethylsilylpropanoic acid at 0 ppm and baseline corrected. (This section was conducted and written by Patrick Edwards, Technical Director, Institute of Fundamental Sciences, Massey University)

5.2.3 Data processing

The 1D NMR spectra were processed quantitatively as individual data sets (Farm Set 1, Farm Set 2, and individual samples) using CHENOMX NMR suite 8.1 (Chenomx Inc, Edmonton, Canada), with a pooled sample from each data set as reference. Compound identification was further conducted on 2D NMR spectra from pooled samples using MestReNova (Mestrelab Research, Galicia, Spain) and Human Metabolome Database as reference databases (Wishart et al., 2013)

Data from each data set were tested for normality and outliers, and statistically explored to test for a difference between the group means. For Farm Set 1 and 2 we used a general linear model that included the random effect of sample collection date throughout the milking period, and the fixed effect of farming system (organic or conventional). For the data set of individual milk samples, we used a mixed model. It included fixed effects of system (organic versus conventional), sampling date (spring versus autumn) and sampling time (morning (AM) versus afternoon (PM)), as well as their interactions, while cow within system was a random

effect. An F-test was used to ascertain the degree of differences, and a multiple range test to compare the interaction combinations. Analysis was carried out with SAS version 9.4 (Cary, NC, USA).

5.3 RESULT AND DISCUSSION

Milk metabolites have been investigated by NMR with a variety of aims. Researchers have identified potential markers regarding animal health (Sundekilde et al., 2013), processing properties (Sundekilde et al., 2014), and to authenticate milk and dairy products. Similarly, to other milk compounds, each metabolite is influenced by a variety of individual factors and their interactions, e.g., stage of lactation, heritability, breed, animal health, and diet. In our study, 34 milk metabolites were identified present in all milk samples (Table 5.1). We quantified 33 of these using trimethylsilylpropanoic acid as internal standard. No metabolite reported in this study was significantly affected by farming system across all three data sets. As a consequence of using defatted milk samples, we were not able to observe differences in polyunsaturated FA profile as reported by other studies, however, milk FA composition for these data sets has been published previously (Schwendel et al., 2015; Schwendel et al., 2017).

5.3.1 Individual milk sample set

No differences between organic and conventionally produced milk were observed, with the exception of β-hydroxybutyrate, which was increased in organic milk (Table 5.2). Sample date and time of milking affected 8 and 11 metabolites, respectively, with acetate, dimethyl sulfone, hippurate and N-acetylglucosamine influenced by both ($P < 0.05$). Interactions between several influence factors (system, date, time of milking) were also observed, with the largest number ($n = 10$) of compounds affected by the interaction between system and sampling date. Hippurate showed the greatest variability and was significantly influenced by all individual factors and their interactions, except the farming system, and is, therefore, indicative of the multitude of factors influencing metabolites in milk. However, these influence factors are presently unknown. In the case of hippurate, factors previously reported to affect its concentration in milk (e.g., somatic cell count, animal genetics) can be excluded as a farm system effects were not detected (Sundekilde et al., 2011; Buitenhuis et al., 2013). Furthermore, difference in Jersey breed percentage can be excluded as an influence factor, as compounds (e.g., carnitine and citrate) previously reported to be increased in milk from Jersey cows, were not significantly different between the two herds.

The effect of stage of lactation has been investigated for a variety of milk metabolites (Klein et al., 2013), especially in regard to negative energy balance of the cow during early lactation. Similar to the compounds observed in regard to changes in energy balance and lactation progression (Lu et al., 2013), N-acetylglucosamine

increased throughout the lactation period in our study, while hippurate decreased. Other metabolites reported (e.g., fumarate glutamate, phophocholine, and succinate (Klein et al., 2013; Lu et al., 2013) were not affected in this trial. For this data set, the first sampling day was 13 weeks into lactation, by which stage negative energy balance and the extreme changes in concentration observed in very early lactation are overcome (Artegoitia et al., 2014).

Time of milking also had an effect on metabolite composition. To our knowledge no other study has investigated diurnal effect on milk compounds other than FA. The amount of most metabolites in milk is not correlated with their levels in plasma (Klein et al., 2013), however, relationships have been reported for a few compounds (e.g., serum valine concentration has been related to milk fumarate concentration (Maher et al., 2013). Influence factors for milk metabolites appear to be more complex than factors known to affect diurnal milk FA composition (e.g., time of grazing, rumen passage rate). Trimethylamine-N-oxide and dimethylsulfone are of microbial origin (Maher et al., 2013) and could potentially be affected by rumen conditions, but causes for these affects are presently unclear, and only dimethylsulfone was affected by time of milking in this data set.

Table 0.1 Assignment of ^{1}H and ^{13}C NMR signals of compounds identified in organic and conventional milk samples

Compound	Chemical shift (δ) in ppm				
	2D NMR[1]		HMDB[2]		
	^{1}H	^{13}C	^{1}H	^{13}C	Assignment
Acetate	1.9	26.1	1.9	26.1	CH_3
Acetone	2.4	33.4	2.2	32.9	
Alanine	1.5	19.1	1.5	19.0	CH_3
Alanine	3.8	53.2	3.8	53.6	CH
Allantoin	5.4	66.2	5.4	66.2	
Butyrate	3.1	55.6	3.3	55.9	$3 \times CH_3$
Carnitine	2.4	45.8	2.4	45.7	CH_2
Carnitine	3.2	56.7	3.2	56.9	$3 \times CH_3$
Carnitine	3.4	72.5	3.4	72.9	$N\text{-}CH_2$
Choline	3.2	56.7	3.2	56.7	$3 \times CH_3$
Choline	3.5	70.2	3.5	70.1	$N\text{-}CH_2$
Choline	4.1	58.5	4.1	58.5	$O\text{-}CH_2$
cis-Aconitate	3.1	46.3	3.1	46.1	CH_2
Citrate	2.5	48.5	2.5	48.7	CH_2
Citrate	2.7	48.5	2.7	48.7	CH_2
Creatine	3.0	39.8	3.0	39.5	CH_2
Creatine	3.9	56.7	3.9	56.4	CH_3
Creatinine	3.0	33.0	3.0	33.0	CH_3
Creatinine	4.1	59.3	4.1	59.2	CH_2
Dimethylsulfone	3.2	44.3	3.1	44.2	
Formate	8.5	ND	8.4	172.4	CH
Fucose	3.7	75.4	3.6	75.7	CH-OH
Fucose	3.8	75.0	3.8	74.8	CH-OH
Fucose	5.2	95.6	5.2	95.1	CH-OH
Fumarate	6.5	138.1	6.5	138.0	CH
Galactose	3.5	74.8	3.5	74.7	CH

Galactose	3.7	75.4	3.6	75.6	
Galactose	3.9	72.1	4.0	72.1	
Galactose	3.9	71.5	3.9	71.6	
Galactose	4.0	72.9	4.1	73.2	CH
Galactose	4.5	98.6	4.6	99.3	CH
Glutamate	2.1	29.9	2.1	29.8	γ-CH_2
Glycine	3.6	44.7	3.5	44.3	
Hippurate	4.0	46.7	4.0	46.8	
Hippurate	7.6	131.6	7.5	131.5	CH_2-3,5
Hippurate	7.6	135.0	7.6	134.9	CH-4
Hippurate	7.8	130.0	7.8	129.9	CH_2-2,6
β-Hydroxybutyrate	1.2	24.6	1.2	24.4	CH_3
Isobutyrate	2.2	40.4	2.4	39.6	
Lactic acid	1.3	22.9	1.3	22.9	CH_3
Lactic acid	4.1	71.3	4.1	71.4	CH
Lactose	3.3	76.8	3.3	77.0	CH-2
Lactose	3.6	75.4	3.6	74.6	CH-2’
Lactose	3.6	77.6	3.6	77.8	CH-3
Lactose	3.6	81.1	3.7	80.9	CH-3
Lactose	3.7	81.2	3.7	80.9	CH-4
Lactose	3.7	78.2	3.7	78.3	CH-5’
Lactose	3.7	63.9	3.8	64.1	CH2-6’
Lactose	3.8	63.9	3.7	64.1	CH-5'
Lactose	4.5	105.7	4.5	106.1	CH-1’
N-Acetylglucosamine	3.5	78.7	3.5	78.7	
N-Acetylglucosamine	3.5	72.9	3.5	72.7	
N-Acetylglucosamine	3.8	63.0	3.8	63.1	
N-Acetylglucosamine	5.2	93.7	5.2	93.5	
N-Acetylglutamate	2.1	29.9	2.1	29.8	γ-CH_2
N-Acetylglutamine	2.1	29.8	2.1	29.3	

N-Acetylcarnitine	2.1	23.3	2.1	23.2	
N-Acetylcarnitine	3.5	70.2	3.6	70.7	
Phosphocholine	3.2	56.7	3.2	56.5	
Phosphocholine	3.6	69.1	3.6	68.9	3 x CH_3
Succinic acid	2.4	36.9	2.4	36.8	
Urea	5.6	ND	5.6	165.5	
Uridine	7.9	144.7	7.9	144.6	
Valine	3.6	63.0	3.6	63.3	
Triethylamine-N-oxide	3.2	62.5	3.3	62.2	CH_3
Orotate[3]	6.2	104.2	6.1	103.2	CH

[1]Identified from pooled samples, [2] References from Human Metabolome Data Base (HMDB), [3]not quantified

Table 0.2 Effect of system, season and time of milking on milk metabolites

Compound	**System**			**Season**			**Time of milking**			***P*-value**						
in mM	**Con**[1]	**Org**[2]	**SEM**	**Spring**	**Autumn**	**SEM**	**Morning**	**Evening**	**SEM**	**System**	**Date**	**Time**	Sys[3] x T[4]	Sys x S[5]	T x S	Sys x S x T
Acetate	0.053	0.054	0.001	0.056	0.052	0.001	0.051	0.057	0.001	NS	< 0.05	< 0.001	NS	NS	NS	NS
Acetone	0.011	0.013	0.001	0.012	0.012	0.001	0.012	0.012	0.001	NS	NS	NS	NS	< 0.001	< 0.05	NS
Alanine	0.0149	0.0141	0.0003	0.0143	0.0147	0.0003	0.0144	0.0146	0.0003	NS	NS	NS	NS	NS	NS	NS
Allantoin	0.018	0.022	0.002	0.021	0.020	0.002	0.022	0.019	0.002	NS	NS	NS	NS	NS	NS	NS
Butyrate	0.116	0.141	0.009	0.127	0.130	0.009	0.092	0.165	0.009	NS	NS	< 0.001	NS	NS	NS	NS
Carnitine	0.044	0.055	0.006	0.050	0.049	0.005	0.061	0.039	0.005	NS	NS	< 0.01	NS	NS	NS	NS
Choline	0.306	0.299	0.008	0.297	0.307	0.008	0.305	0.300	0.008	NS	NS	NS	NS	NS	NS	< 0.1
cis-Aconitate	0.011	0.099	0.068	0.099	0.011	0.067	0.100	0.010	0.067	NS	NS	NS	NS	NS	NS	NS
Citrate	4.21	4.56	0.08	4.48	4.29	0.09	4.50	4.28	0.09	NS	NS	NS	< 0.01	< 0.001	NS	< 0.01
Creatine	0.236	0.250	0.005	0.236	0.250	0.006	0.246	0.240	0.006	NS	< 0.1	NS	NS	NS	NS	NS
Creatinine	0.123	0.117	0.007	0.138	0.101	0.006	0.128	0.112	0.006	NS	< 0.001	< 0.1	NS	< 0.01	NS	NS
Dimethyl sulfone	0.030	0.029	0.001	0.034	0.024	0.001	0.028	0.030	0.001	NS	< 0.001	< 0.05	NS	NS	NS	NS
Formate	0.033	0.034	0.001	0.033	0.033	0.001	0.034	0.033	0.001	NS	NS	NS	NS	NS	NS	NS
Fucose	0.014	0.015	0.001	0.014	0.014	0.001	0.012	0.016	0.001	NS	NS	< 0.05	NS	NS	NS	NS
Fumarate	0.0083	0.0094	0.0003	0.0089	0.0088	0.0003	0.0099	0.0078	0.0003	NS	NS	< 0.001	NS	< 0.05	NS	< 0.01
Galactose	0.364	0.528	0.047	0.412	0.480	0.046	0.490	0.402	0.046	NS	NS	NS	< 0.05	< 0.05	< 0.05	< 0.01
Glutamate	0.121	0.398	0.202	0.404	0.115	0.199	0.376	0.143	0.200	NS	NS	NS	NS	NS	NS	NS
Glycine	18.75	18.54	0.492	19.12	18.16	0.485	18.24	19.04	0.487	NS	NS	NS	< 0.05	< 0.001	NS	< 0.01
Hippurate	0.125	0.136	0.003	0.136	0.125	0.004	0.117	0.144	0.004	NS	< 0.05	< 0.001	< 0.001	< 0.05	< 0.05	< 0.001
β-Hydroxybutyrate	0.016	0.018	0.001	0.016	0.017	0.001	0.016	0.017	0.001	< 0.01	NS	NS	NS	NS	NS	< 0.01

Isobutyrate	0.0023	0.0024	0.0001	0.0023	0.0023	0.0001	0.0025	0.0022	0.0001	NS	NS	< 0.1	NS	NS	NS	NS
Lactate	0.017	0.276	0.199	0.273	0.020	0.198	0.279	0.014	0.197	NS	NS	NS	NS	NS	NS	NS
Lactose	110.34	109.27	4.08	110.01	109.61	4.26	107.43	112.18	4.17	NS	NS	NS	NS	NS	NS	NS
N- Acetyl glucosamine	0.371	0.381	0.013	0.299	0.454	0.013	0.401	0.352	0.013	NS	< 0.001	< 0.01	< 0.05	NS	NS	NS
N-Acetyl glutamate	0.012	0.010	0.001	0.010	0.012	0.001	0.012	0.010	0.001	NS	NS	NS	< 0.1	NS	NS	NS
N-Acetyl glutamine	0.016	0.017	0.001	0.016	0.017	0.001	0.016	0.017	0.001	NS	NS	NS	NS	NS	NS	NS
N-Acetylcarnitine	0.022	0.023	0.001	0.022	0.023	0.001	0.024	0.021	0.001	NS	NS	< 0.05	NS	NS	NS	NS
Phosphocholine	0.196	0.181	0.010	0.198	0.178	0.010	0.184	0.192	0.010	NS	NS	NS	NS	< 0.05	NS	NS
Succinic acid	0.0145	0.0152	0.0004	0.0145	0.0151	0.0004	0.0163	0.0134	0.0004	NS	NS	< 0.001	NS	< 0.1	NS	< 0.01
Urea	0.344	0.314	0.027	0.243	0.415	0.024	0.361	0.296	0.024	NS	< 0.001	< 0.05	NS	< 0.001	NS	NS
Uridine	0.003	0.004	0.000	0.005	0.003	0.000	0.004	0.004	0.000	NS	< 0.001	NS	NS	< 0.1	NS	< 0.1
Valine	0.0058	0.0062	0.0002	0.0060	0.0060	0.0002	0.0062	0.0059	0.0002	NS	NS	NS	< 0.1	< 0.001	NS	< 0.05
Trimethylamine N-oxide	0.125	0.135	0.011	0.097	0.163	0.011	0.141	0.119	0.012	NS	< 0.001	NS	< 0.05	< 0.1	NS	NS

[1]Con – Conventional, [2]Org – Organic, [3]Sys- System, [4]T – Time, [5]S – Season, NS – not significant

5.3.2 Bulk milk sample sets

The number of metabolites influenced by the farming system varied depending on the farm set (Table5.3). In Farm Set 1, 15 of the 33 quantitated compounds were affected, with citrate, creatine, fumarate, and trimethylamine-N-oxide most affected ($P < 0.01$) by farm system, while in Farm Set 2, alanine was the only compound different ($P < 0.05$) between organic and conventionally produced milk. Sampling date showed similar effects on the farm sets, with 29 compounds affected in Farm Set 1, while acetate was the only compound affected in Farm Set 2. Neither alanine nor acetate were significantly affected in Farm Set 1.

The effect of the farming system differed between the two farm sets, with no system effect observed for Farm Set 2. The system effect on Farm Set 1 for the bulk milk samples, contrasted with the results from the individual sample set, which was also collected on Farm Set 1 during the same lactation period.

Many factors known to be different between the organic and the conventional farms in Farm Set 1 are also different in Farm Set 2 (e.g., higher percentage of Jersey breed in organic herds, higher application rates of nitrogen fertilizer on conventional farms), while influence factors like somatic cell count and animal genetics have already been accounted for in the individual milk sample set. To our knowledge, no milk compound other than polyunsaturated FA has been identified as different between organic and conventionally produced milk and cheese when using NMR. Moreover, these differences disappear when comparing milk from conventional grass fed cows and organically raised cows (Prema et al., 2013; Erich et al., 2015). Presumably, differences observed between organic and conventionally produced bulk milk in Farm Set 1 are a result of one or more presently unknown factors effective during early or very late lactation, or both, as these periods were not considered by the individual milk sample set and the bulk milk samples from Farm Set 2. Sampling date is known to significantly affect several milk metabolites (Lu et al., 2013; Artegoitia et al., 2014).

Table 0.3 Milk metabolites in bulk milk samples from conventional (Con) and organic (Org) farms.

Metabolite	**Farm Set 1** (n[1]=104)			**Farm Set 2** (n=40)			**Set 1**	**Set 2**	**Set 1**	**Set 2**
	Con	**Org**		**Con**	**Org**		**System**		**Date**	
in mM	**Mean**		**SEM**	**Mean**		**SEM**	***P-value***			
Acetate	0.112	0.113	0.002	0.074	0.072	0.004	NS	NS	< 0.001	< 0.05
Acetone	0.035	0.038	0.001	0.013	0.016	0.002	NS	NS	< 0.01	NS
Alanine	0.026	0.027	0.001	0.012	0.010	0.001	NS	< 0.05	< 0.01	NS
Allantoin	0.068	0.070	0.002	0.025	0.020	0.003	NS	NS	< 0.001	NS
Butyrate	0.269	0.237	0.011	0.031	0.030	0.003	< 0.05	NS	< 0.001	NS
Carnitine	0.199	0.231	0.008	0.209	0.202	0.025	< 0.05	NS	< 0.001	NS
Choline	0.633	0.673	0.011	0.171	0.166	0.016	< 0.05	NS	< 0.001	NS
cis-Aconitate	0.022	0.025	0.001	0.008	0.010	0.001	< 0.05	NS	< 0.001	NS
Citrate	7.06	7.99	0.19	3.50	3.14	0.16	< 0.01	NS	< 0.01	NS
Creatine	0.539	0.576	0.009	0.146	0.136	0.018	< 0.01	NS	< 0.001	NS
Creatinine	0.159	0.211	0.020	0.147	0.114	0.016	< 0.1	NS	< 0.05	NS
Dimethyl sulfone	0.070	0.064	0.002	0.014	0.014	0.002	< 0.05	NS	< 0.001	NS
Formate	0.069	0.074	0.002	0.085	0.084	0.005	< 0.05	NS	< 0.001	NS
Fucose	0.026	0.018	0.003	0.011	0.010	0.001	< 0.1	NS	NS	NS
Fumarate	0.020	0.024	0.001	0.009	0.008	0.001	< 0.001	NS	< 0.05	NS

Galactose	0.576	0.557	0.067	0.513	0.766	0.151	< 0.05	NS	< 0.1	NS
Glutamate	0.247	0.273	0.009	0.082	0.070	0.012	< 0.05	NS	< 0.01	NS
Glycine	28.38	31.24	0.86	10.89	8.24	1.12	< 0.05	NS	< 0.01	NS
Hippurate	0.225	0.242	0.009	0.092	0.072	0.007	NS	< 0.1	< 0.01	NS
β-Hydroxybutyrate	0.032	0.033	0.001	0.013	0.017	0.001	NS	< 0.1	< 0.1	NS
Isobutyrate	0.0036	0.0044	0.0003	0.0020	0.0012	0.0003	< 0.1	< 0.1	< 0.05	NS
Lactic acid	0.051	0.059	0.002	0.038	0.028	0.004	< 0.05	< 0.1	< 0.001	NS
Lactose	90.92	91.15	1.40	88.792	92.186	3.357	NS	NS	< 0.001	NS
N-Acetylglucosamine	0.324	0.337	0.013	0.262	0.257	0.027	NS	NS	< 0.01	NS
N-Acetylglutamate	0.020	0.023	0.004	0.012	0.014	0.003	NS	NS	< 0.05	NS
N-Acetylglutamine	0.034	0.034	0.009	0.025	0.025	0.012	NS	NS	NS	NS
N-Acetylcarnitine	0.038	0.041	0.002	0.015	0.015	0.002	NS	NS	< 0.05	NS
Phosphocholine	0.151	0.134	0.013	0.087	0.039	0.017	NS	< 0.1	< 0.01	NS
Succinic acid	0.020	0.023	0.001	0.011	0.010	0.001	< 0.05	NS	< 0.05	NS
Urea	1.21	1.12	0.06	0.39	0.36	0.05	NS	NS	< 0.001	NS
Uridine	0.011	0.016	0.002	0.004	0.005	0.001	NS	NS	< 0.001	NS
Valine	0.017	0.016	0.001	0.007	0.007	0.001	NS	NS	< 0.05	NS
Trimethylamine-N-oxide	0.362	0.415	0.011	0.142	0.118	0.034	< 0.01	NS	< 0.001	NS

5.4 CONCLUSION

Organic and conventionally produced milk samples were investigated from two different farm sets that used all–year-round pasture based systems. Differences between milk produced by the two systems were only observed in Farm Set 1 where sampling was conducted throughout the whole lactation period. No individual milk metabolite could be identified to be consistently different between both milk types across all data sets. Changes in metabolite concentration in Farm Set 1 were attributed to differences in stage of lactation. In agreement with previous studies investigating organic and conventional milk metabolites using NMR, we could not identify any potential marker molecules between the two milk varieties. Furthermore, sampling date and time of milking had only minor effects on metabolite concentration. This leads to the overall conclusion that the metabolomic profile in milk of healthy animals that are fed to their requirements is not affected by other farming factors.

REFERENCES

Artegoitia, V. M., J. L. Middleton, F. M. Harte, S. R. Campagna, and M. J. De Veth. 2014. Choline and choline metabolite patterns and associations in blood and milk during lactation in dairy cows. PLoS ONE 9. http://dx.doi.org/10.1371/journal.pone.0103412.

Buitenhuis, A. J., U. K. Sundekilde, N. A. Poulsen, H. C. Bertram, L. B. Larsen, and P. Sørensen. 2013. Estimation of genetic parameters and detection of quantitative trait loci for metabolites in Danish Holstein milk. J. Dairy Sci. 96:3285-3295. http://dx.doi.org/10.3168/jds.2012-5914.

Erich, S., S. Schill, E. Annweiler, H. U. Waiblinger, T. Kuballa, D. W. Lachenmeier, and Y. B. Monakhova. 2015. Combined chemometric analysis of 1H NMR, 13C NMR and stable isotope data to differentiate organic and conventional milk. Food Chem. 188:1-7. http://dx.doi.org/10.1016/j.foodchem.2015.04.118.

Klein, M. S., M. F. Almstetter, N. Nürnberger, G. Sigl, W. Gronwald, S. Wiedemann, K. Dettmer, and P. J. Oefner. 2013. Correlations between milk and plasma levels of amino and carboxylic acids in dairy cows. J. Proteome Res. 12:5223-5232. http://dx.doi.org/10.1021/pr4006537.

Lu, J., E. Antunes Fernandes, A. E. Páez Cano, J. Vinitwatanakhun, S. Boeren, T. Van Hooijdonk, A. Van Knegsel, J. Vervoort, and K. A. Hettinga. 2013. Changes in milk proteome and metabolome associated with dry period length, energy balance, and lactation stage in postparturient dairy cows. J. Proteome Res. 12:3288-3296. http://dx.doi.org/10.1021/pr4001306.

Maher, A. D., B. Hayes, B. Cocks, L. Marett, W. J. Wales, and S. J. Rochfort. 2013. Latent biochemical relationships in the blood-milk metabolic axis of dairy cows revealed by statistical integration of 1H NMR spectroscopic data. J. Proteome Res. 12:1428-1435. http://dx.doi.org/10.1021/pr301056q.

Prema, D., J. L. Pilfold, J. Krauchi, J. S. Church, K. K. Donkor, and B. Cinel. 2013. Rapid determination of total conjugated linoleic acid content in select Canadian cheeses by 1H NMR spectroscopy. Journal of Agricultural and Food Chemistry 61:9915-9921. http://dx.doi.org/10.1021/jf402627q.

Schwendel, B. H., P. C. H. Morel, T. J. Wester, M. H. Tavendale, C. Deadman, B. Fong, N. M. Shadbolt, A. Thatcher, and D. E. Otter. 2015. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. J. Dairy Sci. 98:1411-1425. http://dx.doi.org/10.3168/jds.2014-8322.

Schwendel, B. H., T. J. Wester, P. C. H. Morel, M. H. Tavendale, C. Deadman, N. M. Shadbolt, and D. E. Otter. 2014. Invited review: Organic and conventionally produced milk-An evaluation of influence factors on milk composition. J. Dairy Sci. http://dx.doi.org/10.3168/jds.2014-8389.

Schwendel, B. H., T. J. Wester, P. C. H. Morel, M. H. Tavendale, C. Deadman, N. M. Shadbolt, and D. E. Otter. 2017. Pasture feeding conventional cows removes differences between organic and conventionally produced milk. Food Chemistry. Ref. No: FOODCHEM-D-16-04780 *(accepted for publication)*

Sundekilde, U. K., P. D. Frederiksen, M. R. Clausen, L. B. Larsen, and H. C. Bertram. 2011. Relationship between the metabolite profile and technological properties of bovine milk from two dairy breeds elucidated by NMR-based metabolomics. Journal of Agricultural and Food Chemistry 59:7360-7367. http://dx.doi.org/10.1021/jf202057x.

Sundekilde, U. K., F. Gustavsson, N. A. Poulsen, M. Glantz, M. Paulsson, L. B. Larsen, and H. C. Bertram. 2014. Association between the bovine milk metabolome and rennet-induced coagulation properties of milk. J. Dairy Sci. 97:6076-6084. http://dx.doi.org/10.3168/jds.2014-8304.

Sundekilde, U. K., N. A. Poulsen, L. B. Larsen, and H. C. Bertram. 2013. Nuclear magnetic resonance metabonomics reveals strong association between milk metabolites and somatic cell count in bovine milk. J. Dairy Sci. 96:290-299. http://dx.doi.org/10.3168/jds.2012-5819.

US Government Printing Office. 2014. Electronic Code of Federal Regulations: Regulations of the Department of Agriculture: Subchapter M–Organic Foods Production Act Provisions. Vol. 2014.

Wishart, D. S., T. Jewison, A. C. Guo, M. Wilson, C. Knox, Y. Liu, Y. Djoumbou, R. Mandal, F. Aziat, E. Dong, S. Bouatra, I. Sinelnikov, D. Arndt, J. Xia, P. Liu, F. Yallou, T. Bjorndahl, R. Perez-Pineiro, R. Eisner, F. Allen, V. Neveu, R. Greiner, and A. Scalbert. 2013. HMDB 3.0-The Human Metabolome Database in 2013. Nucleic Acids Res. 41:D801-D807. http://dx.doi.org/10.1093/nar/gks1065

Chapter 6

Discussion

At the outset of this study, I expected that organic milk must be somehow different from conventionally produced milk. This thought originated from the simple believe that if people are prepared to pay premium prices, and governments put production regulations and certification processes in place, then there must be a tangible difference between organic and conventionally produced products.

However, an investigation (Chapter 1) of the research conducted on the composition of organic and conventionally produced milk published prior at the outset of my PhD program painted a misleading picture. The only compound group comprehensively investigated before this thesis were milk fatty acids (FA), however, results varied greatly among studies. This lack of agreement was because most studies that purported to compare organic and conventionally produced milk did not take into account major factors which affect milk composition (e.g., diet and breed). The two general conclusions which could be made before conducting our own study were: milk composition was influenced by a variety of factors, and pasture feeding increased the amount of poly-unsaturated fatty acids. Our study was designed intentionally to minimize and account for a variety of factors known to influence milk composition by using two farm sets that were extensively monitored and similarly managed, >95 % forage based, and utilized Jersey-Holstein-Friesian crossbreed cows on adjacent properties.

As stated above, milk FA composition has been by far the most investigated milk compound group, which is why it was the first compound group I wanted to investigate in this study.

6.1 Fatty acid composition

As diet appeared to have such a big impact, the first point of interest was to investigate whether the FA composition of organic and conventionally produced milk differed in a pasture based system (Chapter 2). Despite factoring in seasonal and diurnal effects, I observed that a large number of FA were affected by the farming system (28 of 51 FA, $P < 0.001$) when analysing milk samples collected from individual cows from one organic and one conventional dairy herd at Massey University, Palmerston North, NZ. The analysis of milk samples from individual animals assured that any differences between herd averages were true and not caused by 'outlier' animals, causing an imbalance of the herd average. Results showed that conventional milk contained a greater amount of vaccenic acid (C18:1 t11; VA) and conjugated linoleic acid (C18:2 c9t11; CLA). These differences were observed even though cows from both herds were fed solely on pasture of similar species diversity and

botanical and chemical composition. Furthermore, these result stand in direct contradiction to the majority of results reported in the literature, where major claims of potential health benefits for humans based on the greater amount of CLA (Lock and Bauman, 2004) are made for organic milk. The cause for the greater amount of CLA and VA were unknown, however, after considering the known influence factors on milk FA composition and the differences observed between the farms on the farm sets in our study, we postulated that these two FA were influenced by differences in nitrogen fertilizer application. Although not usually mentioned, differences in fertilizer application generally can be assumed for most studies comparing organic and conventional milk from pasture grazed cows. Several studies (Elgersma et al., 2005; Arvidsson et al., 2012; Glasser et al., 2013) reported higher content of total FA and α-linoleic acid (ALA) after application of various nitrogen fertilizer levels (30-120 N kg/ha) on the same forage. An approximate increase of 3g ALA /kg DM in ryegrass pasture per 50 kg N/ha has been reported by Elgersma et al. (2005). This would result in a significant increase in amount of ALA taken up by cows feeding solely on fertilized pasture compared to cows feeding on pasture with lower nitrogen fertilizer treatment.

Up to 99% of ALA and LA consumed by cows is bio-hydrogenated in the rumen, with VA being a main derivative (Lee and Jenkins, 2011). VA is then partly desaturated to CLA in the mammary gland, explaining the elevated content of VA and CLA in milk from predominantly grass-fed cows (Destaillats et al., 2005; Leiber et al., 2005). A higher dietary intake of ALA should consequently lead to a higher amount of VA and CLA in milk. Furthermore, I observed that odd and branched chain FA (OBCFA) were significantly affected by farming system, with 13 OBCFA increased ($P < 0.001$) in conventionally produced milk. These differences could have resulted from differences in the rumen ecology between the organic and conventional herd (Collomb et al., 2008). Changes in the OBCFA profile leaving the rumen, which are subsequently reflected in milk FA profile, are largely caused by alterations in the relative abundance of specific bacterial populations in the rumen (Vlaeminck et al., 2006; French et al., 2012). No samples of ruminal contents were taken in this trial and it was, therefore, not possible to assess if there were differences in the rumen microbiota between organic and conventionally farmed cows.

Potential causes for differences in rumen microbiota composition in pasture based diets may have been variations in botanical composition between pastures (Wiking et al., 2010; Larsen et al., 2012). Greater amounts of branched-chain FA in milk have been observed when cows were fed hay that contained a higher amount of herbaceous plant material (Baars et al.). Herbs, like chicory (Molan et al., 2003) and plantain (Jarzomski et al., 2000), contain condensed tannins and secondary plant metabolites that are known to influence the bio-

hydrogenation of FA in the rumen (Patra and Saxena, 2011; Petersen et al., 2011). For our study, the differences in clover and herb content between farms were considered minor and could not be statistically explored due to the lack of repetitive pasture composition measurements. Consequently, we are unable to completely exclude the possibility that slight differences in botanical composition had an effect on milk FA composition.

Another possible cause for the differences observed for OBCFA between organic and conventionally produced milk could have been the effect of the drenching of the organic herd with garlic-cider vinegar. The lack of research on the long-term effects of chronic supplementation with small doses of garlic makes it difficult to determine its possible impact in our study. However, garlic is known for its antimicrobial properties (Feldberg et al., 1988) has been reported to influence ruminal volatile FA composition (Calsamiglia et al., 2007) and can, therefore, not be excluded to have impacted rumen microbiota of the organic herd. Although this first data set of the individual milk samples considered a variety of factors known to influence milk FA composition, the weak point of this first study was that it represented only one farm set and only one milking season. Fortunately, a second farm set was located near Tokoroa, NZ that which had a similar arrangement of one organic and one conventional farm adjacent to each other and managed in a similar manner.

The second research study (Chapter 3) utilized bulk milk samples collected throughout the lactation period from the same farm set (Farm Set 1) as the individual milk samples (Massey University, Palmerston North, NZ) and from Farm Set 2 located at Tokoroa, NZ. In agreement with the results from our first study on individual milk samples (Chapter 2), I observed increased levels of VA and CLA in conventionally produced bulk milk samples collected throughout the lactation period on both farm sets. The detailed knowledge about differences and similarities between both farm sets enabled further elimination of potential causes and supported my hypothesis that the increase in VA and CLA in conventional milk was most probably linked to a greater amount of ALA in the pasture caused by a higher application of nitrogen fertilizer on conventional pastures. Although a high rate of nitrogen fertilizer application is more characteristic of conventionally farmed pasture, it is simply a reflection of the affordability and accessibility of synthetically produced fertilizer compared to certified organic nitrogen fertilizer, rather than regulations limiting fertilizer application on organic farms.

I did not observe the same differences for OBCFA on Farm Set 2. Based on the knowledge about differences and similarities between both farm sets, I postulated that the differences observed for OBCFA are likely caused by the drenching of the organic herd with cider vinegar garlic mixture in Farm Set 1, rather than differences in the amount of secondary plant metabolites.

Most of the differences reported between milk from organic and conventional cows have been in FA profile, however, few studies have investigated compounds other than FA when looking for differences between organic and conventionally produced milk (Payling et al., 2015).

6.2 Other milk components

I constructed a more complete image of organic and conventional milk by investigating the composition of the proteins, oligosaccharides (OS) and volatile compounds in addition to FA (Chapter 3). Protein composition, which is less susceptible than FA to diet, has received little attention in relation to organic and conventional milk production (Kuczyńska et al., 2012). Milk OS may be able to influence on the selection of gut microbiota in infants at a very early stage of life, and the current interest in bovine milk OS is dominated by the desire to create a bovine-based infant formula that mimics human milk in OS composition and concentration (Lee et al., 2015). Individual animal genetics, breed, and stage of lactation are known to influence milk OS composition (McJarrow and Van Amelsfort-Schoonbeek, 2004; Tao et al., 2009). Furthermore, bovine diet may influence OS composition in milk (Asakuma et al., 2010; Liu et al., 2014), with greater total sialic acid concentration observed in milk from grazed cows, although research is limited.

Volatile compounds in milk fat have been investigated primarily in relation to flavor components derived from different diets and the development of off-flavors during heat treatment or storage (Coppa et al., 2011). In this study milk fat volatiles were investigated predominately to see whether it was possible to detect volatile secondary plant metabolites, or traces of the garlic drench in the milk samples. Both secondary plant metabolites (Collomb et al., 2008) and garlic has been shown to affect rumen microbiota, with the possibility to affect OBCFA in milk. However, amount of garlic in the drench and differences in intake of secondary plant metabolites between organic and conventional cows have been questioned for being too low to cause the significant effect on OBCFA observed for organic and conventionally produced milk on Farm Set 1. The assumption was, therefore, that if it were possible to detect either garlic residues (e.g., allicine) or secondary plant metabolites in milk, their concentration might have been high enough to have an impact on the rumen microbiota, which consequently could have caused the effect observed for OBCFA in milk. The analysis of milk fat volatiles as a whole provides further information on the differences between organic and conventionally produces milk.

6.2.1 Oligosaccharide Composition

Eleven chromatographic features of the correct calculated m/z values observed in all milk samples and were putatively assigned as the corresponding bovine milk OS. All OS were affected by sampling date ($P <$

0.001, Farm Set 1; $P \leq 0.05$, Farm Set 2), while four OS concentrations were increased ($P < 0.05$) in organic milk (3 Hex; 3 Hex, 1 NeuAc; 4 Hex, 1 HexNAc; 3 Hex, 2 HexNAc) independent of the farm set, and a further four OS were unaffected by either farming system or farm set. It is presently unknown how milk OS are synthesized in the mammary gland so it's not possible to speculate on why differences were observed. The characteristics of both farm sets indicated that factors specific to our trial, such as, soil type, pasture composition, and milk volume per cow/day had little or no impact on OS concentration found in milk.

6.2.2 Protein Composition

A greater amount of daily milk yield and milk solids was observed for Farm Set 2 compared to Farm Set 1, with pasture composition significantly different between both farms sets. Pasture from Farm Set 2 contained 50% white clover compared to less than 5% for Farm Set 1. Clover content in bovine diet has been associated with greater milk volume and protein yield (Thomson et al., 1985) compared to ryegrass based diets, which reflects our observations. Concentrations of individual milk proteins varied. Differences in the total casein percentage for both farm sets are presumably related to the higher clover content in pastures in Farm Set 2, with previous studies reporting higher contents of α- and β-casein in milk from cows grazing clover instead of ryegrass (Grandison et al., 1985). This increase in casein relative to whey was explained to result from a greater feed intake when grazing clover (Thomson et al., 1985). Breed composition varied between our farm sets and systems, but for Farm Set 2 with a larger disparity between the organic and conventional herds, no system effect for any major protein was observed. I believe that breed can, therefore, be excluded as a cause of differences in protein content. I observed changes in the concentration of κ-casein and β-lactoglobulin in Farm Set 1 throughout the lactation period, however, no farm system related differences could be observed between organic and conventionally produced milk.

6.2.3 Milk fat volatile composition

I observed similar volatile compounds in milk fat in our study to what others have reported (Croissant et al., 2007), except that I did not detect terpenes. The method I used was optimised to quantitate longer chain compounds (> C4:0), such as, alkanes, alcohols, ketones, and FA. It employed a combined extraction and distillation procedure on milk fat followed by a gas chromatographic separation, using a stationary phase suited to longer chain aliphatic compounds. As a consequence, I did not observe the same quantity and variety of aromatic compounds (e.g., terpenes). The greatest agreement with other studies occurred with ketones, where the detection of individual compounds appeared to be independent of sample type, preparation, and analysis type. Hydrocarbons, which made up the second largest group of volatile compounds in this study, have been

reported by others (Toso et al., 2002). However, individual hydrocarbons vary between studies and, by comparison, I observed more long chain compounds, which may reflect my choice of chromatography column. Other classes of compounds observed were aldehydes, non-esterified FA, and lactones, all of which have been reported by other researchers (Vagenas and Roussis, 2012).

None of the individual compounds or classes of compounds showed trends for farm set, system, or sampling date. This indicates that factors other than pasture composition and stage of lactation influence milk fat volatile composition. Similar to my findings, others have reported that most volatile compound classes (e.g., alcohols, aldehyde, hydrocarbons) are largely unaffected by dietary differences (Bugaud et al., 2001). Milk volatile compounds may arise from microorganisms in milk as there is no correlation between volatiles in pasture and those found in in milk (Bugaud et al., 2001). I was not able to detect volatile secondary plant metabolites, or traces of the garlic drench in the milk samples. This could be, because their concentration was below the detection limit of the analytical method, or a reflection of the stationary phase chosen for the chromatographic separation.

6.3 Non-targeted method

After investigating several specific milk compound groups (e.g., proteins, FA, and OS), I decided to use a non-targeted approach rather than concentrating further on specific compounds (e.g., iodine) or selective compound groups (e.g., vitamins), which would only enable the analysis of limited compounds per analytical method. Non-targeted methods provide the opportunity to investigate a large number of compounds originating from a variety of biological pathways. Nuclear magnetic resonance (NMR) spectroscopy is, after mass spectrometry (MS), the analytical platform used to investigate the metabolomics composition of complex biological samples. NMR, although significantly limited by the number of compounds which can be identified per sample, provides a standardised methodology with minimal changes in chemical shift and signal intensity between samples, independent of instrumentation or sample type. It, therefore, allows the analysis of a multitude of samples without run order or batch effects, the direct comparison between different studies, and the creation of extended spectral libraries. Consequently, the ratio of compounds detected vs. compounds identified is presently significantly greater compared to MS analysis.

The number of metabolites influenced by the farming system varied depending on the data (individual or bulk milk samples) and farm set. Bulk milk samples in Farm Set 1 showed 15 of the reported 33 compounds affected by farm system, with citrate, creatine, fumarate, and trimethylamine-N-oxide most affected ($P < 0.01$), while in Farm Set 2 alanine was the only compound different ($P < 0.05$) between organic and conventionally produced milk. Similarly, no differences between organic and conventionally produced milk were observed in

the individual sample set, with the exception of β-hydroxybutyrate, which was increased in organic milk. Sampling date had the greatest influence on Farm Set 1, with 29 of 33 compounds affected, while acetate was the only compound affected in Farm Set 2, and only 8 of compounds were affected ($P < 0.05$) in the individual milk data set. Differences observed between organic and conventionally produced bulk milk in Farm Set 1 are presumably a result of one or more presently unknown factors effective during early or very late lactation, or both, as these are the periods not considered by the individual milk sample set and the bulk milk samples from Farm Set 2. Sampling date is known to significantly affect several milk metabolites. (Lu et al., 2013; Artegoitia et al., 2014).

Chapter 7

Conclusion

The aim of our study was to investigate differences between organic and conventional milk from pasture-based systems. Most studies that purport to compare organic and conventionally produced milk do not take into account the major factors which affect milk composition (e.g., diet and breed). Our study was designed intentionally to minimize these differences. By using two farm sets with similarly managed, pasture-based systems on adjacent properties, we were able to make a true comparison of the impact of organic versus conventional dairy farming on milk composition, and not just a comparison of pasture versus concentrate feeding. We attempted to explain many of the differences observed. Our study investigated four different classes of compounds and applied non-targeted metabolomics to investigate differences in the chemical composition of organic and conventional bovine milk produced on a pasture based system. We accounted for known factors that influence milk composition and selected farms that were >95% forage based and utilized Jersey-Holstein-Friesian crossbreed cows. We demonstrated that VA and CLA are increased in conventional milk, while LA and ALA show greater abundance in organic milk independent of the farm set. Similar to FA, system effects were observed for half of the reported OS, while four OS were not influenced by farm set or system. Presently, influence factors on reported OS in bovine milk are unknown and further research is required.

Overall, none of the factors identified by this study that potentially alter milk composition were unique to either farming system. Nitrogen fertilizer application, clover content in pasture, and garlic drenching are not unique to organic or conventional farming systems. Many conventionally produced dairy products might differ in their chemical composition from organically produced ones as a result of differences in diet, breed, and farm management between the two systems. However, increasing availability of milk from pasture fed conventional cows will reduce and potentially eliminate any overall differences previously reported between organic and conventionally produced milk.

Beyond this study, I had great difficulty with the term ‘organic’ itself. It is not consistently or adequately defined to draw any overall conclusions regarding organic and conventionally produced milk. As outlined in Chapter 1, regulations between countries vary, and within each country regulations allow for further variability. Factors known to influence milk composition change depending on geographical location, season, individual farming practices, and local dairy breeds, and cannot be restricted to either organic or conventional farm systems,

therefore, causing variability between the two milk types. The variations between organic farming regulations of different countries, and the differences in application within each country, lead to the possibility that certified organic cows raised in a geographical area might have significantly less pasture access than conventional cows in another area. Similarly, depending on the regulations, some certified organic cows can have up to three courses of antibiotic treatment in their lifetime, while organic cows in a different region under a different regulatory framework will lose their organic status forever when treated just once. Scientific studies require defined parameters to evaluate the research objectives, with organic milk, these parameters are not defined.

Beyond the regulatory term of 'organic', the greatest difficulty is caused by the apparent discrepancy between 'organic' regulations and what scientific studies can investigate, and what consumers believe the term 'organic' stands for. Many of the characteristics consumers associate with organic products (e.g., improved animal welfare, health benefits for humans, and better environmental footprint) have not been scientifically proven, or even tested. This can be partly linked to the above mentioned differences in organic regulations, however, a lack of consumer knowledge of what organic regulations entail is probably the main cause for the discrepancy. Future consumer education on how organic dairy production actually differs from conventional dairy production might cause a devaluation of organic products, as farming reality and consumer perception are not identical.

Given the problems surrounding organic regulations and the vague consumer assumptions regarding organic products, the New Zealand dairy industry should step away from organic milk production, while developing a premium brand of pasture based conventional milk. Milk powder and dairy products are New Zealand's largest exports (by value) and are presently marketed on the global commodity market to compete with dairy products from other countries where cows are fed readily available concentrates. Those other countries are capable of producing a higher milk volume per cow as a consequence of concentrate feeding. The New Zealand dairy industry can presently only compete with overseas producers because of its low input pasture based production system. Furthermore, the value of New Zealand and its milk production originates from its 'green, clean' and consequently 'safe' image. Therefore, New Zealand's dairy industry should focus on marketing its premium products (similar to 'pasture milk' in other countries), originating from a pasture based farming system, rather than converting South Island sheep stations to dairy, which involves the continuous draining and polluting of rivers in areas not conducive to the style of dairy farming suited to New Zealand.

Chapter 8

Future Outlook

During the progression of this study, a variety of topics raised further research questions. They could not be answered within the present study because 1) they were outside the scope of the present research question, and 2) the necessary tools to conduct the research were not available. However, I have identified below a few areas of future research which would complement the present study.

8.1 Investigate effect of nitrogen fertilizer application on milk FA composition

We observed an increased CLA and VA in conventional milk on both farm sets (Chapter 2 and 3). Based on previous research studies and in depth knowledge about the characteristics of the farms used in this study, I postulated that this increase was caused by a higher application rate of nitrogen fertilizer on the conventional farms, however, this effect has not been examined directly. To investigate this, monitored dairy herds would be grazed on pasture with different levels of nitrogen fertilizer application rate in a crossover design trial. Pasture composition would have to be closely monitored so that it is equal across paddocks to prevent effects on milk composition due to botanical variation in pasture. FA composition would have to be monitored in pasture and milk to ascertain if there are any direct correlations between fertilizer application and ALA concentration in pasture with VA and CLA concentration in milk.

8.2 Investigate long-term effect of of cider vinegar garlic drench and secondary plant metabolites on rumen derived FA

Differences in concentration of OBCFA were observed on Farm Set 1 between organic and conventionally produced milk (Chapter 2 and 3). We speculated that the cause for this could have been either the long-term drenching of the organic herd in Farm Set 1 with cider vinegar garlic solution as the same effect was not observed in Farm Set 2 where this was not practiced, or by differences in intake of secondary plant metabolites with slight differences in pasture composition between farms. To be able to explore the effect of cider vinegar garlic mix, cows would be drenched with various combinations of cider vinegar, garlic, and carrier in a crossover design. Milk FA composition, as well as rumen microbiota and FA composition should be investigated to explore the impact of garlic and cider vinegar, either separately or in combination. It would be worthwhile investigating if there is a minimum dose, and to which degree the production of OBCFA could be

suppressed. A similar study should be conducted with small amounts of secondary plant metabolites to establish effects of low doses on rumen microbiota, rumen FA composition, and consequently milk FA composition.

8.3 Investigation of free OS in colostrum from organic and conventional cows

Current interest in bovine milk OS is dominated by the desire to create a bovine-based infant formula that mimics human milk in OS composition and concentration (Lee et al., 2015). Individual animal genetics, breed, and stage of lactation are known to influence milk OS composition, with diet also a potential influence factor. However in this study, I observed similar trends for minor OS that were increased in organic milk independent of farm set, without any plausible explanation from presently known influence factors. To investigate the causes and significance of this, it may help to investigate whether these observations in milk OS can also be found in bovine colostrum, which contains significantly higher concentrations of OS compared to milk. Other aspects should also be investigated (e.g., nitrogen fertilizer application) to determine factors that influence milk OS.

8.4 Investigation of organic and conventionally produced milk using high resolution MSfor non-targeted metabolomics.

We used NMR in this thesis for a non-targeted approach to investigate large sample sets and look for differences in metabolite composition. The main disadvantage of using NMR was that there was only a small number of compounds that could be resolved and we may be missing differences related to metabolic pathways that we could not detect. Therefore, milk samples should be investigated using a high resolution MS as detector with which is possible to screen for a much wider array of metabolites. However, while the preparation and the analysis of hundreds of samples is not a problem anymore, it is difficult and laborious to try and draw meaningful conclusions from potentially thousands of mass features (putative metabolites). Data processing has to be conducted meticulously with carefully selected settings of signal to noise ratios, elimination of artificial fragments, consideration of shifts in retention time, loss of sensitivity, and run and batch order effects. Results also need to be checked for different isotopic masses. This requires specialised computing tools developed specifically to process the colossal data sets that are created. Only then can the data be statistically analyzed to identify significant mass features. The second step is to identify statistically relevant mass features as chemical compounds. Although metabolomics libraries for data generated by MS are developing, mass spectra differ depending on individual instrument, analysis method, and sample matrix. Consequently, relevant mass features need to be fragmented to create mass artefacts which provide additional information. In the final step, identified

compounds are either spiked into the sample or used as external standards to verify that the observed fragmentation patters match those of the actual compound.

8.5 Investigation of the effect of organic and conventional farming on animal welfare and environmental impact

Beyond the chemical analysis of milk and pasture samples, further investigation should be conducted into the perceived benefits of organic milk production for impacts on animal welfare and the environment. As described in the Introduction (Chapter 1), consumers purchase organic products for a variety of reasons, with differences in milk composition and potential health benefits only one aspect. A major review considering animal welfare and environmental impact needs to be conducted to investigate whether the perceived consumer assumption that organic farming is kinder on the animals and the environment can be shown. This would provide scientific rationale for purchase decisions presently based on consumer assumptions.

REFERENCES

Artegoitia, V. M., J. L. Middleton, F. M. Harte, S. R. Campagna, and M. J. De Veth. 2014. Choline and choline metabolite patterns and associations in blood and milk during lactation in dairy cows. PLoS ONE 9. http://dx.doi.org/10.1371/journal.pone.0103412.

Arvidsson, K., A. M. Gustavsson, V. Fievez, and K. Martinsson. 2012. The effect of N-fertilisation rate or inclusion of red clover to timothy leys on fatty acid composition in milk of dairy cows fed a commercial silage Concentrate ratio. Animal 6:1178-1186.

Asakuma, S., Y. Ueda, F. Akiyama, Y. Uemura, M. Miyaji, M. Nakamura, M. Murai, and T. Urashima. 2010. Short communication: Effect of grazing on the concentrations of total sialic acid and hexose in bovine milk. J. Dairy Sci. 93:4850-4854. http://dx.doi.org/10.3168/jds.2010-3357.

Baars, T., J. Wohlers, D. Kusche, and G. Jahreis. Experimental improvement of cow milk fatty acid composition in organic winter diets. J. Sci. Food Agric. http://dx.doi.org/10.1002/jsfa.5525.

Bugaud, C., S. Buchin, J. B. Coulon, A. Hauwuy, and D. Dupont. 2001. Influence of the nature of alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk. Lait 81:401-414.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90:2580-2595. http://dx.doi.org/10.3168/jds.2006-644.

Collomb, M., W. Bisig, U. Buetikofer, R. Sieber, M. Bregy, and L. Etter. 2008. Fatty acid composition of mountain milk from Switzerland: comparison of organic and integrated farming systems. Int. Dairy J. 18:976-982.

Coppa, M., B. Martin, P. Pradel, B. Leotta, A. Priolo, and V. Vasta. 2011. Effect of a hay-based diet or different upland grazing systems on milk volatile compounds. Journal of Agricultural and Food Chemistry 59:4947-4954. http://dx.doi.org/10.1021/jf2005782.

Croissant, A. E., S. P. Washburn, L. L. Dean, and M. A. Drake. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. J. Dairy Sci. 90:4942-4953.

Destaillats, F., J. P. Trottier, J. M. G. Galvez, and P. Angers. 2005. Analysis of α-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acids. J. Dairy Sci. 88:3231-3239.

Elgersma, A., P. Maudet, I. M. Witkowska, and A. C. Wever. 2005. Effects of Nitrogen fertilisation and regrowth period on fatty acid concentrations in perennial ryegrass (Lolium perenne L.). Annals of Applied Biology 147:145-152.

Feldberg, R. S., S. C. Chang, A. N. Kotik, M. Nadler, Z. Neuwirth, D. C. Sundstrom, and N. H. Thompson. 1988. In vitro mechanism of inhibition of bacterial cell growth by allicin. Antimicrobial Agents and Chemotherapy 32:1763-1768.

French, E. A., S. J. Bertics, and L. E. Armentano. 2012. Rumen and milk odd- and branched-chain fatty acid proportions are minimally influenced by ruminal volatile fatty acid infusions. J. Dairy Sci. 95:2015-2026. http://dx.doi.org/10.3168/jds.2011-4827.

Glasser, F., M. Doreau, G. Maxin, and R. Baumont. 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Anim. Feed Sci. Technol. 185:19-34.

Grandison, A. S., D. J. Manning, and M. Erson. 1985. Chemical Composition, Rennet Coagulation Properties and Flavour of Milks From Cows Grazing Ryegrass or White Clover. J. Diary Res. 52:33-39. http://dx.doi.org/10.1017/s0022029900023864.

Jarzomski, C. M., N. E. Stamp, and M. D. Bowers. 2000. Effects of plant phenology, nutrients and herbivory on growth and defensive chemistry of plantain, Plantago lanceolata. Oikos 88:371-379.

Kuczyńska, B., K. Puppel, M. Gołębiewski, E. Metera, T. Sakowski, and K. Słoniewski. 2012. Differences in whey protein content between cow's milk collected in late pasture and early indoor feeding season from conventional and organic farms in Poland. J. Sci. Food Agric. http://dx.doi.org/10.1002/jsfa.5663.

Larsen, M. K., X. C. Fretté, T. Kristensen, J. Eriksen, K. Søegaard, and J. H. Nielsen. 2012. Fatty acid, tocopherol and carotenoid content in herbage and milk affected by sward composition and season of grazing. J. Sci. Food Agric. 92:2891-2898. http://dx.doi.org/10.1002/jsfa.5620.

Lee, H., V. L. D. MeloSilva, Y. Liu, and D. Barile. 2015. Short communication: Quantification of carbohydrates in whey permeate products using high-performance anion-exchange chromatography with pulsed amperometric detection. J. Dairy Sci. 98:7644-7649. http://dx.doi.org/10.3168/jds.2015-9882.

Lee, Y. J., and T. C. Jenkins. 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. Journal of Nutrition 141:1445-1450. http://dx.doi.org/10.3945/jn.111.138396.

Leiber, F., M. Kreuzer, D. Nigg, H. R. Wettstein, and M. R. L. Scheeder. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids 40:191-202. http://dx.doi.org/10.1007/s11745-005-1375-3.

Liu, Z., P. Moate, B. Cocks, and S. Rochfort. 2014. Simple liquid chromatography-mass spectrometry method for quantification of major free oligosaccharides in bovine milk. Journal of Agricultural and Food Chemistry 62:11568-11574. http://dx.doi.org/10.1021/jf5037849.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197-1206. http://dx.doi.org/10.1007/s11745-004-1348-6.

Lu, J., E. Antunes Fernandes, A. E. Páez Cano, J. Vinitwatanakhun, S. Boeren, T. Van Hooijdonk, A. Van Knegsel, J. Vervoort, and K. A. Hettinga. 2013. Changes in milk proteome and metabolome associated with dry period length, energy balance, and lactation stage in postparturient dairy cows. J. Proteome Res. 12:3288-3296. http://dx.doi.org/10.1021/pr4001306.

McJarrow, P., and J. Van Amelsfort-Schoonbeek. 2004. Bovine sialyl oligosaccharides: Seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. Int. Dairy J. 14:571-579. http://dx.doi.org/10.1016/j.idairyj.2003.11.006.

Molan, A. L., A. J. Duncan, T. N. Barry, and W. C. McNabb. 2003. Effects of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworm and gastrointestinal nematodes. Parasitology International 52:209-218.

Patra, A. K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91:24-37. http://dx.doi.org/10.1002/jsfa.4152.

Payling, L. M., D. T. Juniper, C. Drake, C. Rymer, and D. I. Givens. 2015. Effect of milk type and processing on iodine concentration of organic and conventional winter milk at retail: Implications for nutrition. Food Chem. 178:327-330. http://dx.doi.org/10.1016/j.foodchem.2015.01.091.

Petersen, M. B., K. Søegaard, and S. K. Jensen. 2011. Herb feeding increases n-3 and n-6 fatty acids in cow milk. Livest. Sci. 141:90-94. http://dx.doi.org/10.1016/j.livsci.2011.05.004.

Tao, N., E. J. DePeters, J. B. German, R. Grimm, and C. B. Lebrilla. 2009. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. J. Dairy Sci. 92:2991-3001. http://dx.doi.org/10.3168/jds.2008-1642.

Thomson, D. J., D. E. Beever, M. J. Haines, S. B. Cammell, R. T. Evans, M. S. Dhanoa, and A. R. Austin. 1985. Yield and Composition of Milk From Friesian Cows Grazing Either Perennial Ryegrass Or White Clover In Early Lactation. J. Diary Res. 52:17-31. http://dx.doi.org/10.1017/s0022029900023852.

Toso, B., G. Procida, and B. Stefanon. 2002. Determination of volatile compounds in cows' milk using headspace GC-MS. J. Diary Res. 69:569-577.

Vagenas, G., and I. G. Roussis. 2012. Fat-derived volatiles of various products of cows', ewes', and goats' milk. International Journal of Food Properties 15:665-682. http://dx.doi.org/10.1080/10942912.2010.498542.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389-417. http://dx.doi.org/10.1016/j.anifeedsci.2006.06.017.

Wiking, L., P. K. Theil, J. H. Nielsen, and M. T. Sørensen. 2010. Effect of grazing fresh legumes or feeding silage on fatty acids and enzymes involved in the synthesis of milk fat in dairy cows. J. Diary Res. 77:337-342. http://dx.doi.org/10.1017/s002202991000021x.

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