Cooking Meat effects on Fatty Acid Profiles in Consumed Grass-Finished Meat Products Session

We now move on to preparation of meat and milk products and how this affects fatty acid composition before it comes to the table. This was a two-part session. The first presentation dealt with how cooking meat affects fatty acid composition and consumer appeal. This session focused on some claims that cooking grass-fed and -finished meat negated any change in fatty acid composition that occurred due to finishing cattle on pasture rather than sending them to a feedlot where they are fed liberal amounts of grain. It is important to again look at the omega-6 (n-6) to omega-3 (n-3) ratio as it dramatically drops in the meat when cattle or sheep are finished on pasture and this remains so after the meat is cooked. It is also very important to know the type of pasture the livestock are finished on. If it is over-mature or drought-stricken cool season grass pasture or warm season grass pasture, then the n-6 to n-3 ratio may still be high, but grass-finished animals will still have a much lower ratio than the confinement finished livestock. A legume, cool season grass pasture grazed at the vegetative state before significant yellowing of older leaves occurs will produce n-6:n-3 ratios generally below 4.0. If the raw meat, before it is cooked, comes from livestock on poor quality cool season pasture or from warm season grass pasture, the ratio has already been set and more than likely will be above 4.0. If they were fed hay to keep them well-fed while on pasture, they were not finished properly to get a desirable n-6:n-3 ratio. This ratio is more important than the amount of n-3 alone would have on the health of the person consuming grass-finished meat.

The second presentation was from the USDA Nutrient Data Laboratory (NDL). They highlight the fat content and other nutrients in beef and the effect of cooking on these nutrients in some grass- and grain-fed lamb and beef cuts. They have been working with other institutions that test for fats and other nutrients in meats to build and compile a very comprehensive data base for people to study and share with other interested parties to get the facts on the nutritional value of meat products.

Forage-Finishing Beef: Impact on Consumer Appeal and Meat Quality

Carol L. Lorenzen. Ph.D.

Professor, Division of Animal Sciences, University of Missouri – Columbia

Introduction

The use of forage to replace grains in finishing diets of cattle has been discussed since cattle have been in feedlots. When reviewing the literature, it seems that there is a push to replace grains with alternative rations about every twenty years. Until the use of grains to make fuels, most of the rationale has centered on the use of lands to produce "feed" instead of "food." However, at the times that this topic has been raised in the past, the science and economics have dictated that the practice of finishing cattle on high energy, grain diets remain in place. The challenge that beef cattle industry is facing today is different than the challenges it has faced in the past and warrants revisiting the effects of high levels of forage inclusion in the diet has on meat quality. Additionally, the niche marketing efforts of some cattle producers to produce a forage-finished beef product have attracted major marketing avenues, such as Whole Foods and the USDA grass-fed standard make it more fashionable, if not more profitable, than in the past.

Table 1: Relationships[a] between feeding systems and meat quality attributes[b]

Trait	Feeding Systems					
	Rate of gain	Grazing	Feedlot days	Dietary protein	Dietary fat	Energy source
Producer/feedlot						
Carcass grading		Y				
Consumer/foodservice						
Lean color			N			
Lean texture		N				
Fat color		N			N	N
Fat melting point		N	N		N	N
Marbling	N	N	N		N	N
Tenderness	N		N			
Juiciness					N	
Flavor desirability		N	N		N	N
Flavor intensity		N	N	N	N	N
Acceptability			N			
Shelf life					N	

[a] Relationships: Y – indicates factor affects trait according to published literature; N – indicates factor does not affect trait according to published literature
[b] Adapted from Owens and Gardner, 1999.

Meat quality has different meanings to different segments of the beef cattle industry. For the producer/feedlot operator, meat quality relates to carcass characteristics, such as dressing percentage, and the USDA yield and quality grade traits. To the consumer /food-service operator, meat quality is defined as product attributes such as fat content, meat and fat color, and cooking yield. A summary of the effects of different feeding systems on meat quality traits is found in Table 1. Most of these columns reveal that the feeding systems do not affect meat quality when comparisons are made at the same body compositional endpoint. However, decreased grain in the finishing diet can alter the body composition at harvest and result in meat quality differences. This brief review will focus on areas where changes from a high grain diet can positively or negatively affect meat quality.

Nutritional Composition

Nutritional composition, an aspect of consumer satisfaction, changes with deviations in feeding regimes. Current nutritional labeling for meat requires calories, calories from fat, total fat, saturated fat, cholesterol, sodium, total carbohydrates, dietary fiber, sugars, protein and vitamins and minerals. Four of these categories specifically deal with the fat component. There are four distinct fat depots in cattle that are laid down during growth and development in the following order: perinephric (internal), subcutaneous (external), intermuscular (seam), and intramuscular (marbling). Marbling is fat of the highest value because it largely determines USDA quality grade and it is not deposited until the cattle have excess energy in their diet and their maintenance and growth needs have been met. Fat is made up of phospholipids and triglycerides. Triglycerides have three fatty acids attached to a glycerol back bone.

Fatty acids (FA) are either **saturated**, no double bonds within the chemical structure, or **unsaturated**, one or more double bonds within the chemical structure. Consuming large quantities of certain saturated FA has been implicated in heart disease. All animal fats are a mixture of saturated and unsaturated FA with ruminant animals having a higher percentage of saturated FA than monogastric animals. Many studies have reported the differences in FA composition between forage- and grain-finished cattle. FA composition affects the flavor of the meat, melting point of the fat, and fatty acid oxidation. One FA group that has gained a lot of attention is total conjugated linoleic acid (CLA) because of its potential role in human health reducing body fat and improving immune function. Total CLA content (isomers *cis-9, trans-11*; *trans-10, cis-12*; *cis-9, trans-11*; *trans-9, trans-11*) is increased in meat and milk products when cattle are fed substantial levels of forage (Table 2). Table 2 shows that even the inclusion of forage in the diet with a small supplementation of grain can increase the CLA content in both raw and cooked meat. Others have shown an increase in CLA content with forage-feeding (Jiang *et al*., 2010; Duckett *et al*., 2009; Leheska *et al*., 2008). Another group of FA that have been shown to have health benefits is omega-3, noted as n-3 in the table. Human research points out that the health benefits of the n-3s mainly come from fish and nut sources; however, beef does contribute to the total amount of n-3s in the diet. The data in Table 2 point to an increase in n-3 content (n-6 remains the same or drops) when forage is used to finish cattle which is supported by other researchers (Jiang *et a*l., 2010; Duckett *et al*., 2009; Leheska *et al*.,

2008). When marketing for forage-finished beef claims an increase in "good" fatty acids, it refers to CLA and n-3s.

Table 2: Effect of feeding regime on conjugated linoleic acid isomers (CLA) content (mg/g fat) and omega-6 to omega-3 ratio[1]

Fatty Acids	Feedlot	Pasture + Grain	Pasture
Raw			
Fat (%)	5.7[a]	3.7[b]	3.7[b]
n-6:n-3	53.67	16.71	10.42
Total CLA	6.10[b]	6.68[b]	9.95[a]
Cooked			
Fat (%)	8.1[a]	5.3[b]	4.6[b]
n-6:n-3	40.84[a]	12.29[b]	9.26[b]
Total CLA	3.97[b]	6.15[a]	7.36[a]

[1]Adapted from Lorenzen *et al*., 2007
[a, b] Means within a row lacking a common superscript differ ($P < 0.05$)

Fat location on the carcass causes changes to the ratio of monounsaturated to saturated fatty acids regardless of feeding regime (Kerth *et al*., 2015). Fatty acids composition also differs by cut and is largely, but not solely, related to different percentages of fat within different muscles in trimmed cuts of beef (Table 3) reflecting the importance of having specific cuts tested before making marketing claims.

Table 3: Effect of muscle on conjugated linoleic acid isomers (CLA) content (mg/g fat) and omega-6 to omega-3 ratio[1]

Fatty Acids	Ribeye[2]	Inside round[2]	Shoulder Clod[2]
Raw			
Fat (%)	4.2[b]	3.8[b]	5.3[a]
n-6:n-3	22.44	32.44	22.70
Total CLA	8.64	9.83	9.25
Cooked			
Fat (%)	7.2[a]	4.6[b]	6.4[b]
n-6:n-3	17.81[b]	26.80[a]	16.64[b]
Total CLA	6.60[b]	7.18[ab]	7.93[a]

[1]Adapted from Lorenzen *et al*., 2007
[2]Ribeye = *Longissimus lumborum*, Inside round = *Semimembranosus*, Clod = *Triceps brachii*
[ab] Means within a row lacking a common superscript differ ($P < 0.05$)

Cooking method and degree of doneness can affect nutritional value and are important considerations since that is the way meat is consumed. During cooking, the outside of the meat browns and there is a progressive loss of the red color internally due to changes in myoglobin, the pigment responsible for meat color. The browning on the surface and breakdown of protein components during cooking contribute to changes in flavors at

different degrees of doneness. In addition, collagen shrinkage and protein hardening are responsible for decreased tenderness at higher degrees of doneness, like well done. There are two major components of meat that are lost during the cooking process, water and fat, which can affect the perception of juiciness. Therefore, as the degree of doneness increases, moisture content decreases and fat and protein contents increase on a percentage basis (Smith *et al*., 2011; Alfaia *et al*., 2010). The change in composition during cooking also impacts the nutritional value of the meat. Cooking increases saturated and monounsaturated fatty acid content while decreasing polyunsaturated fatty acids (Alfaia *et al*., 2010). Tables 2 and 3 show that cooking elevated fat percentages and decreased n-6:n-3 ratios and total CLA on a mg/g of fat basis compared to raw samples. The decreases are partially explained by the increase in fat during cooking and the stability of CLA during thermal processes (Alfaia *et al*., 2010).

Fat and Meat Color

Consumers make meat purchasing decisions based on color. It is the only indicator that they have that meat is wholesome and of high eating quality. One of the main objections to finishing cattle on forage is the increase in yellow color of the fat and darker color of the muscle due to the consumption of carotenoid pigments (Kerth *et al*., 2007; Dunne *et al*., 2006). Both Leheska *et al*. (2008) and Kerth *et al*. (2007) reported increased yellowness of subcutaneous fat in cattle finished on pastures compared to those finished on concentrate diets. Research has addressed the question of how long a high concentrate diet would have to be fed to cattle that have received a high forage diet to reverse the negative effect on fat and lean color. Dunne *et al*. (2006) reported marked decrease in the yellow fat color with 28 days of feeding a concentrate diet, but they noted that the exact time to mitigate the effects of forage would be dependent on diet. It has been reported that generally, the exclusion of forage for 90 days before harvest is recommended to mitigate the effects of forage feeding on both fat and lean color (Miller, 2002).

In addition to the feeding regime, the chronological age of the cattle also plays a role in the color of the fat and lean and the amount of time it will take for the negative effects due to forage-feeding to be changed. It is well established that as cattle get older their lean gets coarser and darker and their fat becomes more yellow. This typically does not happen with cattle less than 30 months of age. Bidner *et al*. (1981) reported in cattle less than 30 months of age no difference in fat color between forage, forage supplemented with grain, and feedlot finishing diets. However, Bidner *et al*. (1981) did report darker lean color scores for forage finished cattle compared to feedlot finished cattle.

Carcass Grading

In experiments when cattle are fed for the same number of days but at different energy levels, the cattle fed at the higher energy level tend to have heavier carcasses and increased levels of fatness (Kerth *et al*., 2007; Cox *et al*., 2006; Sinclair *et al*., 2001; Camfield *et al*., 1997, Moody, 1976). This decrease in carcass fatness by lower energy or forage-based diets can be viewed positively regarding a decreased USDA yield grade. The increased energy level in the diet when fed for greater periods of time also leads to

increased USDA quality grade factors (Owens and Gardner, 1999; Camfield *et al*., 1997; Moody, 1976). Significant, acceptable differences (achieving a USDA Select grade) can be seen in as little as 60 days on a high energy diet (Camfield *et al*., 1997).

Table 4: Effects of feeding regimes on USDA grade traits

Trait	Feedlot	Pasture + Grain	Pasture
Cool Season Grass[1]			
Hot carcass weight (lbs.)	732[a]	619[b]	561[c]
Fat thickness (in)	.44[a]	.21[b]	.14[b]
Ribeye area (in^2)	12.0[a]	10.9[b]	10.3[b]
Kidney, pelvic & heart fat (%)	3.0[a]	2.3[b]	1.9[c]
USDA yield grade	3.2[a]	2.4[b]	2.1[b]
Marbling score	Small[68a]	Slight[60b]	Slight[11b]
USDA quality grade	Choice[-a]	Select[-b]	Select[-b]
Ryegrass[2]			
Hot carcass weight (lbs.)	729[b]	767[a]	502[c]
Fat thickness (in)	.42[a]	.41[a]	.25[c]
Ribeye area (in2)	12.6[a]	12.8[a]	10.5[b]
Kidney, pelvic & heart fat (%)	2.3[a]	2.2[a]	1.5[b]
USDA yield grade	3.0[a]	2.9[a]	2.3[b]
Marbling score	Slight[99]	Slight[80]	Slight[51]
USDA quality grade	Select[+]	Select[+]	Select[-]

[1]Adapted from Lorenzen *et al*., 2007
[2]Adapted from Kerth *et al*., 2007
[a, b, c] Means within a row lacking a common superscript differ ($P < 0.05$)

In experiments when cattle are fed to same body compositional endpoint, meaningful bio-logical differences in USDA yield and quality grade traits are not seen (Bowling *et al*., 1977). In general, it takes forage finished cattle more time on feed to reach the same body compositional endpoint as compared to cattle finished on a high energy concentrate ration (Lorenzen *et al*., 2007). Often the increased length of time to finish the cattle on forage also had a negative impact on the economics of finishing the cattle.

When cattle are fed a combination of diverse cool season grasses (Lorenzen *et al*., 2007) or ryegrass (Kerth *et al*., 2007) and grain, acceptable levels of USDA yield and quality grade traits are reported (Table 4). These data show that the benefit of leaner cattle can be combined with an acceptable USDA quality grade when cattle finished on pasture are supplemented with grain at 1.2% of their body weight. It could be argued that a Select⁻ average quality grade is not optimum and supplementation higher than 1.2% of the body weight may achieve more desirable results.

Meat Palatability

Palatability is commonly described as tenderness, juiciness and flavor, all the things that lead to consumer acceptability of the meat. Of these three, tenderness has the greatest potential to control with feeding regime. Some researchers have found forage-finishing has a negative impact on instrumental measures of tenderness (Schroeder *et al*., 1980; Bowling *et al*., 1977). Others report no differences in tenderness due to feeding regime (Lorenzen *et al*., 2007; Sinclair *et al*., 2001; Bidner *et al*., 1981) Kerth *et al*. (2007). They, however, reported increased values for mechanical tenderness in strip loins but not in ribeyes; this is an interesting finding because both cuts come from the same muscle and highlight the tenderness gradient that runs through most muscles. It should also be noted that many studies, even if they find a difference in tenderness, do not report values that reach the threshold for meat to be considered tough (Kerth *et al*., 2007; Lorenzen *et al*., 2007); indicating that acceptably tender meat can be produced by finishing beef on pastures. Chronological age of the cattle used in the experiments that reported no differences in tenderness is difficult to discern from the literature, whereas the ages of the cattle used in three other experiments were less than 30 months. As cattle mature, they get tougher due to reduced collagen solubility. Feeding cattle high energy diets prior to harvesting has been shown to increase collagen solubility regardless of the cattle's age (Miller *et al*., 1983; Aberle *et al*., 1981). The greatest increase in tenderness is found when cattle are fed a high energy diet for a minimum of 70 days (Aberle *et al*., 1981).

Table 5: Trained panel ratings for ribeye steaks from cattle on different feeding regimes[1,a]

Attribute	Feedlot	Ryegrass Pasture + Grain	Ryegrass Pasture
Initial juiciness	6.17[b]	5.35[c]	5.73[c]
Sustained juiciness	5.75[b]	5.15[c]	5.38[bc]
Initial tenderness	6.17	5.52	5.05
Sustained tenderness	5.73	5.00	4.49
Flavor intensity	6.02[b]	5.48[c]	5.44[c]
Beef flavor	5.92[b]	5.54[c]	5.28[c]

[1]Adapted from Kerth *et al*., 2007
[a] Based on an 8-point hedonic scale with 1 = dislike dry, tough, bland and 8 = extremely juicy, tender, flavorful
[b, c] Means with a row lacking a common superscript differ ($P < 0.05$)

Flavor is another aspect of palatability that can be controlled by feeding regime but to a lesser extent than tenderness. Some studies have reported lower flavor ratings for cattle finished on forages (Tables 5 and 6; Kerth *et al*., 2007; Lorenzen *et al*., 2007; Cox *et al*., 2006; Schroeder *et al*., 1980), while other studies have detected no differences (Sinclair *et al*., 2001; Bidner *et al*., 1981). However, except for the study conducted by Schroeder *et al*. (1980), all the other ratings were within the acceptable range. Data presented in Table 5 indicate a greater amount of juiciness and flavor in meat from cattle finished on

grain. All scores were above 4.0 on an 8-point scale (Table 5), which indicates that all attributes were on the positive part of the scale for the trait. While the data presented in Table 6 indicate that cattle finished on pasture or pasture with grain supplementation have lower consumer panel ratings for overall like, liking of flavor, and liking of juiciness; mean scores were above 5 on a 9-point scale (Table 6) indicating that the consumers found the samples to be acceptable in all traits. In both tables samples were matched for marbling scores between feeding regimes to reduce the known effect of marbling score on trained and consumer panel ratings where higher marbling scores are preferred. In another aspect of consumer preference, Cox *et al.* (2006) asked consumers about their intent to purchase meat and reported 65.9% of consumers would purchase steaks from cattle finished on grain compared to 34.1% that would prefer steaks from cattle finished on forage. Kerth *et al.* (2007) reported that consumers not only ranked grain-finished steaks higher but also would pay more money for them.

Table 6: Consumer panel ratings for ribeye steaks from cattle on different feeding regimes[1,a]

Attribute	Feedlot	Cool Season Pasture + Grain	Cool Season Pasture
Overall like	6.5[b]	5.8[c]	5.8[c]
Liking of tenderness	6.3	6.1	5.8
Liking of flavor	6.4[b]	5.7[c]	5.7[c]
Liking of juiciness	6.5[b]	5.5[c]	5.7[c]

[1]Adapted from Lorenzen *et al.*, 2007
[a] Based on a 9-point hedonic scale with 1 = dislike extremely and 9 = like extremely
[b, c] Means with a row lacking a common superscript differ ($P < 0.05$)

Recommendations

Forage-finished cattle have an improved nutritional profile and have expectable eating quality. Cattle finished on forage should be supplemented, which can still provide the nutritional marketing claims, for a minimum of 70 days to reduce negative effects of forage on fat color, promote tenderness associated with collagen turnover, and achieve a USDA quality grade acceptable to the current marketplace. In addition, cattle fed a forage diet supplemented with grain should be slaughtered at less than 30 months of age to help decrease the potential negative effects of fat color and decreased tenderness.

Literature Cited

Aberle, E. D., E. S. Reeves, M. D. Judge, R. E. Hunsley, and T. W. Perry. 1981. Palatability and muscle characteristics of cattle with controlled weight gain: time on a high energy diet. J. Anim. Sci. 52:757-763.

Alfaia, C. M. M., S. P. Alves, A. F. Lopes, M. J. E. Fernades, A. S. H. Costa, C. M. G. A. Fontes, M. L. F. Castro, R. J. B. Bessa, and J. A. M. Prates. 2010. Effect of cooking

methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. Meat Sci. 84:769-777.

Bidner, T. D., A. R. Schupp, R. E. Montgomery, and J. C. Carpenter. 1981. Acceptability of beef finished on all-forage, forage-plus-grain or high energy diets. J. Anim. Sci. 53:1181-1187.

Bowling, R. A., G. C. Smith, Z. L. Carpenter, T. R. Dutson, and W. M. Oliver. 1977. Comparison of forage-finished and grain finished beef carcasses. J. Anim. Sci. 45:209-215.

Camfield, P. K., A. H. Brown, P. K. Lewis, L. Y. Rakes, and Z. B. Johnson. 1997. Effects of frame size and time-on-feed on carcass characteristics, sensory attributes, and fatty acid profiles of steers. J. Anim. Sci. 75:1837-1844.

Cox, R. B., C. R. Kerth, J. G. Gentry, J. W. Prevatt, K. W. Braden, and W. R. Jones. 2006. Determining acceptance of domestic forage- or grain-finished beef by consumers from three southeastern U.S. states. J. Food Sci. 71:S542-S546.

Duckett, S. K., J. P. D. Neel, J. P. Fontenot, and W. M. Clapham. 2009. Effects of winter stocker growth rate and finishing systems on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content. J. Anim. Sci. 87:2961-2970.

Dunne, P. G., F. P. O'Mara, F. J. Monahan, and A. P. Moloney. 2006. Changes in colour characteristics and pigmentation of subcutaneous adipose tissue and *M. longissimus dorsi* of heifers fed grass, grass silage or concentrate-based diets. Meat Sci. 74:231-241.

Kerth, C. R., K. W. Braden, R. Cox, L. K. Kerth, and D. L. Rankins, Jr. 2007. Carcass, sensory, fat color, and consumer acceptance characteristics of Angus-cross steers finished on ryegrass (*Lolium multiflorum*) forage or on a high-concentrate diet. Meat Sci. 75:342-331.

Kerth, C. R., A. L. Harbison, S. B. Smith, and R. K. Miller. 2015. Consumer sensory evaluation, fatty acid composition, and shelf-life of ground beef with subcutaneous fat trimmings from different carcass locations. Meat Sci. 104:30-36.

Leheska, J. M., L. D. Thompson, J.C. Howe, E. Hentges, J. Boyce, J. C. Brooks, B. Shriver, L. Hoover, and M. F. Miller. 2008. Effects of conventional and grass-feeding systems on nutrient composition of beef. J. Anim. Sci. 86:3575-3585.

Lorenzen, C. L., J. W. Golden, F. A. Martz, I. U. Gruen, M. R. Ellersieck, J. R. Gerrish, and K. C. Moore. 2007. Conjugated linoleic acid content of beef varies by feeding regime and muscle. Meat Sci. 75:159-167.

Miller, R. K. 2002. Factors affecting the quality of raw meat. In Kerry J., J. Kerry, and D. Ledward (Eds), *Meat Processing – Improving Quality*(pp. 27-63). Cambridge, UK: Wood head Publishing Ltd.

Miller, R. K., J. D. Tatum, H. R. Cross, R. A. Bowling, and R. P. Clayton. 1983. Effects of carcass maturity on collagen solubility and palatability of beef from grain-finished steers. J. Food Sci. 48:484-486 & 525.

Moody, W. G. 1976. Quantitative and qualitative differences in beef from various energy regimes. Reciprocal Meat Conference Proceedings. 29:128-149.

Owens, F. N. and B. A. Gardner. 1999. Ruminant nutrition and meat quality. Reciprocal Meat Conference Proceedings. 52:25-36.

Schroeder, J. W., D. A. Cramer, R. A. Bowling, and C. W. Cook. 1980. Palatability, shelf life, and chemical differences between forage and grain-finished beef. J. Anim. Sci. 50:852-859.

Sinclair, K. D., G. E. Lobley, G. W. Horgan, D. J. Kyle, A. D. Porter, K. R. Matthews, C. C. Warkup, and C. A. Maltin. 2001. Factors influencing beef eating quality 1. Effects of nutritional regimen and genotype on organoleptic properties and instrumental texture. Animal Science. 72:269-277.

Smith, A. M., K. B. Harris, A. N. Haneklaus, and J. W. Savell. 2011. Proximate composition and energy content of beef steaks as influenced by USDA quality grade and degree of doneness. Meat Sci. 89:228-232.

Exploring Nutrient Content of Meats Using Research Protocols at the Nutrient Data Laboratory

Janet Roseland[1], Quynhanh Nguyen[1], Kristine Patterson[1], Pamela Pehrsson[1], Dale Woerner[2], Cody Gifford[2], Jennifer Leheska[3]

[1]USDA Nutrient Data Laboratory, [2]Colorado State University, [3]Consultant, Canyon TX

Scientists at USDA's Nutrient Data Laboratory (NDL) study and report the nutrient content of foods. This paper highlights the content of lipids (fats) and other nutrients in beef and the effect of cooking on these nutrients in some grass- and grain-fed lamb and beef cuts. Data from NDL studies are available in the USDA National Nutrient Database for Standard Reference (USDA, 2017a). The data from direct analysis of nutrient components found in over 9000 foods are used for national intake surveys, labeling, policy, and other purposes (Ahuja et al., 2013).

NDL meats research study methods include these key steps:

a. Determine what research is needed, often in cooperation with industry/university collaborators, based on objective information such as market research, consumption data, mandatory labeling cuts/nutrient, and market shares.

b. Develop protocols and quality control procedures using standard operating procedures, validated analytical methods and analytical labs.

c. Produce a statistical sampling plan specifying number and type of samples required from representative sources.

d. Collect and prepare representative samples of meat from sources such as packing plants or suppliers or retail stores, using the sampling plan. Experienced university meat scientists fabricate samples into retail cuts when necessary. They weigh and dissect the meat sample components such as bone, cartilage, fat, and lean meat. This is done for both raw and cooked samples. The fat and the lean components are then separately homogenized and packaged before being sent to laboratories for nutrient analysis.

e. Analyze nutrient content at USDA-validated laboratories using official analytical methods such as AOAC (AOAC, 2000) and quality assurance procedures such as standard reference materials (SRMs), in-house control materials, and blind duplicates.

f. Evaluate data for consistency and for detecting potential outliers.

g. Prepare data products available for public dissemination.

GRASS- AND GRAIN-FINISHED LAMB STUDY

Colorado State University (CSU) conducted a study with input from NDL and the American Lamb Board for the purpose of obtaining nutrient and composition data for 11 widely purchased retail domestic lamb cuts. The estimated per capita intake of lamb in

the US was 1.0 pound in 2015, with higher popularity among specific population groups (USDA ERS, 2017).

Samples for cuts of domestically-raised grain-finished and grass-finished lamb were collected during all four seasons from retail suppliers providing the majority of the market. Grass-finished lamb cuts were obtained from the two representative sources which had seasonal supply: The Intermountain West region and the West Coast region. Grain-finished cuts were obtained from three sources: 2 in the Intermountain West region and 1 from the West Coast region.

Raw samples (n=24 per grain-finished and n=10 per grass-finished cut) were dissected using standard protocols. Each cut's total weight and the weight of each component, including separable lean, separable fat, and refuse, were recorded. ["Separable lean" pertains to muscle, connective tissue, and intramuscular fat that are considered edible. "Separable fat" is the seam fat and the fat on the outside of the cut.]

Cuts designated to be grilled were prepared on a two-sided grill preheated to 195° C until a 60° C internal temperature was attained. Cuts assigned to be roasted were cooked on racks in roasting pans in preheated 160° C convection oven to 60° C internal temperature. Ground lamb was pan-grilled in a non-stick anodized skillet preheated to 195° C and removed from heat at 74° C internal temperature. Post-cooking weights for all cuts were recorded. Cuts were refrigerated for at least 12 hours. Cooked samples were dissected and weighed using standard protocols for the components previously described.

Laboratories were validated by NDL as having the ability to accurately analyze samples using established methodology in order to participate in the study. Nutrient data quality protocols included use of quality control samples in each analytical batch of samples, in-house laboratory control materials, and random blind duplicates. The separable lean, seam fat, and external fat components were homogenized and analyzed at CSU for proximates, fatty acids, and cholesterol. Minerals and vitamins were analyzed at other laboratories. Estimated nutrient values were developed for raw and cooked cuts as "separable lean only" and "separable lean and fat" profiles.

In this study, saturated fatty acids (SFA) were the sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0. Monounsaturated fatty acids (MUFA) were summed 14:1, 15:1, 16:1, 17:1, 18:1, 20:1, 22:1, and 24:1. Polyunsaturated fatty acids (PUFA) were the sum of 18:2 n-6, 18:2 CLA, 18:3 n-3 (ALA), 20:2 n-6, 20:4, 20:5 n-3 (EPA), 22:2, 22:4, 22:5 n-3 (DPA), 22:6 n-3 (DHA). *Trans* fatty acids (TFA) were summed 16:1t, 18:1t, and 18:2t.

Grass- and grain-finished lamb results

For these results, data for separable lean from all cuts were combined to create datasets for cooked grass-finished, cooked grain-finished, raw grass-finished, and raw grain-finished. Results for total fat were expressed as g/100 g edible tissue, while fatty acids were expressed as g/100 total fat.

The primary SFA were 16:0 (palmitic) and 18:0 (stearic). The primary PUFA was 18:2 n-6 (linoleic). The primary MUFA was 18:1 (oleic). The main TFA was 18:1t, primarily 18:1 t11 (vaccenic acid). Initial results indicated that total fat for raw was lower in grass-finished (5.2 g) compared to grain finished (5.4 g) expressed as g/100 g edible tissue. For these raw and cooked cuts, SFA, PUFA, total CLA, & TFA (especially vaccenic acid), were higher in grass- compared to grain-finished, expressed as g/100 g fat. MUFA was lower in grass- compared to grain-finished. When the effect of cooking was examined, total fat was 7.8 g in cooked grain-finished compared to 5.2 g grass-finished raw (per 100 g edible tissue), which appeared to be 32% higher in cooked than the raw counterpart. In grain-finished, total fat was 8.7 g in cooked and 5.4 g in raw, making cooked appear to be 63% higher than raw. Differences were observed for cooked compared to raw for SFA, MUFA, PUFA, and TFA, as well. However, sample size was not large enough to determine statistical significance when comparing data among cuts from this study; thus, these should be viewed as preliminary results. Further studies are necessary.

Our general observations for total fat, SFA and MUFA are similar to those of a meta-analysis by Popova et al. (2015), in which SFA increased (p<0.05), while total fat and MUFA decreased (p<0.05) in grass- compared to grain-finished. Results suggest that pasture raising can be a successful strategy for improving lamb's nutritional quality (Popova et al. 2015). Applications from this study and a grass-fed beef study will be discussed later in this report. Regarding the effect of cooking, a higher concentration of fat and other specific nutrients compared to raw has been observed in other studies, as well. Possible reasons for this occurrence are the infiltration of fat from adjacent fatty tissue (removed after cooking) (Slover et al., 1987) and higher percent moisture loss in relation to the degree of fat lost (Garrett and Hinman, 1971). Concerning *trans*-fat, however, cooking had a minimal effect when the concentration of intramuscular fat due to cooking was considered, in a study of pasture-fed lamb and beef (Purchas et al., 2015).

Gifford et al (2016) evaluated data for each individual cut in this study, finding that total fat content varied among cuts. For example, total fat was higher in raw separable lean from grass-finished shoulder arm chops, whole shoulder, frenched rib chops, rib chops and sirloin chops than their grain-finished counterparts. However, the total fat in the 6 other cuts was lower in grass-finished compared to grain-finished. In the cooked cuts in the study, total fat content was higher in each grain-finished cut than its grass-finished counterpart (Gifford et al., 2016). In contrast, a meta-analysis published in 2015 found that grazing lambs were lower in total fat than lambs raised indoors, in most studies (Popova, Gonzales-Barron and Cadavez, 2015). Gifford et al. (2016) suggested that a reason for the cuts having higher fat in the grass-finished compared to grain-finished in their study was that they may have possessed a greater proportion of phospholipids compared to the other cuts. Gifford et al (2016) found that the majority of fatty acid content in the separable lean was composed of palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1 n9) for the grass-finished and grain-finished lamb cuts. Of the separable lean's total fatty acid profile, 59% of the profile for raw grain-finished cuts and 57% of the profile for raw grass-finished cuts was composed of polyunsaturated fatty acids (PUFA), mono-unsaturated fatty acids (MUFA), and stearic acid (Gifford et al., 2016).

GRASS-FED BEEF STUDY

This study was conducted to determine the nutrient composition of US-raised grass-fed beef, in collaboration with the Beef Checkoff Program, America's Beef Producers, Texas Tech University (TTU), and NDL, and was published in 2008 (Leheska et al., 2008). The estimated per capita intake of beef in the US was 53.9 pounds in 2015, (USDA ERS, 2017). While grass-fed beef represents <2% of total beef sales, grass-fed beef demand grew by 40% in 2016 (Johnson, 2017). Consumer research suggests that increased demand for grass-fed beef will not slow in the near future (Williams, 2013). Grass-fed ground beef and strip steak samples were obtained on 3 occasions from 15 producers representing 13 states (AL, AR, CA, CO, GA, ID, KY, MN, MO, MT, NM, TX, VA). Two steaks were obtained from 3 different animals for each of the 3 times, from each producer. Steaks were fabricated from the 13th rib area of the strip loin. Similarly, 85% lean ground beef was collected from 3 different carcasses at 3 different times from each producer. If 85% lean was unavailable, the next leanest ground beef (e.g., 88%) was provided. Control ground beef and strip steak samples were obtained at 3 different times in each of 3 US regions.

Steak samples were weighed and dissected to separate the lean, fat, and refuse components for each steak. All components were weighed and the edible portions were homogenized for analysis. Aliquots for steak and ground beef were prepared for analysis using study protocols. TTU analyzed proximate nutrients, while validated commercial laboratories analyzed fatty acids, cholesterol, thiamin, vitamin B12, and mineral content. Quality control was monitored using certified reference materials and blind duplicates. NDL scientists validated all data. Nutrient data for raw grass-fed ground beef and strip steaks, along with study documentation, were released in SR in 2008.

In this study, SFA was the sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 22:0. MUFA was the sum of 14:1, 15:1, 16:1, 18:1, and 20:1. PUFA was the sum of 18:2, 18:3n-3 (ALA), 18:4, 20:2n-6, 20:3n-6, 20:4n-6, 20:5n-3 (EPA), 22:5n-3 (DPA), 22:6n-3 (DHA). TFA was the sum of 16:1t, 18:1t, and 18:2t.

Ground beef and strip steaks: Grass-fed and control results

Total fat was significantly lower in grass-fed steak (n=41) than in the control (n=9) (p <0.05) and was also lower in grass-fed ground beef (n=42) than in the control (n=9) (Figure 1). Total SFA, n-3 fatty acids, total CLA, and vaccenic acid were significantly higher in grass-fed than control (p<0.05; Figures 2-4). Total MUFA was significantly lower in grass-fed than controls (p<0.05; Figure 2). N-6, total *trans*, total PUFA fatty acids and cholesterol results showed no significant difference between grass-fed and controls (Figures 3, 5-7). (Editor's note: However, the n-6:n-3 ratio is quite different between grass-fed and controls when comparing bars in figure 3. Grass-fed n-6:n-3 ratio is less than 4 [≈2.0] due to the elevated level of n-3.) The primary SFAs were stearic and palmitic acids. The primary MUFA was oleic acid. The primary PUFA was linoleic acid.

The effect of grass-feeding on beef fatty acids seems to be influenced somewhat by breed, the response of different muscles to the diet, growing season, harvest time, and other factors (Van Elswyk and McNeill, 2014; Duckett et al., 2009). Despite these variables, these results (Leheska et al., 2008) are similar to others comparing grass- to grain-finished beef for total fat, SFA, MUFA, PUFA, and cholesterol (Van Elswyk and McNeill, 2014).
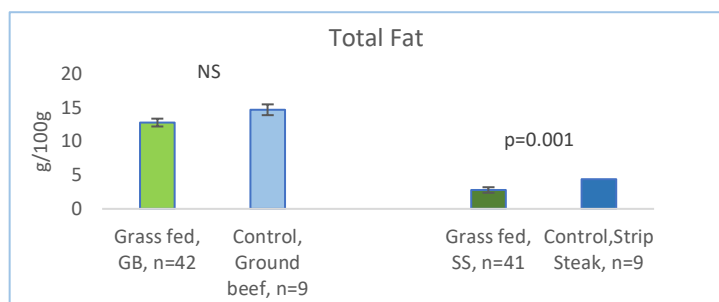

Figure 1. Total fat (g/100 g edible tissue) content of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3 occasions. (Data from Leheska et al., 2008).
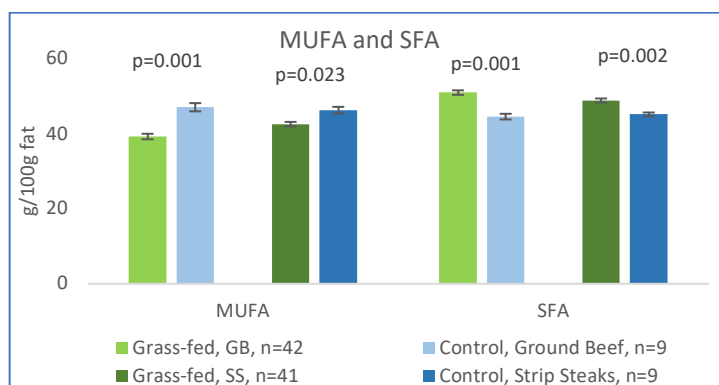
Figure 2. MUFA and SFA (g/100 g fat) content of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3 occasions. (Data from Leheska et al., 2008).
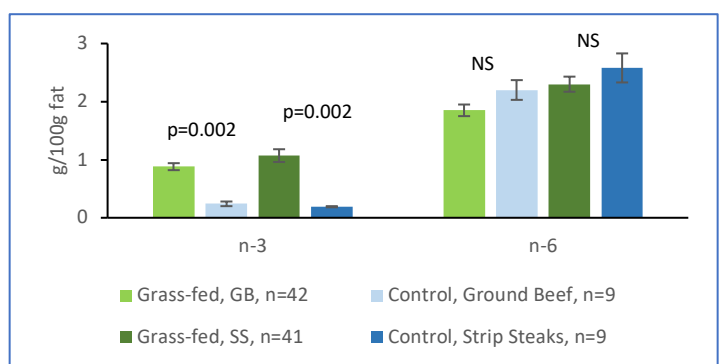
Figure 3. N-3 and n-6 (g/100 g fat) content of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3
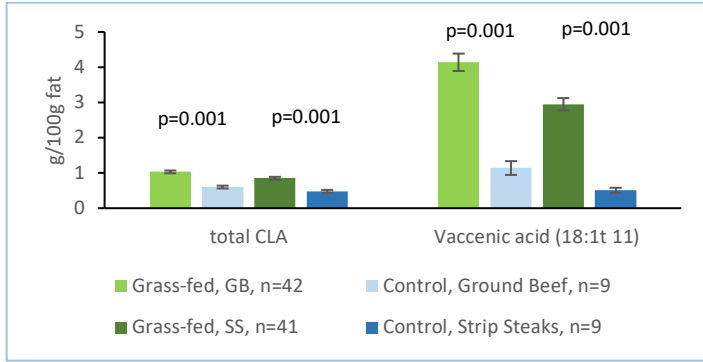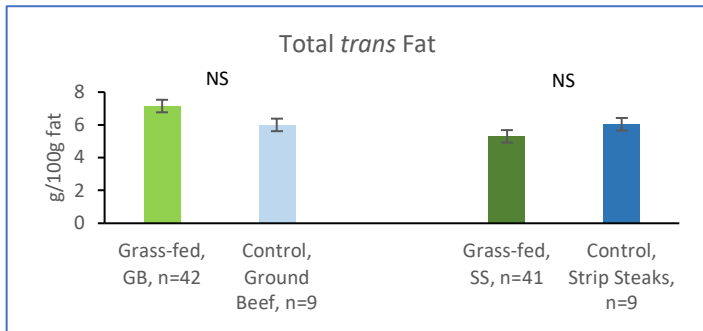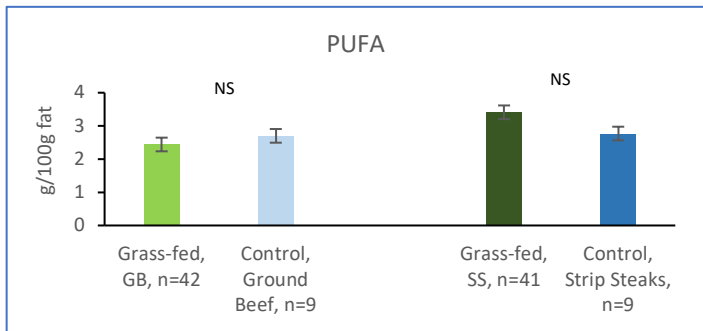
Figure 4. CLA and vaccenic (g/100 g fat) content of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3

Figure 5. Total trans content (g/100 g fat) of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3

Figure 6. PUFA content (g/100 g fat) of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB included 3 composite samples from 13 grass-fed producers plus 1 composite sample from 2 grass-fed producers. Grass-fed SS included 3 composite samples from 13 grass-fed producers, 1 composite from another producer, plus 2 composite samples from 1 grass-fed producer. Control GB and SS included samples from 3 US regions on 3 occasions. (Data from Leheska et al., 2008).
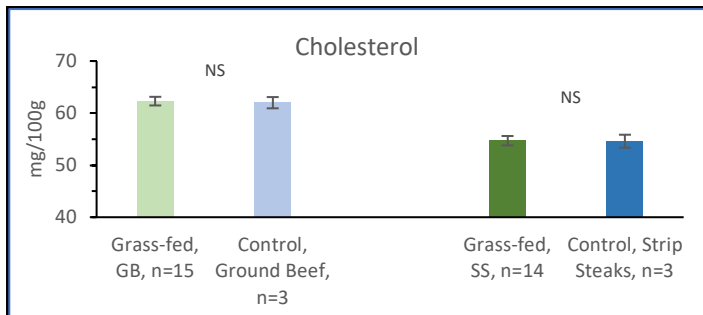

Figure 7. Cholesterol content (mg/100 g edible tissue) of grass-fed and control ground beef (GB) and strip steaks (SS). Grass-fed GB and SS included 1 composite sample from each grass-fed producer. Control GB and SS included a single composite sample for each region from which samples were collected. (Data from Leheska et al., 2008).

Lamb and beef: Grass vs grain nutrient trends and health applications

The main trends observed between grass- and grain-fed in these specific studies that were common to beef and lamb were: a) Lower total fat and lower total MUFA (as % total fat) in grass-fed compared to controls; b) Higher total SFA, vaccenic acid, and total CLA (as % total FA) in grass-fed compared to controls. Additional research is needed to confirm these observations.

Individual fatty acids are worth noting, because although reduction in total fat and SFA intake has been recommended based on specific correlations between diet and health, specific individual fatty acids seem to vary in their effect (Daley et al., 2010). For example, the primary SFA in beef and lamb regardless of feeding regime are stearic acid (the only SFA which shows a neutral effect on LDL cholesterol) and palmitic acid (which shows less cholesterol-raising effect than other SFAs in these meats) (Daley et al., 2010).

The primary MUFA in beef and lamb, oleic acid, is known for its cholesterol-lowering effect (Daley et al., 2010). Lower MUFA concentration in grass-fed compared to grain-fed beef and lamb, as well as the role of MUFA intake in promoting cardiovascular (CV) health, is well documented (Popova et al., 2015; Van Elswyk and McNeil, 2014).

PUFA content in beef and lamb in both feeding regimes is low, primarily present as omega-6 fatty acid linoleic acid (C18:2 n-6) (Van Elswyk and McNeill, 2014). Among the omega-3s, small increases of ALA and trace or no increases in EPA, DHA, and DPA in grass- vs grain-fed were noted in ours and other studies; therefore, lean cuts from either feeding method could provide modest amounts of omega-3s (Van Elswyk and McNeill, 2014).

The main TFA in animal products is usually vaccenic acid (C18:1 t11) (VA). VA is produced in ruminants and a precursor of conjugated linoleic acid (CLA) (C18:2 c9 t11). While some studies suggest CLA and VA may have health benefits (Purchas et al., 2015, Van Elswyk and McNeill, 2014), others indicate that effects of VA require further investigation (Gebauer et al., 2015). Although higher VA and total CLA concentrations expressed as percent fat were seen in grass-fed, the amounts are modest when converted to intakes per serving. Thus, amounts in grass- and grain-fed meat are nearly the same, since grass-fed meat is typically lower in total fat (Van Elswyk and McNeill, 2014). For SFA as well, although higher in grass-fed than grain-fed when expressed as percent total fat, it can translate to a lower amount per serving (Van Elswyk and McNeill, 2014).

The cholesterol content of beef and lamb are similar to that of other meats in the USDA food composition database between grass- vs grain-fed beef the difference was significant in only one US study (Rule et al., 2002; Van Elswyk and McNeill, 2014).

BEEF NUTRIENT DATA IMPROVEMENT STUDY

Following changes in the beef industry in feeding practices, age of animal at harvest, breeds, and new retail cuts, the Nutrient Data Improvement Study (NDI) was conducted through advice from the beef industry to obtain nutrient data for selected contemporary nationally representative retail beef cuts. A comparison of the lower fat levels of sirloin

steak in 2010 to those in 1963 and 1990 (Figure 8; NCBA, 2014) exemplifies the magnitude of the changes over time.

The research team collaborating with USDA included National Cattlemen's Beef Association (NCBA), Texas A & M University (TAMU), TTU, CSU, and a statistician. Samples were obtained at packing plants in 6 different states (TX, WI, NE, KS, CO, AZ) from at least 12 carcasses in each state. The effects of cooking method and cut on cooking yield were reported as % of each cut's raw to cooked weight. The effects on fat concentration were reported as a percentage (g/100 g) of each cut's total edible lean and separable fat. Full details were reported by Roseland et al., 2015.
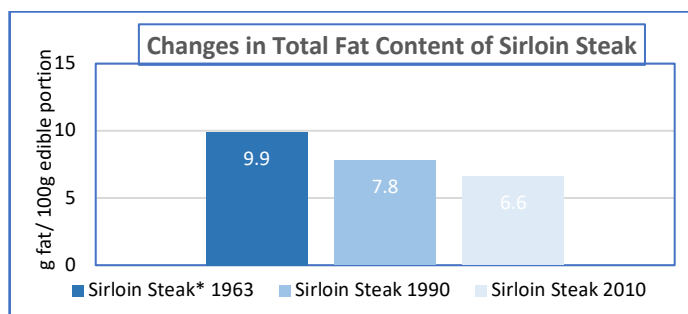
Figure 8. Total fat levels of sirloin steak compared over time. Sirloin Steak* 1963 data reported by Watt and Merrill (1963); 1990 and 2010 data reported by USDA food composition database (USDA, 2018). (Graph used by permission, NCBA, 2014)

Effect of cooking on cuts comparing chuck, round, and loin

The effect of cooking methods on different cuts had varied effects on nutrient content and cooking yield, with some effects significant ($p<0.05$). During cooking, most cuts lost fat and all cuts lost moisture. Cooked cuts had a higher concentration of fat and other nutrients compared to raw cuts, likely due to the higher percent moisture lost than percent fat lost in each cut (Acheson, 2013; Garrett and Hinman, 1971; Martin et al., 2013; Roseland et al., 2015; West et al., 2014).

Cooking yields differed among the 3 roasted cuts studied ($p<0.05$; Figure 9). Roasted chuck eye and tenderloin had the highest yields (84% and 82%, respectively) compared to ribeye roast (76%). In contrast, among the 3 grilled cuts, ribeye had the highest cooking yield (83%) compared to chuck eye and tenderloin ($p<0.001$; Roseland et al., 2015). Fat and moisture concentrations were different among the roasted cuts ($p<0.001$) (Figure 10). As fat increased, moisture decreased for the roasted cuts and the grilled cuts.

The ribeye cuts were highest in total fat, SFA, MUFA, PUFA, and TFA (g/100 g of cut's edible lean and fat), while the tenderloin cuts were lowest (both steaks and roasts) when ribeye, chuck, and tenderloin were compared. Detailed results of this study have been published (Roseland et al., 2018).
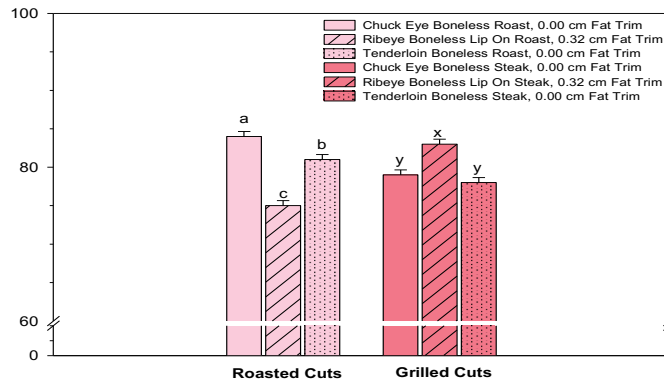
Figure 9. Cooking yields for roasted and grilled beef cuts from 3 primals. N=36 per cut. (Roseland et al., 2015)
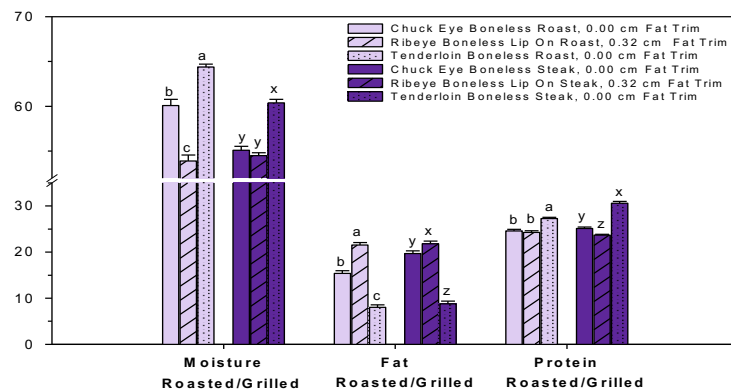

Figure 10. Proximate content of roasted and grilled beef cuts from 3 primals. N=36 per cut. (Roseland et al., 2015)

Effect of cooking on pairs of chuck, round, and loin cuts

Cooking methods affected cooking yields, fat, and fatty acid concentrations when comparing roasting, grilling, and braising. A pair-wise evaluation of comparable cuts confirmed that roasted chuck and tenderloin cuts had higher cooking yields (p<0.05) than their respective grilled steaks. Conversely, roasted ribeye had lower cooking yields than the grilled steak counterparts, whether boneless or with bone (Figure 11; Nguyen et al., 2014).

Fat concentrations were lower in roasted cuts than in corresponding grilled cuts. Fat was lower in grilled cuts than in corresponding braised cuts. For example, fat was 23% lower in grilled shoulder steak than in its corresponding braised cut (p<0.001) and was lower in three roasted cuts (chuck eye, tenderloin, and ribeye) than in corresponding thinner grilled steaks (Figure 12; Roseland et al., 2015).

The lower cooking yield of the roasted ribeye compared to grilled was unexpected, since higher final endpoint temperatures and higher cooking temperatures, as in grilling, are typically associated with lower cooking yield due to higher endpoint temperature (Wahrmund-Wyle et al., 2000a). The unusual finding could be due to the ribeye's composition, since the fat and moisture concentrations of the ribeye steak vs roast were not significantly different. On the other hand, grilled tenderloin and chuck steaks had higher fat and low-

98

er moisture than roasted counterparts, coinciding with lower cooking yields for these steaks compared to roasts (p<0.05). Thus, the higher moisture levels in the tenderloin and chuck roasts, plus the lower endpoint temperatures in roasting, were related to these roasts' higher cooking yields (Roseland et al., 2015).

Total SFA, MUFA, and TFA were lower in roasted ribeye, chuck eye, and tenderloin compared to grilled counterparts. Conversely, braised shoulder values were higher than or equal to grilled shoulder for SFA, MUFA, PUFA, and TFA, but only PUFA was significantly different (p<0.05). The higher concentrations observed in the braised cuts could be the result of using higher internal temperatures (85°C) than for other methods, possibly causing a relatively greater concentration effect, compared to cuts cooked to lower internal temperature (70°C). Detailed results of this study will be published.
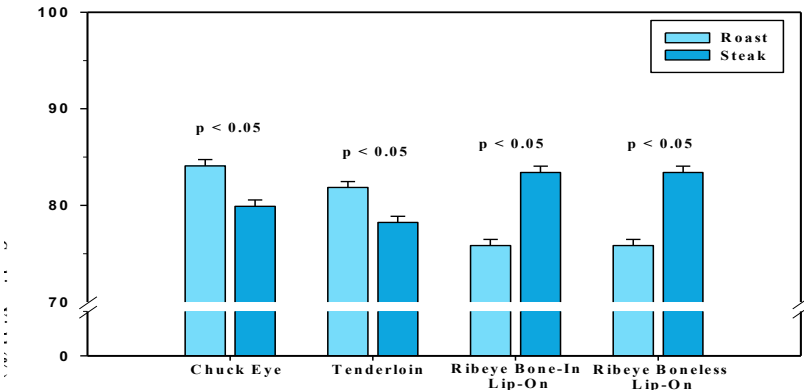

Figure 11. Cooking yields for pairs of roasted and grilled beef cuts from 3 primals. N=36 per cut. (Nguyen et al., 2014)
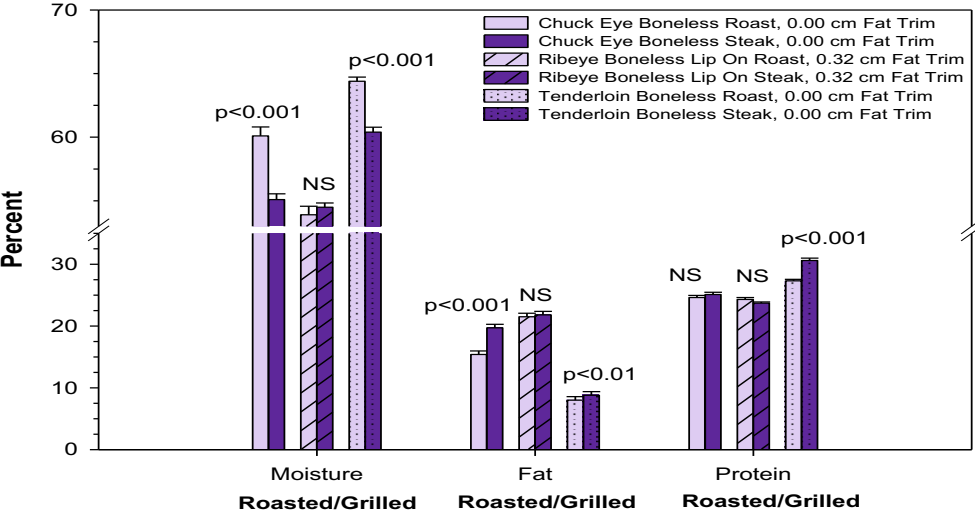

Figure 12. Proximate content of paired roasted and grilled beef cuts from 3 primals. N-36 per cut. (Roseland et al., 2015)

NATIONWIDE GROUND BEEF STUDY

A study was designed by NDL in collaboration with America's Beef Producers, University of Wisconsin (UW), and Texas Tech University to obtain ground beef nutrient data over a range of fat levels. A goal was to establish the mathematical relationship between the total fat content of raw ground beef and various nutrients, using regression techniques (USDA, 2017b). Retail samples of ground beef (n=72) labeled from 4 to 30% fat were purchased using a sampling plan developed for NDL's National Food and Nutrient Analysis Program (Pehrsson et al., 2000; Perry et al., 2003). The basis of this plan divided the US into 4 regions, each having 3 consolidated metropolitan statistical areas (CMSA), where samples were collected from stores in each CMSA in 2000 and in 2011. Samples were cooked as broiled patties, pan-broiled patties, loaves, and crumbles. Patties were made from 112 g samples pressed into molds and oven-broiled for 8.7 minutes or pan-broiled in an electric skillet for 11.75 minutes. Crumbles were pan-browned for 5.3 minutes; loaves were baked in 325°F/163°C oven for 41 minutes. All samples were cooked to 160°F/71°C internal temperature.

Raw and cooked samples were chemically analyzed for proximates, cholesterol, fatty acids, vitamins, and minerals by qualified laboratories using AOAC or other validated methodology (AOAC, 2000), duplicate samples, and reference materials. Data were evaluated using mixed model regression analysis with SAS (SAS, 2004) to obtain prediction equations. Estimated mean values for each nutrient covered the range of products from 3-30% labeled fat, showing the relationship between analytical raw fat and analytical nutrient values.

Ground beef study results

As fat in raw cuts increased, values for all 3 fatty acid classes increased as positive linear relationships ($p<0.05$; Roseland et al., 2016a). The effect of cooking showed a non-linear relationship between analytical raw fat and cooked fat, and also for cooked SFA, MUFA, and PUFA, reflecting the result of fat and moisture loss. Values for cooked fat (g/100 g) varied by cooking method. For example, cooked fat levels ranged from 3.65-16.44 in pan-broiled patty and from 4.0-16.50 in loaf (Roseland et al., 2016b).

Nutrient values for ground beef in the raw form and for four cooking methods, from samples analyzed in 2001 and 2012, been made available in the USDA food composition database (USDA, 2017a) for selected fat levels (3, 5, 7, 10, 15, 20, 25, and 30%) of raw ground beef. In addition, a ground beef calculator was developed by NDL, providing predicted nutrient profiles for raw and cooked ground beef at fat levels from 3 to 30%.

COOKING YIELD STUDIES

Cooking yield data are useful tools for making decisions regarding food plans and food preparation, such as cases where maximizing cooking yields is a desired outcome (Roseland et al., 2014). Cooking yields are gauges of changes in food weights due to moisture loss or fat gain/loss during cooking. NDL studies allow use of raw data to estimate cooked values and to determine amounts to purchase. Also, these available data--for over 175 cuts of beef, lamb, pork, poultry, and other meats--benefit researchers, scientists, nutrition

professionals, industry officials, and consumers by providing valuable information regarding the impact of cooking methods, meat type, and fat content on total cook-ing yield.

In an NDL study of cooking yields, data for three different beef and three different pork cuts were evaluated. Results varied according to cooking method, with broiling having the highest and braising having the lowest cooking yields (p<0.0001; Figure 13). Among the pork cuts, although cooking yields and moisture changes differed according to cut/cooking method (p<0.0001), no difference in fat was observed although all three cuts increased in fat concentration after cooking (Roseland et al., 2012).

In an NDL study of 7 types of ground broiled meats (i.e., beef, pork, bison), cooking yields were generally inversely related to cooked fat levels. Among the types analyzed, ground pork had lowest cooking yield, which was significantly different than all the other meats (p<0.0001) except ground beef (Figure 14) (Roseland et al., 2012).
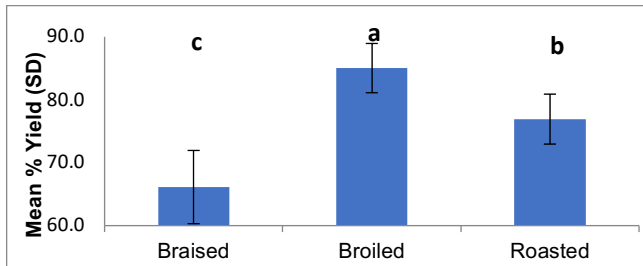
Figure 13. Cooking yields and standard deviations (SD) for beef and pork cuts prepared using 3 different methods. N=83 for Braised (71 beef shoulder roasts + 12 pork shoulder roasts); N=49 for Broiled (36 beef ribeye steaks + 12 pork loin chops); N=47 for Roasted (35 beef ribeye roasts + 12 pork center loin roasts).
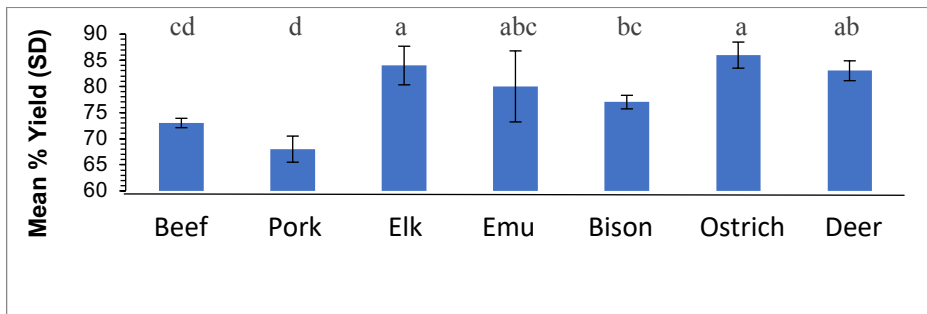
Figure 14. Cooking yields and standard deviations (SD) for seven types of ground broiled meat. N=6 for Emu, Bison, Ostrich; N=5 for Elk; N=4 for Beef, Pork; N=3 for Deer.

IMPACT OF NDL STUDIES

Obtaining nutrient composition and cooking yield data for specific cuts and cooking methods supports research examining nutrient intake and health. Further research into factors affecting nutrient composition, variability, and yield can benefit researchers, purchasers, and other database users. These studies provide "reference" data, which may be used to make general comparisons with other global sources to support trade and research, both domestically and abroad. Collaborative research protocols have been developed to conduct these studies, which have yielded representative data for scientists' use in conducting subsequent studies. Current data from the studies can be helpful for estimating US intake, conducting further research, and establishing nutrition guidelines. The data and user-friendly tools developed by the USDA/Nutrient Data Library are accessible at: https://www.ars.usda.gov/ba/bhnrc/ndl, including a) On-line nutrient analytically-based data for over 9000 foods in SR and brand name information for over 175,000 foods in the

Branded Food Products Database; b) USDA Nutrient Data Sets for Beef and Lamb Retail Cuts to assist retailers with nutrient labeling including 28 nutrients (USDA, 2017c; USDA, 2013); c) Ground beef calculator; d) USDA Cooking Yields Tables for Meat and Poultry (USDA, 2014). Future research plans at NDL will encompass investigations of factors affecting nutrient content and variability of meat and dairy products, such as source, breed, season, animal diet and other agricultural practices, in order to attain better health outcomes in the US populations particularly among vulnerable populations.

References

Acheson, R.J., D.R. Woerner, J.N. Martin, K.E. Belk, T.E. Engle, T.R. Brown, C.J. Brooks, A.M. Luna, L.D. Thompson, H.L. Grimes, A.N. Arnold, J.W. Savell, K.B. Gehring, L.W. Douglass, J.C. Howe, K.Y. Patterson, J.M. Roseland, J.R. Williams, A. Cifelli, J. Leheska, and S.H. McNeil. 2015. Nutrient database improvement project: Separable components and proximate composition of raw and cooked retail cuts from the beef loin and round. Meat Sci. 110:236-244.

Ahuja, J.K., A.J. Moshfegh, J.M. Holden, and E. Harris. 2013. USDA food and nutrient databases provide the infrastructure for food and nutrition research, policy, and practice. J. Nutr. 143(2):241S–249S.

AOAC, Official Methods of Analysis of AOAC International. 2000. 17th edition, AOAC International, Gaithersburg, MD. Daley C.A., A. Abbott, P.S. Doyle, G.A. Nader, S. Larson. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr. Journal 9:10

Duckett, S.K., J.P.S. Neel, J.P. Fontenot, W.M. Clapham. 2009. Effects of winter stocker growth rate and finishing system on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content. J. Anim. Sci. 87:2961-2970.

Garrett, W.N. and N. Hinman. 1971. Fat content of trimmed beef muscles as influenced by quality grade, yield grade, marbling score and sex. J. Anim. Sci. 33(5):948-957.

Gebauer, S.K., F. Destaillats, F. Dionisi, R.M Krauss, D.J. Baer. 2015. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. Am. J. Clin. Nutr. 102:1339-46.

Gifford, C.L., D.R. Woerner, J.N. Martin, T.E. Engle, K.E. Belk, and M.A. Harris. 2016. Complete Nutrient Analysis of Grain Finished and Grass Finished Lamb. Master's Thesis. Retrieved from Colorado State University, Digital Repository.

Johnson, N. September 22, 2016. The steaks have never been higher. Retrieved February 17, 2017 from http://grist.org/briefly/grass-fed-   beef-sales-jumped-40-percent-in-2015/

Leheska, J.M., L.D. Thompson, J.C. Howe, E. Hentges, J. Boyce, J.C. Brooks, B. Shriver, L. Hoover, and M.F. Miller. 2008. Effects of conventional and grass-feeding systems on the nutrient composition of beef. J. Anim. Sci. 86(12):3575–3585.

Martin, J.N., J.C. Brooks, L.D. Thompson, J.W. Savell, K.B. Harris, L.L. May, A.N. Haneklaus, J.L. Schutz, K.E. Belk, T. Engle, D.R. Woerner, J.F. Legako, A.M. Luna, L.W. Douglass, S.E. Douglass, J. Howe, M. Duvall, K.Y. Patterson, and J.L. Leheska. 2013. Nutrient database improvement project: the influence of U.S.D.A. Quality and Yield Grade on the separable components and proximate composition of raw and cooked retail cuts from the beef rib and plate. Meat Sci. 95(3):486-494.

National Cattlemen's Beef Association (NCBA). 2014. Lean Matters: Chronicling Beef's Change from Gate to Plate – A Distinctive Public–Private Collaboration. Retrieved December 27, 2016 from http://www.beefissuesquarterly.com/CMDocs/BeefResearch/Nutrition/LeanMatters_Web.pdf

Nguyen, Q.V., J.M. Roseland, and J.R. Williams. 2016. Fat and other key nutrients in retail lamb cuts in the United States, Australia and New Zealand. Abstract and poster for National Nutrient Databank Conference, May 2016, Alexandria, VA.

Nguyen, Q.V., J.M. Roseland, J.R. Williams, J.C. Howe, and L.W. Douglass. 2014. Comparison of cooking yields and fat and moisture retentions in retail beef cuts. Abstract and poster for National Nutrient Databank Conference, May 2014, Portland, OR.

Pehrsson, P.R., D.B. Haytowitz, J.M. Holden, C.R. Perry, and D.G. Beckler. 2000. USDA's National Foods and Nutrient Analysis Program: food sampling. J. Food Compos. Anal. 23:379-389.

Perry, C.R., P.R. Pehrsson, and J. Holden, 2003. A revised sampling plan for obtaining food products for nutrient analysis for the USDA National Nutrient Database. Proceedings of the American Statistical Association. Section on Survey Research Methods [CD-ROM], Alexandria, VA. American Statistical Association. San Francisco.

Popova, T., U. Gonzales-Barron, and V. Cadavez. 2015. A meta-analysis of the effect of pasture access on the lipid content and fatty acid composition of lamb meat. Food Res. Intl. 77:476-483.

Purchas, R.W., Wilkinson, B.H.P., Carruthers, F., Jackson, F. 2015. A comparison of the *trans* fatty acid content of uncooked and cooked lean meat, edible offal and adipose tissue from New Zealand beef and lamb. J. Food Compos. Anal. 41:151-156.

Roseland, J.M., J.R. Williams, M.B. Duvall, B.A. Showell, K.Y. Patterson, J.C. Howe, J.M. Holden. 2012. Effect of meat type and cooking method on meat yields.

International Congress of Meat Science and Technology. August 14, 2012. Montréal, Canada.

Roseland, J.M., J.C. Howe, K.Y. Patterson, J.R. Williams, J.M. Holden, L.W. Douglass, J.C .Brooks, L.D. Thompson, J.W. Savell, K.B. Harris, D.R. Woerner, S.H. McNeill, A.M. Cifelli. 2014a. Nutrient results from a collaborative nationwide beef study to update data in the USDA database, 2007 to 2013. Abstract and poster for National Nutrient Databank Conference, May 2014, Portland OR,

Roseland, J.M., Q.V. Nguyen, J.R. Williams, L.W. Douglass, K.Y. Patterson, J.C. Howe, J.C. Brooks, L.D. Thompson, D.R. Woerner, T.E. Engle, J.W. Savell, K.B. Gehring, A.M. Cifelli, S.H. McNeill. 2015. Protein, fat, moisture, and cooking yields from a U.S. study of retail beef cuts. J. Food Compos. Anal. 43:131-139.

Roseland, J.M., Q.V. Nguyen, J.R. Williams, K.Y. Patterson, L.W. Douglass, J.C. Howe. 2016a. Nutrient values for ground beef products   ranging from 3 to 30% fat for four cooking methods, from USDA research study. Abstract and poster for Reciprocal Meat Conference, June 19-22, 2016, San Angelo, TX.

Roseland, J.M., Q.V. Nguyen, J.R. Williams, K.Y. Patterson, L.W. Douglass. 2016b. Retail Ground Beef Identified as "Lean" from USDA Research Study. Abstract and poster at Academy of Nutrition and Dietetics conference, October 15-18, 2016, Boston, MA.

Roseland, J.M., Q.V. Nguyen, L.W. Douglass, K.Y. Patterson, J.C. Howe, J.R. Williams, L.D. Thompson, J.C. Brooks, D.R. Woerner, T.E. Engle, J.W. Savell, K.B. Gehring, A.M. Cifelli, S.H. McNeill. 2018. Fatty acid, cholesterol, vitamin, and mineral content of cooked beef cuts from a national study. J. Food Compos. Anal. 66:55-64.

Rule, D.C., K.S. Broughton, S.M. Shellito, G. Maiorano. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80:1202-1211.

SAS System (version 9.1). SAS Institute Inc. Cary, NC.

Slover, H.T., E. Lanza, R.H. Thompson Jr., C.S. Davis, G.V. Merola. 1987. Lipids in raw and cooked Beef. J. Food Compos. Anal. 1: 26-37.

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2014. USDA Table of Cooking Yields for Meat and Poultry. Retrieved February 9, 2017 from https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/yield-and-retention

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2013. USDA Nutrient Data Set for Retail Beef Cuts From SR, Release 3.0. Retrieved December 27, 2016 from https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Beef/Retail_Beef_Cuts03.pdf

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2017a. USDA National Nutrient Database for Standard Reference. Version Retrieved February 20, 2017 from http://www.ars.usda.gov/nutrientdata

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2017b. Ground Beef Calculator. Retrieved February 9, 2017 from https://ndb.nal.usda.gov/ndb/beef/show

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. 2017c. USDA Nutrient Data Set for Retail Lamb Cuts from SR. Retrieved February 9, 2017 from https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Meat/Lamb_Labeling_Doc.pdf

US Department of Agriculture, Economic Research Service 2017. Livestock and meat domestic data. Quarterly red meat supply and disappearance and per capita disappearance Retrieved February 2, 2017 from https://www.ers.usda.gov/data-products/livestock-meat-domestic-data

Van Elswyk, M.E. and S.H. McNeill, 2014. Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: The U.S. experience. Meat Sci. 96:535-540.

Wahrmund-Wyle, J.L., K.B. Harris, J.W. Savell. 2000a. Beef retail cut composition: 1. Separable tissue components. J. Food Compos. Anal. 13(3):233-242.

Watt, B.K., and A. Merrill 1963. Composition of foods: Raw, processed, prepared. Department of Agriculture, Agriculture Handbook No. 8. Washington, DC, USA: United States Department of Agriculture.

West, S.E., K.B. Harris, A.N. Haneklaus, J.W. Savell, L.D. Thompson, J.C. Brooks, J.K. Pool, A.M. Luna, T.E. Engle, J.S. Schutz, D.R. Woerner, S.L. Arcibeque, K.E. Belk, L. Douglass, J.M. Leheska, S. McNeill, J.C. Howe, J.M. Holden, M. Duvall, K. Patterson. 2014. Nutrient database improvement project: the influence of USDA quality and yield grade on the separable components and proximate composition of raw and cooked retail cuts from the beef chuck. Meat Sci. 97(4):558-567.

Williams, A. The future of grassfed: Laying out the promise and challenges. Graze e-magazine November 1, 2013. Accessed 29 November 2016. http://www.grazeonline.com/grassfedpromisechallenge

Processing Milk effects on Fatty Acid Profiles in Consumed Grass-Fed Milk Products

If pastured cows do produce a better fatty acid composition in their milk, how does milk processing affect the composition once it is pasteurized, homogenized, and skimmed? How does milk in its various forms affect human digestion? These questions are being researched by the three people at this session. One of the biggest problems has been the penchant of nutritionists to tout fat-free or low-fat milk because of the saturated fat content regardless of some new facts about stearic and palmitic fatty acids having no or little effect on cardiovascular disease. The problem with skimming off the fat (cream) is that it removes other good fatty acids as well, such as omega-3 (n-3). According to the USDA standard reference database, an eight-fluid ounce cup (244 g) of 3.25% fat milk has 0.183 grams of omega-3s. This is a small amount of n-3 and is from confinement-fed cow's milk. If the milk is non-fat or skim, the amount goes down to 0.0049 grams of n-3 or essentially zero. Now you can buy non-fat milk with an added n-3 derived from algae, so much for "natural". Perhaps it would be better just to eat some salmon or trout.

Variations in milk lipids

Michael H. Tunick, Ph.D., Diane L. Van Hekken, Ph.D., Peggy M. Tomasula, D.Sc.

Dairy and Functional Foods Research Unit, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA 19038

Structure

Fats (if solid at room temperature) and oils (if liquid) are in the class of compounds known as lipids. Around 95-98% of the lipids in milk are comprised of triacylglycerols, which are abbreviated TG and better known as triglycerides (McGibbon & Taylor, 2006) (Figure 1).
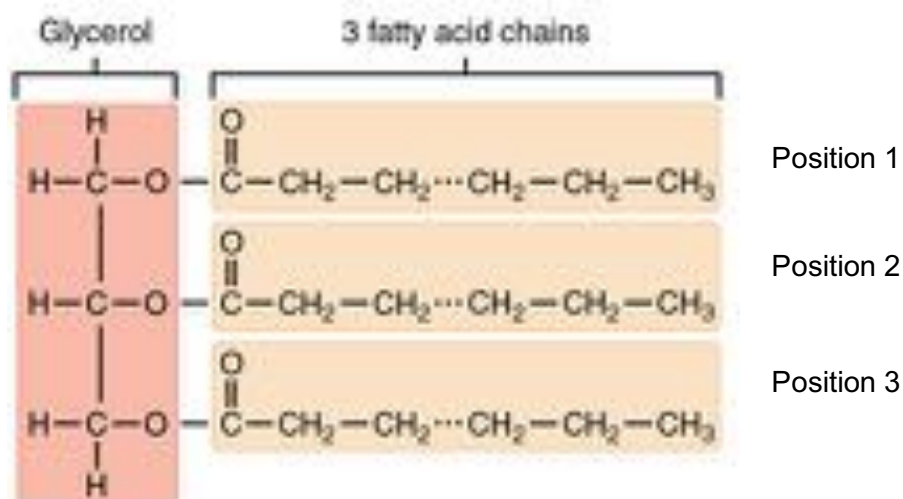

Figure 1. A triglyceride molecule, showing three fatty acids attached to a glycerol backbone.

TG are composed of a glycerol backbone with three fatty acid (FA) molecules attached. TG in milk contain 26-54 carbon atoms with a myriad of possible arrangements of FA on the backbone (Jensen, 2002). The typical FA profiles of cow, goat, and sheep milk are shown in Table 1.The abbreviations correspond to the number of carbon atoms in the molecule and the number of double bonds it has, separated by a colon. Note that roughly half of the FA in milk is comprised of palmitic (16:0) and oleic (18:1) acids. The *cis* and *trans* designations refer to whether the hydrogen atoms along the double bond are on the same side or on opposite sides. Oleic and vaccenic acids both contain 18 carbon atoms with one double bond, but the atoms are arranged differently. Elaidic acid (18:1 *trans*-9), identified as the primary *trans*-fat in hydrogenated fat, is found in miniscule amounts in milk and is not shown. CLA, conjugated linoleic acid, comprises a class of nearly 30 similar FA; the predominant one in milk is rumenic acid, abbreviated as 18:2 *cis*-9, *trans*-11. Humans convert vaccenic acid to rumenic acid. The double bonds in CLA are closer together than in α-linoleic acid.

FA are saturated if they do not contain a double bond (they are saturated with hydrogen

atoms) and are unsaturated if they contain at least one double bond. Monounsaturated fatty acids (MUFA) contain one double bond and polyunsaturated fatty acids (PUFA) contain more. Some structures are shown in Figure 2.

Table 1. Principal fatty acids in milk of cows, goats, and sheep, in grams of fatty acid per 100 g of fat (Chouinard et al., 1999; Park et al., 2007).

Fatty acid		Concentration (g/100 g)		
Common name	Abbreviation	Cow	Goat	Sheep
Butyric	4:0	4.2	2.2	3.5
Caproic	6:0	2.2	2.4	2.9
Caprylic	8:0	1.2	2.7	2.6
Capric	10:0	2.7	10.0	7.8
Lauric	12:0	3.1	4.0	4.4
Myristic	14:0	11.1	9.8	10.4
Pentadecanoic	15:0	1.2	0.7	1.0
Palmitic	16:0	27.0	28.2	25.9
Palmitoleic	16:1	1.5	1.6	1.0
Margaric	17:0	0.6	0.7	0.6
Stearic	18:0	11.0	8.9	9.6
Oleic	18:1 *cis*-9	23.9	19.3	21.1
Vaccenic	18:1 *trans*-11	1.9	0.7	1.0
α-Linoleic	18:2 *cis*-9, *cis*-12	2.5	3.2	3.2
Rumenic	18:2 *cis*-9, *trans*-11	0.7	0.7	0.7
Linolenic	18:3	0.4	0.4	0.8

Stearic acid (18:0)

Oleic acid (18:1)

α-Linoleic acid (18:2)

Rumenic acid (18:2), a CLA
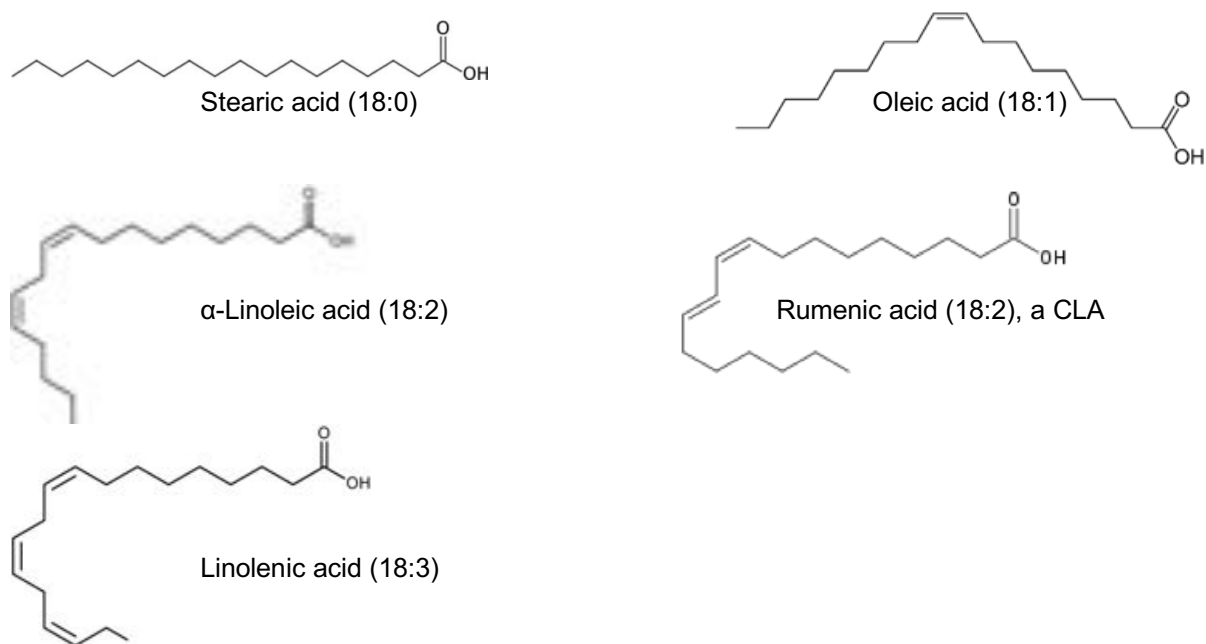
Linolenic acid (18:3)

Fig. 2. Skeletal structures of major 18-carbon fatty acids found in milk.

FA and TG in milk are assembled in the mammary gland and are derived from feed and from microbial activity in the rumen of the animal. Variations occur because of species, diet, season, health of the animal, stage of lactation, and other factors. The mammary gland in ruminants synthesizes FA containing an even number of carbons from 4 to 14,

along with some 16:0. The remaining 16:0 and the longer FA arise from dietary lipids and breakdown of TG in adipose tissue. Bacterial flora in the rumen synthesize the relatively small numbers of FA with an odd number of carbons. FA may be desaturated in the mammary gland to form unsaturated acids (Månsson, 2008).

The positioning of FA on the TG molecule is not random: nearly all of 4:0, 6:0, and 8:0 are found at position 1, most of the 14:0 and 16:0 occur in position 2, and most of the FA containing 18 carbons are located at the positions 1 and 3 (Blasi et al., 2008). TG in milk from goats and sheep appear to be similar to bovine TG in this regard (Park et al., 2007). FA may be broken away from the backbone by activity of lipase enzymes, thus becoming free fatty acids (FFA). When we consume dairy products, lipases in the mouth and stomach preferentially attack the TG molecule at position 3 (Williams 2000). Therefore, the shorter and longer FA are much more likely to become FFA in the digestive system than the medium-size FA.

Milkfat floats in milk in the form of globules surrounded by a membrane composed of two-thirds lipid and one quarter protein. Most of the lipid in the milkfat globule membrane consists of TG, but some 40% is phospholipid (Fong et al., 2007), a molecule that is similar to a TG except that a phosphate group is attached to the glycerol backbone instead of the third FA. The phosphate groups, which are aligned on the outside of the membrane, are water-soluble and the FA, which point toward the globule, are not. This emulsification prevents globules from coalescing in the fluid portion of the milk and also protects their contents from the action of lipases. Milkfat also contains a small amount of mono- and di-glycerides, which have only one or two FA, as well as fat-soluble vitamins (A, D, E, and K), sterols (such as cholesterol), and FFA.

Saturated and Unsaturated Fatty Acids

Dairy fats account for around 21% of the saturated fat intake in the US, but there is no consistent evidence that milkfat levels are associated with an increased risk of cardio-vascular disease, coronary heart disease, or stroke (Huth and Park, 2012). Detailed metabolic studies have shown that short-chain and medium-chain FA have minimal effect on plasma LDL and cholesterol levels, only 12:0, 14:0, and 16:0 contribute to higher levels, and 18:0 is considered neutral (Williams 2000). It is not clear whether these effects are due to TG structure, the FA themselves, or some other factor (Mensink, 2005).

MUFA do not appear to influence inflammatory effects in the body, but various aldehydes produced in the oxidation of PUFA, as well as sugars, are known to initiate or advance inflammation, cancer, asthma, type 2 diabetes, and atherosclerosis (Lawrence, 2013). Saturated fats alone might not be responsible for many of the adverse health effects with which they have been associated, but oxidation of PUFA may be the cause of any association that have been found (Lawrence, 2013).

CLA and Omega-3 Fatty Acids

Dairy products contribute about 75% of the total CLA in the human diet. CLA has been identified as a factor against cancer, obesity, diabetes, and atherosclerosis, while helping with modulation of the immune system and bone growth (Lock and Bauman, 2004). A study in our laboratory of milk from adjacent farms, one with cows on pasture and other with cows fed conventionally, revealed that grazing increased the rumenic acid content in milk by 29-36% (Tunick et al., 2016); a nation-wide study of conventional and organic milk from 14 processors showed an 18% increase (Benbrook et al., 2013).

Much research has been directed toward omega-3 FA, which contain a double bond located three carbon atoms from the end farthest from the glycerol backbone. The omega-3 FA of note in milk is α-linolenic acid (18:3); it and linolenic acid (18:2, an omega-6 FA) serve as precursors to other FA that the body requires, namely EPA (20:5) and DHA (22:6). In fact, 18:2 and 18:3 are regarded as essential FA since the body needs them and must obtain them from the diet (Simopoulos, 2006). Our comparison of two farms indicated that milk from pasture-fed cows contained 28-56% more 18:3 than milk from the adjacent conventional farm (Tunick et al., 2016). A nation-wide survey of milk revealed that organic milk averaged 60% more 18:3 than conventional milk (Benbrook et al., 2013).

Omega-6 FA cannot be converted to omega-3 FA in the body since mammals lack the enzyme required. Humans used to consume the two in about equal amounts, but in today's Western diets the ratio is around 16 to 1 (Simopoulos, 2006). In milk, the ratio is within the recommended 4 to 1.

Trans-Fatty Acids

Trans-FA have been linked to coronary heart disease, but the harmful types of these (especially elaidic acid, 18:1 _trans_-9) are found in very low levels in milk (Mozaffarian et al., 2006). Vaccenic acid (18:1 _trans_-11) is a _trans_-FA that occurs in milk, but it is a precursor of rumenic acid (the main CLA in milk) and is considered beneficial (Park et al., 2007).

Flavor and Mouthfeel

People do not consume dairy products simply because of the health aspects – they also enjoy the flavor. TG do not contribute to flavor because their large size makes them non-volatile. In contrast, short- and medium-chain FA (containing up to 12 carbon atoms) are volatile, have low perception thresholds (we can detect them at parts-per-million concentrations), and are responsible for some of the characteristic flavors of dairy products (Curioni and Bosset, 2002). Goat and sheep milk contain higher levels of short- and medium-chain FA than cow milk, resulting in stronger cheese flavors. Branched-chain FA come from breakdown of proteins instead of lipids and are also noted components of goat and sheep milk cheeses. FFA are precursors of other compounds that result from action of lipases (Curioni and Bosset, 2002), and lipids serve as solvents for these and other compounds that provide flavors.

Mouthfeel, which results from physical stimulation of receptors in the mouth, is also an important part of the eating experience. Milkfat melts just below body temperature (35°C) and exhibits gradual and complete melting in the mouth. It is perceived to have smooth mouthfeel and imparts a desirable cooling sensation in the mouth as it melts. Perceived aroma is related not only to volatile compounds in the nose but also to sensations of taste and mouthfeel (de Roos, 2006). Studies using TG containing 8:0 and 10:0 have shown that lipid deposition on the tongue and other oral surfaces is related to sensory perception (Pivk et al., 2008).

Summary

The FA in milkfat have different lengths, levels of saturation, and molecular arrangements. They also have some effects on human health, although the influence of saturated fats appears to have been overstated. CLA and omega-3 FA found in dairy products are essential to health and are increased in milk from grass-fed animals.

References

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:e82429.

Blasi, F., D. Montesano, M. De Angelis, A. Maurizi, F. Ventura, L. Cossignani, M.S. Simonetti, and P. Damiani. 2008. Results of stereospecific analysis of triacylglycerol fraction from donkey, cow, ewe, goat and buffalo milk. J. Food Compos. Anal. 21:1-7.

Chouinard, P.Y., L. Corneau, D.M. Barbano, L.E. Metzger, and D.E. Bauman. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579-1584.

Curioni, P.M.G., and J.O. Bosset. 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int. Dairy J. 12:959-984.

deRoos, K.B. 2006. How lipids influence flavor perception. p. 145-158. *In* F. Shahidi and H. Weenen (ed.) Food Lipids: Chemistry, Flavor, and Texture. ACS Books, Washington, DC.

Fong, B.Y., C.S. Norris, and A.K. MacGibbon. 2007. Protein and lipid composition of bovine milk-fat-globule membrane. Int. Dairy J. 17:275-288.

Huth, P.J., and K.M. Park. 2012. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. Adv. Nutr. 3:266–285.

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

Lawrence, G.D. 2013. Dietary fats and health: dietary recommendations in the context of scientific evidence. Adv. Nutr. 4:294-302

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.

Månsson, H.L. 2008. Fatty acids in bovine milk fat. Food Nutr. Res. 52. DOI: 10.3402/fnr.v52i0.1821.

McGibbon, A.K.H., and M.W. Taylor. 2006. Composition and structure of bovine milk lipids. p. 1-42. *In* P.F. Fox and P.L.H. McSweeney (ed.) Advanced Dairy Chemistry, Vol. 2: Lipids, 3rd edn. Springer, New York, NY.

Mensink, R.P. 2005. Effects of stearic acid on plasma lipid and lipoproteins in humans. Lipids 40:1201-1205.

Mozaffarian, D., M.B. Katan, A. Ascherio, M.J. Stampfer, and W.C. Willett. 2006. *Trans*-fatty acids and cardiovascular disease. New Engl. J. Med. 354:1601-1613.

Park, Y.W., M. Juárez, M. Ramos, and G.F.W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Res. 68:88-113.

Pivk, U., N. Godinot, C. Keller, N. Antille, M.-A. Juillerat, and P. Raspor. 2008. Lipid deposition on the tongue after oral processing of medium-chain triglycerides and impact on the perception of mouthfeel. J. Agric. Food Chem. 56:1058-1064.

Simopoulos, A.P. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed. Pharmacother. 60:502-507.

Tunick, M.H., D.L. Van Hekken, M. Paul, E.R. Ingham, and H.J. Karreman. 2016. Case study: Comparison of milk composition from adjacent organic and conventional farms in the USA. Int. J. Dairy Technol. 69:137-142.

Williams, C.M. 2000. Dietary fatty acids and human health. Ann. Zootech. 49:165-180.

Impact of processing on the healthy fatty acids in milk and other dairy products

Diane L. Van Hekken, Ph.D., Michael H. Tunick, Ph.D., Peggy M. Tomasula, D.Sc.

Dairy and Functional Foods Research Unit, USDA, ARS, ERRC, 600 East Mermaid Lane, Wyndmoor, PA 19038

Abstract

Common dairy processing protocols can alter the composition of fluid milk and dairy foods in ways that affect the amount of healthy fats in the final product. The total amount of fat in dairy products is affected by controlling the fat content of the milk and making different dairy foods that concentrate the fat, for example, whole milk contains 3.25% fat, while butter contains 80% fat. The profile of the fatty acids that make up the fat in milk can be altered by some processing conditions and through animal feeding practices. A comparison of fatty acids in milk and dairy foods coming from pasture-fed or confined dairy herds illustrates the differences in levels of healthy fats. Milk from pasture-fed cows typically exhibits higher levels of the healthy fatty acids and is preferably used in products where the fats will contribute to the daily total of healthy fats in the diet.

Introduction

With increasing consumer interest in sustainable agriculture, organic foods, and grass-fed dairy products, many farmers have backed away from the conventional practices of feeding high grain total mixed rations to confined cows and have opted for practices that incorporate significant amounts of grazing. Certified organic herds must obtain a minimum average of 30% of their dry matter intake from pasture during the grazing season and can include some silages and grains (all grown according to organic guidelines), while the diet of grass-fed herds cannot include grains. Of the 3.8 billion gallons of milk sold in the U.S. in 2016, organic and grass-fed milk accounted for 4.9 and 0.04% of the sales, respectively (DMI, 2016).

Inclusion of grazing as a major source of nutrients for the milking herd has been found to have a tremendous effect on the distribution of fatty acids (**FAs**) that make up the fat found in milk. Milk from grazing cows contains higher levels of the specific FAs that are beneficial to human health, such as the omega-3 poly-unsaturated fatty acids (**PUFA**) and the conjugated linoleic acids (**CLA**). However, milk must be processed to some degree before it reaches the consumer and there is limited, and often contradictory, information on the processing stability of the healthy fats found in milk. The question we address

in this paper is: Once milk leaves the farm, what are the effects of common dairy processing practices on the amount and distribution of the healthy FAs in milk and dairy products?

Fatty acids (FAs) in milk

Almost all (98%) of the fat in milk is in the form of triglycerides with three FAs bound to a glycerol backbone (McGibbon and Taylor, 2006). Over 400 individual FAs have been identified in bovine milk fat so the combinations of the 3 FAs in the triglyceride molecule can be quite extensive (Mansson, 2008). There are 20 FAs that contain 2-18 carbons (C2 - C18) in length that make up 90% of the total FAs present in milk fat, while the rest of the FAs are present in trace amounts and require sophisticated isolation methods for identification. A typical FA profile identifies and quantifies the FAs. Their distribution can be correlated to a variety of factors, from changes in diet to the health of the animal.

Although many of the FAs are known to have specific functions in the body, the healthy fats targeted in this work are the longer chain C18 PUFA. The major omega-3 FA in milk is alpha-linolenic acid, C18:3, with one of its three double bonds located at the 3rd carbon from the end of the chain. Linoleic acid, C18:2, is the predominant omega-6 FA in milk with one of its two double bonds found at the 6th carbon from the end. Isomers of linoleic acid, the CLAs, also designated as C18:2, have a single bond between the two double bonds. The predominant CLA in milk is rumenic acid (70 - 90%) and is the one most beneficial to human health out of the C18:2 isomers. Vaccenic acid, C18:1, contains only one double bond, and is tracked because it is a precursor of rumenic acid. See other papers in this proceedings that describe the FA in more detail and summarize their functions in human health.

Milk processing

Raw milk undergoes many processing steps en route to its final product, which may include altering the fluid product, separating or concentrating the fat, or concentrating the protein and fats (Figure 1). One of the most common steps alters the amount of components in milk by removing or adding proteins, fats, sugar (lactose), and water.
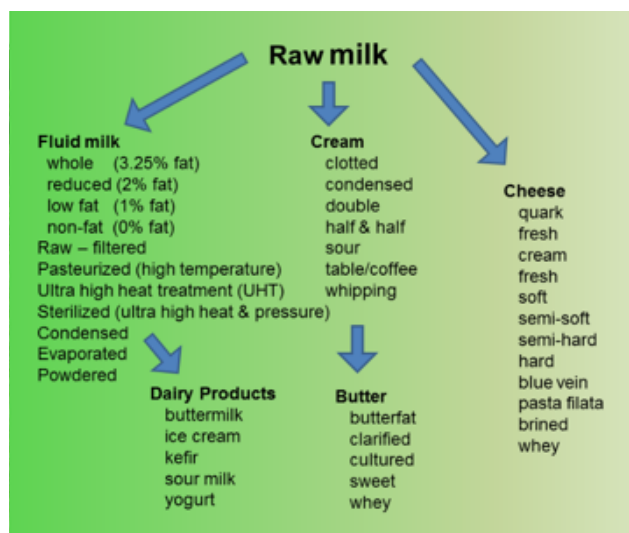
Figure 1. Flow chart of some of the many products manufactured from raw milk

Another step is to expose milk to a variety of stresses, such as heat, pressure, shear, and vacuum. Other modifications, including the addition of enzymes, cultures, and other ingredients. All approaches can affect the specific properties of the dairy food, such as texture, flavor/aroma, and functionality. Two common heat treatments expose milk to high temperatures: high temperature, short time (HTST) pasteurization at 161°F (72°C) for a minimum of 15 seconds and ultra high temperature (UHT) heating at 280°F (138°C) for 2 seconds (21CFR131.3, 2016). Cream, which must contain at least 18% milk fat, requires slightly higher pasteurization temperatures (5°F or 3°C higher). Homogenization is usually conducted in one or two stages, passing milk through a small orifice at pressures between 10 and 25 MPa. This removes the membrane around the fat droplets and breaks them into smaller spheres that stay suspended in the milk. Of the 3.8 billion gallons of fluid milk sold in the U.S. in 2016, raw (not pasteurized) and non-homogenized milk accounted for only 0.01 and 0.03% of sales, respectively (DMI, 2016).

The fat content of dairy products has significant impact on the amount of healthy fats present. Non-fat or skim milk versions contain no fat of any kind and do not contribute to the daily total of healthy fats consumed. In low fat products, the quantity of the healthy fats, although lower than full fat products, still contributes to the daily total in the diet.

There is limited information on the impact of processing on the profile of healthy FAs in milk. One study reported that heating milk at 85°C for 16 seconds or 95°C for 5 minutes did not alter the level of CLA in milk, while UHT heating (140°C for 4 seconds) and microwaving for 5 min decreased CLA content by 15 and 20%, respectively (Herzallah et al., 2005). Batch pasteurization (63°C for 30 min) and microwave heating of milk also increased the distribution of CLA *trans* isomers. Heating milk fat to 200-225°C, slightly higher than typical baking and frying temperatures, decreased CLA by 20-32%, while cooking at >300°C, slightly higher than typical of broiling and commercial pizza oven temperatures, led to isomerization and oxidation of FAs and up to a 60% decrease in CLA levels (Precht et al., 1999). Fermented skim milk products fortified with omega-3 FAs from milk fat were fairly heat stable at 80°C for 30 min (Luna et al., 2004), while fortifying with CLA from sunflowers decreased CLA content by 10% after heating at 73°C for 15 seconds

(Campbell et al., 2003). Unfortunately, not all researchers handle the milk samples the same way before measuring the initial level of FAs before processing. Some studies specifically state that they conducted FA analysis using fresh or frozen raw milk, others heated the milk to 60-100°C to extend shelf life and destroy bacteria, and still others do not mention how the milk was handled. Many of the survey studies report the FA profiles in dairy products without any information of the fat profile in the starting milk. Therefore, a better understanding of the impact that the processing of milk has on the healthy fatty acid profile of milk is needed.

ARS-DFFRU Processing Study

In a recent study conducted in the Dairy & Functional Foods Research Unit (**DFFRU**), fresh raw milk was collected from neighboring grazing certified organic (**ORG**) and confined non-grazing conventional (**CONV**) herds in Berks County, Pennsylvania, over an 8-week period during the grazing season (Van Hekken et al., 2017). Raw milk was standardized to 3.25% fat (Wr) and 1) homogenized (Wh), 2) HTST pasteurized (Wp), 3) homogenized and HTST pasteurized (Whp), or 4) homogenized and UHT heated (Whu). Quantities of the healthy FAs determined in the milk before (Wr) and after processing (Whr, Wp, Whp, and Whu) are shown in Table 1. Compared to the CONV milk, ORG milk contained higher levels of vaccenic acid (C18:1), linolenic acid (C18:3), and CLA. Milk from both farms contained similar amounts of linoleic acid (C18:2). The mean omega-6:omega-3 ratio (linoleic acid: linolenic acid) was lower for ORG milk than CONV milk, 4.9 and 7.3, respectively, and was closer to the ratio of <5 targeted by Simopoulos (2008) to aid in the prevention of many chronic diseases such as heart disease, arthritis, diabetes, and inflammatory disorders. Compared to the starting raw milk, processing did not significantly alter the quantities of the healthy FAs in the milk, therefore, ORG milk continued to contain higher levels of C18:1, C18:3, and CLA as well as lower ratios of omega-6:omega-3 FA. Results indicated that the healthy FAs were stable under common dairy processing conditions of homogenization, HTST pasteurization, and UHT heating.

Table 1. Levels of healthy fatty acids in whole milk fat from grazing organic and confined conventional herds before and after processing. *Samples were raw (Wr), raw homogenized (Whr), HTST pasteurized (Wp), homogenized and HTST pasteurized (Whp), and homogenized and UHT heated (Whu).*

Fatty acids	Vaccenic C18:1 *trans*	Linoleic C18:2	Conjugated Linoleic Acid C18:2 isomers	Linolenic C18:3	omega-6: omega-3 FA ratio
			(g fatty acid/100 g milk fat)		
Grazing organic					
Wr	3.52 [a]	3.31 [a]	0.90 [a]	0.72 [a]	4.6
Whr	3.29 [ab]	3.61 [a]	0.95 [a]	0.75 [a]	4.8
Wp	3.49 [a]	3.58 [a]	0.99 [a]	0.75 [a]	4.8
Whp	3.39 [ab]	3.56 [a]	1.01 [a]	0.68 [a]	5.2
Whu	3.43 [a]	3.51 [a]	0.92 [a]	0.69 [a]	5.1
Non-grazing conventional					
Wr	2.72 [c]	3.60 [a]	0.74 [b]	0.43 [b]	8.4
Whr	2.55 [c]	3.71 [a]	0.74 [b]	0.57 [b]	6.5
Wp	2.83 [bc]	3.51 [a]	0.91 [a]	0.50 [b]	7.0

Whp	2.62 [c]	3.50 [a]	0.81 [ab]	0.53 [b]	6.6
Whu	2.67 [c]	3.36 [a]	0.70 [b]	0.41 [b]	8.2

[a-c] *Means not sharing the same letter within a column are significantly different (P < 0.05).*

We also examined the digestibility of raw and processed milk from the grazing organic and confined conventional herds using *in vitro* digestion techniques (Van Hekken et al., 2017). Overall, milk was digested for one hour in gastric conditions [pepsin enzyme, pH 1.5, 98°F (37°C)] and two hours in intestinal conditions [phosphate buffer, bile salts, mix of enzymes for fats and proteins, 98°F (37°C)]. At the initial pH adjustment and addition of pepsin (G-0), milk formed large protein clots that entrapped the fat. The clots quickly broke down into smaller clots within 15 min of gentle shaking and steadily decreased in size as the *in vitro* digestion progressed. After 3 hours in a simulated gastrointestinal system, 85-94% of the proteins had been digested.

Milk fat was not hydrolyzed during gastric digestion, but the fat droplets tended to coalesce into larger ones as the protein clots broke apart and hydrolyzed. Once intestinal conditions were in place (lipases were included in the enzyme mix), lipolysis occurred as measured by the release of free fatty acids (FFA) from the milk triglycerides (Figure 2). Based on the total amount of FFA measured at 120 min (I-120), 50-60% of the FFA were released within the first 15 min. The rate slowed then stabilized as FFA accumulated and inhibited the lipase activity by blocking the enzyme access to new substrate. This inhibition would not occur in the body where the FFA would be absorbed or moved farther
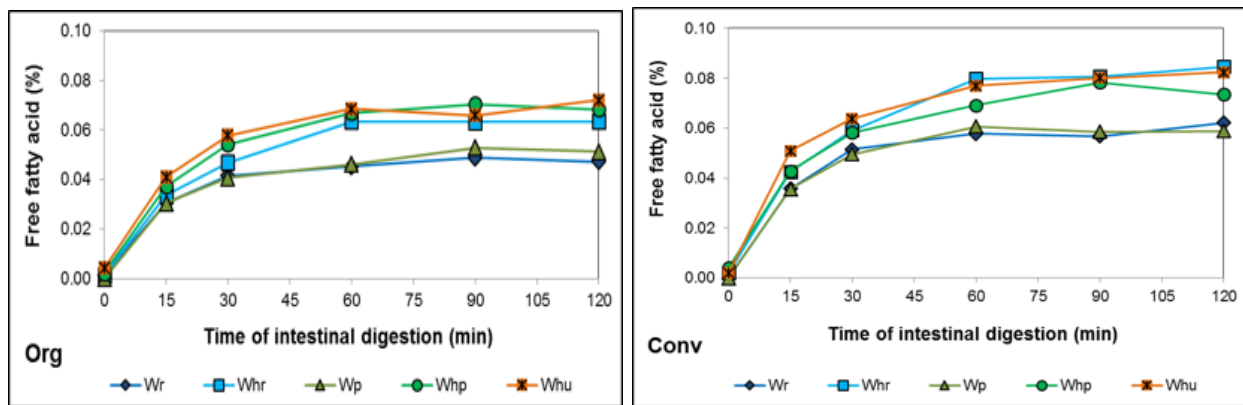

Figure 2. Release of free fatty acids during 120 min in vitro intestinal digestions of milk from grazing organic (ORG) and confined conventional (CONV) herds.

down the digestive tract. Homogenized samples (Whr, Whp, and Whu) released more FFA than the Wr and Wp samples, because homogenization removed the membrane surrounding the lipid droplets and shattered the large droplets into smaller spheres, thus increasing the surface area and accessibility of the lipase to the milk fat substrate. Compared to the ORG samples, the CONV samples released more FFA, in part because they contained higher amounts of C16:0 and C18:0 than the ORG samples, 43.1 and 40.5 mg FA/100 g milk fat, respectively. These saturated FAs are located at the first and third positions of the triglyceride, which are preferred sites for lipase activity. The size and quantity of the fat droplets in the digested sample decreased with time. More research is needed to understand the release and digestion of the healthy fatty acids in the intestinal tract.

Processing of dairy products

Regular yogurt is similar in fat content to milk and can contain 0-3.25% fat. The milk is pasteurized at 203°F (95°C) for 10 min and homogenized before cultures are added and the mix fermented at 107°F (42°C) for several hours. Once the yogurt reaches pH 4.5, it is chilled to 45°F (7°C). Depending on the final product, the yogurt base may have sweeteners and fruit mixed in before packaging.

Research has shown that the basic yogurt processing steps do not alter the CLA levels or distribution of the isomers in the final product (Boylston and Beitz, 2003; Dave et al., 2002; Herzallah et al., 2005; Shantha et al., 1995). Most studies claimed that CLA and omega-3 FAs levels were stable after 7-42 days of refrigerated storage (Boylston and Beitz, 2003; Luna et al., 2004; Dave et al., 2002; and Shantha et al., 1995); only one study reported that CLA content decreased after 7 days (Herzallah et al., 2005).

Cheese is a dairy product that concentrates milk fat (15-45% of the final product) and protein (7-36% of the final product). Cheese making protocols are as diverse as the hundreds of different varieties and styles of cheese made around the world. Briefly, milk (raw or pasteurized, seldom homogenized) is inoculated with cultures to slightly ferment the milk before coagulating with enzymes, primarily chymosin. The milk gel is cut into cubes and cooked before the whey is drained and the curd is salted. Lastly, the curd is packed into molds for pressing and then aged.

Although cheeses have high fat contents, studies have reported that processing protocols had no effect on the distribution of CLA in the fat fraction (mg per gram of fat basis) of Cheddar (Shantha et al., 1995), French Emmental (Gnadig et al., 2004), Gouda, Mozzarella (Shantha et al., 1995), and Swedish-Swiss-type cheeses (Jiang et al., 1997). Studies involving three different varieties of Cheddar, using different starter cultures and slightly

different manufacturing protocols, reported that total CLA levels were stable after 13 months of aging but that the CLA isomer distributions were different among the different brands (Werner et al., 1992; Lin et al., 1999). Another study reported that the CLA content of processed cheese increased 14% during manufacture and was attributed to the cooking step (Garcia-Lopez et al., 1994). One study demonstrated the variety of approaches and issues involved in enhancing omega-3 FA levels in Cheddar, Mozzarella, and Queso Fresco by adding fish or plant oils at different stages of cheesemaking (Bermúdez-Aguirre, 2012).

Research has shown that some dairy cultures can convert free linoleic acid (C18:2) to its isomers (CLA) in media and even less can do so in a milk environment (Bisig et al., 2007). The primary limitation is that the linoleic acid in milk is bound within the triglycerides and the cultures can convert it only when it is released in its free form. Therefore, to increase the CLA content in cheese or yogurt, non-dairy oils from plants and fish that are rich in unbound C18:2 are added, which the dairy cultures then convert to CLA (Kim and Liu, 2002; Bisig et al., 2007). Research continues to screen dairy cultures to identify ones that can increase CLA in dairy products.

Butter is the most fat-dense dairy product and typically contains 80% fat. Processing starts by pasteurizing cream (38% fat) at 203°F (95°C) for 15 seconds, aging for 12 hours and then using mechanical stress (churning) to remove the milk fat globule mem-brane from the fat droplets and coalesce the fat.

Because of the higher fat content, butter contains the highest amounts of PUFAs of any dairy food but the distribution of the healthy fats does not change significantly during processing or storage (Butler, et a., 2011; Mallia et al., 2008; Shantha et al., 1995; Bisig et al., 2007). However, butter is prone to fat oxidation, which causes off-flavors (rancidity) and degradation of the C18 FAs.

Processing does not appear to alter the CLA distribution in sour cream, ice milk, and ice cream. No differences have been reported between the starting raw milk and finished products (Shantha et al., 1995).

The only known process that can significantly alter the distribution of FAs in the final dairy product is cold fractionation. This process uses the low melting properties of the C18s, to concentrate PUFAs into a soft fraction. The method takes melted anhydrous milk fat from 140 to 50°F (60 to 10°C) to get hard and soft fractions, with the soft fraction containing 63% more CLA and 28% more vaccenic acid than the starting fat fraction (O'Shea et al 2000). Another approach is to manipulate the pressure and temperature within a supercritical carbon dioxide chamber to fractionate anhydrous milk fat. At 3500 psi (24 MPa) and 104°F (40°C), anhydrous milk fat yielded five fractions with one fraction containing 89% of the CLA (Romero et al., 2000).

Summary

Processing can alter the amount of total fat in a product, which will determine the total quantity of healthy fats in the food. Most of the processing steps used to make dairy products will not affect the distribution of the fatty acids on a mg/g of fat basis found in the starting milk. However, microwaving and extreme heat processing can alter the distribution of isomers of the C18:2. There are still only two ways to increase the distribution of healthy fats within the FA profile of dairy products:

- Manipulate the cow's diet to increase the level of naturally-occurring healthy fats in the milk.
- Add healthy fatty acids from plant or fish sources to milk or dairy products, which introduces other issues involving incorporation, stability, added cost, and off-flavors.

References

Bermúdez-Aguirre, D., Barbosa-Cánovas, G.V. 2012. Fortification of queso fresco, cheddar and mozzarella cheese using selected sources of omega-3 and some nonthermal approaches. Food Chem. 133:787-797.

Bisig, W.,Eberhard, P., Collomb, M., and Rehberger, B. 2007. Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products – a review. Lait 87:1-19.

Boylston, T.D., and Beitz, D.C. 2002. Conjugated linoleic acid and fatty acid composition of yogurt produced from milk of cows fed soy oil and conjugated linoleic acid. J. Food Sci.67:1973-1978.

Butler, G., Nielsen, J.H., Larsen, M.K., Rehberger, B., Stergiadis, S., Canever, A., and Leifert, C. 2011. The effects of dairy management and processing on quality characteristics of milk and dairy products. NJAS- Wageningen J. Life Sci. 58:97-02.

Campbell, W., Drake, M.A., Larick, D.K. 2003. The impact of fortification with conjugated linoleic acid (CLA) on the quality of fluid milk. J. Dairy Sci. 86:43-51.

21CFR131.3. 2016. Milk and cream. Definitions. Code of Federal Regulations, Title 21, Part 131. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/crcre/CFRSearch.ctm?fr=131.3. Accessed 02/01/2017.

Dave, R.I., Ramaswamy, N., and Baer, R.J. 2002. Changes in fatty acid composition during yogurt processing and their effects on yogurt and and probiotic bacteria in milk procured from cows fed different diets. Aust. J. Dairy Technol. 57:197-202.

Dairy Management Inc. (DMI). 2016. Retail monthly milk snapshot. 12/25/2016. IRI Custom DMI Market Advantage Database. pg 2.

Garcia-Lopez, S., Echeverria, E. Tsui, I., and Balch, B. 1994. Changes in the content of conjugated linoeic acid (CLA) in processed cheese during processing. Food Res. Int. 27:61-64.

Gnadig, S., Chamba, J. F., Perreard, E., Chappaz. S., Chardigny, J.M., Rickert, R., Steinhart, H. Sebedio, J. L. 2004. Influence of manufacturing conditions on the conjugated linoleic acid content and the isomer composition in ripened French Emmental cheese. J. Dairy Res. 71:367-371.

Herzallah, S. M., Humeid, M. A., Al-Ismail, K. M. 2005. Effect of heating and processing methods of milk and dairy products on conjugated linoleic acid and trans fatty acids isomer content. J. Dairy Sci. 88:1301-1310.

Jiang, J., Bjorck, L., Fonden, R. 1997. Conjugated linoleic acid in Swedish dairy products with special reference to the manufacture of hard cheese. Int. Dairy J. 7:863-867.

Kim, Y. J. and Liu, R. H. 2002. Increase of conjugated linoleic acid content in milk by fermentation with lactic acid bacteria. J. Food Sci. 67:1731-1838.

Lin, H., Boylston, T.D., Luedecke, L.O., and Shultz, T.D. 1999. Conjugated linoleic acid content of Cheddar-type cheese as affected by processing. J. Food Sci. 64:874-878.

Luna, P., Martin-Diana, A. B., Alonso, L., Fontecha, J., de la Fuenete, M. A., Requena, T., and Juarez, M. 2004. Effects of milk fat replacement by PUFA enriched fats on n-3 fatty acids, conjugated dienes and volatile compounds of fermented milks. Eur. J. Lipid Sci. Technol.106:417-423.

Mallia, S., Piccinali, P., Rehberger, B., Badertscher, R., Escher, F. and Schlichtherle-Cerny. 2008. Determination of storage stability of butter enriched with unsaturated fatty acids/conjugated linoleic acids (UFA/CLA) using instrumental and sensory methods. Int. Dairy J. 18:983-993.

Mansson, H. L. 2008. Fatty acids in bovine milk. Food Nutr. Res. 52:PMC2596709. Doi:10.3402/fnr.v52i0.1821.

McGibbon, A.K.H., and Taylor, M.W. 2006. Composition and structure of bovine milk lipids. p. 1-42. *In* P.F. Fox and P.L.H. McSweeney (ed.) Advanced Dairy Chemistry, Vol. 2: Lipids, 3rd edn. Springer, New York, NY.

O'Shea, M., Devery, R., Lawless, F., Keogh, K., Stanton, C. 2000. Enrichment of the conjugated linoleic acid content of bovine milk fat by dry fractionation. Int. Dairy J. 10:289-294.

Precht, D., Molkentin, J., Vahlendieck, M. 1999. Influence of the heating temperature on the fat composition of milk fat with emphasis on cis-/trans-isomerization. Nahrung 43:25-33.

Romero, P., Rizvi, S. S. H., Kelly, M. L., Bauman, D. E. 2000. Short communication: Concentration of conjugated linoleic acid from milk fat with a continuous supercritical fluid processing system. J. Dairy Sci. 83:20-22.

Shantha, N.C., Ram, L.N., O'Leary, J., Hicks, C., and Decker, E.A. 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Sci. 60:695-697.

Simopoulos, A.P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardio-vascular disease and other chronic diseases. Exp. Biol. Med.2008. 233:674-688.

USDA, ARS. 2017. National nutrient database for standard reference. Release 28. http://ndb.nal.usda.gov/ndb/ Accessed Feb 20, 2017.

Van Hekken, D. L., Tunick, M.H Ren, D.X., and Tomasula, P.M. 2017. Comparing the effect of homogenization and heat processing on the properties and in vitro digestion of milk from organic and conventional dairy herds. J. Dairy Sci. 100:6042-6052.

Werner, S. A., Luedecke, L. O., Schultz, T. D. 1992. Determination of conjugated linoleic acid content and isomer distribution in three cheddar-type cheeses: effects of cheese cultures, processing, and aging. J. Agric. Food Chem. 40:1817-1821.

A New Way to Look at the Impact of Dairy Foods on Health

Peggy Tomasula, D.Sc., Jenni Firrman, LinShu Liu

Dairy and Functional Foods Research Unit, Eastern Regional Research Center, Agricultural Research Service, USDA, 600 E. Mermaid Lane, Wyndmoor PA 19038
Peggy.tomasula@ars.usda.gov

MILK – PART OF A HEALTHY DIET

Bovine milk has long been a staple of human nutrition. Milk and milk products are rich sources of several bioactive and functional compounds such as the casein and whey proteins, along with lactoferrin and the immunoglobulins found in whey; milkfat; lactose and oligosaccharides; vitamins and minerals; and enzymes. Digestion of milk yields bio-active peptides from the milk proteins and free fatty acids and monoglycerides from milk-fat. The Dietary Guidelines for Americans 2015-2020 suggest that a healthy adult consume the equivalent of 3 cups of fat-free or low-fat milk per day (USHHS and USDA, 2015**).** The effects of milk consumption on overall human health have been well documented (Haug et al., 2004; Ebringer et al., 2008; Visioli and Strata, 2014). The bioactive peptides of milk are encrypted in the casein or whey proteins and are released upon contact with digestive enzymes in the stomach and small intestine (Tunick et al., 2016), or released during lactic acid fermentation of milk products such as yogurt and cheese or other proteases (Fitzgerald and Murray, 2006; Korhonen and Pihlanto, 2006). The bio-active peptides from casein or whey have shown a broad spectrum of activities such as antihypertensive, antimicrobial, opioid-like, and antioxidative. Casein phosphopeptides (CPP), probably the most studied of the bioactive peptides of casein, are known for their mineral-carrying capacity and have already found commercial applications in oral-health products. Most research on the effects of bioactive peptides has been conducted in the laboratory (*in vitro*) but not yet applied *in vivo* (in animal or human clinical trials) to a large extent. For most of the bioactive peptides, it is not known if the reported bioactivities will persist *in vivo*. Information on the fats and lipids of milk is found in the proceedings of this conference and also in Tunick et al. 2015.

The benefits of dairy consumption on human health have been well documented, and supporting literature can be found in the USDA Nutrition Evidence Library, Center for Nutrition Policy and Promotion, https://www.cnpp.usda.gov/nutritionevidencelibrary and in the proceedings of this conference.

DIGESTION OF MILK – A MATTER OF PROCESSING?

Commercial fluid milk is processed from raw milk to create 0.1% (skim), 1%, 2% and 3.25% (whole) pasteurized milk. The fat in milk exists as globules, 3 to 5 µm in size, with a protective membrane known as the milk fat globule membrane (MFGM). To prevent a cream layer from forming, the fat is first separated from milk and then added back to create milk of the desired fat content. It is then homogenized, which removes the MFGM and reduces the sizes of the fat globules to less than 1 µm (Michalski and Januel, 2006),

increasing their numbers. The MFGM fragments have also been proposed as nutraceuticals (Spitsburg, 2005) but little research has been done to isolate and explore the benefits of the MFGM and its fragments on human health.

The homogenized milk is then treated by high temperature short time pasteurization (HTST) at the minimum conditions of temperature and holding time of 71.7°C (161°F) for 15 seconds. Vat pasteurization may be used in on-farm operations and requires a minimum temperature of 63°C (145°F) and hold time of 30 minutes. Ultra-pasteurization (UP) is conducted at the minimum conditions of 138°C (280°F) for 2 seconds and is typically used for specialty milk products and has a longer shelf-life than HTST-treated milk. The milk must be refrigerated because it was not aseptically packaged. This is referred to as ultra-high temperature (UHT) processing if packaged aseptically and then does not require refrigeration. HTST eliminates human pathogens of concern and extends shelf – life of milk. UP is not considered commercially sterile but has a shelf life up to 90 days. UHT milk is commercially sterile and has a shelf-life up to 6 months (FDA, 2017).

The effects of homogenization and pasteurization on the digestibility of raw milk was tested using an *in vitro* digestion model (Tunick, et al. 2016). Standardized raw whole milk was subject to either homogenization, HTST, homogenization plus HTST, or homogenization plus UHT processing. Raw skim milk was subject to HTST or UHT processing. Next, the processed samples were exposed to *in vitro* gastric digestion at pH 1.5, 38°C, using a simulated gastric fluid containing pepsin followed by intestinal digestion at pH 7.0 using a simulated intestinal fluid containing lipase, pancreatin and bile salts. Afterwards, the samples were run on a sodium dodecyl sulfate-PAGE (SDS-PAGE) gel to follow the disappearance of the individual casein and whey proteins **(Figure 1)** in the stomach and remaining proteins or peptides and lipids **(Figure 2)** in the small intestine as a function of time.

After 60 minutes of *in vitro* gastric digestion, the intact casein and minor whey proteins were digested, and casein and whey peptides (sizes represented at below approximately 10 kDa) and the major whey proteins, alpha-lactalbumin (α-LA) and beta-lactoglobulin (β-LG), remained. (See Lane 4, Figure 1a). Some degradation is seen in the homogenized raw whole, homogenized UHT, and UHT skim raw milk samples **(Figure 1a)**. After intestinal digestion for 120 minutes, low molecular weight peptides or amino acids persisted for the samples containing fat and the raw whole homogenized sample, with multiple bands remaining even after the 2 hours of digestion (Lane 6, Figure 1b). Confocal microscopy of samples during digestion showed fat droplets remaining after intestinal digestion for raw milk samples that were homogenized **(Figure 2)**. Skim milk and raw whole milk showed complete digestion after 3 hours and homogenization with HTST and UHT processing showed possible formation of fat-protein aggregates (Tunick, et al, 2016), an indication that processing may affect the digestibility of milk.

These findings are supported by a similar *in vitro* study showing that most bovine milk proteins and peptides, including pepsin resistant proteins, were completely digested by the end of the 3-hour process (Gallier, et al, 2012). β-LG and a few other peptides were still detected, although at a lower concentration. Fat globules were shown to digest at
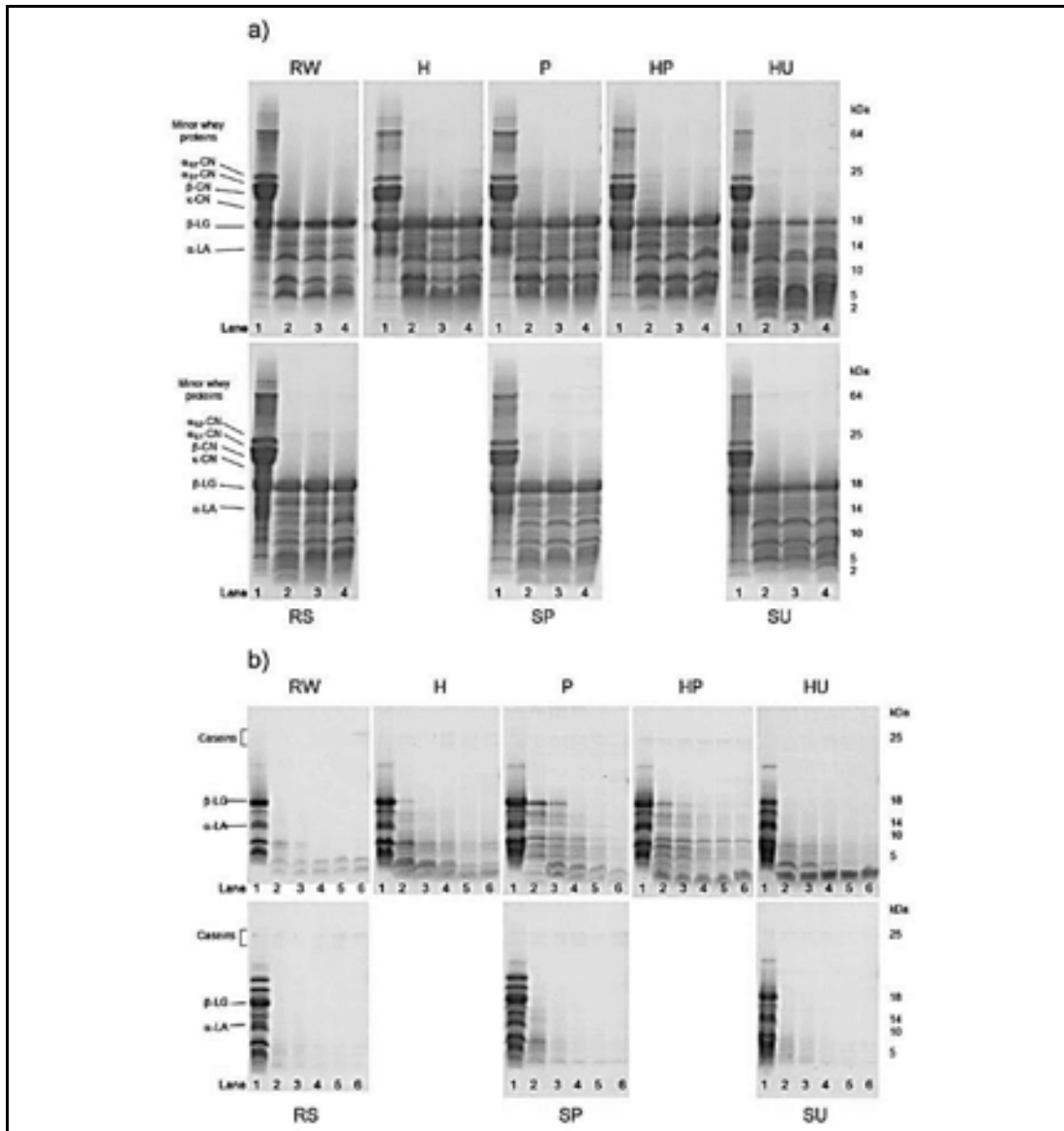
Figure 1: SDS-PAGE of processed milk during in vitro gastrointestinal digestion
RW, raw whole milk; H, homogenized raw whole milk; P, HTST-pasteurized whole milk; HP, homogenized and HTST pasteurized milk; HU, homogenized and UHT-processed milk; RS, raw skim milk; SP,HTST-pasteurized skim milk; SU, UHT-processed skim milk. The proteins corresponding to the bands are listed on the left- hand side of the panels. The molecular weights of the proteins and peptides are listed on the right-hand side of the panels. Panel a (top) Gastric digestion of whole milk raw and processed samples; Panel a (bottom) Gastric digestion of skim milk raw and processed samples; Lane 1 shows the processed sample after initiation of gastric digestion at time=0, Lane 2 15 min, Lane 3 30 min., Lane 4 60 min. Panel b (top) Intestinal digestion of whole milk samples from Panel a (top); Panel b (bottom) Intestinal digestion of skim milk samples from Panel a (bottom); Lane 1 of each series shows the processed sample from Lane 4 of Panel a) adjusted to 0 time. Lane 2 15 min., Lane 3 30 min., Lane 4 60 min., Lane 5 90 min., Lane 6 120 min.

different rates and depending on their size were protected by MFGM glycosylated pro-teins during gastrointestinal digestion. Therefore, it may be possible that after milk consumption, fat globules, along with some protein, will not be absorbed by the small intes-tine, and will enter the large intestine.
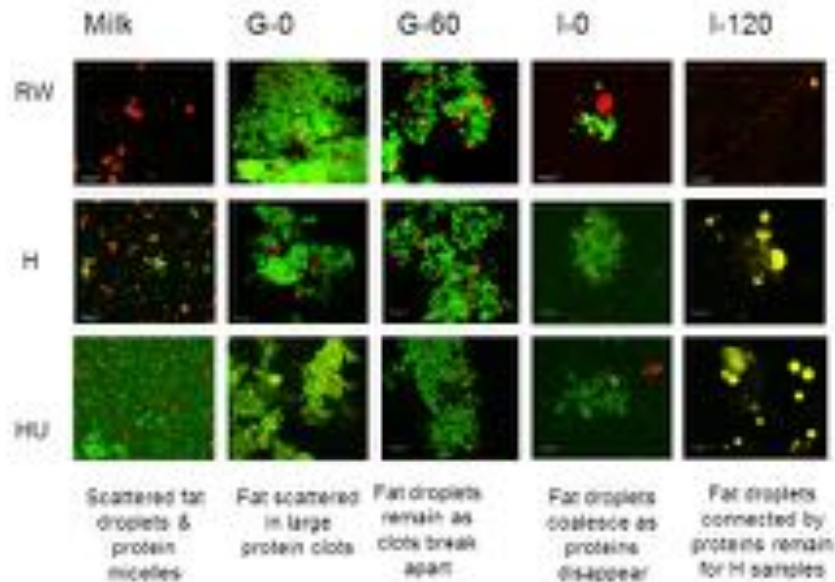
Figure 2: In vitro gastrointestinal digestion of milk – confocal microscopy

RW = raw whole milk; H = homogenized, raw whole milk; HU = Homogenized, UHT-processed whole milk; G= gastric digestion; I = intestinal digestion. The colors red and yellow are fats, the color green is protein. RW, raw whole milk; H, homogenized, raw whole milk; P, HTST-pasteurized whole milk; HP, homogenized and HTST pasteurized milk; HU, homogenized and UHT-processed milk; RS, raw skim milk; SP, HTST-pasteurized skim milk; SU, UHT-processed skim milk.

MILK AND THE GUT MICROBIOTA- IS FAT A NECESSARY COMPONENT?

A number of studies of the effect of human milk on the development of the gut microbiota in infants have demonstrated that it provides an array of irreplaceable benefits to the infant that may persist throughout life **(**Jost, et al., 2015; De Leoz, et al., 2015; Pacheco, et al., 2015). Other studies demonstrated the positive effects of probiotic-containing fermented milk on the human gut microbiota **(**Unno et al., 2015; Ceapa et al., 2013**).** Yet, the effect of bovine milk consumption on the composition and metabolome**,** the Biochem-ical composition of small molecules resulting from gene expression, of the human gut microbiota in the individual intestinal regions- the ascending, transverse and descending colon - remains undefined. In particular, the effect of fluid milk on the gut microbiota is of interest. We are interested in comparing the effects of fat-free to full fat milk using an artificial gastrointestinal system.

The current opinion in the Dietary Guidelines for Americans 2015-2020 is that fat-free and low-fat milk retain the same nutrients as full-fat milk, making these products a more desir-able dietary addition, and the recommended form of milk consumption (USHHS and USDA, 2015). However, it is recognized that milk fat contains many types of fatty acids and lipids, and fat-soluble vitamins; all of which play a beneficial role in human health (Haug, et al., 2004; Ebringer, et al., 2008). Previous studies have demonstrated that and lipids, and fat-soluble vitamins; all of which play a beneficial role in human health (Haug,

et al., 2004; Ebringer, et al., 2008). Previous studies have demonstrated that consuming full-fat milk is inversely correlated to both global and abdominal obesity (Crichton and Alkerwi, 2014; Holmberg and Thelin, 2012), and that milk fat plays a role in the release of gastrointestinal peptides, which function to slow gastric emptying (Panahi, et al., 2014). The gut microbiota is composed of bacteria from the phyla: Firmicutes, Bacteriodetes, Actinobacteria, and Proteobacteria. One study analyzing fecal samples of humans consuming a diet supplemented with either fat-free or full-fat yogurt revealed that the addition of milk fat resulted in changes to not only the Firmicutes/Bacteroidetes ratio, a ratio that changes with the fat content of a diet, but also a change with respect to the relative abundance of class Bacilli and family Streptococcaceae (Walsh, et al, 2016). These results indicate that the fat component of dairy products is able to modify the gut microbiota composition (Walsh, et al., 2016). However, the extent and location of these effects remains unknown.

THE GUT MICROBIOTA - RELATIONSHIP TO FOOD

The relationship between human health and diet is well documented. However, studies relating the diet to only the human host are one-dimensional because they ignore the gut microbiota, which consists of over 10^{14} bacterium, representing 500-1000 individual species (Xu and Gordon, 2003; Payne et al., 2012; Konturek, et al., 2015). Dietary components provide the substrates that maintain the gut microbial community (Power, et al., 2014; Venemaa and Van den Abbeele, 2013). The metabolites produced from this community serve as substrates for human cells, thereby contributing to host physiological status (Krishnan, et al., 2015; LeBlanc, et al., 2013). Accordingly, the gut microbiota can be described as the mediator between diet and human health (Sonnenburg and Backhed, 2016). Therefore, it is important to understand the effect of diet on the gut microbiota, because changes to the gut microbiota, and the quantity and/or type of metabolites produced, can directly and indirectly influence human health (Maga, et al., 2013; Sonnenburg and Backhed, 2016)*.*

HOW TO STUDY THE GUT MICROBIOTA - *IN VITRO* vs. *IN VIVO*

Studying the gut microbiota is a challenging endeavor (Power, et al., 2014). Most of the species that comprise the gut microbiota are obligate anaerobes (grow in the absence of oxygen) (Konturek, et al., 2015) and many strains are considered unculturable (cannot be grown under laboratory conditions) (Lau, et al., 2016; Feria-Gervasio, et al., 2014). In order to study all aspects of the gut microbiota, a system must be used that will provide the precise environmental conditions necessary for a comprehensive gut microbial community to form. This requires utilizing either an *in vivo* system which relies on a living organism, or an *in vitro* system designed to mimic the physiological conditions of the colon.

While *in vivo* studies are typically considered more significant, the relevance of animal data in the context of the human gut microbiota remains in question (Payne, et al., 2012). Application of an *in vivo* model is complicated, since each species has a unique community which has evolved based on dietary components and are anatomically different from humans (Muegge, et al., 2011; Nguyen, et al., 2015). The use of humans as an *in*

vivo model is also limited due to the complexity of the gastrointestinal tract (GIT) environment, limitations with accessing different parts of the intestine, and difficulty removing samples (Payne, et al., 2012; Feria-Gervasio, et al., 2014; Stearns, et al., 2011). Also, any *in vivo* study must adhere to stringent ethical parameters, restricting the type of research that can be performed (Venemaa and Van den Abbeele, 2013; Guerra, et al. 2012; Payne, et al., 2012). Because of these constraints, many gut microbial studies rely overwhelmingly on fecal sample analysis (Payne, et al., 2012; Van den Abbeele, *et al*., 2010). However, data have revealed that there is a discrepancy between the com-position of the microbiota in a fecal sample and the microbiota found in the individual intestinal regions (Eckburg, *et al*., 2005; Feria-Gervasio, *et al*., 2014). Data generated from fecal sample analysis does not provide information on the micro-environment of the gut or the site of fermentation (Van den Abbeele, et al., 2010; Venemaa and Van den Abbeele, 2013). Therefore, in gut microbial research, the *in vitro* model remains essential, providing a method to measure that which cannot be examined using an *in vivo* system (Guerra, et al. 2012). *In vitro* studies of the gut microbiota are superior to *in vivo* studies in a number of ways. They have no ethical constraints; therefore, they can be used to study microbial modifiers that may be considered hazardous, and/or permit experimental parameters that would be unacceptable for *in vivo* work (Van den Abbeele, et al., 2010; Venemaa and Van den Abbeele, 2013; Lacroix, C., et al., 2015). During the experiment, multiple sample types can be harvested at any time point with no restraints on volume, frequency, or sample site (Venemaa and Van den Abbeele, 2013). Importantly, with *in vitro* systems, the physiological conditions can be altered to mimic any region of the large intestine, controlling factors such as pH, anaerobiosis, and transit time (Venemaa and Van den Abbeele, 2013; Van den Abbeele, et al., 2010). Strict control over these environmental parameters makes results from *in vitro* systems more recordable and reproducible (Van den Abbeele, et al., 2010). For over 20 years, such *in vitro* model systems have been developed and validated, including the Simulator of the Human Intestinal Microbial Ecology (SHIME®) (Feria-Gervasio et al., 2014).

SIMULATOR OF THE HUMAN INTESTINAL MICROBIAL ECOLOGY (SHIME®)

The Simulator of the Human Intestinal Microbial Ecology, more commonly referred to as the SHIME, was developed as an *in vitro* tool to study the gut microbiota (Molly, et al., 1993). It is a five-stage, sequential and continuous bio-reactor system that mimics the GIT, starting with the stomach and ending with waste removal **(Illustration 1)** (Molly, et al., 1993; Van de Wiele, et al*.,* 2015). Within the system are three individual reactors that mimic the ascending, transverse and descending regions of the large intestine that work together to reproduce the physiological conditions of the large intestine and maintain microbial diversity along the intestinal tract (Molly, et al*.,*1994; Van de Wiele *et al.,* 2015). In order to provide a surface for bacterial colonization, porous beads coated with mucin agar are added into each reactor. This allows for complex biofilms to form increasing complexity of the community (Van den Abbeele, et al., 2010)**.** The SHIME system has been previously verified to maintain colon diversity over several months (Molly, et al*.,* 1994; Van de Wiele, et al., 2015). The resulting community has been validated and claimed to be similar to the human large intestine in respect to type of microorganisms located in each region and their metabolic activities (Molly et al*.,* 1994). The TWINSHIME system

was developed to consist of two SHIME systems that are run in parallel, providing for both experimental and control systems in a single experiment.

The most valuable component of the SHIME system is that it encompasses the entirety of the GIT, while also incorporating the large intestinal regions on an individual level. This feature is paramount because the enzymatic and bacterial digestions of dietary components along the GIT is a cascade-like, dynamic and enduring process. During transit, a metabolite of bacterial digestion upstream can serve as a substrate for bacterial metabolism downstream, function in cell signaling, or stimulate another reaction in a separate region. Locating the origin of the substrate, where the downstream reaction occurs, and the final product of the reaction, is possible through the application of SHIME technology. Utilizing the SHIME system, the processes occurring in the specific large intestinal regions, including changes to the gut microbial community and metabolome, can accurately be delineated. Three times a day, feed is added to the stomach and pancreatic juice to the small intestine. Pumps connecting to the reactors are turned on to move the fluid through the system, mimicking the movement through the gastro-intestinal tract. The ability to demonstrate that metabolites from one part of the large intestine, potentially function as substrates in a different intestinal region, will provide significant insight for the fields of medicine, pharmacy, and health care.

Illustration 1. Diagram of the SHIME system
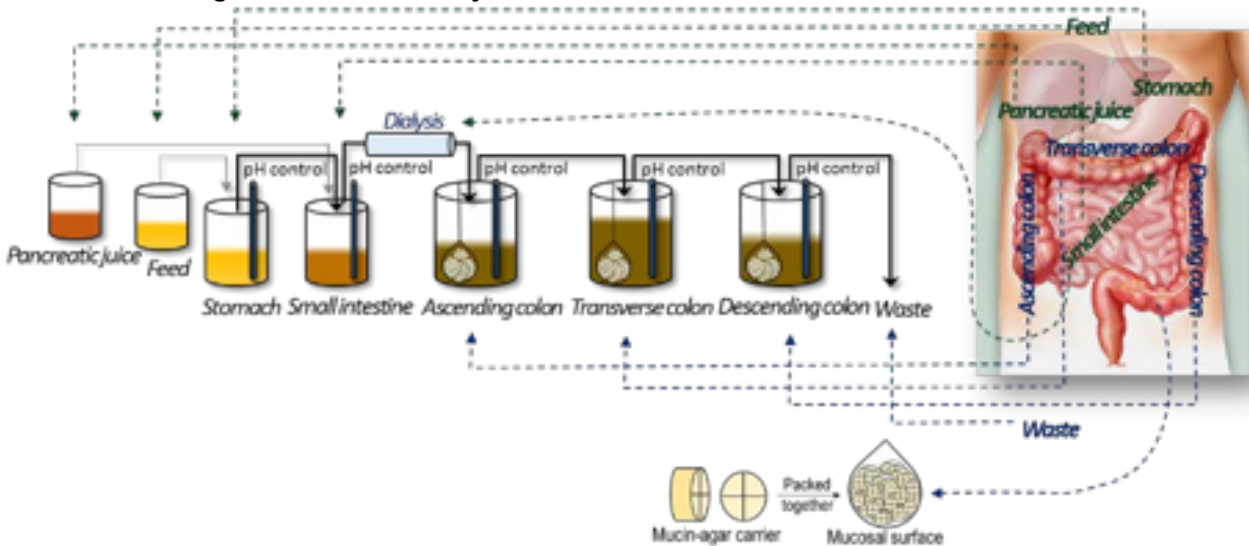
In contrast to the design representing the large intestine, the design for the simulator of the small intestine is far from concrete, where there is only one bioreactor representing the three segments of the small intestine, and its nutrition absorption function is assigned to a geo-separated column to remove small molecules. The column used for the absorption process is a dialysis membrane that functions to separate out the nutrients generated and allows the larger non-digested material to move to the large intestine. Therefore, it is not surprising that most publications using the TWINSHIME® system are related to the use of its lower GIT simulator.

Illustration 2. The TWINSHIME® system in the Dairy and Functional Foods Research Unit; Eastern Regional Research Center; Agricultural Research Service, USDA, Wyndmoor, PA

EXPERIMENTAL DESIGN - WHAT IT TAKES TO RUN A TWINSHIME® EXPERIMENT

The TWINSHIME apparatus is composed of two complete independent SHIME systems, both containing five water-jacketed bioreactors set up in sequence to represent the stomach (ST pH 2), small intestine (SI pH 6.6-6.9), ascending colon (AC pH 5.4-5.6), transverse colon (TC pH 6.2-6.4), and descending colon (DC pH 6.6-6.9) **(Illustration 3)**. To initiate this system, the colon regions are filled with a "defined medium" (DM), to maintain the fecal homogenate, and then inoculated with 5% fecal homogenate. Transfer tubes are set up in between each reactor to allow for movement of the culture in a sequential manner, and the transfer tube height is fixed so that the volume of each colon reactor does not change. Three times a day, DM is pumped into the ST and allowed to incubate for 1 hour. The DM is then pumped into the SI along with pancreatic juice con-taining bile salts, leaving the ST empty. The resulting slurry incubates in the SI reactor for 30 minutes. At this point the systems are flushed with nitrogen to remove any oxygen that may have entered the system or been released during the feeding cycle. After incubation, the pumps are turned on, and all the slurry from the SI is slowly pumped into the AC over a period of 1.5 hours. As the slurry from the SI moves into the AC, the added volume is pumped into the TC, and the added volume in the TC is pumped into the DC. Finally, the added volume is pumped from the DC to the waste container. During each cycle, the ST and SI reactors are completely emptied, while the volume of the AC, TC, and DC remain the same and only the excess amount added from the SI is removed. In this way the movement of slurry through the system is able to mimic the cascade-like reaction in the large colon, where the residence time is 24 to 48 hours and the metabolites generated up stream can be the substrate of the bacteria downstream, and/or serve as stimuli.

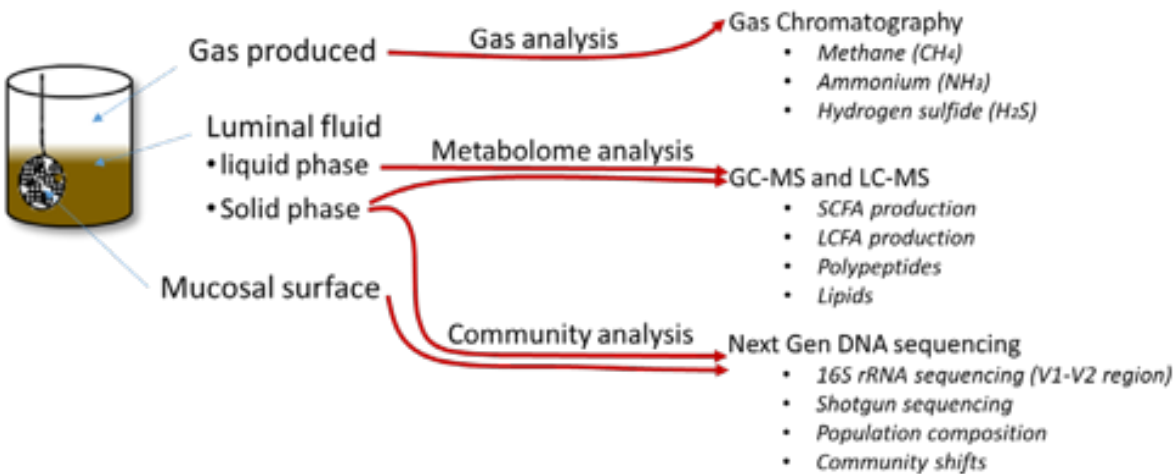*Illustration 3: System set- up for a TWINSHIME® experiment*
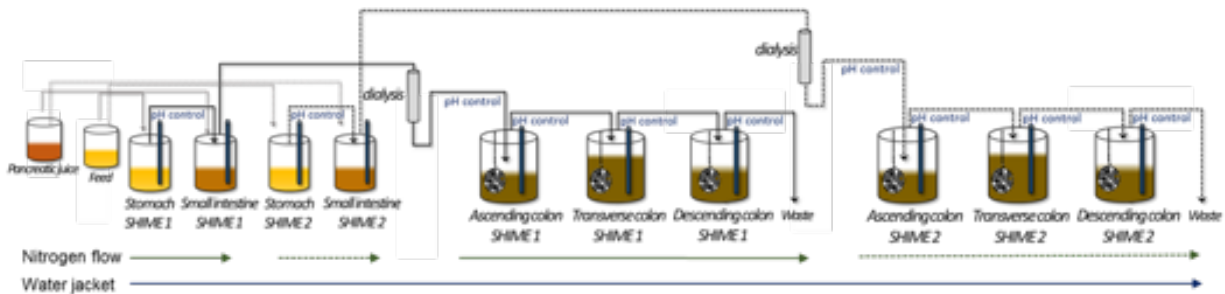
Figure 3: Types of samples and analysis performed for each experiment

The TWINSHIME system is maintained at 37°C by water flowing through the jackets of the bioreactors, and anaerobic conditions sustained by sealing the reactors and using nitrogen flow. The pH of each bioreactor is computer-controlled to match the physiological conditions of the colon regions using 0.5 M HCl and 0.5 M NaOH. The three-colon bioreactors in both SHIME systems also contain porous carriers pre-coated with mucin to provide a surface for bacterial attachment and growth. Samples are harvested from each of the bioreactors every 3-4 days **(Figure 3)**. 16S rRNA Next Generation DNA sequencing is used to define the population of both the luminal and mucin phases for each bioreactor at each time point. LC (liquid chromatography)- and GC (gas chromatography)-MS (mass spectrometry) are used to identify the short chain fatty acids (SCFA) and/or other metabolites in each sample, generating a profile of metabolic activity for the community in each bioreactor over time. Computational methods to analyze the sequencing information, such as QIIME, Unifrac, PCoA, richness/diversity, are applied for data interpretation.
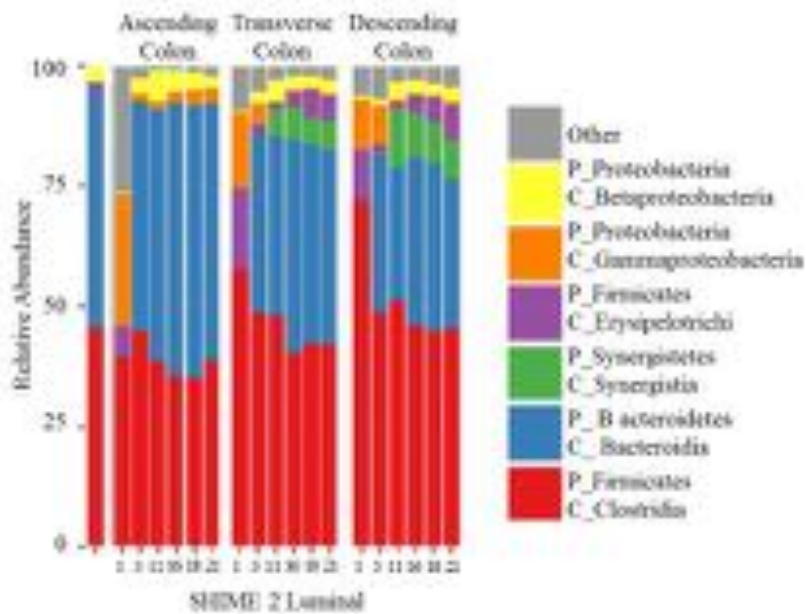
a)
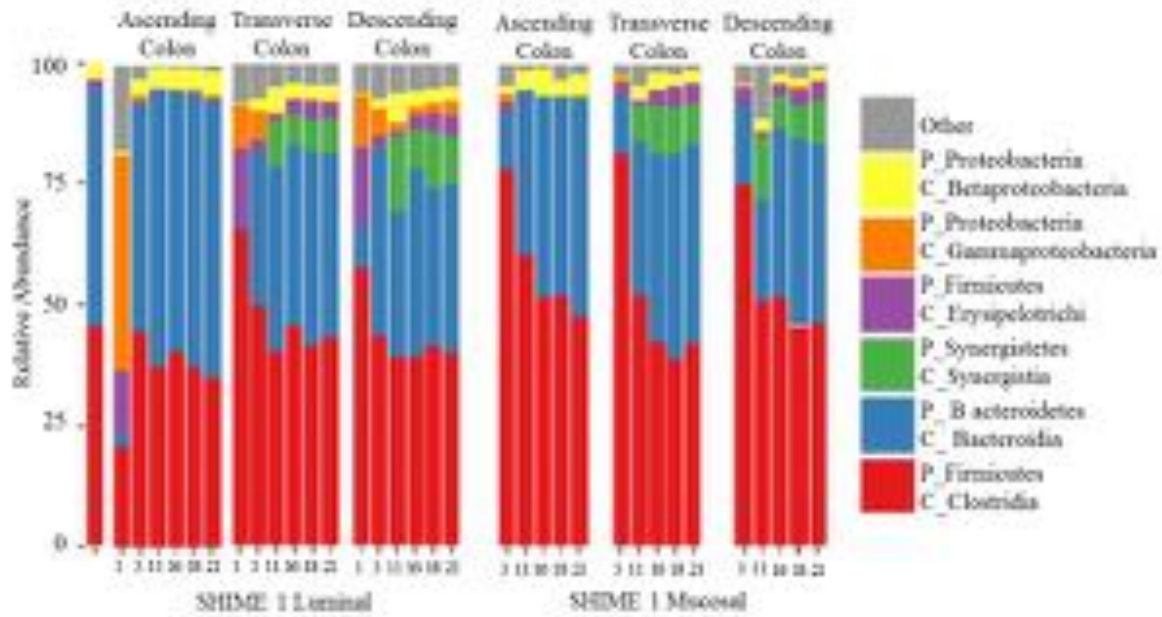
b)

Figure 4: Community composition of a TWINSHIME® experiment
Community composition was determined based on relative abundance at the class level for each region and phase of the SHIME system over time. **a)** Community composition of the SHIME 1 system, which had both a luminal and mucosal phase. **b)** Community composition of the SHIME 2 system, which had only a luminal phase.

RESULTS OF AN INITIAL TWINSHIME® EXPERIMENT- WHAT CAN BE EXPECTED

The ability of the TWINSHIME® system to reproduce a human gut microbiota was tested in an initial experiment. In order for the TWINSHIME® system to be functional it must first, be able to produce a community that is similar to the original fecal homogenate used to inoculate the system, and second, this community must reach a point of stability where there is no fluctuation in composition or metabolites with time. In this experiment, the SHIME 1 had mucin agar carriers added, and SHIME 2 had no mucin carriers added.

The community composition in terms of relative abundance at the class level was determined using 16S rRNA sequencing. The community composition in all three colon regions for both the SHIME 1 and SHIME 2 were similar to the original fecal homogenate (See the first lane of Figures 4a and 4b) used to inoculate the system **(Figures 4a and 4b)**. Whether or not these communities reached a point of stability was evaluated by generated PCoA plots based on both weighted and unweighted UniFrac distances (not shown). These results demonstrated that the communities achieve a mature and unchanging state between days 11 and 15. Total short chain fatty acid (SCFA) levels were also measured for the luminal phase of each system over time (Figure 5, Table 1). These results demonstrate that initially the levels of the SCFAs are fluctuating in each colon region, however, by day 15 these levels begin to even out and enter into a steady state **(Figure 5)**. Based on these initial findings it can be concluded that the TWINSHIME® system can reproduce a stable gut microbiota, similar to the fecal sample used for inoculation.
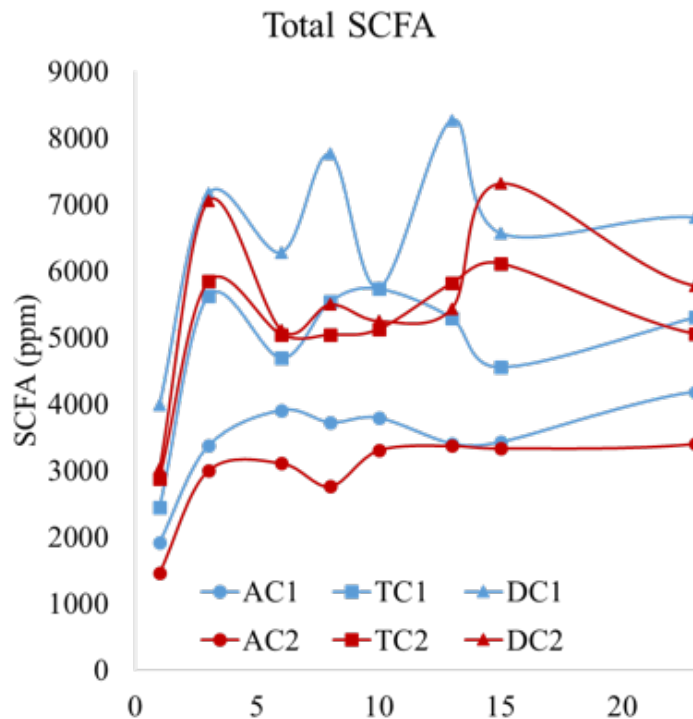

Figure 5. Total SCFA production over time.
A GC-MS was used to determine the total amount of SCFA produced in each colon region over time.

Table 1. Short Chain Fatty Acids

Reference : Short Chain Fatty Acids
(SCFA)

Acetate: propionate: butyrate
- 60:20:20 ratio in colon and stool

Acetate
- Substrate for fatty acid synthesis and cholesterol in the liver
- Increases colonic blood flow and oxygen uptake
- protects from enteric infections

Propionate
- Related with specific health benefits
- Induces satiety and may play an important role in obesity

Butyrate
- energy source for colonocytes
- protects from inflammatory disorders
- suppress the growth of colonic tumors

MILK RESEARCH AND THE TWINSHIME® SYSTEM - A PERFECT COMBINATION

The effect of milk consumption on the gut microbiota is unclear, even though research using *in vitro* digestion models, which only extend from the stomach to the small intestine, have suggested that after bovine milk consumption, fat globules, along with some protein will make it through the small intestine and possibly enter the large intestine (Gallier, et al., 2012; Tunick, et al., 2016). It is possible that some of the health benefits attributed to milk consumption are related to the gut microbiota. The TWINSHIME® system will be used to study the effects of both fat-free and full-fat milk on the gut microbiota. In this experiment, the DM will be supplemented with either fat-free or full-fat milk three times a day. Samples will be harvested and analyzed to detect both changes to the community composition and to evaluate changes in the metabolome **(Figure 6)**. The results of these experiments should clearly define the effect that milk has on the gut microbiota, and whether or not these interactions may contribute to the health benefits associated with milk.
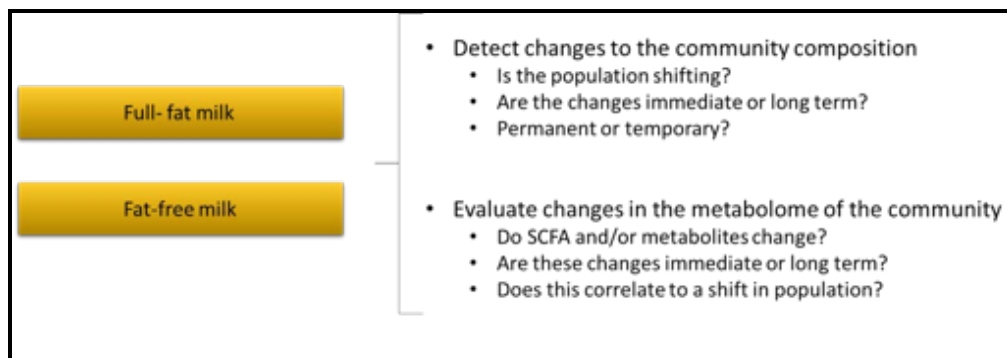
Full- fat milk

Fat-free milk

- Detect changes to the community composition
  - Is the population shifting?
  - Are the changes immediate or long term?
  - Permanent or temporary?

- Evaluate changes in the metabolome of the community
  - Do SCFA and/or metabolites change?
  - Are these changes immediate or long term?
  - Does this correlate to a shift in population?

Figure 6: Testing the effects of milk on the gut microbiota

Literature Cited

Ceapa C, Wopereis H, Rezaïki L, Kleerebezem M, Knol J, and Oozeer R. 2013. "Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health." *Best Practice and Research. Clinical Gastroenterology* 27 (1): 139-55.

Crichton G, and Alkerwi A. 2014. "Whole-fat diary food intake is inversely associated with obesity prevalence: findings form the Observation of Cardiovascular Risk Factors in Luxembourg study." *Nutrition Research* 34 (11): 936-43.

De Leoz M, Kalanetra K, Bokulich N, Strum J, Underwood M, German J, Mills D, and Lebrilla C. 2015 . "Human milk glycomics and gut microbial genomics in infant feces show a correlation between human milk oligosaccharides and gut microbiota: a proof-of-concept study." *Journal for Proteome Research* 14 (1): 491502.

Ebringer L, Ferencík M, and Krajcovic J. 2008. "Beneficial health effects of milk and fermented dairy products-review." *Folia Microbiologia* 53 (5): 378-94.

Eckburg P, Bik E, Bernstein C, Purdom E, Dethlefsen L, Sargent M, Gill S, Nelson K, and Relman D. 2005. "Diversity of the Human Intestinal Microbial Flora." *Science* 308: 1635-38.

FDA, Food and Drug Administration. 2017. Department of Health and Human Services, Grade A, Pasteurized Milk Ordinance. 21 CFR part 131.3 Milk and Cream, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=131 Accessed 8/01/2017.

Feria-Gervasio D, Tottey W, Gaci N, Alric M, Cardot J, Peyret P, Martin J, Pujos E, Sébédio J, and Brugère J. 2014. "Three-stage continuous culture system with a self-generated anaerobia to study the regionalized metabolism of the human gut microbiota." *Journal of Microbiological Methods* 96: 111-8.

Fitzgerald, R.J., Murray, B.A. 2006. "Bioactive peptides and lactic fermentations." 2006. *International J. Dairy Technology*. 59: 118-125.

Gallier S, Ye A, and Singh H. 2012. "Structural changes of bovine milk fat globules during in vitro digestion." *Journal of Dairy Science* 95: 3579-92.

Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, and Alric M. 2012. "Relevance and challenges in modeling human gastric and small intestinal digestion." *Trends in Biotechnology* 30 (11): 591-600.

Haug A, Høstmark A, and Harstad O. 2004. "Bovine milk in human nutrition- a review." *Lipids in Health and Disease* 6:1-16.

Holmberg S, and Thelin A. 2013. "High dairy fat intake related to less central obesity: a male cohort study with 12 years' follow-up". *Scandinavian Journal of Primary Health Care* 31(2): 89-94.

Jost T, Lacroix C, Braegger C, Chassard C. 2015. "Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health." Nutrition Reviews 73 (7): 426-37.

Konturek P, Haziri D, Brzozowski T, Hess T, Heyman S, Kwiecien S, Konturek S, and Koziel J. 2015. "Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases." *Journal of Physiology and Pharmacology* 66 (4): 483-91.

Korhonen, H. and Pihlanto, A. 2006. "Bioactive peptides: Production and functionality", *International Dairy Journal*, 16: 945-960.

Krishnan S, Alden N, and Lee K. 2015. "Pathways and functions of gut microbiota metabolism impacting host physiology." *Current Opinion in Biotechnology* 36: 137-45.

Lacroix C, de Wouters T, and Chassard C. 2015. "Integrated multi-scale strategies to investigate nutritional compounds and their effect on the gut microbiota." *Current Opinion in Biotechnology* 32: 149-55.

Lau J, Whelan J, Herath I, Lee C, Collins S, Bercik P, and Surette M. 2016. "Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling." *Genome Medicine* 8 (1): 72.

LeBlanc J, Milani C, Savoy de Giori G, Sesma F, van Sinderen D, and Ventura M. 2013. "Bacteria as vitamin suppliers to their host: a gut microbiota perspective." *Current Opinion in Biotechnology* 24 (2): 160-68.

Maga E, Weimer B, and Murray J. 2013. "Dissecting the role of milk components on gut microbiota composition." *Gut Microbes* 4 (2): 136-9.

Michalski, M-C and C. Januel. 2006. "Does homogenization affect the human health properties of cow's milk?" *Trends. Food Sci. Technol.* 17: 423-437.

Molly K, Vande Woestyne M, De Smet I, and Verstraete W. 1994. "Validation of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) Reactor Using Micro-organism-associated Activities ." M*icrobial Ecology in Health and Disease* 7: 191-200.

Molly K, Vande Woestyne M, and Verstraete W. 1993. "Development of a S-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem." *Applied Microbiology and Biotechnology* 39: 25458.

Muegge B, Kuczynski J, Knights D, Clemente J, González A, Fontana L, Henrissat B, Knight R, Gordon J. 2011. "Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans." *Science* 332 (6032): 970-4.

Nguyen T, Vieira-Silva S, Liston A, and Raes J. 2015. "How informative is the mouse for human gut microbiota research?" *Disease Models and Mechanism* 8 (1): 1-16.

Pacheco A, Barile D, Underwood M, and Mills D. 2015. "The impact of the milk glycobiome on the neonate gut microbiota." *Annual Review of Animal Biosciences* 3: 419-45.

Panahi S, El Khoury D, Kubant R, Akhavan T, Luhovyy B, Goff H, Anderson G. 2014. "Mechanism of action of whole milk and its components on glycemic control in healthy young men." *Journal of Nutritional Biochemistry* 25(11): 1124-31.

Payne A, Zihler A, Chassard C, and Lacroix C. 2012. "Advances and perspectives in in vitro human gut fermentation modeling." *Trends in Biotechnology* 30 (1): 17-25.

Power S, O'Toole P, Stanton C, Ross R, and Fitzgerald G. 2014. "Intestinal microbiota, diet and health." *British Journal of Nutrition* 111: 387-402.

Sonnenburg J and Bäckhed F. 2016. "Diet-microbiota interactions as moderators of human metabolism." *Nature* 6 (535): 56-64.

Spitsberg, V.L. 2005. Invited review: "Bovine milk fat globule membrane as a potential nutraceutical," *Journal of Dairy Science,* 88: 2289–2294.

Stearns J, Lynch M, Senadheera D, Tenenbaum H, Goldberg M, Cvitkovitch D, Croitoru K, Moreno-Hagelsieb G, and Neufeld J. 2011. "Bacterial biogeography of the human digestive tract." *Scientific Reports* 1 (170): 1-9.

Tunick, MH, Van Hekken, DL, and M. Paul. 2015. "Leveraging the Beneficial Compounds of Organic and Pasture Milk" in *Emerging Dairy Processing Technologies: Opportunities for the Dairy Industry*. Eds. N Datta and PM Tomasula, Wiley Online Library.

Tunick M, Ren D, Van Hekken D, Bonnaillie L, Paul M, Kwoczak R, and Tomasula P. 2016. "Effects of heat and homogenization on in vitro digestion of milk." *Journal of Dairy Science* 99; 4124-39.

Unno T, Choi J, Hur H, Sadowsky M, Ahn Y, Huh C, Kim G, and Cha C. 2015. "Changes in human gut microbiota influenced by probiotic fermented milk ingestion." *Journal of Dairy Science* 98: 3568–3576.

USHHS and USDA. 2015. "Dietary Guidelines For Americans 2015-2020; 8th edition." *https://health.gov/dietaryguidelines/2015/guidelines/.*

Van de Wiele T, Van den Abbeele P, Ossieur W, Possemiers S, and Marzorati M. 2015. "The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)." Chapter in the book *The Impact of Food Bioactives on Healthin vitro and ex vivo models*, edited by Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, Mackie A, Requena T, Swiatecka D, and Wichers H. 305-3017. SpringerLink.

Van den Abbeele P, Grootaert C, Marzorati M, Possemiers S, Verstraete W, Gérard P, Rabot S, Bruneau A, El Aidy S, Derrien M, Zoetendal E, Kleerebezem M, Smidt H, and Van de Wiele T. 2010. "Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX." *Applied Environmental Microbiology* 76 (15): 5237-46.

Venemaa K and Van Den Abbeele P. 2013. "Experimental models of the gut microbiome." *Best Practice & Research Clinical Gastroenterology* 27 (1): 115-26.

Visioli F and Strata A. 2014. "Milk, Dairy Products, and Their Functional Effects in Humans: A Narrative Review of Recent Evidence." *Advances in Nutrition* 5: 131–43.

Walsh H, Haq H, Cersosimo L, Kien C, and Kraft J. 2016. "Decreased Abundance of Firmicutes in the Gut Microbiota After Consumption of a Diet Containing Milk Fats." *The FASEB Journal* 30(1): 683.

Xu J and Gordon J. 2003. "Honor thy symbionts." *PNAS* 100 (18): 10452-59.

Human Health Implications of Consuming Grass-fed Meat and Milk Products

After we fed our dairy and beef animals a high green or ensiled forage diet and took care not to mess things up cooking meat or processing milk, was this all-in-vain? Probably not, but it depends on if we believe the old nutrition science or the new one. We have had more recent research that tends to indicate that saturated fats or at least certain ones have very little to no impact on cardiovascular health. The old science is what led everyone to believe that all saturated fats clogged arteries.

Then, there is the issue of omega-3 not being changed dramatically in quantity even if the percentage change between grass-fed meat and milk and confinement-fed animals is two-fold or more. As true as that might be, it is actually the n-6:n-3 ratio that is most important. The typical America diet has ratio well above the maximum level thought to be healthful – 4.0. A diet with grass-fed full fat milk products in it would help bring the n-6:n-3 ratio down to 4.0 or less. Add some grass-finished red meat in the diet, and it would lower the overall ratio down.

CLA is thought to be healthful, but we are still waiting for a definitive study that involves human trials, not lab animals. We can raise livestock that have more CLA in their meat and milk. The trouble is that we still do not know if it is worth the trouble to produce meat and milk products from pastured livestock. In countries where meat and milk products are not shunned, but embraced, they consume enough CLA to be likely enough to reduce cancer incidence if human trials bear out what has been learned from animal trials.

The saturated fat controversy has gone on far too long with little to no true resolution. We seem to be in a 20th century time warp. It impedes dietary recommendations as long as saturated fats of any type are restricted to less than 10 percent of calories per day. Omega-3 (n-3) is stripped out of skim milk products so some of us resort to fish oil supplements and the like. Demand for dairy products continues to slip; driving more dairy farmers out of the business. Beef prices at the farm have been reasonably good, but only due to prolonged droughts that caused some beef producers to reduce their cattle numbers significantly. Meanwhile, we consume way too much n-6 in relation n-3 even with lightening up on meat and dairy. It is the n-6 that some say is the real culprit to clogged arteries, not saturated fats. Who is right? The American public needs to know now, not years from now.

Another issue with fatty acids is the origin of *trans* fats. From healthyforgood.heart.org, "There are two broad types of trans fats found in foods: naturally-occurring and artificial *trans* fats. Naturally-occurring *trans* fats are produced in the gut of some animals, and foods made from these animals (e.g., milk and meat products) may contain small quantities of these fats. Artificial *trans* fats (or *trans* fatty acids) are created in an industrial pro-

cess that adds hydrogen to liquid vegetable oils to make them more solid. The primary dietary source for *trans* fats in processed food is "partially hydrogenated oils." In November 2013, the U.S. Food and Drug Administration (FDA) made a preliminary determination that partially hydrogenated oils are no longer **G**enerally **R**ecognized **a**s **S**afe (GRAS) in human food." Some jurisdictions around the World outright ban them from being in food.

Reducing the milk fat content also strips out a lot of other bioactive fatty acids in milk that have a great impact on human health. We need to look at the side effects of our actions. Tunnel vision that focuses on one thing to the detriment of everything else is not good scientific work. Even worse, if the focal point was predicated on limited or flawed data or a questionable cause/effect relationship, it should be restudied exhaustively to make sure the original premise had merit in the first place. Then, be willing to admit to a mistake.

In this session, we have three presentations that speak to these issues. The first presentation is an overview. The second presentation takes a look at ruminant fatty acids in relation to industrially hydrogenated. The third presentation focuses in on milk fatty acids and their effect on human health.

Dairy and Meat: Implications for Human Health

Naomi K. Fukagawa, MD Ph.D.

USDA ARS Beltsville Human Nutrition Research Center, Beltsville, MD 20705

Introduction

The landscape for food production has changed dramatically over the last 70 years and continues to evolve as both populations and technology boom. Adaptation to the growing demands for the production of "healthy" food in a sustainable manner presents challenges beyond the pasture and the table. The implications for dietary guidance necessitates a better understanding of how production practices affect the nutrient quality of food and ultimately human health. The World Health Organization (WHO) presented its program for health for 2014-2019 that extended beyond just the prevention of disease. It focused on the changing world and the need to integrate solutions across disciplines to meet the needs of the rising population and changing demographics.
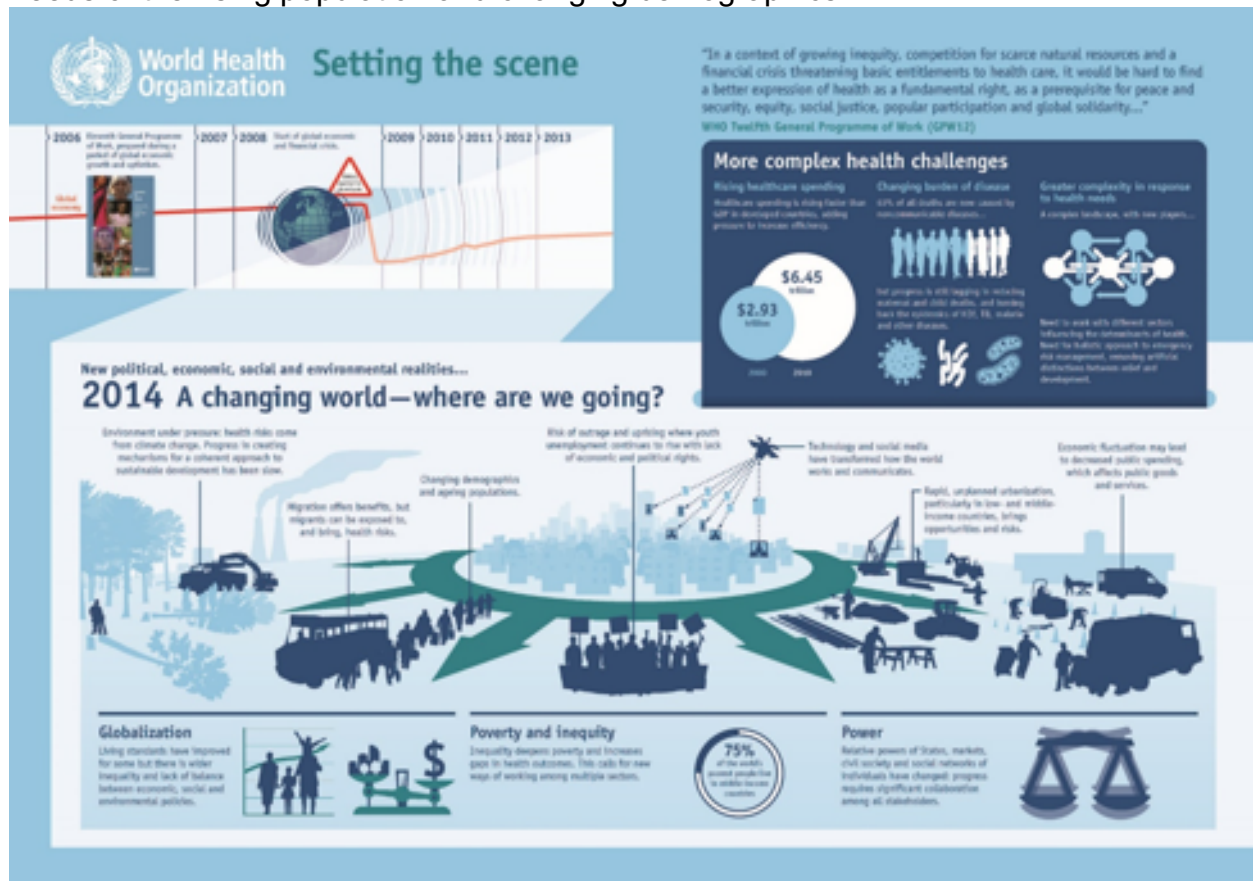

Figure 1. WHO 12th General Programme of Work 2014-2019 (Chapter 1)

Challenges Beyond Pasture and Table

The world population hit 7 billion in 2011 (United Nations 2017) and of those people, ~13% are hungry and ~32% of children in developing countries are malnourished (World Hunger Organization 2015). This plight starts during pregnancy in both the U.S. and the rest of the world. We know that, in general, food production is adequate but often not where food is needed most because of access, poverty, and social/political issues. Another challenge comes from the concern about the environmental impact of agricultural practices and climate change that affect soil quality, water availability, and choices that farmers must make. It is important to recognize that there is room for farms of all sizes, but how to maintain the workforce and public trust in where their food comes from remains unanswered.

Equally challenging is the uncertainty that people have about what constitutes a "healthy" plate. Scientists and nutritionists have recommended a shift to plant-based diets, but it is not clear whether this addresses the need to be culturally sensitive and whether this shift assures improved outcomes for people and the planet. Considerations for the beef and dairy industry include the ability to genetically select for specific traits in animals, how management of the herds influences the nutritional quality of the products and impact on the environment, and new products that meet evolving consumer choices/demands. From the human health perspective, beef and dairy provide a good source of protein, vitamins and minerals, bioactive compounds and potentially important oligosaccharides (complex sugars that link lactose with other monosaccharide building blocks such as glucose, galactose, fucose, sialic acid and N-acetylglucosamine). These sugar chains are reported to modulate immunity, act as prebiotics, and protect against some pathogens (Bode et al 2016).

Despite the many positive attributes related to beef and dairy foods and recent reports re-evaluating relationships between fat and health outcomes (Pimpin et al 2016; Mozaffarian 2016), it will take time to change decades of dietary guidance that have recommended a reduction in intakes of saturated fats and red meat (e.g. Kritchevsky 1998; Dietary Guidelines 2015-2020). Monounsaturated fatty acids (MUFA) have long been reported to be associated with lower risk for coronary artery disease (Mensink et al 2003; Shingfield et al 2008). The beef and dairy industry have supported research on healthful aspects of meat and dairy and explored ways to alter nutrient content in their products. Relatively recently, Lehnert et al (2015) identified a genetic mutation that resulted in lower saturated fat and higher content of monounsaturated fatty acids (MUFA) in milk produced by the mother and her offspring. This demonstrates the feasibility of selecting for specific heritable traits that lead to production of milk with a preferred fatty acid profile. Figure 2b shows the mother and Figure 2c shows the lower saturated fat (blue) and higher MUFA (red) in milk from the daughters carrying the mutation.
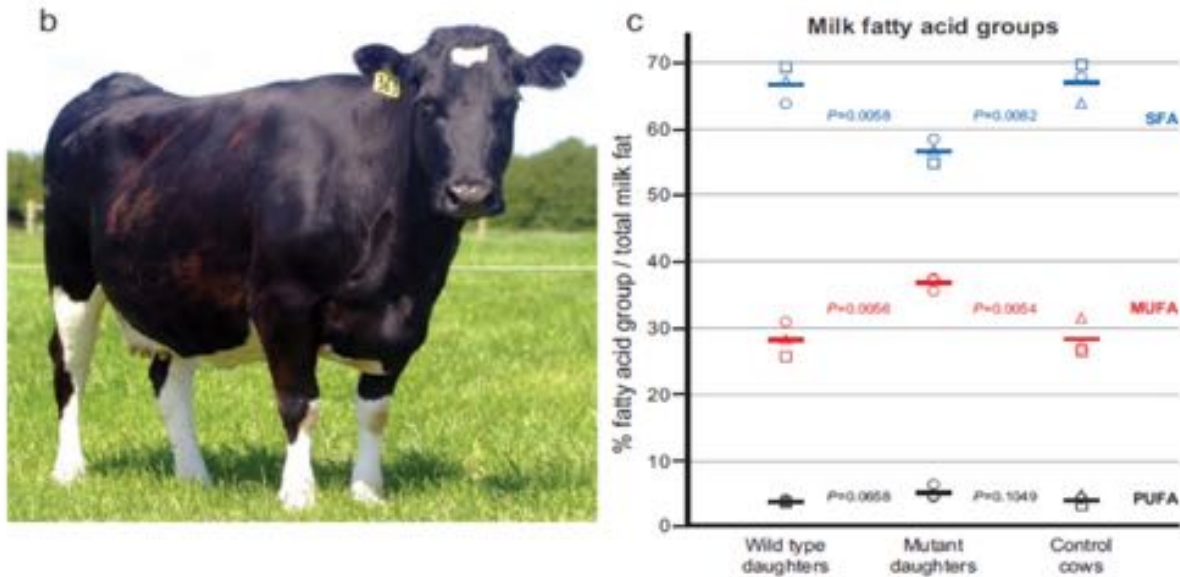
Figure 2. b. Cow with heritable mutation responsible for low milk fat content. c. Segregation of milk fatty acid composition in the F1 generation.
Milk contents of saturated (SFA, blue), monounsaturated (MUFA, red), and polyunsaturated fatty acids (PUFA, black) for three individual mutant and wild-type daughters of cow 363, and for three unrelated, breed-matched control cows in the same herd, are indicated by open symbols. Means are indicated by bold horizontal bars, and P values (two-tailed Student's t-test) for fatty acid groups differences are stated between genotype groups. Differences between wild-type daughters and control cows were not significant (PSFA50.96, PMUFA50.96, PPUFA50.93).

Another potential way to influence the nutrient profiles of milk is through the diet. Rumen microbiota are active in lipid metabolism, and it is known that the composition of the diet and type of lipid supplements interact to affect fatty acid profiles in milk fat (Shingfield et al 2008). Bioactive fatty acids in dairy products include alpha-linolenic acid (ALA), conjugated linoleic acid (CLA) and vaccenic acid (VA); all reported to have beneficial health effects such as reducing inflammation and lowering risk for type 2 diabetes mellitus and cardiovascular diseases (Bainbridge et al, 2016; Shingfield et al, 2008)). Forage species in pastures and degree of maturity have been shown to alter the fatty acid composition of grazing animals (Shingfield et al, 2008; Daley et al, 2010). Bainbridge et al (2016) recently reported that the content of bioactive fatty acids in milk is influenced by the breed of the cow, time of lactation, as well as the diet provided during lactation, supporting the contention that the number of bioactive compounds in in milk can be modulated through breeding and specific feeding regimens. Branched-chain fatty acids (BCFA) are another class of bioactive fatty acids in milk reported to exert anti-tumor effects, reduce the incidence of necrotizing enterocolitis in infants and improve pancreatic beta-cell function (Bainbridge et al 2016). Total BCFA concentrations in human milk at 4 weeks postpartum have also been recently examined and shown to differ between mothers from different parts of the world and influenced by diet (Dingess et al, 2017). In other work, branched short-chain fatty acids (e.g. isobutyric and isovaleric acids) generated by fermentation of branched chain amino acids from undigested protein reaching the colon were found to modulate glucose and lipid metabolism in fat cells (Heimann et al 2016). The interactions between food and the microbiota of the rumen as well as the gut need to be better understood but will likely serve as novel ways to influence the nutritional value of dairy products.

Other components in milk that may contribute to beneficial health effects include complex sugars known as oligosaccharides. A range of health benefits are attributed to human milk oligosaccharides (HMO) but research has been limited because of difficulty in its isolation or synthesis. Nevertheless, work continues on the use of nonhuman oligosaccharides and efforts are focusing on ways to use bovine milk oligosaccharides as precursors for synthesis of HMO's (Bode et al, 2016).

Red meat remains an important source of essential amino acids (building blocks of protein), and important vitamins (A, B_6, B_{12}, D and E) and minerals (iron, zinc and selenium). Although concerns have been raised about saturated fat in red meat, finishing diets can alter the lipid profile in a way to improve upon the final nutrient profile as reviewed by Daley et al in 2010. In addition, Bainbridge et al (2016) suggested that the content of BCFA could be modulated by both breed and diet. Clearly, more research through partnerships between academia, producers, consumers, and government is needed.

References

Bainbridge ML, Cersosimo LM, Wright ADG, Kraft J. Content and composition of branched-chain fatty acids in bovine milk are affected by lactation stage and breed of dairy cow. PLoS One. 2016. 11(3): e0150386. doi:10.1371/journal. pone.0150386

Bode L, Contractor N, Barile D, Pohl N, Prudden AR, Boons GJ, Jin YS, and Jennewein S. Overcoming the limited availability of human milk oligosaccharides: challenges and opportunities for research and application. Nutr Rev. 2016. 74(10):635.

Daley CA, Abbott A, Doyle PS, Nader GA and Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr J. 2010; 9:10.

Dingess KA, Valentine CJ, Ollberding NJ, Davidson BS, Woo JG, Summer S, Peng YM, Guerrero ML, Ruiz-Palacios GM, Ran-Ressler RR, McMahon RJ, Brenna JT, Morrow AL. Branched-chain fatty acid composition of human milk and the impact of maternal diet: the Global Exploration of Human Milk (GEHM) Study. Am J Clin Nutr. 2017 Jan;105(1):177-184. doi: 10.3945/ajcn.116.132464. Epub 2016 Nov 30.

Heimann E, Nyman M, Pålbrink AK, Lindkvist-Petersson K, Degerman E. Branched short-chain fatty acids modulate glucose and lipid metabolism in primary adipocytes. Adipocyte. 2016 Oct 28;5(4):359-368. eCollection 2016 Oct-Dec.

Kritchevsky D. History of recommendations to the public about dietary fat. J Nutrition. 1998. 128 (2): 449S.

Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003 May;77(5):1146-55.

Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. Circulation. 2016 Jan 12;133(2):187-225. doi: 10.1161/CIRCULATIONAHA.115.018585.

Pimpin L, Wu JH, Haskelberg H, Del Gobbo L, Mozaffarian D. Is Butter Back? A Systematic Review and Meta-Analysis of Butter Consumption and Risk of Cardiovascular Disease, Diabetes, and Total Mortality. PLoS One. 2016 Jun 29;11(6): e0158118. doi: 10.1371/journal.pone.0158118. eCollection 2016. Review.

Shingfield KJ, Chilliard Y, Toivonen V, Kairenius P, Givens DI. *Trans* fatty acids and bioactive lipids in ruminant milk. In Bioactive components of milk, Advances in experimental medicine and biology (ed. Z Bösze), 2008. 606:3-65. Springer, New York, U.S.

U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary Guidelines for Americans 2015-2020, 8th Edition. https://health.gov/dietaryguidelines/2015/guidelines/

United Nations, Department of Economic and Social Affairs, Population Division. (2017). *World Population Prospects: The 2017 Revision, Key Findings and Advance Tables*. Working Paper No. ESA/P/WP/248.

World Health Organization. The 12th General Programme of Work - Not merely the absence of disease. GPW/2014-2019. Chapter 1. Setting the Scene. 2014. http://apps.who.int/iris/bitstream/10665/112792/1/GPW_2014-2019_eng.pdf?ua=1

World Hunger Organization. http://www.worldhunger.org/2015-world-hunger-and-poverty-facts-and-statistics/

Does dietary source of trans fatty acids affect risk for cardiovascular disease in humans?

David J. Baer, Ph.D.

USDA, ARS Beltsville Human Nutrition Research Center, Beltsville, MD 20705

Dietary trans fatty acids (TFA) come from partially hydrogenated oils (sometimes referred to as "industrial TFA" or iTFA) or from the fat of ruminants (for example, cows, sheep, goats and buffalo) (sometimes referred to as "natural TFA" or rTFA). Industrial TFA are formed during the hydrogenation of vegetable oils using catalysts. Depending on the hydrogenation conditions, a mixture of isomers is formed, however, elaidic (*trans*-9 18:1) acid is the primary isomer formed (Lock and Bauman). Under some industrial conditions, vaccenic (*trans*-11 18:1) acid (VA) can be formed as well. In contrast, ruminant animals produce primarily vaccenic acid through the biohydrogenation of linoleic and alpha-linolenic acids in the rumen. Through further hydrogenation, ruminants also produce stearic acid (a fully saturated fatty acid). On the other hand, vaccenic acid can further be desaturated in the rumen or extraruminal tissue (for example, mammary gland) to produce rumenic acid (cis-9, trans-11, 18:2), a conjugated linoleic acid (CLA) (Lock and Bauman). CLA from ruminants has been shown in some animal (preclinical studies) to reduce risk for cancer (Gebauer et. al.[1]). Thus, dietary sources of CLA could be used to decrease risk for cancer. However, animal feeding practices that increase CLA also increase vaccenic acid (Lock and Bauman).

In addition to differences in the isomers between iTFA and rTFA, there are differences in the amount of TFA formed. In the production of industrial TFA, the amount of TFA formed depends on several factors including the extent of the hydrogenation process. In partial hydrogenation of vegetable oils, the TFA content can range from 1 to 60% of total fatty acids. However, in ruminants, biohydrogenation in the rumen is tightly regulated and the concentration of *trans*-18:1 acids range naturally from 2 to 5% of total fatty acids but this can be manipulated by the type of diet fed to the animal (Lock and Bauman).

The physiological effects of iTFA on chronic disease risk factors, specifically risk factors for coronary heart disease, are well established. However, the health effects of rTFA are less studied and less known. Animal studies (preclinical studies) and studies of cells (in vitro studies) suggest that the effects of rTFA may differ from those of iTFA (Gebauer et. al.[1]). Further, results from these studies suggest that VA and c9,t11-CLA may lower cholesterol and reduce risk for coronary heart disease (Gebauer et. al.[1]). Further, results of some epidemiologic studies are consistent with the results from preclinical and in vitro studies (Ascherio et. al.) (Liu et. al.) (Pietinen et al.) (Willet et. al.). Other studies suggest that risk for coronary heart disease is similar for all isomers of TFA, regardless of dietary source (Oomen et. al.).

There are few human clinical studies (Chardigny et. al.) (Lacroix et. al.) (Motard-Belanger et. al.) (Tholstrup et.al.), and these studies are heterogenous with respect to study design.

Some studies lack a proper control group making comparisons difficult (Chardigny et. al.). Other studies appear to be underpowered (Motard-Belanger et. al.). Several studies have been conducted with a free-living cohort without sufficient control of the diet (Tholstrup et. al.) (Chardigny et. al.) whereas other studies had controlled diets but the saturated and unsaturated fatty acid composition of the diets was not adequately matched to eliminate their effect on cholesterol concentration (Motard-Belanger et. al.). For some studies, dairy cattle diets were manipulated to produce dairy products enriched with rTFA (Bauman et. al.); however, this approach changes the concentration of other fatty acids (for example, decreasing saturated fatty acids that are hypercholesterolemic (heightens blood cholesterol levels) and increasing fatty acids that are neutral and hypocholesterolemic (lowers blood cholesterol levels) (for example, stearic acid).

The current mean estimate of TFA intake in the US is 1.3 g/person/day (Doell et.al.) and has decreased from 4.6 g/person/day. However, current estimates of rTFA intake have remained stable over the past decade at approximately 1.2 g/person/day in the United States. Based on current estimates, rTFA is now 48% of total TFA intake and has increased from 21% due to the decrease in iTFA intake in the United States. Given the shift in dietary source of TFA, it has become increasing important to understand differences in how different TFA isomers effect risk factors for coronary heart disease, especially as it may impact food labeling, other regulatory processes, and trade.

A human feeding study was conducted to compare the effects of iTFA and rTFA (VA) on risk factors of coronary heart disease. Vaccenic acid was used as it is the predominant isomer of rTFA and this approach eliminates the effect of changes from other fatty acids when rTFA enriched dairy fat is used.

The study is registered with ClinicalTrials.gov (NCT00942656) and details of study design and results have been previously described (Gebauer et. al. [2]; Gebauer et. al. [3]). This was a double-blind study with investigators, subjects, phlebotomists, analysts, and statisticians blinded to the treatments until after statistical analyses were completed. For the feeding, there were four treatment periods, representing 4 treatments. Each treatment period lasted 24 days. During each treatment period, volunteers received a controlled diet for which stearic acid was replaced with 1) 3.3% energy from VA, 2) 3.3% of energy from mixed isomers of TFA from partially hydrogenated vegetable oil (PHVO; iTFA), and 3) 0.9% energy from c9,t11-CLA (rumenic acid, RA). Stearic acid was used as the fatty acid to be replaced among the diets since changes in intake of stearic acid do not affect circulating cholesterol concentration.

Characteristics of the volunteers who completed the intervention are presented in Table 1. Of the 119 volunteers who were randomized, partial or complete data were obtained from 106. Composition of the four diets is presented in Table 2. Effect of the different diets on LDL cholesterol, HDL cholesterol and triglycerides is presented in Table 3. Effect of dietary intake on LDL cholesterol, HDL cholesterol and triglycerides from different TFA

Table 1. Baseline characteristics of study participants[1]

	Mean ± SD
Sex, n M F	 47 59
Age, y	47 ± 10.8
Body weight, kg	80.5 ± 13.9
BMI, kg/m^2	28.5 ± 4.0
TC, mmol/L	5.00 ± 0.74
LDL-C, mmol/L	3.25 ± 0.63
HDL-C, mmol/L	1.24 ± 0.30
TG, mmol/L	1.12 ± 0.50
SBP, mm Hg	127.6 ± 13.6
DBP, mm Hg	76.1 ± 9.2
Glucose, mmol/L	5.17 ± 0.5

[1]n = 106. Values are expressed as mean ± SD, n = 106. DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol.

Adapted from (Gebauer et. al. [2]).

isomers is presented in Table 3. The increase in LDL cholesterol concentration after consumption of the PHVO (iTFA) diet compared to the control diet is consistent with many published studies – iTFA increases LDL cholesterol concentration. Similarly, HDL cholesterol concentration after consumption of the iTFA diet compared to the control diet is consistent with published literature – iTFA do not alter HDL cholesterol, at least at modest intakes of iTFA. rTFA (VA) increased LDL cholesterol concentration compared to the control, and LDL cholesterol concentration was higher after consumption of the rTFA diet compared to the iTFA diet. On the other hand, whereas iTFA did not change HDL cholesterol concentration compared to the control diet, rTFA increased HDL cholesterol concentration compared to the control and compared to the iTFA diet. LDL cholesterol and HDL cholesterol concentrations were not different after consumption of the RA diet compared to the control but triglyceride concentration was lower.

There has been uncertainty about differentiating rTFA from iTFA for food labeling, regulations, and dietary guidance. Results from this published study suggest that rTFA (VA) and iTFA have similar LDL cholesterol raising effects, at least at higher than usual intakes. The results from the present study demonstrate that at higher than usual intakes, isolated VA and iTFA both adversely affect LDL cholesterol concentrations when replaced with energy from stearic acid. However, rTFA raise HDL cholesterol whereas iTFA do not. The results of this large, well-controlled dietary intervention support the current labeling regu-

lation, with the requirement of VA, but not c9,t11-CLA, to be listed under TFA on the Nutrition Facts Panel.

Table 2. Chemical analysis of treatment diets (% of energy)[1]				
	Control[2]	iTFA	VA	c9,t11-CLA
Protein	17.0±0.13	17.2±0.07	17.0±0.12	17.1±0.12
Fat	33.3±0.31	33.0±0.19	33.4±0.23	33.3±0.17
Carbohydrate	49.7±0.25	49.8±0.15	49.5±0.20	49.6±0.15
Saturated fatty acids	15.9±0.04	12.5±0.05	12.4±0.02	14.9±0.04
Lauric	0.43±0.006	0.42±0.007	0.40±0.003	0.43±0.005
Myristic	0.05±0.005	0.05±0.003	0.05±0.002	0.05±0.004
Palmitic	5.74±0.055	5.90±0.104	5.93±0.025	5.84±0.063
Stearic	9.24±0.105	5.68±0.115	5.61±0.058	8.18±0.126
Other	0.44±0.002	0.44±0.002	0.38±0.002	0.44±0.003
Monounsaturated fatty acids	9.85±0.070	9.77±0.089	9.74±0.047	9.87±0.048
Oleic	9.17±0.138	8.92±0.176	8.94±0.090	9.18±0.092
Other	0.68±0.002	0.86±0.002	0.81±0.003	0.69±0.004
Polyunsaturated fatty acids	5.67±0.015	5.89±0.026	5.77±0.031	5.81±0.017
Linoleic	5.10±0.037	5.30±0.062	5.20±0.080	5.23±0.041
Alpha linolenic	0.45±0.005	0.46±0.012	0.45±0.009	0.46±0.005
Other	0.13±0.002	0.13±0.004	0.13±0.003	0.12±0.004
Trans fatty acids	0.28±0.002	3.26±0.014	3.93±0.012	0.32±0.009
Palmitelaidic	0.02±0.000	0.02±0.001	0.02±0.001	0.02±0.001
Elaidic	0.21±0.007	2.87±0.050	0.00±0.000	0.24±0.032
trans-Vaccenic	0.02±0.001	0.30±0.005	3.86±0.044	0.02±0.003
Other	0.03±0.000	0.06±0.000	0.05±0.001	0.04±0.001
Conjugated linoleic acids	0.04±0.002	0.06±0.002	0.04±0.002	0.84±0.018
Linoelaidic	0.02±0.001	0.04±0.001	0.02±0.001	0.02±0.001
Conjugated linoleic	0.02±0.002	0.02±0.003	0.03±0.003	0.82±0.034

[1]Values are presented as mean ± SEM, *n*=8 samples for each treatment diet. Chemical composition of diets from chemical analyses of weekly composites of food collected throughout the study intervention period. c9,t11-CLA indicates *cis*-9, *trans*-11 conjugated linoleic acid; iTFA, industrially produced *trans* fatty acids; VA, vaccenic acid.

[2]Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,t11-CLA.

Adapted from (Gebauer et. al. [2]).

Table 3. Effect of treatment diets on lipids[1]								
	Control[2]	iTFA	VA	c9,t11-CLA	*P*-values			
					VA vs iTFA	VA vs Control	iTFA vs Control	CLA vs Control
LDL-C, mmol/L	2.94 ± 0.04	3.04 ± 0.04*	3.12 ± 0.04*†	2.93 ± 0.04	0.0114	<0.0001	0.0028	0.6054

HDL-C, mmol/L	1.40 ± 0.02	1.40 ± 0.02	1.43 ± 0.02*†	1.39 ± 0.02	0.0026	0.0110	0.6315	0.2927
TG, mmol/L	1.13 ± 0.03	1.11 ± 0.03	1.16 ± 0.03†	1.06 ± 0.03*	0.0290	0.1488	0.4518	0.0026

[1] All values are means ± SEM, n=106. All statistical analyses were performed with SAS (ver. 9.2, Statistical Analyses System, Cary, NC) using a mixed model analysis (PROC MIXED) to determine whether effects were significant ($P \leq 0.05$). Contrast statements were used to make the following comparisons: iTFA vs control, VA vs control, iTFA vs VA, and c9,t11-CLA vs control.
*Significant difference vs control as defined by $P \leq 0.05$;
†significant difference between iTFA and VA as defined by $P \leq 0.05$.
c9,t11-CLA, *cis*-9, *trans*-11 conjugated linoleic acid; HDL-C, high-density lipoprotein-cholesterol; iTFA, industrially produced *trans* fatty acids; LDL-C, low-density lipoprotein-cholesterol; *P*-value, *P*-value of overall treatment effect; SEM, standard error of the mean; TG, triacylglycerol; VA, vaccenic acid.
[2]Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,t11-CLA.

Adapted from (Gebauer et. al. [2]).

References

Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. Trans-fatty acids intake and risk of myocardial infarction. Circulation 1994;89(1):94-101.

Bauman DE, Barbano DM, Dwyer DA, Griinari JM. Technical note: production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J Dairy Sci 2000;83(11):2422-5.

Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues JB, Chaumont P, et al. Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. Am J Clin Nutr 2008;87(3):558-66.

Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. Food Addit. Contam. Part A. Chem Anal Control Expo Risk Assess 2012;29(6):861-74.

Gebauer SK [1], Chardigny JM, Jakobsen MU, Lamarche B, Lock AL, Proctor SD, Baer DJ. Effects of Ruminant trans Fatty Acids on Cardiovascular Disease and Cancer: A Comprehensive Review of Epidemiological, Clinical, and Mechanistic Studies. Adv Nutr 2011;2(4):332-54.

Gebauer SK [2], Destaillats F, Dionisi F, Krauss RM, Baer DJ. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. Am J Clin Nutr 2015;102(6):1339-46.

Gebauer SK [3], Destaillats F, Mouloungui Z, Candy L, Bezelgues JB, Dionisi F, Baer DJ. Effect of trans fatty acid isomers from ruminant sources on risk factors of cardiovascular disease: study design and rationale. Contemp Clin Trials 2011;32(4):569-76.

Lacroix E, Charest A, Cyr A, Baril-Gravel L, Lebeuf Y, Paquin P, Chouinard PY, Couture P, Lamarche B. Randomized controlled study of the effect of a butter naturally enriched in trans fatty acids on blood lipids in healthy women. Am J Clin Nutr 2012; 95(2):318-25.

Liu XR, Deng ZY, Hu JN, Fan YW, Liu R, Li J, Peng JT, Su H, Peng Q, Li WF. Erythrocyte membrane trans-fatty acid index is positively associated with a 10-year CHD risk probability. Br J Nutr 2013;109(9):1695-703.

Lock AL, Bauman DE. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 2004;39(12):1197-206.

Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 2008;87(3):593-9.

Oomen CM, Ocke MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet 2001;357 (9258):746-51.

Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Am J Epidemiol 1997; 145(10):876-87.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am J Clin Nutr 2006;83(2):237-43.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 1993;341(8845):581-5.

Rumen-derived Fatty Acids - What Makes Them Special

Jana Kraft, Ph.D.

Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT

(Editor's Note: This paper is being summarized from the PowerPoint presentation given at the 2017 Conference. Narrative is limited as most of the slides were self-explanatory.)
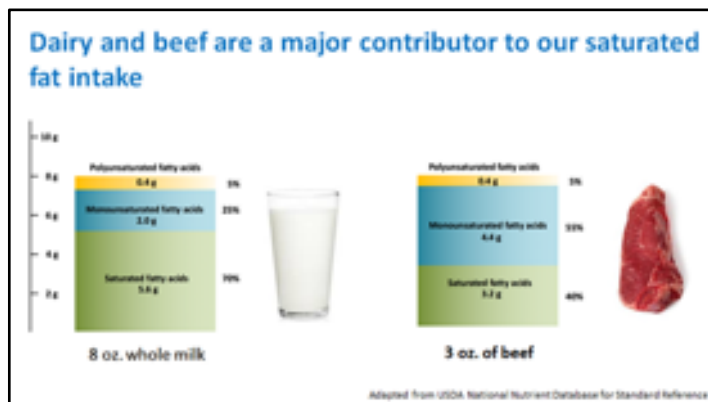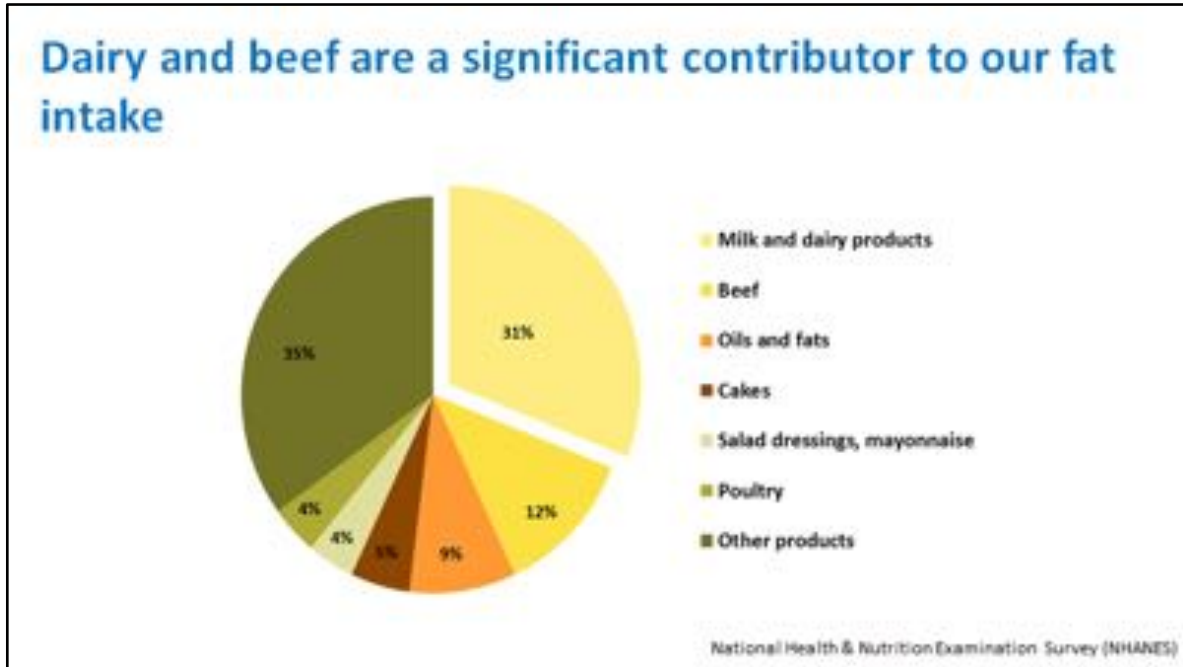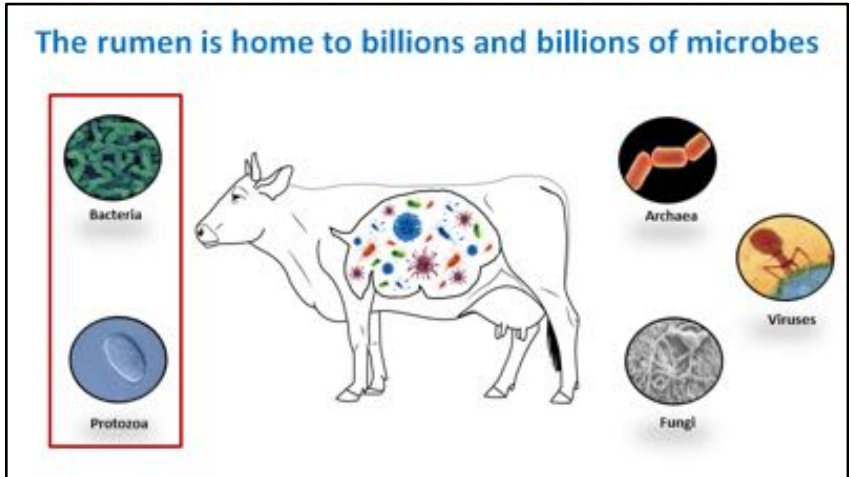

Fats from ruminant-derived products have been suffering from a negative nutritional image. Saturated fats have been under constant scrutiny for their potential role in the development of chronic diseases. Health authorities/agencies promote fat-reduced or fat-free dairy products of as part of a healthy diet. However, dairy and beef are a versatile source of bioactive nutrients from these major nutrient types: protein(peptides), vitamins,

minerals, and fatty acids. Since we are looking particularly at fatty acids, they can be broken down further into these categories:

- Short-/medium-chain fatty acids
- Odd-chain fatty acids (OCFA)
- Branched-chain fatty acids (BCFA)
- Vaccenic acid
- Conjugated linoleic acids (CLA).

The last four listed are derived from the rumen of dairy and beef cattle.

The rumen is home to billions and billions of microbes

Bacteria   Archaea   Viruses   Protozoa   Fungi

Rumen-microbe derived bioactive fatty acids are incorporated into meat and milk. Odd chain fatty acids (OC-FAs); pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), originate from rumen microbial biosynthesis. These two odd chain fatty acids are of particular interest in that they have been associated with reduced risk of coronary heart disease and type 2 diabetes, and for developing multiple sclerosis and Alzheimer's disease (Jenkins et al. 2015). Picture below shows the range of the two odd chain fatty acids in an 8 ounce glass of whole milk and a 3 ounce serving of beef.

Content per serving:

Whole milk (8 oz.)
C15:0 85 - 110 mg
C17:0 48 - 60 mg

Beef (3 oz.)
C15:0 63 - 80 mg
C17:0 19 - 40 mg

Vaccenic Acid
Content per serving:

Whole milk (8 oz.)
70 - 330 mg

Beef (3 oz.)
35 - 290 mg

CLA Content per serving:

Whole milk (8 oz.)
30 - 170 mg

Beef (3 oz.)
20 - 160 mg

Vaccenic acid (C18:1 trans-11) is a principal ruminant trans fatty acid. It originates from rumen biohydrogenation of C18 unsaturated fatty acids [linolenic acid (C18:3 n-3), linoleic acid (C18:2 n-6), oleic acid (18:1 cis-9). First adjacent picture shows the range of vaccenic acid in an 8 ounce glass of milk and a 3 ounce serving of beef. Vaccenic acid is a dietary precursor to cis-9,trans-11 CLA. Cis-9,trans-11 CLA is the principal form of CLA found in ruminant-derived products. It originates from rumen biohydrogenation of linoleic acid and tissue synthesis from vaccenic acid. Second adjacent picture displays the range of CLA in an 8 ounce glass of milk and a 3 ounce serving of beef. CLA has several health benefits for those who consume it in their diet. These benefits are shown in the diagram below.

Conjugated linoleic acids (CLA) have various health effects

Beneficial effects on immune and inflammatory responses

Suppresses tumorigenesis in several cancer types

Maintains bone mass
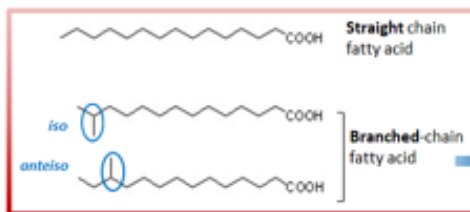
Reduces risk for coronary heart diseases

Anti-obesity effect and may improve body composition

Reviewed by Dilzer and Park 2012

Branched-chain fatty acids (BCFA) are major constituents in bacteria and protozoa cell membranes

COOH   **Straight** chain fatty acid

iso   COOH

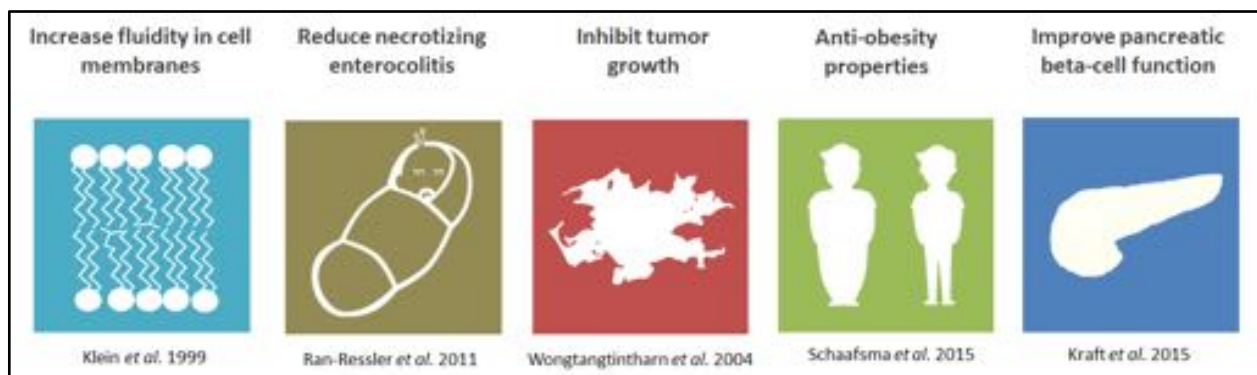anteiso   COOH   **Branched**-chain fatty acid

153
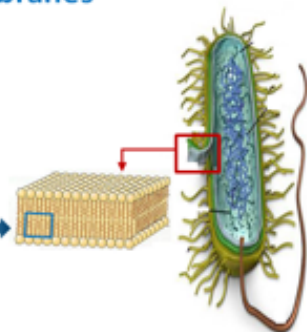
Ruminant products are a rich source of many branched-chain fatty acids (BCFA). Typical BCFA found in ruminant products are:

- iso 13:0
- anteiso 13:0
- iso 14:0
- iso 15:0
- anteiso 15:0
- iso 16:0
- iso 15:0, and
- anteiso 17:0.

BCFA Content per serving:

Whole milk (8 oz.)
120 - 240 mg

Beef (3 oz.)
100 - 180 mg

Branched-chain fatty acids are an emerging class of bioactive fatty acids that exert various positive health effects. The picture below depicts the health promoting benefits.

Increase fluidity in cell membranes	Reduce necrotizing enterocolitis	Inhibit tumor growth	Anti-obesity properties	Improve pancreatic beta-cell function
Klein *et al.* 1999	Ran-Ressler *et al.* 2011	Wongtangtintharn *et al.* 2004	Schaafsma *et al.* 2015	Kraft *et al.* 2015

Two main factors influence the content of bioactive fatty acids in ruminant-derived products, the animal and their diet (ration).

On the Animal side, these traits and body conditions impact the content of bioactive fatty acids in their products:
- ✓ Breed, genetics within breed
- ✓ Stage of lactation and type
- ✓ Diseases, udder infections
- ✓ Ruminal fermentation
- ✓ Activity and composition of the microbial populations

On the Diet side, it is the composition of the diet and the environmental conditions the animal is subjected to:
- ✓ Forage and grain intake
- ✓ Amount, composition, and type of dietary fat
- ✓ Dietary protein intake
- ✓ Energy intake
- ✓ Seasonal and regional effects

How does grazing pasture influence the content of bioactive fatty acids? Milk from cows grazing pasture is a very rich source of bioactive fatty acids. Table 1 characterizes herd size and milk production of confinement and summer pastured dairy herds in western Europe. Note milk yield and herd size is lower for dairy farms pasturing their milk cows in summer versus confinement dairy farms.

TABLE 1
Short Characterization of Cow Herds

Location	Farming	Altitude (m)	Milk yield (kg/yr)	Number of cows
1. Germany, Thuringia	Indoor-fed cows, silages and high concentrate rations, typical plain situation[a]	~200	>6000	>300
2. Germany, Thuringia	Organic farming, pasturing during the summer, only small amounts of concentrate[b]	~500	4000–5000	120–200
3. Switzerland, Alps	Summer pasturing without concentrate[c]	>1200, different places in Switzerland;	~4500	20–500
4. Switzerland, Alps	Summer pasturing without concentrate[c]	1275–2200, only L´Etivaz	~4500	30–50

[a]Most of the milk in Germany is produced under these conditions.
[b]About 5% of cows in Germany are stocked in organic farms.
[c]Summer pasturing of cows is practiced in the Alps regions of Switzerland, Austria, and Germany.

Kraft et al. 2003, Lipids 38 (6): 657-664.

Green leaves of immature pasture plants contain more lipid extract than leaves from mature forage. Due to the short vegetation period, the meadows at higher altitude in the Alps are physiologically young. Furthermore, under the lower environmental temperatures typical of the highlands, plant tissues contain a higher percentage of α-linolenic acid (Hawke, 1973). This is why when one examines Table 2 below, there is such a great increase in vaccenic acid and CLA, as much as tenfold higher, for high altitude (L'Etivaz) pastured cow's milk than for confined cows at a much lower altitude. Total BCFA is also significantly higher in high altitude pastured cow's milk than that of confined cow's milk, just not as dramatic of a difference. Most harvested forages, whether as ensilage or hay, are harvested at a later maturity and α-linolenic acid content in them is thereby lessened considerably. It also argues against grazing tall headed-out pasture grasses.

Table 2
Bioactive fatty acids in milk fat (values in mg/g fat)

| Location	Germany		Switzerland	
	Indoor	Organic farming	Different Places	L'Etivaz
Vaccenic acid	3.48 ± 0.08[a]	14.28 ± 6.68[b]	32.31 ± 4.18[c]	38.57 ± 3.41[c]
c9,t11 CLA	2.76 ± 0.12[a]	8.72 ± 3.50[b]	22.94 ± 2.33[c]	26.70 ± 1.08[d]
ΣBCFA	35.87 ± 1.57[a]	40.70 ± 1.89[b]	45.12 ± 1.95[c]	48.06 ± 2.08[c]
C15:0	10.81 ± 0.18[a]	10.83 ± 0.61[a,c]	12.63 ± 0.21[b,c]	13.22 ± 1.48[b]
C17:0	5.93 ± 0.07[a]	5.88 ± 0.14[a]	7.49 ± 0.24[b]	6.94 ± 0.42[b]

Values in a row not sharing a common superscript roman letter differ, P < 0.05.

Kraft et al. 2003, Lipids 38 (6): 657-664.

Work done at the University of Vermont (shown below) also found milk contents of vaccenic acid, CLA, and omega-3 (n-3) fatty acids were higher when cows grazed on a cool season pasture as opposed to a summer annual, pearl millet, a warm season grass.
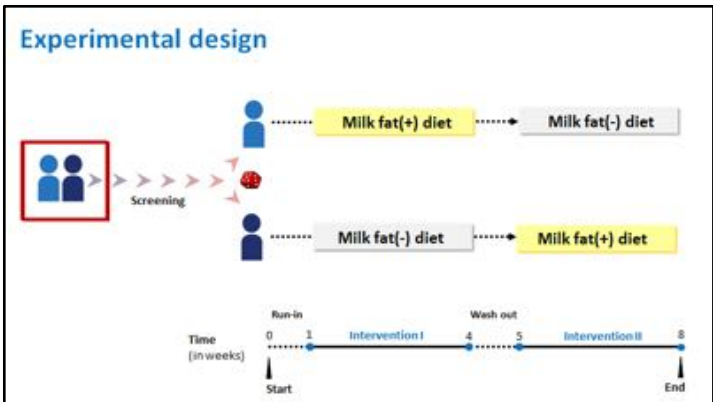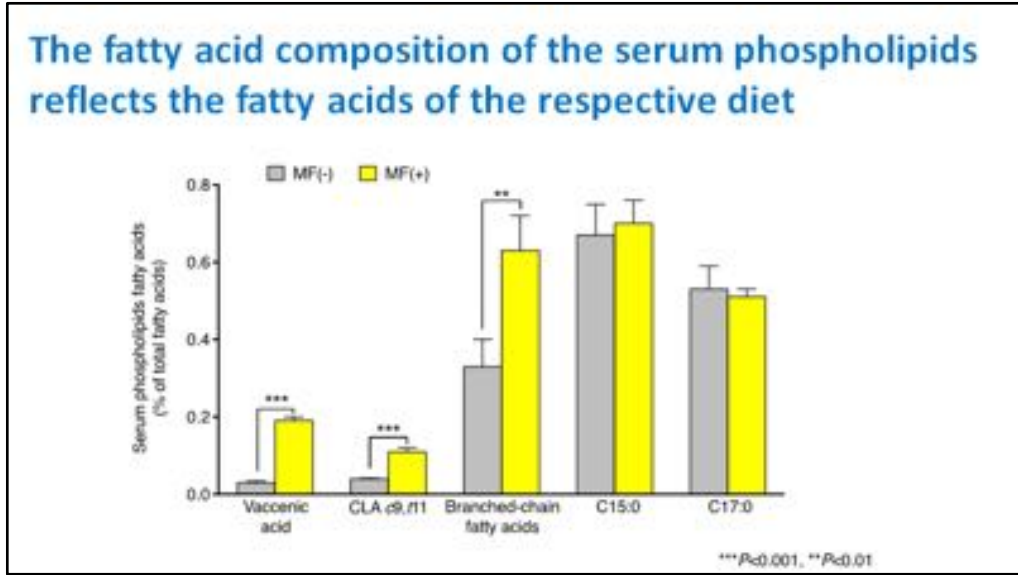

Bainbridge *et al.* 2017, *Food Chem* 217: 750-755.
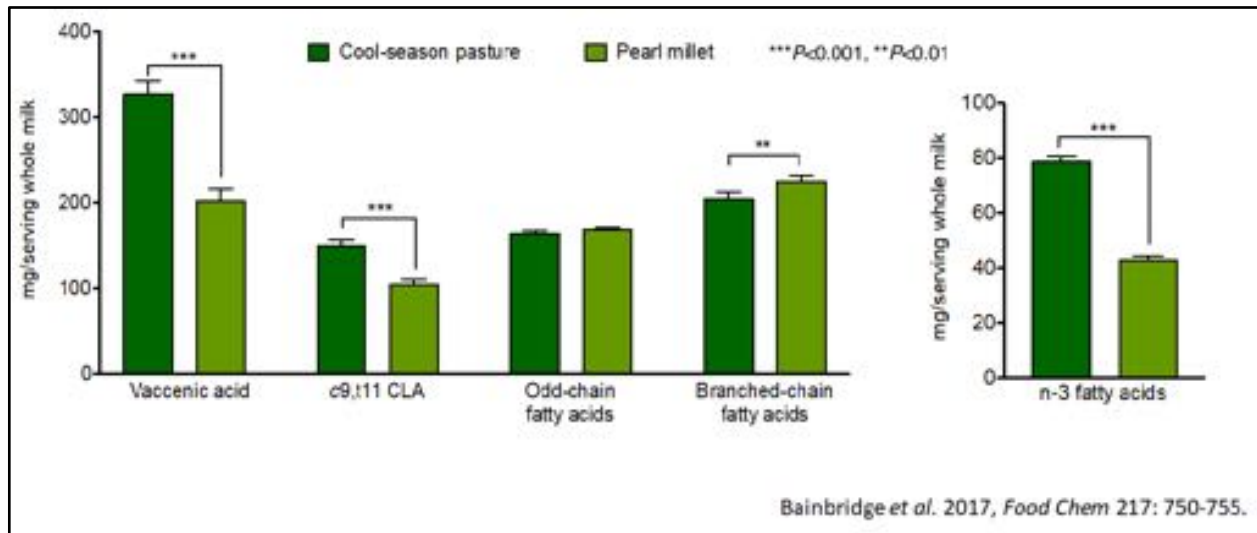
The figure below displays a Pearson correlation matrix between bacterial and protozoal taxa and milk fatty acids of cows grazing a cool-season pasture and pearl millet. The scale of the colors is denoted as follows: the more positive the correlation (closer to 1), the darker the shade of blue; the more negative the correlation (closer to −1), the darker the shade of red. Data were used from the last week of each period (n = 5 for CSP; n = 10 for PM). Un, Unclassified; VA, Vaccenic acid; RA, Rumenic acid; CLA, Conjugated linoleic acids; ALA, a-Linolenic acid; PUFA, Polyunsaturated fatty acids; OBCFA, Odd-and-Branched-chain fatty acids. The proportion of 17:0 in milk was negatively correlated with Butyrivibrio (R = −0.42; P < 0.05 (medium dark red). The milk proportions of 15:0 and 17:0 were positively correlated with the bacterial genus, Prevotella (R = 0.43, and 0.43, respectively; P < 0.05) (medium blue). Milk VA, RA, and total CLA positively correlated with bacteria of the genus Butyrivibrio (R = 0.58, 0.50, 0.47, respectively; P < 0.01 (medium dark blue). The upshot of this is rumen microorganisms synthesize unique FA such as OBCFA, and create biohydrogenation intermediates (e.g., VA and CLA) that are incorporated into milk fat, making it the most distinctive dietary fat in nature. These FA impart beneficial health effects in humans consuming ruminant-derived-food products. Altering microbial communities and their FA metabolism through diet modification can potentially enhance the quantity and profile of these bioactive FA that are available for incorporation into milk and meat (Bainbridge, 2018).

Microbial taxa correlate to bioactive milk fatty acids

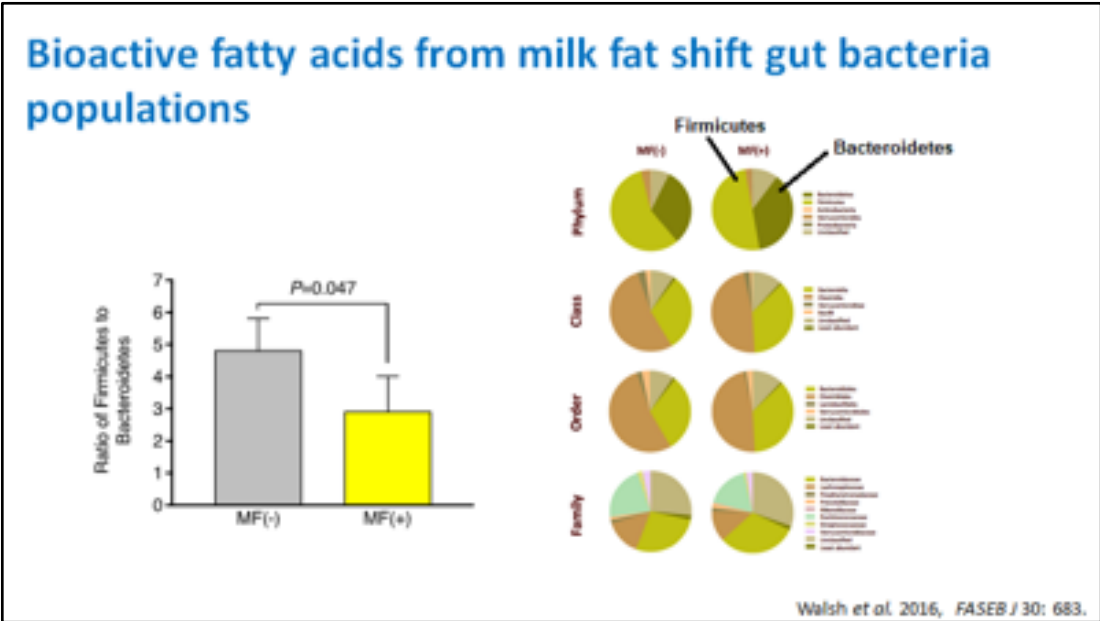Bainbridge et al. 2018, Front Microbiol
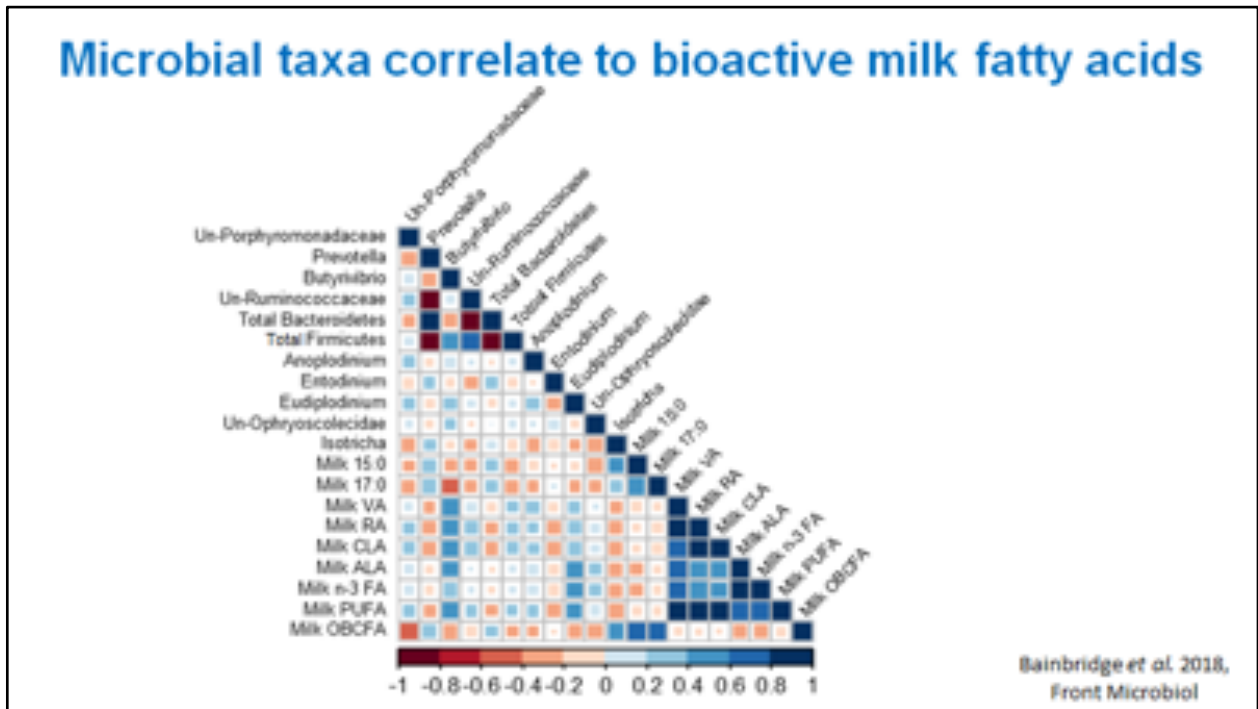
We examined the effects of consuming a diet comprised of bioactive FA from milk fat on metabolic health markers by doing a study whose purpose was to determine if unique, rumen bacteria-derived FA found in dairy fat *per se* alter glucose homeostasis. The study population comprised of 10 women and 11 men who were healthy and of normal weight. Their ages ran from 18 to 40 years old. Experimental design diagram is shown above. A standardized diet was used during the study to carefully ensure the diet differences was only based on the presence or absence of milk fat in the diet. Study endpoints (outcome measurements) were:

➢ Primary endpoints
   o Blood glucose and insulin levels *via intravenous glucose tolerance test
   o Blood triglyceride and cholesterol levels
➢ Explanatory endpoints
   o Gut microbes
   o Inflammation markers

Experimental design

The fatty acid composition of the serum phospholipids reflects the fatty acids of the respective diet

The outcome of the study was to show that vaccenic acid, CLA, and branched-chain FA were significantly elevated in the blood of people who had milk fat in their diet over those deprived of milk fat. These 3 fatty acids promote good health.

Bioactive fatty acids from milk fat shift gut bacteria populations

Walsh et al. 2016, FASEB J 30: 683.

A study done by Walsh et al. (2016) demonstrated that bioactive fatty acid can shift gut bacteria populations. In the figure above, a diet with milk fat in it changed the ratio of Firmicutes to Bacteroidetes over that of a diet without milk in it. Why is this important? These two types of bacteria regulate fat absorption in the gut. Researchers observe a higher ratio of Firmicutes to Bacteroidetes in obese humans, while in leaner humans, a higher ratio of Bacteroidetes to Firmicutes is found. Therefore, the right type of milk fats in the human diet can make it easier to lose fat weight by changing the gut's bacterial makeup. Take Home Messages:

- ✓ Ruminant-derived products are unique as they consist of a variety of bioactive fatty acids with health promoting attributes.
- ✓ The content of rumen-derived bioactive fatty acids can be altered via nutritional strategies – especially pasture regimes.
- ✓ Understanding the complex interplay between rumen microbes and their contributions to the fatty acid pool of ruminant products is key to establishing novel strategies to optimize the content of bioactive fatty acids in milk and meat for human health maintenance and promotion.

References

Bainbridge, M.L., Egolf, E., Barlow, J.W., Alvez, J.P., Roman, J., Kraft, J. Milk from cows grazing on cool-season pastures provides an enhanced profile of bioactive fatty acids compared to those grazed on a monoculture of pearl millet, Food Chemistry, 2017; 217: 750-755.

Bainbridge ML, Saldinger LK, Barlow JW, Alvez JP, Roman J, Kraft J. (2018) Alteration of rumen bacteria and protozoa through grazing regime as a tool to enhance the bioactive fatty acid content of bovine milk. Front Microbiol 9:904. pp.1-13

Dilzer A. and Park Y. Implication of conjugated linoleic acid (CLA) in human health. Critical Reviews in Food Science and Nutrition 2012, 52(6): 488–513.

Hawke, J.C. (1973) Lipids, *in* Chemistry and Biochemistry of Herbage (Butler, G.W., and Baily, R.W., eds.) Vol. 1, pp. 213–263, Academic Press, London.

Jenkins, Benjamin J., James A. West, and Albert Koulman. A Review of Odd-Chain Fatty Acid Metabolism and the Role of Pentadecanoic Acid (C15:0) and Heptadecanoic Acid (C17:0) in Health and Disease. Molecules 2015; 20(2), 2425-2444.

Klein, Wolfgang, Michael H. W. Weber, and Mohamed A. Marahiel. Cold Shock Response of Bacillus subtilis: Isoleucine-Dependent Switch in the Fatty Acid Branching Pattern for Membrane. Journal of Bacteriology, Sept. 1999; p. 5341–5349 Vol. 181, No. 17.

Kraft, Jana, Marius Collombb, Peter Möckela, Robert Sieberb, and Gerhard Jahreisa. Differences in CLA Isomer Distribution of Cow's Milk Lipids, 2003; 38 (6): 657-664.

Kraft, J, Jetton, T, Satish, B, Gupta, D. Dairy-derived bioactive fatty acids improve pancreatic ß-cell function. FASEB J. 2015; 29: 608.25.

Ran-Ressler, Rinat R., Ludmila Khailova, Kelly M. Arganbright, Camille K. Adkins-Rieck, Zeina E. Jouni, Omry Koren, Ruth E. Ley, J. Thomas Brenna, and Bohuslav Dvorak. Branched Chain Fatty Acids Reduce the Incidence of Necrotizing Enterocolitis and Alter Gastrointestinal Microbial Ecology in a Neonatal Rat Model. 2011; PLoS ONE 6(12): e29032.

Schaafsma, Gertjan and Joanne L. Slavin. Significance of Inulin Fructans in the Human Diet. Comprehensive Reviews in Food Science and Food Safety. January 2015; Volume 14, (1): 37-47.

Walsh, H, H. Haq, L. Cersosimo, C.L. Kien, J. Kraft. Decreased Abundance of Firmicutes in the Gut Microbiota After Consumption of a Diet Containing Milk Fats. The FASEB Journal, 2016; 30: 683

Wongtangtintharn, S., Oku, H., Iwasaki, H., Toda, T. Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. J. Nutr. Sci. Vitaminol., 2004; 50, 137–143.

Mob grazing (Ultra-High Stocking)

This session is reported at the end to lend continuity to the issue of fatty acid composition of pasture-fed meat and milk products of the previous sessions. How to change the fatty acid composition through primarily changing their diet. How to keep fresh vegetative forage in front of the livestock for the longest time possible to get the most favorable fatty acid composition as we know it to be today. How to market pasture-fed and -finished meat and milk products to fully participate in reaping the benefits of a value-added product. How to maintain that favorable fatty acid composition when cooking meat or processing the milk. Lastly, do we really know what is the most favorable fatty acid composition that is best for the human diet? It appears that we may have some good indications with some fatty acids and not with others. This is critical because we can only do so much to change the fatty acid composition. If we spend a lot of time, effort, and treasure to adhere to current health guidelines only to find out perhaps they were off the mark, we have wasted time, careers, and money chasing an ephemeral Holy Grail.

Mob grazing, as it is practiced today for cow-calf beef operations, is to allow the forage to go to late maturity. Yet, if we look back at the very first paper in these Proceedings, this is not going to bode well for the right type of plant fatty acids to promote enhanced fatty acid composition in the meat and milk of the livestock grazing them. Finishing beef feeders on late maturity grasses is not going to achieve a low n-6 to n-3 ratio. Some dairy farmers have begun experimenting with allowing the grasses to get more mature. As one dairy farmer has told me, he wants to be able to test for fatty acids so he knows that he is getting a low n-6 to n-3 ratio. If it starts to widen, the adjustment is to graze earlier, not later. He has remarked that he sees a ratio of less than one. This indicates that his cows are eating young vegetative grass and forbs. This is an outstanding low ratio. Presently, the cost of the milk test though is prohibitively high to do on a routine basis throughout the grazing season. Since the quality of the pasture is not the same the whole growing season, especially if switching from cool season grasses to warm season ones, it is really necessary to test at least at key times as forage growing conditions or species change. This way we either know we are producing a consistent fatty acid composition or we need to figure out how to keep it consistent by planning to have ample lush, vegetative pasture throughout the grazing season, or if that is not possible each season, find feed supplements that can be fed to keep fatty acid composition most consistent.

The other problem with eastern US pastures is, that as the forage matures, leaf senescence (leaf yellowing) begins to occur as the leaves get older. They begin to yellow as they shutdown to make way for new leaves or in reaction to drought. Leaving grass to grow ungrazed for 6 weeks or longer will cause as much senesced leaf as green leaf in the grazing zone, severely reducing forage quality. In drier climates, this becomes standing hay that remains edible (cured on the stem). In the subhumid and humid eastern US, it most often becomes moldy fast and is inedible and decays to mush. Even leaves that are not so old can become infected with various leaf diseases due to high humidity in the grass canopy that promotes their spread. Headed out grasses of any species will be avoided if there is something else (vegetative) available to eat. Orchardgrass has often

been the bane of many a grazier if it is allowed to head out before it is grazed. However, any headed out grass will be avoided if vegetative material is available. Tall fescue, timothy, bromegrass, sweet vernalgrass, redtop, or whatever when headed out is going to be avoided unless it is all that is left to graze. This is not ideal, if trying to get good average daily gain or milk production. It can also lead to pink eye infections.

Two presentations were given. One emanating from Virginia in humid pasture country and the other from Missouri in subhumid pasture country with the dominant grass being tall fescue since both of them are in the Upper South tall fescue belt. As you will learn, there are various levels of stock density. This too plays a role on what is achieved in mob stocked pastures.

Summary of Mob Grazing in Virginia

Benjamin Tracy, Ph.D.

Associate Professor, Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA

Introduction

This paper summarizes several research studies that addressed mob-type grazing in Virginia. The studies were conducted from 2012 to 2016 and compared three grazing systems: mob, rotational and continuous grazing. Various system characteristics were measured including forage productivity, forage nutritional value, animal performance, clover populations and indices of soil health.

Mob vs Rotational Grazing

Mob grazing is a type of rotational or managed grazing that involves intensive decision-making to control livestock stocking rates and forage removal from pasture to produced desired outcomes (Allen et al., 2011). Mob type grazing was first promoted by Allan Savory in the 1980s as part of a more holistic approach to rangeland management (Savory, 1988) and then adopted to some extent in eastern pasturelands (Salatin, 2008). With mob grazing, a large number of animals are restricted to a small area, either eating or trampling all the plants before being moved to new grass - sometimes just after a few hours. Grazing usually starts later in the season (e.g., late May/June in Virginia) when pastures have more growth. Mob grazing is then followed by a long recovery period – usually 90 days or longer. Mob grazed pastures may be grazed just once or twice per season as a consequence. By comparison, typical rotational grazing uses recurring periods of grazing and rest among three or more paddocks. It is similar in principle to mob grazing except stocking density is lower and pasture recovery periods are much shorter – e.g., 15-30 days. However, typical grazing management in Virginia usually involves minimal management of stocking rate or control of forage removal. This management is often called continuous grazing.

Research and observational studies from pastures have described the benefits of mob and rotational stocking methods (Jones, 2000, Salatin, 2008). They include:
1. Healthy soil, with high organic matter, water-holding capacity, and an abundance of microorganisms, earthworms and dung beetles.
2. An even distribution of recycled soil nutrients and organic matter across pastures from the intensive management of animal stocking density.
3. Desirable plant diversity with few weeds and consistent seasonal ground cover that will help builds organic matter and reduces soil erosion.

Although various studies have compared rotational with continuous grazing, less formal research has been done on mob grazing. Nonetheless, mob grazing methods have been embraced increasingly by researchers and livestock producers (Earl and Jones, 1996,

Jones, 2000, Salatin, 2008, Tietz, 2011). Part of our goal was to collect field data to help evaluate the potential benefits of mob grazing in an environment like Virginia. The main objective of our work was to compare **mob, rotational and continuous grazing** methods to determine how they affected forage productivity, forage nutritional value, animal performance, indices of soil health, and clover populations.
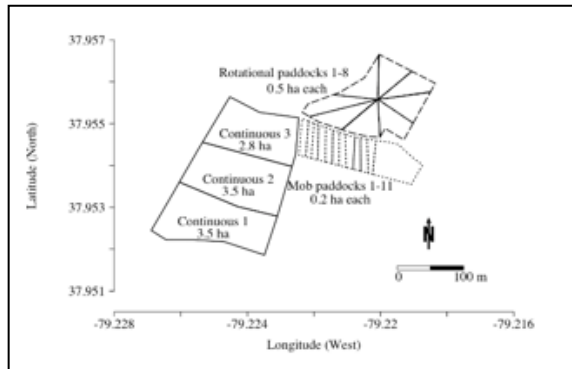
Study Sites and Measurements

Figure 1. Stocking method layout at Raphine.

Research was conducted at three locations: two demonstration farms in Blacksburg and Raphine, Va. from 2013 to 2015 and an additional site at the Virginia Tech Shenandoah Valley Agriculture Research and Experiment Center in Steeles Tavern, VA (SVAREC) from 2014 to 2016. Mob, rotational and continuous grazing systems were installed at all locations. Grazing treatments were not replicated at the demonstration farms (Figures 1 and 2). The grazing systems were replicated 3 times at the

SVAREC site **(Figure 3)**. Detailed site descriptions will not be provided here, however, soils were predominately silt loams and the vegetation at each location was dominated by tall fescue, orchardgrass, and Kentucky bluegrass. Commercial fertilizer and lime was applied according to soil test recommendations before the studies began. Ladino clover (*Trifolium repens* L. 'Will') and medium-sized red clover (*Trifolium pratense* L. 'Cinnamon Plus') were broadcast in February 2013 and 2014 at 1 and 2.5 kg ha^{-1} (3 and 6 lbs. acre^{-1}), respectively to all systems.
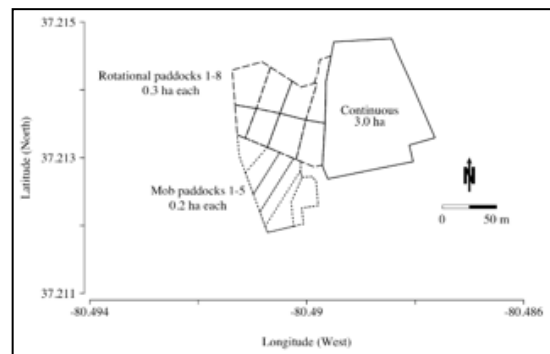

Figure 2. Blacksburg site.

Cattle and Grazing

Beef cows (aver 610kg/1300 lbs.) and steers (aver 310kg/ 680 lbs.) were stocked at the Blacksburg and Raphine locations, respectively. Stocking rates were similar (~1 Animal Unit (AU)/2 acre) where; 1 AU = 454 kg/1000lbs live BW). Water and mineral were offered *ad libitum*. At the Raphine and Blacksburg sites, mob stocking consisted of two stocking periods each year of 12- to 16-h duration, stocking densities were 138,000-155,000 kg live BW ha^{-1} (125,000 - 140,000 lbs. LW/acre on 0.1 - 0.2-ha (0.25-0.50 ac) paddocks, and rest

Figure 3. Stocking method layout at the SVAREC site. Steels Tavern Va.

periods were 90- to 120-d during the growing season. Rotational stocking consisted of 6 to 7 stocking periods of 3- to 4-d duration on 0.3 to 0.8 ha (0.75 to 2 ac) paddocks with fixed 28-30-d rest periods.

'Mob' grazing at the SVAREC location consisted of three stocking periods each year, on 0.1 ha paddocks that were allocated to the cattle every 24 h. Paddocks were not back-fenced to allow access to water at a fixed location on one end of the pasture. Each pasture was rested for a fixed period of 64-d. Stocking density of approximately 43,000 kg live BW ha^{-1} (~ 40,000 lbs. /ac.) was maintained on the paddocks. Rotational and continuous grazing protocols were similar to the demonstration sites. Beef cattle cow-calf groups grazed the SVAREC site.
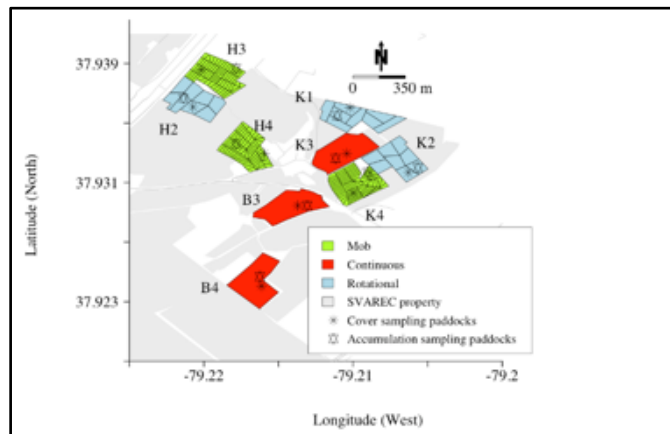
Measurements

Forage mass and nutritional value samples were taken monthly from April to October each year of the study and analyzed using standard procedures. Plant species composition was taken using a percent ground cover method and done 3x each year – spring, summer and fall. Only clover abundance will be reported in this summary paper. The soil samples to evaluate soil carbon pools and health indices were collected in late May 2015 at the two demonstration farms. For each stocking method, samples were collected along 2 transects at 3, 5, 10, 20, 40, 60, and 80m from water sources. Transects were in two directions from the water in the continuous pastures and in two paddocks in the rotational and mob pastures, allowing for any differing slopes and aspects. Soils were returned to Virginia Tech and analyzed for basic soil nutrients and pH and along with soil carbon and nitrogen pools. Soil compaction at each location was measured in early spring 2015 using a soil penetrometer at 20-30 locations within each grazing system. Animal performance only could be measured at the SVAREC site using the replicated treatments. Cow weights and BCS were taken in December before breeding. Calves were weighed at birth (October) and weaning (early May).
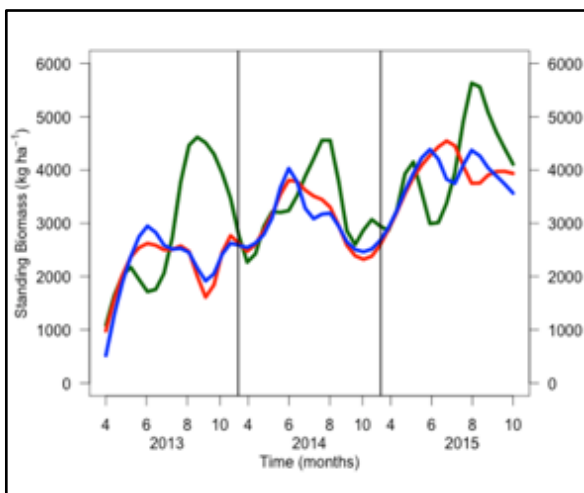
Results and Discussion

Figure 4. Forage mass measured over the three years of the study. Green line = mob, red line= Cont. and Blue line= rotational

Forage production and nutritional value: At Blacksburg and Raphine, the amount of forage mass differed among the stocking methods **(Figure 4)**. Mob grazed paddocks contained on average 600 kg ha^{-1} (540 lbs. /ac.) more forage than rotationally or continuously stocked paddocks. Forage mass was about 350 kg ha^{-1} (315 lbs. /ac.) greater at Blacksburg compared to Raphine. Mob grazed pastures tended to accumulate more forage during the late summer compared with the other stocking methods (green line in Figure 4). Forage accumulation did not differ among the stocking methods, but disappearance (i.e. use by cattle) was lower under mob stocking (data not shown). Overall, these findings suggest that mob grazed pastures accumulated more forage mainly because cattle ate less probably due to much of the grass being trampled down making it difficult to graze.

The main effects of grazing method on for-age nutritional value were not different until 2014 and 2015. As shown in **Figure 5,** for crude protein (CP), continuous pastures generally had higher concentrations especially in 2014 and 2015 (red line on graph). The higher nutritive values were mainly due to the higher amount of white clover in continuous pastures. Cattle often preferentially select clover because of their high protein content and palatability (Mourino et al., 2003) (Chapman et al., 2003). Trends for fiber components (ADF and NDF) were similar to CP so were not shown. Nutritive values did not dip below the limiting threshold set for cow maintenance (e.g., 90 g kg^{-1} or 9% for crude protein). However, values were getting close to falling below the threshold for mob grazed pastures in 2015. The findings suggest forage nutritive values under mob grazing are reasonable for dry cows but may worsen over time since grasses were allowed to become excessively over-mature each year before grazing. Forage production and nutritive value data at the SVAREC site has not been completely analyzed, but preliminary data show similar trends to those at the demonstration farms.
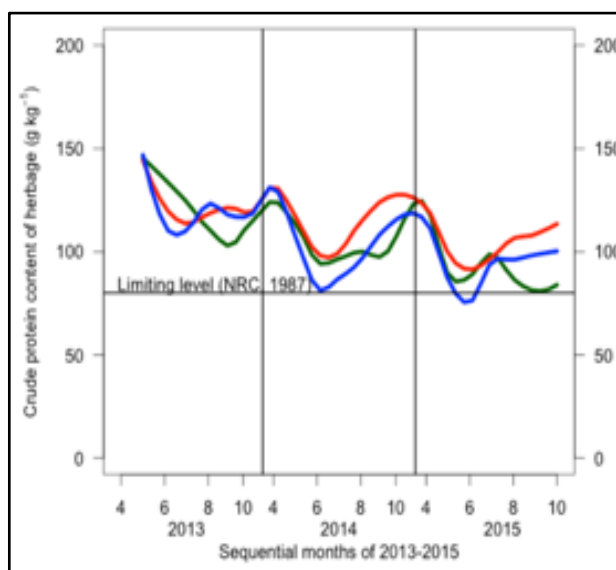

Figure 5. Forage crude protein measured over the three years of the study. Green line = mob, red line= continuous and Blue line= rotational.

Plant Species: Clover Abundance: A major interest in the plant species composition measurements was to evaluate how clovers would establish after overseeding them. As shown in **Table 1**, continuous pastures had more white clover than other stocking methods. Continuous pastures were grazed shorter than the other systems, which tends to favor white clover establishment especially if rainfall is adequate (Schlueter and Tracy, 2012). The amount of bare ground was lowest under mob grazing likely due to the high amount tall grass that was trampled during grazing. Bare ground was low in all stocking methods, however. The upright growth habit of red clover likely helped reduce shading by grasses during mob and rotational stocking and allowed it to establish relatively well (Taylor and Smith, 1995). White clover also tends to colonize bare ground via stolon growth. This situation would explain why continuously stocked areas had greater white clover cover than mob grazed areas. Species composition data from the SVAREC pastures show a similar trend (data not shown). Red clover appeared to establish particularly well under mob grazing at the SVAREC, possibly due to above average rainfall in spring and early summer. In terms of other plants (e.g. weed species), we found no real notable differences among the grazing systems. Overall, it appears that clovers can establish reasonably well under mob grazing – especially red clover when rainfall is adequate.

Table 1. The ground cover percentage of white clover, red clover and bare ground averaged over the growing seasons at the two demonstration farms. SE is standard error of the mean.

	Cover type		
Grazing method	**White clover**	**Red clover**	**Bare**
	———————% ———————		
Continuous	7.5	4.2	3.3
Mob	2.5	3.6	1.1
Rotational	3.0	3.1	3.3
SE	2.0	1.2	1.2

Soil Health Indices: Another objective of the study was to evaluate how the grazing methods would change soil nutrients and health over time. Soil variables were measured only at the demonstration farms. In terms of soil health, we were particularly interested in indices that could be linked to potential carbon sequestration (e.g., soil organic matter). To do this, we took soil samples at the start and end of the study in geo-referenced grids at each site. Soils were analyzed for pH, macro and micronutrients and soil organic matter (SOM). SOM averaged between 3-3.5% at both sites. Organic matter concentrations did not change at the Raphine site, but they increased about 10% in Blacksburg (data not shown). Overall, mob or rotational grazing did not increase SOM or other nutrients substantially more than continuous grazing over this three-year period.
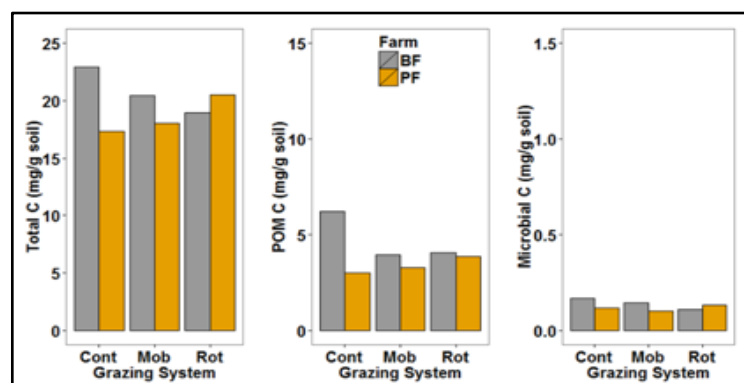

Figure 6. Three of the soil carbon pools measured in 2015 at the Raphine site (BF, left bars) and Blacksburg (PF, right bars). Note the variation between sites.

Several indices of soil health were measured in the study mostly associated with soil carbon and nitrogen pools. These pools have a major impact of soil nutrient availability for growing plants so can influence the productivity of pasturelands. Soil compaction was also evaluated in 2013 and 2015 as a physical index of soil health. **Figure 6** shows data on three soil C pools (total, particulate, and microbial C) and how they varied by site. Soil C pools appeared to be more strongly affected by site that grazing system. The similarity among grazing systems was not surprising given the three-year duration of the study. However, it should be noted that these grazing systems were being imposed on soils that had been in pasture for many years. In all likelihood, soil C pools were probably at or close to saturation in the surface soil layers where we sampled (top 10-15 cm, 4-6 inches). Given the natural site differences and high soil C concentrations, we speculate that it may take 5-10 years to begin to see significant soil changes associated with grazing methods. Soil compaction was comparatively low under mob stocking in 2015 **(Figure 7)**. Soil compaction was actually greatest under rotational grazing, but this was mainly a reflection

of pre-existing soil conditions at the Raphine location. Soil compaction measured when grazing began in 2013 also showed high compaction in the rotational area (data not shown). Although differences were found among the three systems, soil compaction was not severe enough reduce forage growth (Drewry, 2006; Flores and Tracy, 2012).

Soil Nutrient Distribution near Watering Areas: In pastures, it is common to find nutrient build up (especially for P, K, and N) near water or shade areas where livestock conger-gate and deposit manure and urine (West, et al., 1989; Mathews, et al., 1994). We hypothesized that high-density grazing in the mob system might prevent this from happening. To test this idea, we took soil samples along transects in each system starting from water sources to mid pasture. Interesting trends were found for net nitrogen mineralization, which is an index of plant available N in soil. We expected high N mineralization rates near watering areas and a gradual decline as distance increases. This trend would be expected when cattle congregate near water areas and deposit of manure and urine. This pattern was seen under continuous and rotational stocking but not mob stocking (data not shown). Under mob stocking, N mineralization was relatively constant

Figure 7. Soil compaction measured in 2015. Green line (left most) = mob, red line= Cont. and Blue line (right) = rotational.

across the pasture. In fact, N mineralization rates from 0-10m from waters was almost twice as high under continuous and rotational grazing compared with mob grazing. Although not shown, data for particulate organic C (POM-C) show a similar trend. POM-C is a carbon pool that represents easily decomposable organic matter and is usually more sensitive to management changes than total carbon. Overall, the patterns might suggest different cattle behavior with less congregation near water areas under mob grazing and hence less urine and manure deposition there. This result supports the idea the mob grazing with high cattle densities may generate a more even distribution of soil nutrients across pastures rather than the usual high concentration of waste depositions that occur near water or loafing areas.
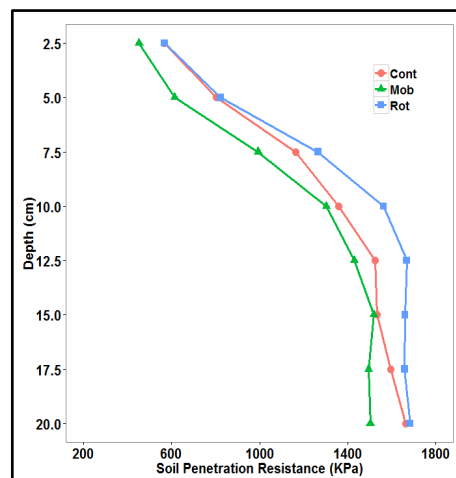
Animal Performance: Animal performance could be measured only at the SVAREC site. Cow and calf data were taken in 2014 and 2015. Cows at breeding (December) were significantly lighter than cows from the other systems especially in 2015 (Table 2). Body condition scores (BCS) taken at the same time also reflect these differences. Calf birth weights were actually lowest in the rotational systems (Table 2). The difference in birth weights did not carry over to weaning weights as these were con sistently lower for calves in mob grazed pastures. We can only speculate on why cattle performed more poorly in the mob grazed systems. One idea is that the long rest periods in the mob paddocks created a situation where most tall fescue plants (70-90% of all grasses) had produced seed heads before grazing. Tall fescue seeds have the highest alkaloid toxin concentrations within the plant (Roberts and Andrae, 2004). Possibly, cows could have been consuming more tall fescue seed and, in turn, more alkaloid toxins in the mob

169

Table 2. 2014 and 2015 animal performance data. Data are means from both yrs. *** P < 0.001, ** P < 0.01, * P < 0.05.

	Continuous	Rotational	Mob	Statistical Difference (P < 0.05)
Cows				
Breeding Wt. (lbs)	1490	1403	<u>1367</u>	**
Body Condition Score	7.1	6.4	<u>5.9</u>	***
Calves				
Birth Wt. (lbs)	84	<u>75</u>	82	**
Wean Wt. (lbs)	440	426	<u>407</u>	*

grazed paddocks. If this was the case, the alkaloids might have had a carry-over effect not only on cow performance but calves as well – possibly though reduced milk production (Thompson and Stuedemann, 1993). The possibility that cows had less available forage due to trampling also could have contributed to the lower production values observed. The higher performance on continuous grazed pastures may have been the result of several factors: the conservative stocking rate (1 cow/2 acres pasture), a high abundance of clover especially white clover, and above average growing season rainfall in 2014/15. Regardless of the specific mechanism, our findings suggest that mob-type grazing where tall fescue is the predominant grass could lead to sub-standard cow-calf performance.

Summary and Conclusions

We learned much about application of mob-type grazing in Virginia from these studies. Although mob grazed pastures can accumulate more forage than continuous or rotational systems, significant forage mass is trampled down and not eaten. Forage quality in mob grazed pastures was reasonably good despite high amounts of over-mature grasses and probably suitable for dry cows. We hypothesized that mob grazing would suppress clover establishment due to shading effects, however, red clover established well in all systems. Rainfall was high in especially in early spring and summer during these studies and that likely benefitted clover establishment. Indices of soil health were measured mostly to evaluate soil carbon sequestration potential. Overall, we found few differences in the soil variables across grazing systems. We did, however, find some evidence to suggest that mob grazing may help spread out manure and urine derived nutrients across pastures better than continuous grazing. Cow-calf performance was significantly poorer under mob grazing in 2014 and 2015 possibly because cows were consuming more highly toxic tall fescue seeds and less forage overall than in the other systems. In summary, we found little evidence to support broad adoption of mob grazing in Virginia over standard rotational grazing practices. Mob grazing efforts appear to be better suited to specific, short-term management tasks (e.g., vegetation control) rather than year-round grazing in our tall fescue-based systems.

Acknowledgments

Robert Bauer and Stephanie Yamada contributed to this work.

References

Allen, V.G., C. Batello, E.J. Berretta, J. Hodgson, M. Kothmann, X. Li. 2011. An international terminology for grazing lands and grazing animals. Grass and Forage Science 66: 2-28. doi:10.1111/j.1365-2494.2010.00780.x.

Chapman, D.F., M.R. McCaskill, P.E. Quigley, A.N. Thompson, J.F. Graham, D. Borg. 2003. Effects of grazing method and fertiliser inputs on the productivity and sustainability of Phalaris-based pastures in Western Victoria Australian Journal of Experimental Agriculture 43: 785–798

Drewry, J.J. 2006. Natural recovery of soil physical properties from treading damage of pastoral soils in New Zealand and Australia: A review. Agriculture, Ecosystems & Environment 114: 159-169. doi:DOI: 10.1016/j.agee.2005.11.028.

Earl, J.M. and C.E. Jones. 1996. The need for a new approach to grazing management - is cell grazing the answer? Rangeland Journal 18: 327-350.

Flores, J.P. and B. Tracy. 2012. Impacts of winter hay feeding on pasture soils and plants. Agriculture, Ecosystems & Environment 149: 30-36. doi:http://dx.doi.org/10.1016/j.agee.2011.12.009.

Jones, C.E. 2000. Grazing management for healthy soils. Stipa Inaugural National Grasslands Conference 'Better Pastures Naturally', Mudgee, NSW Australia.

Mathews, B.W., L.E. Sollenberger, P. Nkedi-Kizza, L.A. Gaston and H.D. Hornsby. 1994. Soil Sampling Procedures for Monitoring Potassium Distribution in Grazed Pastures. Agron. J. 86: 121-126. doi:10.2134/agronj1994.00021962008600010023x.

Mourino, F., K.A. Albrecht, D.M. Schaefer and P. Berzaghi. 2003. Steer Performance on Kura Clover-Grass and Red Clover-Grass Mixed Pastures. Agron J 95: 652-659.

Roberts, C. and J. Andrae. 2004. Tall fescue toxicosis and management. Online. Crop Management doi: 10.1094/CM-2004-0427-1001-MG.

Salatin, J. 2008. An aggressive approach to controlled grazing: Tall grass mob stocking. Acres U.S.A. Vol. 38, No. 5.

Savory, A. 1988. Holistic resource management Island Press, Covelo, CA, USA.

Schlueter, D. and B. Tracy. 2012. Sowing Method Effects on Clover Establishment into Permanent Pasture. Agron. J. 104: 1217-1222. doi:10.2134/agronj2012.0035.

Taylor, N.L. and R.R. Smith. 1995. Red Clover. *In*: R. Barnes, D. Miller and C. Nelson, editors, Forages: Volume I An Introduction to Grassland Agriculture. Iowa State University Press, Ames, IA. p. 217-227.

Thompson, F.N. and J.A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. Agriculture, Ecosystems and Environment 44: 263.

Tietz, N. 2011. Mob Grazing Produces Prime Pastures. Hay and Forage Grower (Feb 2, 2011).

West, C.P., A.P. Mallarino, W.F. Wedin and D.B. Marx. 1989. Spatial Variability of Soil Chemical Properties in Grazed Pastures. Soil Sci. Soc. Am. J. 53: 784-789. doi:10.2136/sssaj1989.03615995005300030026x.

Healing the Land with High Stock Density

Mr. Doug Peterson

Iowa/Missouri Regional Soil Health Specialist, USDA-Natural Resources Conservation Service, Des Moines, Iowa

Healing the Land really means improving soil health. This is a talk about the soil but here is a freebie on economics for you. In case you hadn't noticed, fertilizer prices are a little higher than they have been in the past. If we want to be profitable in most cases, we must get away from annual expenses. Purchased soil amendments (fertilizer, lime, etc.) should be a capital investment, NOT an annual expense. What is a capital investment? It is an investment in property, buildings, or equipment that usually remain in use for several years. What is an annual expense? Seed, feed, and labor.

Animal Impact

How can we improve our soil without purchasing additional inputs? To quote Allan Savory from his book *Holistic Management*, "The only known tool to heal the land is animal impact." (Editor's note: I might add the word "pasture" in place of "the".) What is "Animal Impact"? Animal impact is everything that livestock do to the land. This includes dunging, urinating, hoof action, rubbing, salivating, and grazing. Animal impact is the most powerful tool we have to manage grassland resources. It effects utilization, reduces spot grazing, controls weed and brush competition, improves manure distribution, improves mineral cycling, water infiltration, and produces good seed/soil contact. Most of all it improves pasture soil health and other lands pastured a portion of the year or in a crop rotation.

Figure 13. Buffalo herd on rangeland

Building Soil

How did nature make all that soil in the first place on North American grasslands? The bison roamed around eating the grass. Primarily it was warm season grasses and forbs, but there was a tremendous amount of diversity. There is still discussion about exactly how the bison grazed. There were a lot of factors that came into play. Time of year, growing or dormant grasses, available water, what areas burned, what didn't burn, and a host of other things. Some writings and accounts say they were in small groups grazing only in the burned areas for the entire year. These burned areas would have been grazed pretty hard while unburned areas were almost ungrazed, and then the next year they moved to another burned area. There are also accounts of large herds numbering in the hundreds of thousands. As you can imagine when a large herd like this moved through

an area everything probably got grazed and/or trampled pretty hard. In either of these scenarios the grasslands were severely grazed and then rested for a long period of time, severely grazed, and then rested again. It was this type of grazing regime that developed some of the healthiest soils in the world. I think that we can use different forms of this type of grazing intensity and rest to repair our eroded and worn-out soils of today.

Stock Density

Stock density is a powerful tool. It can do lots of things. I believe that Allan Savory was probably the first person to really talk about the effects of stock density on the soil. He called it "Herd Effect". How do we measure "Animal impact"? By using stock density. Let's say we have 40 head of 1250 lb. cows. That is 50,000 pounds of beef on the hoof.

Table 1. Stock density as affected by size of paddock.

$$\text{Stock Density} = \frac{50{,}000 \text{ lbs Beef (40 cows)}}{10 \text{ acres}} = 5000 \text{ lbs live weight / acre}$$

$$\text{Stock Density} = \frac{50{,}000 \text{ lbs Beef (40 cows)}}{1 \text{ acre}} = 50{,}000 \text{ lbs live weight / acre}$$

$$\text{Stock Density} = \frac{50{,}000 \text{ lbs Beef (40 cows)}}{1/4 \text{ acre}} = 200{,}000 \text{ lbs live weight / acre}$$

I want to make sure everyone is really clear on this point. We are not talking about how many cows that we have on the entire farm. We are talking about how many pounds of livestock that we have concentrated on a given amount of land at any one time.

Can we keep these cows on the three different areas for the same length of time? No. Stock density really doesn't deal with time. It does affect how long something can be grazed, but the calculation of "Stock Density" doesn't figure in time.

Table 2. Rules of thumb for stock density.
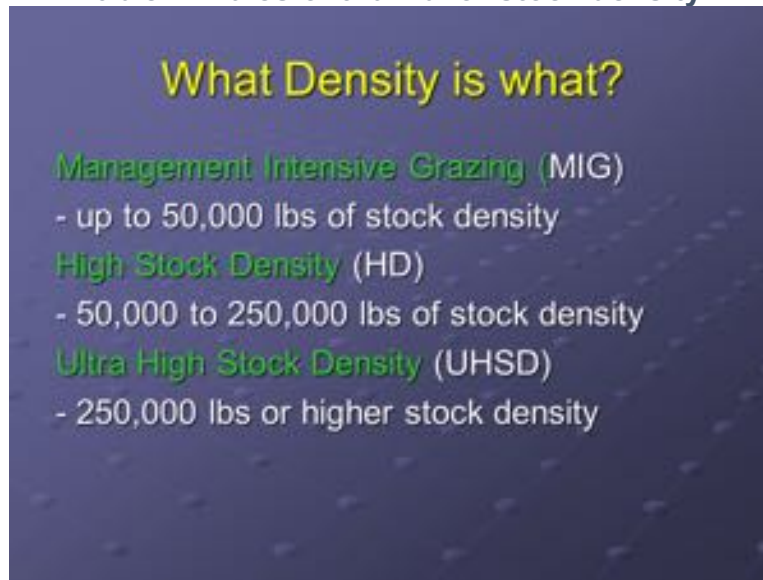
These are just some guidelines that I sort of go by. There are no hard and fast rules. Seldom does MiG get above 50,000, and for 95% of folks, they don't get above 25,000.

UHSD can be called MiG, but MiG cannot always be called UHSD.

High stock density grazing is characterized as:
Grazing by relatively large numbers of animals at a high stock density for a short period of time
  ■ Paddock Numbers: Infinite
  ■ Grazing Period: Minutes – 1 day
  ■ Rest period: months – years
  ■ Stock Density: 50,000 lbs. – 1,000,000 lbs.
  ■ Utilization: 20 – 80%
  ■ Lowest selectivity

The picture below has a stock density that is close to 100,000 lbs. to the acre. Note the cow spacing. The muddy area in the foreground is where 200 pair were camped for the night when it rained almost 4 inches. Today you can't even see the area. You can see the 4 different strips in the picture. Take notice of how much is left after the cows move. We are not making them eat everything. These cows were March calving contract cows. This was June, and we were trying to get them bred so we were not being very hard on them. Utilization was about 50%. We were not back fencing every day. In MO, you can get away without back grazing about 4 days since regrowth will not start right away. The water source is located behind and below the camera location.

Figure 2. Beef cow/calf pairs at a high stock density.

Figure 3. 250 cow/calf pairs on 3 acres.

Figure 3 above shows a set of cow/calf pairs at almost 150,000 stock density. Actually, the photo appears to be at a little higher stock density than that because the 250 pairs have access to 3 acres, but they are standing on only about 2 acres of the field. Again, we are looking at how closely they are spaced. When I talk to people about putting that many cattle on 2-3 acres they always ask, "Can they even fit on that small of an area"? Do they look smashed together to you? Do they look stressed to you?

Figure 4. Yearling beef cattle on summer annual pasture in Texas. Photos by Kirk Gadzia.

Photos were taken 10 minutes apart. Livestock moves were every 20 minutes. Cattle still have almost 10 minutes left to graze in the lower right photo.

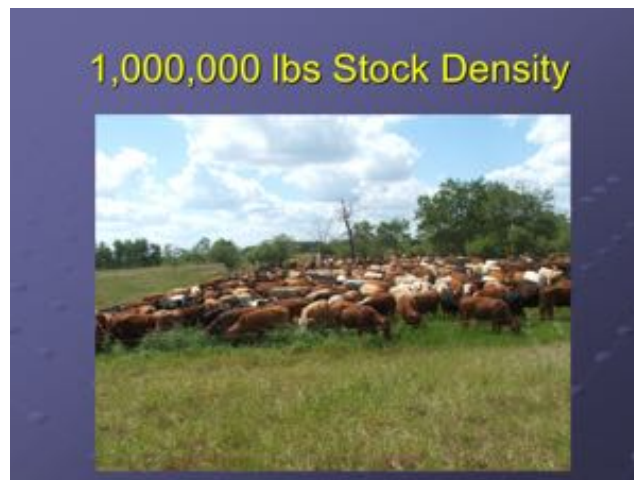500 head of 750 lbs. yearlings on ONE ACRE = 375,000 lbs. to the acre stock density.

Figure 5. What a million pounds per acre looks like, right cozy.

How does High Density Stocking "heal the land"?

So, we have shown you exactly what High Density or Mob Grazing is. What is all the hoopla about "Mob" grazing??? Why are folks talking about it so much? Is it better than MiG? We are going to take a quick look at 5 areas of concern that I think make it significantly better than MiG. We begin with below ground (soil health improvement).

Table 3. How does High Density or Mob grazing differ from Management Intensive Grazing?

Figure 14. Clover growing where a hay bale had been fed previously.

Above is a photo of a little spot of clover in a mob grazed Missouri pasture. What made it? Any guesses? Yep, the rancher fed a bale of hay right there. You all have probably seen something very similar. You unrolled a bale and got a strip of clover or trefoil. What happened that stimulated the clover to grow there? Well, it got an increase in the soil organic matter (SOM), fertility, and water infiltration through the addition and trampling of plant material into the soil. The clover seed may have been in the soil seed bank or in the hay. Some of the original sod was smothered out too by the leftover hay residue. SOM

has a neutralizing effect on the soil pH. It can bring acid soil up and alkaline soil down towards neutral. So, the SOM helped correct the pH, got the microorganisms going, and then you have clover.

Can we heal the land just by adding carbon to the soil? In the photo below **(Figure 7)**, a field is shown that was rested from March through August in 2008. There was a lot of plant material here. Grass, broadleaf plants, just lots of stuff! Then, it was grazed and trampled at a stock density of about 150,000 lbs. per acre for about 12 hours. Not the half a million pounds that you may have read about, but higher than most MiG systems use. Did the cattle "waste" some grass? Well, they didn't eat everything that's for sure! They didn't eat it all, but it was all used for a specific purpose. It was trampled onto the surface of the soil creating a layer of mulch, just like the hay pile, that allowed the clover to

Figure 7. Pasture in August that was ungrazed since March of the same year.

germinate and grow **(Figure 8)**. The hoof action of the cattle is critical to getting the material in contact with the soil. In case you are wondering, no clover was broadcast on this field; no lime and no fertilizer applied for many, many years if ever.

Figure 8. Same field as above with soil seed bank clover released by 2 seasons of mob grazing.

Recently, I had the opportunity to talk to a USDA Soil Microbiologist. He said that it typically takes a couple years for the soil microorganisms to fully respond to the increase in decaying plant material. The mulch is a food source for all the microorganisms in the soil. The field pictured in **figures 7 and 8** has been managed for two years in this manner. I think because of the trampling, the natural nutrient cycle is starting to really kick in, and that is why we are seeing the clover increase.

Figure 9. Grazing residual heights of plants and length of rest between grazing affect root growth greatly.

In Missouri, we take soil samples to a depth of 6-8 inches. Originally it was done because that was the "plow layer" for annual crops. It is still a valid depth because most cool-season grass pastures are managed in a continuous grazing system and the plants only have root systems a few inches deep. There isn't much need in going deeper because, for the most part, the plants just will not pull significant amounts of nutrients much deeper than 6 to12 inches. In areas that have deeper-rooted warm-season grasses, that will change some. Yet, almost all forage plants' root mass reflects their above-ground bio-mass. Short tops mean short roots. In a really well-managed grazing system with fairly long rest periods, we can get cool-season plant roots that are several feet deep barring any root restrictive soil layers or bedrock.
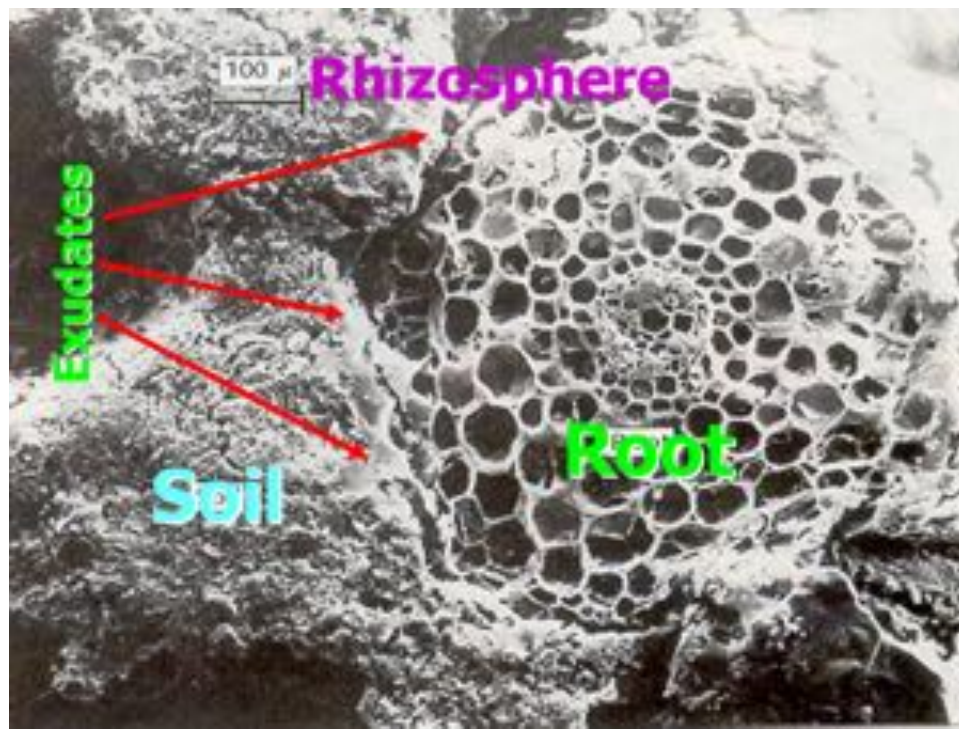

Figure 150. Electron microscope cross sectional view of a live root in soil oozing out exudates that feed soil microorganisms.

Here is how much soil "livestock" there is in a healthy soil: 8000 lbs. What do they all do? I am pretty sure I can't tell you what they all do. I do not even know if I can tell you how many there are. See table 4 below. For those of you unfamiliar with higher mathematics, 15 zeros are a quadrillion and 18 zeros are a quintillion.

Can you tell me how many there are? Well, there are 800 quintillion bacteria, 20 quintillion actinomycetes, 200 trillion fungi, 4 billion algae, 2 trillion protozoa, 80 million nematodes, 40 thousand earthworms, and 8.16 million insects/arthropods.

I can tell you they are ALL critical to the soil health. For example, fungi act as root extensions. They attach to the roots and can extend 30-40 feet. Some of the others make minerals more available to plants. In effect, they make the plant roots 40 feet long. Each one is like a link in a chain. If one is killed or destroyed the entire chain will not work.

So, if we all have soil, do we all have these microorganisms and at these levels?????

Table 4. Good soil health expressed in terms of amount of soil lifeforms present.

The second area of concern is above ground. Since high density grazing leaves a lot of residue on the soil surface: the soil is better protected from erosion, has improved water infiltration, is cooler and moister allowing for better plant and soil microorganism growth.

Figure 11. High density grazing of tall vegetation keeps the soil covered better than MiG grazing at lower stocking levels.

Figure 12. Soil temperatures at low ground cover versus high. 14 degrees F. cooler under high cover.

The sod slices in this rainfall simulation demonstration **(Figure 13)** were collected from actual pastures. The heavy rain was simulated on a 15% slope. Tray 3 is from an over-grazed and overstocked pasture, tray 2 is good rotational grazed and rested pasture system with only 4 inches of cover, and tray 1 same rotational grazed and rested system with only 6+ inches of cover after regrowth. The front jugs are runoff from surface; the rear jugs collected infiltration. Pretty graphic. This has been a great teaching tool for me at outdoor pasture training and education events.
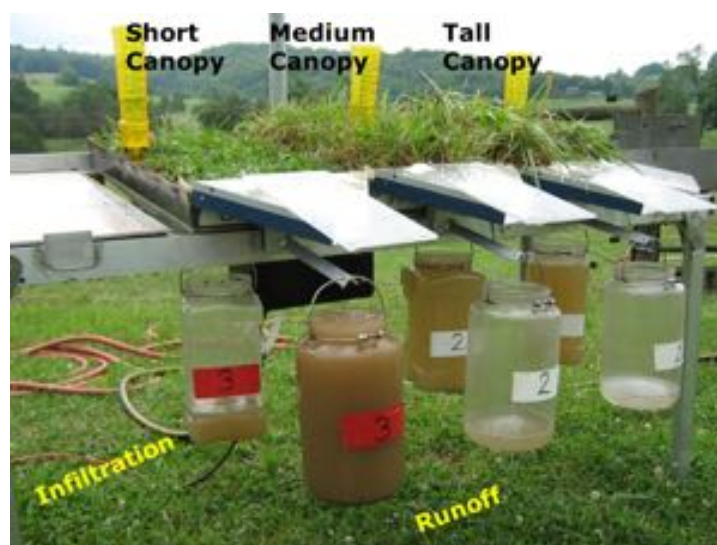

Figure 16. Rainfall simulator showing the differences in runoff, sediment loss, water infiltration from pasture sods taken from 3 differently managed pastures.

The short canopy pasture sod has filled the front jug to overflowing with runoff. The jug also has collected a lot of suspended sediment that washed off the soil surface of the

short canopy sod. Meanwhile, the back jug that collects infiltrated water through the sod has only 2-3 inches of water in it. Contrast this to the medium canopy pasture sod. Very little runoff has occurred. Its front jug has collected perhaps an inch of runoff and the water has very little suspended sediment in it. Meanwhile, its back jug is filled to its brim with infiltrated water. Some sediment has colored this infiltrated water. This would be much reduced if more of the soil profile was under the sod and the root system less disturbed during collection. Finally, with tall canopy pasture sod, runoff is even less and clearer than the medium canopy sod as can be seen in the front jug. The back jug inter-estingly has less infiltrated water in it and is slightly clearer. This can be a result of at least 3 things: more organic matter in the soil (retains more water) if the pasture has been managed this way a long time, more root mass binding the soil particles together better, and perhaps a bit more canopy interception of the applied rainfall.

High density grazing increases rest periods, the third area of concern. This causes:
- Lengthening rest periods means less grazing events per year in a paddock.
- Increases number of paddocks as they must be smaller to get desired density.

Utilizing all the plants helps extend recovery periods. This can help get rid of some unde-sirable brush and weeds along with the forage plants as selectivity is reduced when com-petition for the available herbage is keen among competitive grazers.

Figure 14. Sumac growing in a native pasture in MO.

Figure 15. Same native pasture after high density grazing occurred.

This rancher had been rotating twice daily at about 100,000 stock density. The cattle learned to eat sumac. No, he wasn't starving them. They didn't eat it last. They ate it right along with everything else. If it was in the front of the strip, they ate it first. If it was in the back of the strip, they ate it later on. They learned to JUST EAT whatever was there. Grass, legumes, sumac, weeds, whatever was available. Therefore, high density grazing can be a great vegetation control tool.

Figure 176. Ironweed grazed at 120,000 lbs. stock density.

There seems to be a change in grazing habits and animal behavior as you approach and get over 100,000 lbs. They will graze almost anything. But at 100,000, they still don't knock everything down. At higher densities, they will actually knock everything down to the ground, even coarser weeds and small woody plants. I think higher densities are better for the land, but it just takes more time. It requires more paddocks, more monitoring of forage availability, and more livestock moves. However, it takes less time to get pastures into better condition.

The fourth area of concern is mineral cycling. Again, high density grazing has the advantage. The most important factor is nutrient distribution. As livestock numbers or weight increase per unit of area, dung and urine are excreted much closer together and more uniformly since the livestock are more bunched and graze the entire area.
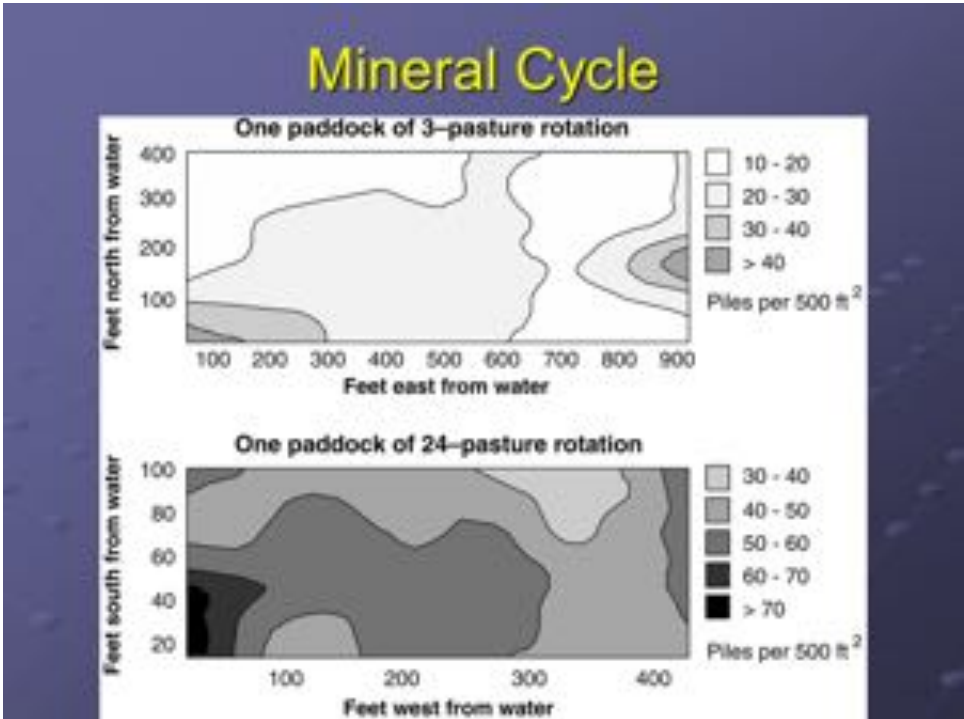

Figure 17. Distribution of dung piles as affected by number of paddocks with same herd size.

In the 3-paddock system (analogous to continuously grazed pastures) very few manure piles are deposited in the main paddock area. There is a concentration of manure near shade and water. I frequently ask audiences to identify the areas of the field where manure piles are most densely concentrated (shade, east end; water, southwest corner). Note its dimensions versus the 24-paddock dimension. This paddock is 360,000 square feet in size. While the 24-paddock system's paddock is only 45,000 square feet or 1/8th of the of 3-pasture rotation paddock.

In the intensively grazed 24-paddock system, there is a much more even distribution of manure piles in the pasture creating a higher density of manure piles in the main paddock area. There is still a concentration of nutrient near water, but the trends are less pronounced. Same head of livestock but stock density is 8 times denser.

Table 5. Rotation frequency effect on how many years to get 1 dung pile/sq. yard.

Mineral Cycle

Rotation Frequency	Years to Get 1 Pile/sq. yard
Continuous	27
14 day	8
4 day	4 – 5
2 day	2
1 time a day	??
2-6 times a day	??

The table above shows some statistics in how long it takes to get 1 dung pile per square yard over the whole pasture. This all has to do with stock density. As rotation frequency increases, the less time it takes to get dung spread at 1 dung pile per square yard. In all likelihood, continuously grazed pastures may never see each square yard receive a dung pile. If the livestock are attracted to shade trees, hay bunks, water troughs, or gate openings, more and more dung piles will end up in the same few square yards repeatedly while other areas of the pasture will become more nutrient deficient as dung and urine are rarely placed there.

Figure18. Dung distribution at a stock density of 120,000 lbs.

Figure 18 is a photo taken of a once-a-day move at 120,000 lbs. density pasture. How close are the manure piles? How much of the area got covered with urine or manure?

The fifth and last area of concern is animal performance. More mature forage will tend to have a better energy to protein ratio that is more in line with certain livestock needs. Lactating dairy cows and pasture finished livestock require a higher plane of nutrition to keep milk flow or weight gain at desired levels. Thus, a younger, vegetative growth stage of more nutritious forage types is required for them. Care must be taken to be ready to move livestock piles. How long will it take every square yard to get a manure pile? There are 2-4 times as many urine patches as manure piles. They show up as green patches in this once they have reached the level of vegetation removal that keeps them and the pastures in good condition regardless of stock density levels.

Table 6. Animal performance on high density grazing must be closely monitored to avoid hurting their performance and their pastures.
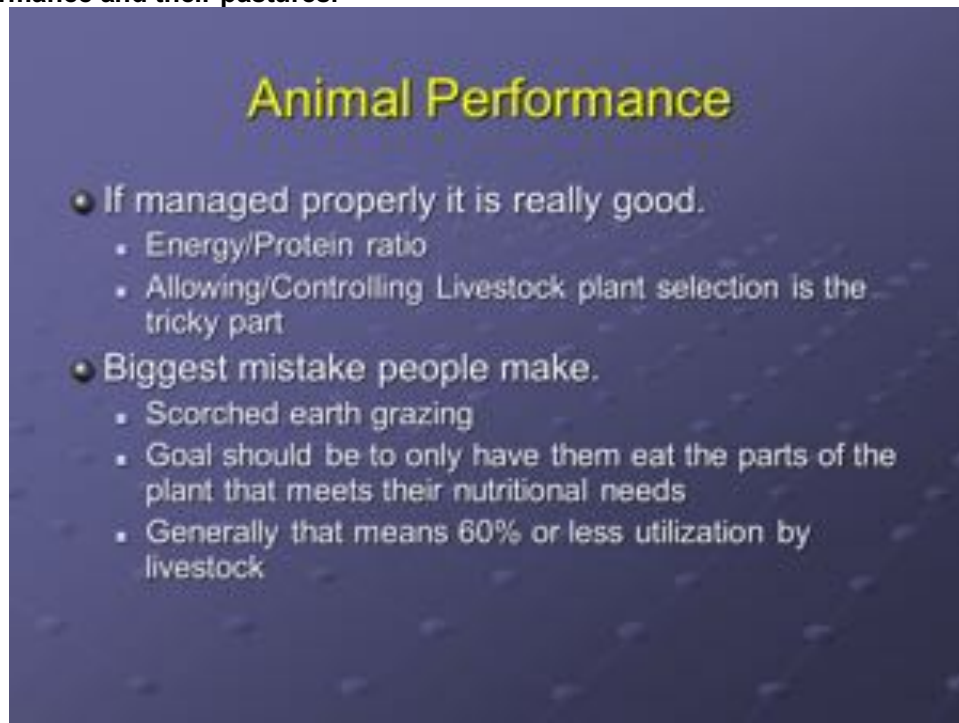
The soil is the basis of everything. I believe the soil is the most important thing we have to take care of. HOWEVER, we have to consider the "whole" when making management decisions. They are:
- Animal performance goals
- Financial goals
- Personal goals.