

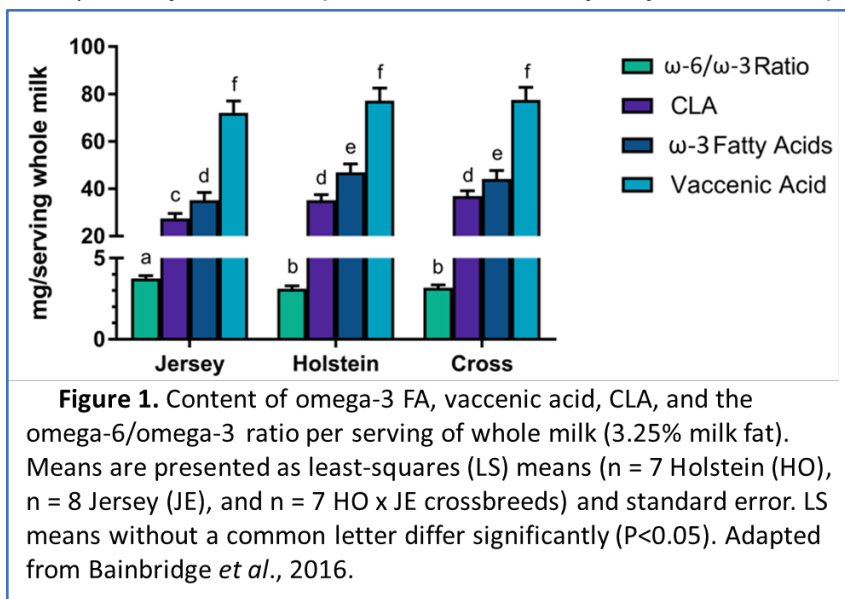
Improving Milk and Forage Fatty Acids in Pasture-Based Dairies

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Introduction

Consumer awareness of the link between dietary fats and health outcomes has led to increased demand for food products enriched with bioactive fatty acids (FA). This demand for foods with added nutritional value is reflected by the 25% increase in sales of organic whole-milk from 2014 to 2015, in comparison to a <1% increase for low-fat and fat-free versions (Maltby, 2016). Milk fat contains more than 400 different FA (Jensen, 2002), many of which possess bio-activity beyond that of providing nutritional value and



are termed bioactive FA (Kris-Etherton *et al.*, 2004). Bioactive FA in dairy products, such as alpha-linolenic acid (ALA; 18:3 c9, c12, c15 omega-3), conjugated linoleic acids (CLA), and vaccenic acid (VA; 18:1 t11), are typically present in low percentages in milk (<5%), but may have beneficial effects on human health (Parodi, 1997). CLA have been shown to have anti-carcinogenic effects in

animal models (Moon, 2014), and research suggests VA can reduce tumor growth and the risk of cardiovascular diseases (CVD) (Field *et al.*, 2009), while omega-3 (n-3) FA have demonstrated protective effects against inflammation (Zhao *et al.*, 2004), neurological disorders (Pan *et al.*, 2012), and CVD (Fleming and Kris-Etherton, 2014). This is supported by several observational studies and diet-intervention trials that associated milk consumption with a lower risk for CVD and type 2 diabetes (Rice *et al.*, 2013; Vatten *et al.*, 2013). Increasing the content of bioactive FA in dairy products offers an opportunity for dairy producers to increase their income by marketing dairy products with added nutritional value (*i.e.*, functional foods).

Strategies to improve the bioactive FA content of milk

There are several factors that affect the FA composition of milk fat, such as breed, lactation stage, season, and diet. Our research has evaluated the milk FA composition of seven Holstein, eight Jerseys, and seven Holstein x Jersey crossbreeds across a lactation and demonstrated that Holsteins produce milk with higher proportions of bioactive FA

when compared to Jerseys and Holstein x Jersey crossbreeds (Figure 1) (Bainbridge *et al.*, 2016). Other research has corroborated this observation, showing milk fat from Jersey cows has a higher content of saturated FA (SFA) and a lower content of CLA (Kelsey *et al.*, 2003). This has been shown to be an effect of the differing mammary enzyme expression of delta-9 desaturase, which converts VA to CLA, between these two breeds (Kelsey *et al.*, 2003).

Diet is the most prominent factor to affect milk FA composition. However, there are several challenges in increasing the content of bioactive FA in milk through dietary modifications. In ruminants, dietary FA are first subject to microbial digestion in the rumen, resulting in a vastly different profile between dietary FA and FA to be incorporated into milk (Pappritz *et al.*, 2011). The FA composition of forages is highly unsaturated, with most of forage FA being polyunsaturated FA (PUFA) (Glasser *et al.*, 2013), yet milk fat is comprised of predominantly SFA. This is a result of the biohydrogenation mechanisms bacteria have developed to mitigate the bacteriostatic effects (kept from reproducing) of PUFA in the rumen (Jenkins *et al.*, 2008). Biohydrogenation is the action of adding hydrogens to double bonds, ultimately leading to a saturated FA product, this process is rapid and extensive often exceeding a rate of 85% (Jenkins *et al.*, 2008). Yet, incomplete biohydrogenation does allow for accumulation and escape of biohydrogenation intermediates from the rumen. Thus, biohydrogenation of dietary FA by rumen bacteria is both the origin of unique rumen-derived bioactive FA (e.g., VA and CLA) and the cause of the low content of PUFA in milk fat.

Nonetheless, a strong positive correlation exists between the content of ALA in animal diet and the content of CLA and ALA in milk fat. Organic dairy products have been shown to contain higher proportions of bioactive FA (Benbrook *et al.*, 2013; Schwendel *et al.*, 2015) because organic dairy producers are required to have 30% of their cows' dry matter intake come from pasture for a 120-day grazing season (U.S. Department of Agriculture, 2010). The attribution of this effect to pasture feeding, and not organic status was recently supported by research of Schwendel *et al.* (2017).

While the inclusion of fresh forage in the diet of dairy cows leads to an improved milk FA profile, not all forage species affect the milk FA profile in the same way. Turner *et al.* observed a higher content of n-3 FA in milk when cows grazed birdsfoot trefoil (*Lotus corniculatus* L.) in comparison to ryegrass (*Lolium* sp. L.; Turner *et al.*, 2005), and Soder *et al.* demonstrated an increased content of CLA in milk with additional forage species in the pasture (3, 6, and 9 species mixtures vs. 2 species mix) (Soder *et al.*, 2006). Bainbridge *et al.* evaluated a summer annual species, pearl millet (*Pennisetum glaucum* L.), which grows rapidly during the hot-summer months when cool-season perennial pastures experience less growth (Bainbridge *et al.*, 2017). The contents of CLA and n-3 FA in a serving of whole milk (3.25% fat) increased when cows grazed a cool-season pasture compared to pearl millet (Figure 2), with no difference in milk production. This observation can mainly be attributed to the 2-fold increase in ALA content of cool-season pasture in comparison to the pearl millet (12.0 vs. 6.5 mg/g dry matter) (Bainbridge *et al.*, 2017). Therefore, strategies to increase the content of bioactive FA in milk fat should be focused

on improving pastures to contain higher contents of ALA in addition to maximizing grazing throughout the pasture season.

Factors affecting forage FA profile

When considering herbage FA profile, ALA is the FA of greatest interest for several reasons. Firstly, ALA is the predominant FA in leaf tissue, and therefore in vegetative forage in general. Secondly, ALA content of feed is strongly correlated with ALA and CLA content of milk (*i.e.*, is a precursor for milk omega-3 FA as well as CLA). And thirdly, ALA is highly susceptible to lipolysis and oxidation, therefore it is the individual forage FA that has the greatest potential for losses, before forage has even been fed to an animal.

For dairy producers hoping to maximize the ALA available to their cows from forage, there are four primary management considerations: I) vegetation stage (maturity), II) season, III) wilting time of conserved forage and, IV) nitrogen fertility.

Vegetation stage is the greatest determinant of ALA content in forages because ALA is primarily found in chloroplast membranes. As such, young leaf tissue predominant in early vegetative stages is relatively rich in ALA, but advancing maturity brings an increase in non-leaf components (leaf sheath /petiole, culm, seedhead) and increased fiber content and eventually decreased chloroplast content as nitrogen is translocated to seedheads (Boufaïed *et al.*, 2003; Glasser *et al.*, 2013; Khan *et al.*, 2012). Increasing regrowth intervals from 20 to 38 days in *Lolium* species was shown by Dewhurst *et al.* (2001) to reduce ALA content 1.5 - 2.3-fold and total FA content 1.4 - 2-fold.

Forage ALA content has repeatedly been found to be highest in the early spring and autumn, with a marked decline in the mid-summer (Dewhurst *et al.*, 2001; Glasser *et al.*, 2013). The effect of season on forage ALA content is likely a combination of vegetation stages at different times of the year, and the alteration of chloroplast membrane FA by plants in response to heat and cold. The amount of saturated FA relative to unsaturated FA in chloroplast membranes will influence the plasticity of the membrane at various ambient temperatures. Greater levels of unsaturation FA (*i.e.*, ALA), lend greater flexibility to membranes at lower temperatures. Conversely, increasing saturated FA (primarily 16:0) content and

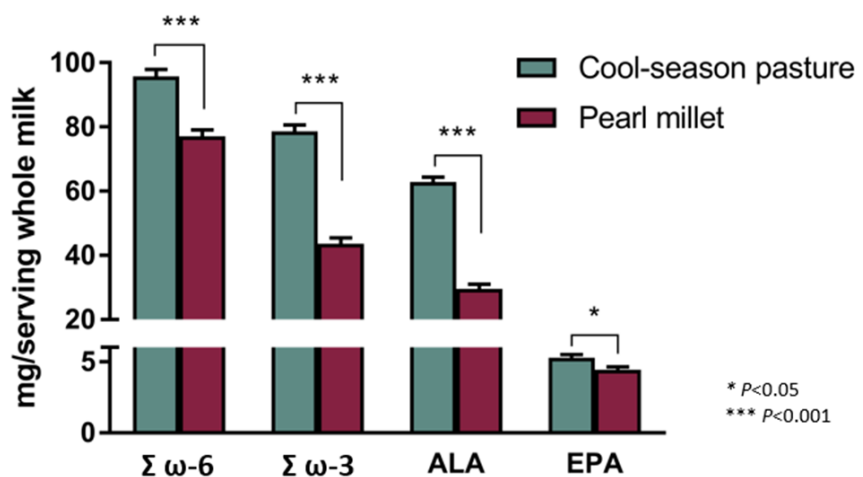


Figure 2. Content of total omega-6 FA, total omega-3 FA, ALA, and eicosapentaenoic acid (EPA) per serving of whole milk (3.25% milk fat). Means are presented as least-squares (LS) means (n = 8) and standard error. Adapted from Bainbridge *et al.*, 2017.

reducing ALA content is necessary to maintain photosynthetic thermo-stability at higher temperatures (Falcone *et al.*, 2004; Xu and Siegenthaler, 1997). Because of this seasonal variability, it may be desirable from a FA perspective to prioritize the feeding of early and later cuts to milking animals, when appropriate.

In conserved forages, increases in wilting time are correlated with decreases in ALA content (Glasser *et al.*, 2013; Khan *et al.*, 2012). Lipolytic enzymes are present and active in plants as chloroplast membrane lipids are constantly being replaced (Falcone *et al.*, 2004). Additionally, ALA is oxygenated in response to stressors (such as wounding, *i.e.*, harvest) to produce jasmonates (a class of oxidized lipids (oxylipins) that derive from α -linolenic acids (α -LAs)) and green leaf volatiles (Dar *et al.*, 2015; Sofo *et al.*, 2016; Turner *et al.*, 2002; Venkatesan, 2015; Vu *et al.*, 2012). As such, ALA content will decline in mowed forage until moisture content is low enough to inhibit enzyme activity, or anaerobic conditions prevent further losses. Because anaerobic conditions inhibit the degradative enzyme activity, dried hay may have a 31% - 78% decrease in ALA content depending upon quality, relative to ensiled forage (Glasser *et al.*, 2013).

Susceptibility to wilting losses of ALA may vary between species and even cultivar (Chow *et al.*, 2004) and may be reduced in cultivars with the stay-green trait (Dewhurst *et al.*, 2002).

Nitrogen fertility is another management decision that can affect the ALA content of forages. Insufficient nitrogen for a forage plant's needs may lead to decreased leaf tissue relative to the entire plant mass, as well as a reduction in the metabolic content of plant cells (*e.g.*, chloroplasts) and an increase in the structural content of plant cells (*e.g.*, neutral detergent fiber) (Boufaïed *et al.*, 2003; Elgersma *et al.*, 2005; Glasser *et al.*, 2013).

In summary, the best management practices for desirable forage fatty acid content are generally consistent with the pursuit of high quality grazing and conserved ensiled forage, namely maximizing access to vegetative stage grazing and putting up conserved feed in a timely manner, while ensuring adequate fertility for pasture and haycrop species.

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