Markers of health status in pasture-fed versus total mixed ration-fed Jersey dairy cattle

Megan Lee Seneca
University of New Hampshire, Durham

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Abstract
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Keywords
Agriculture, Animal Culture and Nutrition

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MARKERS OF HEALTH STATUS IN PASTURE-FED VERSUS TOTAL MIXED RATION-FED JERSEY DAIRY CATTLE

BY

MEGAN LEE SENeca

Bachelor of Science, Michigan State University, 2004

THESIS

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in
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This thesis has been examined and approved.

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ABSTRACT

MARKERS OF HEALTH STATUS IN PASTURE-FED VERSUS TOTAL MIXED RATION-FED JERSEY DAIRY CATTLE

By

Megan Lee Seneca

University of New Hampshire, December, 2012

Milk from pastured cows contains more beneficial fatty acids than milk from total mixed ration (TMR) fed cows. No studies have been done comparing health status in cows from each system. This study used pastured vs. TMR fed Jersey cows to measure levels of cortisol, haptoglobin (Hp), somatic cells, and production within and between farms over time.

Cortisol was significantly different between farms with means of 126.78 and 93.64 ng/mL for pastured and TMR groups, respectively. Levels in pastured cows trended with temperature and humidity. There was no significant difference in Hp levels between farms though levels did change over time within farms. All animals had Hp levels considered normal. Somatic cell counts were not significantly different, though the pastured group had a numerically lower average, 57.7 vs. 113.8 x 10^3 cells/mL. Results indicated that environmental factors caused added stress to cows but feeding TMR vs. grass did not.
CHAPTER I

INTRODUCTION

Dietary Evolution

What we eat can have profound effects on our health. The Western diet is a well-known term that describes a high caloric diet, high in carbohydrates and fat, and low in fiber, antioxidants, and some vitamins. This diet is prevalent in the United States, but other countries have also started to move away from traditional diets towards the Western diet, influenced by Western culture. The amount of seed grains in our diet and in the diet of our livestock has shifted the ratio of essential dietary fatty acids away from the traditional ratio that our ancestors ingested before the onset of agriculture, approximately 10,000 years ago (Simopoulos, 1999a). This type of diet has been linked to diseases such as atherosclerosis, hypertension, obesity, diabetes, and cancer by numerous studies over the decades.

One of the hypothesized reasons the Western diet has had negative health effects in humans is that it is dramatically different to the hunter-gatherer, Paleolithic diet that humans ate over the hundreds of thousands of years, low in caloric density, high in protein, low in fat and carbohydrates, and higher in antioxidants including vitamins A, C, and E (Simopoulos, 1999a). The ten thousand years that have passed since humans have started growing and eating food crops is not sufficient time for our DNA to change and adapt to this shift in diet. Considering the spontaneous mutation rate of DNA has been estimated at 0.5% per million years, our DNA has changed very little since the
Agricultural Revolution, perhaps 0.005% (Eaton and Konner, 1985). Our genes today are still very similar to our ancestors from the Paleolithic period, forty-thousand years ago, yet our diet today is different when you consider the composition of fatty acids (FAs) we consume, especially the omega-6 and omega-3 FAs and their ratio. The ratio of omega-6 to omega-3 FAs was closer to 4:1 or lower when humans lived a hunter-gatherer lifestyle, whereas today there are estimates of 12:1-16.7:1 (Simopoulos, 1999a) and even higher in some populations and individuals.

Some populations still maintain a lower ratio, such as Japan, which has an estimated omega-6 to omega-3 FA ratio of 4:1 (Sugano, 2000). Historically, the Japanese have had a record of long, healthy lives which may be attributed to not only their low fat diet, but also the type of fats they ingest. Commonly eaten fats from fish and seaweed are both high in omega-3 FAs. Japan has been increasing their amount of dietary omega-6 FAs as Western influence permeates the culture. One of the resulting health effects is the major causes of death shifting from cerebrovascular disease, to cancer and heart disease (reviewed by Sugano, 2000). Other traditional societies have also seen health effects from Western influences on diet. A study from 1984 took middle-aged, urban, diabetic Australian Aborigines and returned them to their native lands for seven weeks to live their traditional hunter-gatherer lifestyle. Results showed marked improvements in fasting glucose, a decrease in fasting insulin and plasma triglyceride levels, and a decrease in very low density lipoproteins. All ten participants also lost an average of 8kg over the seven weeks (O'Dea, 1984). The "Inuit Paradox" documents that native Inuit and Eskimo populations had a lower incidence of cardiovascular disease, arthritis, and diabetes even though they routinely ate a diet high in meat and fat. This is in contrast to
research that found a diet high in saturated fat is a risk factor for cardiovascular disease. It was hypothesized that this difference might be because the meat and fats in the natives’ diet were high in omega-3 and low in omega-6 FAs. Fats from fish and marine mammals are known to be sources of omega-3 FAs, since omega-3 synthesizing algae is at the base of this food chain. Other studies found no difference in the occurrence of these diseases between Inuit or Eskimo and other western populations (Bjerregaard et al., 2003). The answer may be that cardiovascular risk is determined by type as well as quantity of dietary fat.

It has been shown that diets lower in omega-6 and higher in omega-3 with few processed components have beneficial effects on health and longevity. Two prominent examples of these are the Mediterranean diet and the Paleolithic diet. The Mediterranean diet is characterized by having monounsaturated olive oil as the dominant fat source, high to moderate consumption of fruits and vegetables, cereals, fish, wild herbs, and little meat, usually eaten along with wine. Epidemiological studies have shown mortality rates decrease for coronary heart disease and cancer while eating this type of diet (Kok and Kromhout 2004; Estruch et al 2006). The Paleolithic diet consists of foods that humans had access to prior to the establishment of agriculture, primarily meat from wild animals, uncultivated plants, fish, vegetables, fruits, roots, eggs, and nuts. Not included in this diet are grains, legumes, dairy products, salt, refined sugar, and processed oils. Studies show this diet also has beneficial effects on type 2 diabetes and risk factors for cardiovascular disease (Jönsson et al. 2009, Frassetto et al. 2009). Thorsdottir et al., (2004) found a negative correlation with type-2 diabetes in men and coronary heart disease mortality in
women and the supply of omega-3 FA in the diet. There was also a positive correlation with these conditions and the omega-6:omega-3 ratio in milk.

**Fatty Acids and Inflammation**

Alpha-linolenic acid (ALA), an omega-3 FA, and Linoleic Acid (LA), an omega-6 FA, are the two essential fatty acids (EFA) for humans, which means they must be obtained in the diet since they cannot be synthesized by the body. The designations omega-3 and omega-6 indicate that there is a double bond at the third or sixth carbon from the terminal end of the carbon chain. The number and location of double bonds determine FA structure, including how it bends and interacts with other FAs and its properties such as melting point. These structural differences can have very different physiological effects. Fats, or lipids, not only provide a rich source of concentrated energy but also act as major components of all cell membranes and as precursors to hormones, eicosanoids, and other signaling molecules. These FAs are incorporated into tissue membranes as phospholipids and are converted through enzymatic oxygenation to eicosanoids. Omega-6 and omega-3 FAs obtained in the diet compete for incorporation into tissue membranes and the products of their metabolism are involved in immune function and the inflammatory response.

Omega-6 FAs are converted to arachidonic acid (AA) which is the physiologically active omega-6 FA present in tissue membranes. Arachidonic acid and its omega-3 counterpart, EPA, derived from ALA, are parent compounds for two different series of eicosanoids, each with varying physiological activities.
Metabolism of AA can follow either of two pathways, the 5-lipoxygenase (5-LOX) or the cyclooxygenase (COX) pathway. In the COX pathway, cyclooxygenase transforms AA into the three classes of series 2 eicosanoids. One class is the prostaglandins, specifically prostaglandin G_2 (PGG_2), which is converted to prostaglandin

**Figure 1**

**Fatty Acid Structures**

**Alpha-Linolenic Acid (18:3), an Omega-3 FA**

**Linoleic (18:2), an Omega-6 Fatty Acid**

**Conjugated Linoleic Acid (18:2), cis 9, trans 11**

**Stearic Acid (18:0), a Saturated Fatty Acid**
H$_2$(PGH$_2$) with release of a free radical of oxygen. Oxygen free radicals can cause DNA, lipid, and protein damage in tissues.

Prostaglandin D$_2$ (PGD$_2$) is derived from PGH$_2$ and causes bronchoconstriction and vasodilation whereas PGE$_2$ causes fever, vasodilation and pain, and PGF$_{2\alpha}$ causes vasoconstriction. PGH$_2$ is also the precursor to a second type of eicosanoid, thromboxane A$_2$ (TXA$_2$), which is the most potent biochemical responsible for vasoconstriction and platelet aggregation, the essence of blood clotting. The third type of eicosanoid resulting from AA metabolism is prostacyclin (PGI$_2$) which decreases platelet aggregation, causes vasodilation, and potentiates edema. Prostacyclin acts as the innate balance for the pro-aggregatory effects of TXA$_2$. The 5-LOX pathway, the second pathway by which AA can be metabolized, gives rise to hydroperoxyeicosatetraenoic acid (HPETE) and its derivatives which include the series 4 leukotrienes (LT). Leukotriene B$_4$ (LTB$_4$) is a potent chemotactic agent that causes aggregation of leukocytes, and LTC$_4$, LTD$_4$ and LTE$_4$ all cause vasoconstriction, bronchospasm, and increased vascular permeability. The series 2 eicosanoids from the COX pathway and the series 4 eicosanoids from the LOX pathway produced from AA metabolism mentioned above are biologically active in small amounts, and if formed in large amounts can contribute to chronic inflammatory and allergic disorders, the formation of thrombi, and cell proliferation (i.e., cancer). Since AA is derived from ALA, omega-6 FAs are considered pro-inflammatory. If omega-3 derived EPA is the primary fatty acid incorporated in cell membranes, the eicosanoids resulting from both the COX and LOX pathways are produced in smaller quantities than those of AA and of series 3 and 5 instead of 2 and 4. The series 3 and 5 eicosanoids are less biologically active than the series 2 and 4 from AA metabolism,
therefore their response to pro-inflammatory stimuli is weaker. For example, PGD₃ will cause less bronchoconstriction and vasodilation than PGD₂. The functional differences in these EPA-derived eicosanoids result in omega-3 FAs being considered anti-inflammatory.

**Figure 2**

Fatty Acid Metabolites

<table>
<thead>
<tr>
<th>Omega-6</th>
<th>Omega-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic Acid</td>
<td>A-Linolenic Acid</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>Eicosapentaenoic Acid</td>
</tr>
<tr>
<td>PGD₂, PGE₂, PGF₂, PGI₂, TXA₂</td>
<td>PGD₃, PGE₃, PGF₃, PGI₃, TXA₃</td>
</tr>
<tr>
<td>LTA₆, LTB₄, LTC₄, LTD₆, LTE₄</td>
<td>LTA₅, LTB₅, LTC₅, LTD₅, LTE₅</td>
</tr>
</tbody>
</table>

**Fatty Acids and Disease**

A balance of these two classes of FAs constitutes the ideal physiological condition where the inflammatory response reacts as needed during stress or injury yet is controlled in a healthy condition; however, because of the increased amounts of omega-6
in the typical Western diet, the products of AA metabolism are synthesized in a much larger proportion to the products of EPA metabolism. This shift to a pro-inflammatory physiological state may increase risk of cardiovascular disease, hypertension, diabetes, Alzheimer's, arthritis, and cancer (Calder, 2006; Berquin et al., 2008). According to the Centers for Disease Control (CDC), chronic diseases are responsible for 70% of deaths in the United States. Atherosclerosis, an inflammatory disease of the arterial wall, is the major cause of cardiovascular disease, the number one cause of morbidity and mortality in the United States.

Another important class of FAs, although not considered either omega-3 or omega-6 class, are conjugated linoleic acids (CLA). CLAs are isomers of LA with the cis-9, trans-11 isomer being one of the more common and bioactive forms. CLA has been found to have both anti-carcinogenic and anti-inflammatory effects (Pariza and Hargraves, 1985; Ha et al., 1987). Other studies found CLA to be anti-diabetogenic, anti-atherogenic, anti-obesity, and to modulate the immune system and bone growth (Belury, 2002). An example of the anti-inflammatory effects was noted in a study where one group of mice were fed a high-CLA diet had a reduced inflammatory response when septic shock was induced, compared to the group on a low-CLA control diet (Reynolds et al., 2009). It is still unknown by what exact physiological mechanism CLA causes these effects, but it is thought that CLA may compete with LA and thereby modulate FA metabolism and eicosanoid production (Belury and Kempa-Steczko, 1997). Less LA in tissues would yield less AA production and the subsequent pro-inflammatory cytokines derived from this pathway.
Ruminants

CLA is also called rumenic acid because it is found predominantly in ruminant tissue and milk. When cattle ingest polyunsaturated FAs (PUFAs) such as ALA and LA, these FAs can have toxic effects on the rumen microbes. The microbes therefore attempt to saturate the carbon chain through a process called biohydrogenation until the fully saturated, stearic acid is formed. This is the reason that dairy products have higher levels of saturated fat (SA) than other animal products. However, in the process of biohydrogenation, intermediates are formed before the entire biohydrogenation is complete. These intermediates include cis-9, trans-11 CLA and trans-11(18:1) or vaccenic acid. Though most of the fat ingested becomes saturated in the rumen by the microbes, some of the intact FAs and intermediates pass out of the rumen before full biohydrogenation is complete, continuing into the intestines where they can be absorbed and enter the bloodstream. Escape rate from the rumen depends on intake amount and rate of passage of digesta. Short and medium chain FAs diffuse directly into intestinal capillaries, whereas long chain FAs such as LA, CLA, and ALA, first enter the intestinal villi where they are assembled into triglycerides and further into chylomicrons. Chylomicrons then enter the lymphatic system and eventually the bloodstream. Chylomicrons transport triglycerides to tissues where they can be stored, metabolized, or, in the case of RBC membranes, incorporated into the phospholipid bilayer. The more long-chain FAs ingested, the more partially hydrogenated FAs are available to escape the rumen and enter the bloodstream. Upon reaching the mammary gland, circulating cis-9, trans-11 CLA in the bloodstream can be directly incorporated into milk or trans-11
Vaccenic acid can be converted to *cis*-9, *trans*-11 CLA by the endogenous enzyme, Δ9 desaturase. CLA production is therefore influenced by long-chained PUFAs ingested in the diet and intermediates that escape the rumen during the process of biohydrogenation, especially vaccenic acid.

The amount of CLA produced in milk and meat is heavily dependent on diet. Numerous studies have reported increases in CLA in milk (Dhiman et al., 1999; Jahreis et al., 1997) and meat (French et al., 2000; Ponnampalam et al., 2006) with an increase in the amount of forages in the diet versus concentrates. A study by Elgersma et al. (2004) showed that significant changes in milk fat composition could be achieved in 14 days after transitioning from a grass based diet to a silage diet where short-chained FA increased and long-chained FAs and CLA decreased.

A study by Elgersma et al. (2003b) found feeding fresh cut grass decreased saturated fat (SFA) and increased PUFA and CLA in milk, but not as much as when cows were allowed to graze on pasture. This suggests that there is an effect of grazing *per se* in addition to the nutrient content of the grass itself. There has been some research on increasing CLA without grazing by feeding oil seeds or fish oil that are high in omega-3 FA. A study in which cows were fed marine algae, a source of long-chain FAs, found significant increases in CLA, trans-vaccenic acid, and another omega-3, docosahexaenoic acid (DHA), in milk from both the group that ingested unprotected algae and the group that ingested rumen protected algae over the control group (Franklin et al., 1999). However, to decrease biohydrogenation, these fats need to be coated or protected to increase rates of escape and pass unaltered into the intestines for absorption into the blood stream.
Sources of Omega-6

The major contributors to the increase of omega-6 in the Western diet are seed grains such as corn and soybeans, and seed oils such as corn, soybean, cottonseed, and sunflower. Most of these have a high omega-6 to omega-3 ratio compared to fats from fish or grass-fed meat and were not a part of the diet of our ancestors.

Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Corn Oil</th>
<th>Soybean Oil</th>
<th>Cottonseed Oil</th>
<th>Sunflower Oil</th>
<th>Canola Oil</th>
<th>Olive Oil</th>
<th>Wild Salmon</th>
<th>Grass-fed Beef</th>
<th>Grain-fed Beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:3 ratio</td>
<td>83:1</td>
<td>13:1</td>
<td>259:1</td>
<td>19:1</td>
<td>2:1</td>
<td>13:1</td>
<td>1:11</td>
<td>2:1</td>
<td>4:1</td>
</tr>
</tbody>
</table>


Along with the dietary changes in what we ingest directly, a change has also occurred over the past 150 years in what we feed to our livestock animals. Prior to 1850, almost all cattle in the United States were free ranged or pastured and took four to five years to reach market weight. Today, cattle are brought to slaughter at only 14 months and finished for the last 6 months on a feedlot where they are fed an energy dense ration of mainly corn, some corn silage, and hay. This diet offers extremely fast growth and deposition of intramuscular fat, known as marbling, a desired trait of meat in Western countries. Grain fed beef has been shown to have 3-4 times less omega-3 FA and an omega-6 to omega-3 ratio 2 times higher than that of beef from a grass fed animal (Cordain, 2002). The commonly used diets for feedlot cattle can cause negative health effects for the animal due to the acidic conditions it causes in the digestive system, especially the rumen (Nagaraja and Chengappa, 1998). In current animal production
systems, animals stay in the finishing feed-lot only long enough to gain sufficient weight and marbling before slaughter. While rumen microflora can adapt to some grain in the diet by altering certain populations of bacteria, high grain diets for an extended amount of time are not generally well tolerated by the rumen. The products of grain fermentation by microbes are volatile fatty acids (VFAs). High levels of these VFAs can lower the pH of the rumen enough to cause discomfort in the animal and reduce feed intake, further compounding the acidic environment in the rumen and possibly causing systemic metabolic acidosis.

The diets of dairy cattle have similarly changed by way of zero-grazing practices, where cows have been moved from the fields into buildings, are confined into pens or individual stalls, and meal-fed a ration that is formulated for maximum milk production. This diet is usually fed as a total mixed ration (TMR), where all components are mixed together and fed as a complete diet. This corn-based diet high in easily fermentable carbohydrates allows dairy cows to produce the highest quantity of milk possible and has helped make the Holstein breed the most common dairy breed in conventional production dairies. Comparing diets, Kolver and Muller (1998) found that Holsteins transitioned to an all pasture diet produced 33% less milk, 0.19 percentage points less protein, and weighed an average of 35kg less, than their herd mates that were kept on a total TMR diet. To demonstrate how these changes have affected milk production, the following data are from the United States Department of Agriculture (USDA) archives on milk production. Records show a U.S. average of 12.46 lbs/cow/day in 1931 versus 70 lbs/cow/day in 2011. The USDA uses all cows, including dry cows, in the milk production averages (US Department of Agriculture, National Agricultural Statistics
Service). This constitutes a nearly six fold increase in milk production over 80 years, due mostly to management (housed vs. pastured) and diet (grazed vs. TMR-fed), but also to breed development.

To manage milk production and animal health, numerous dairy nutrition professionals have recommended guidelines for feeding cattle. It has been suggested to feed high producing dairy cows in a confinement system no more than 60% of their diet as concentrate with a minimum of 40% forage, as a high grain diet may cause metabolic disorders such as acidosis, bloat, and laminitis (University of Minnesota Extension). The forage portion usually consists of majority of corn silage, followed by hay or haylage. There are many different kinds of feed that can be fed to the dairy cow, including cottonseed meal, corn gluten meal, sunflower meal, bakery waste, blood meal, poultry litter, and beet pulp.

The University of New Hampshire conventional dairy feeds a TMR with 55:45 forage to concentrate ratio and approximately 65% of the forage portion is corn silage. A common high producing dairy cow diet is approximately 75% corn, derived from both the grain concentrate portion and the whole plant silage portion.

Conversely, pastured dairy cows typically eat a high forage diet with forage to concentrate ratio closer to 75:25, where the forage portion includes fresh grass from pasture and supplemental and winter feed is more commonly grass haylage versus corn silage.

As with meat from beef cattle raised on pasture, dairy cows on pasture have shown a different FA composition in their milk as a result of the higher forage content and different FA ratio in their diet compared to TMR fed cows. A study comparing milk
from the five Nordic countries found higher omega-3 and a lower omega-6 FA in milk from Iceland compared to all the other countries tested, attributed to both the widespread feeding of fishmeal, which is high in omega-3 FA, and to pasturing over the summer months (Thorsdottir, 2004). Increasing PUFAs, due to increases in rumenic acid in milk fats was seen, from the lowest values in a lowland corn and concentrate feeding system, intermediate in lowland pasture, to the highest in highland pasture (Collomb et al. 2002).

**Diet Modification and Health**

Lower omega-6: omega-3 FA ratio in the diet, as well as intake of CLA has multiple health benefits for humans [4,6,7,8,9,10]. Cattle that graze ingest more omega-3 FAs than their confined counterparts, which modify the FA composition of their meat and milk. Among the studies in the literature reporting health effects of omega-3s and CLA, studies investigating the health effects on cows eating this type of diet are lacking. If animals that eat more omega-3 FAs experience the same anti-inflammatory effects and health benefits that human beings experience, these animals may experience fewer of the inflammatory and metabolic conditions than their TMR- fed counterparts.

Mastitis, inflammation of the mammary gland, is the number one cause of revenue loss to the dairy industry. Mastitis is caused when pathogens enter the teat canal, damage mammary tissues and cause an inflammatory response in the infected quarter. The extent of inflammation is measured by counting the somatic cells (white blood cells or leukocytes) in milk. While all milk contains white blood cells, levels increase dramatically with infection, damage the lining of the udder, and decrease both milk quantity and quality. The protein composition of milk changes as milk proteins decrease and serum proteins increase due to damaged mammary epithelial cells. This decreases
milk quality for cheese production, one of the most profitable milk products. Milk fat can also be degraded as leukocytes release enzymes which break down micelles to release free fatty acids (FFAs). Free fatty acids cause milk to become rancid, and the presence of excess FFAs can cause an unacceptable flavor (Akers, 2002).

Normal milk is defined as having a somatic cell count (SCC) less than 200,000 cells/mL. However SCC are commonly lower, especially in first lactation cows. A count greater than 200,000 cells/mL indicates probable infection. Other factors that contribute to a higher milk SCC include being late in the lactation cycle, old age, and environmental stress. Although these factors may cause slight elevations of SCC, such increases are usually inconsequential when compared to the elevation which results from infection (The National Mastitis Council Online, http://nmconline.org/dhiscc.html). Subclinical mastitis may not cause observable symptoms of infection but SCC may increase from normal. Counts above 400,000 cells/mL are likely caused by inflammation due to mastitis-causing organisms. Severe infections can cause counts to be much higher, greater than $1 \times 10^6$ cells/mL (Akers, 2002). At high SCC levels, milk may be clotted, stringy, discolored, bloody, thin, or translucent, all of which are unacceptable for Grade A fluid milk. Chronically infected cows may suffer decreased production and decreased milk quality depending on intramammary damage. In some cases, production may never recover to previous levels and the animal may be culled from the herd. As environmental stress can be a factor in increasing SCC, measuring stress as well as immune status can give a producer an idea of a cow’s susceptibility to future infections.

Leg and hoof health affect TMR raised cattle more than pastured cattle (Rutherford et al, 2008), not only because of the hard material on which they spend most
of their time (concrete, slotted floors, stalls, etc. versus pasture), but also from injuries from these surfaces and equipment, lack of exercise, confined spaces, and a higher grain diet. A high grain diet has been associated with laminitis, an inflammation of the lamina deep to the hoof wall. Dairy cattle can also suffer reproductive problems such as retained placenta or metritis after calving. These conditions may contribute to infertility in subsequent breeding attempts. Mastitis, lameness, reproductive problems, and metabolic conditions such as acidosis and bloat lead to profit losses from reduced production, increased veterinary and treatment costs, increased time and labor, and sometimes require culling of the animal. It is advantageous to keep an animal healthy and productive for as long as possible to maintain a profitable and quality product.

More research is needed to evaluate the relationship between diet and disease in dairy cattle.

**Health Markers and Stress**

Acute stress responses are normal and typically the body soon returns to homeostasis once the stressor is removed. Chronic stress, where the stressor remains for a long period of time, is more detrimental to the health and welfare of the animal. Chronic stress may cause decreased function of the immune system and increased risk of disease. Markers of both acute and chronic stress are used to evaluate the health condition and stress of cattle.

The most general and least invasive indicators are behavioral and performance-related. Some behavioral indicators for acute stress are vocalization, restlessness, aggression, pain response, and cessation of forward movement (Broom, 2003). For
chronic stress, another behavioral indicator is stereotypy, the persistent repetition of an act for no obvious purpose (Broom, 2006). Performance indicators for acute stress include reduction in milk yield, interference with milk ejection, or milk let-down (Rushen et al. 2001). Milk samples can also be tested for SCC, which as mentioned above, can indicate infection in the mammary gland. Milk urea nitrogen (MUN) is an established reflection of blood urea nitrogen, which can decrease reproductive success at high levels (>18mg/dL) and may be indicative of having too much protein in the diet. Performance indicators of chronic stress include reduction in the ability to grow, breed and produce (e.g. milk, meat), a reduction in body condition score (BCS) or weight (Bertoni, 1999), and an increase in the incidence of infectious and metabolic diseases (Broom, 2006).

Clinically relevant markers of both acute and chronic stress can be measured in the blood. White blood cells in whole blood are used as a measure of systemic inflammation and stress, especially the neutrophil/lymphocyte ratio (Zahorec, 2001). The hormone cortisol (hydrocortisone) has been examined in both acute and chronic stress measurements. Cortisol is a steroid hormone, specifically a glucocorticoid that is produced by the adrenal gland cortex, secreted throughout the day with diurnal variations. Cortisol levels are usually at their highest in the morning and at their lowest between midnight and 4 am. The adrenal gland is stimulated to release cortisol under conditions of stress, or when blood glucocorticoid levels are low. The main functions of cortisol are to: 1) increase blood sugar through gluconeogenesis while sparing it from uptake by most tissues, 2) stimulate fat, protein, and carbohydrate metabolism, and 3) suppress the immune system, preventing the release of substances that cause inflammation. This last function is particularly important because during a stressful event, it is imperative that
cortisol redirect resources and energy, enabling the animal to survive during the emergency. However, prolonged elevated cortisol levels and the resulting immunosuppression may be harmful as it increases the chances of infection and decreases the body’s ability to heal.

Cortisol has been used as a marker for acute stress in cows because of its quick response to stressors such as transportation (Lay et al., 1996), re-grouping/re-penning (Friend et al., 1977), or temperature, where it was found to return to near basal levels after only 5 minutes of removal of thermal exposure (Abilay et al., 1975). In situations of chronic or repeated stress, cortisol levels remain high which is evidence of a failure to restore homeostasis. Higher basal cortisol levels in individuals can increase sensitivity and reaction to future stresses. Broom (1988) observed a hyper-reactive response after adrenocorticotropic hormone (ACTH) challenge in cows that were under chronic stress, supporting a relationship between high basal levels and a chronic stressful condition.

Cortisol can be a highly variable hormone. Cortisol levels follow a diurnal rhythm, so care must be taken to collect samples from the same time of day for comparison. Basal levels can vary greatly per individual yet still follow a consistent pattern within the individual. In some studies, sampling procedures themselves caused a rise in cortisol as the animal can be stressed during handling. Reference values for normal baseline cortisol levels in cattle are still to be established and values in the literature vary widely. There are breed differences, individual variation, and different assays for a variety of sources including: plasma, serum, saliva, and feces. Hopster et al., (1999) measured values of plasma cortisol by fluoroimmunoassay in Holstein Friesian dairy cows after repeated jugular venipuncture and reported values ranging from 1.43-22.6
ng/mL. Lefcourt et al., (1993) measured plasma cortisol levels by radioimmunoassay in Holstein dairy cows at 15 minute intervals over 48 hours and found a weak circadian rhythm with minimum of 3.1 ng/mL at 1800 hr and maximum of 4.5 ng/mL at 0530 hr. However, they found a strong ultradian rhythm of 120 minutes with a peak to trough amplitude of 1-17 ng/mL. Another study by West et al., (1991) investigated the effects of bovine somatotropin (bST) on serum cortisol versus control in Holstein and Jersey dairy cows. The two treatment groups were not significantly different from controls at 9.96 ng/mL (0 g/day bST) and 8.13 ng/mL (20 mg/day bST). Serum cortisol measured by enzyme-linked immunosorbent assay (ELISA) in Holstein calves before and after weaning was found to increase from 6.74 ng/mL to 10.21 ng/mL one day post weaning, to 17.10 ng/mL three days post-weaning (Myung-Hoo Kim et al., 2010). A study measuring the effects of pre-slaughter handling of beef cattle by Cockran and Corley (1991) reported maximum values, determined by radioimmunoassay, of 162 ng/mL in plasma in a steer that was upside down in a chute. Because of the variation throughout the literature, the researchers involved with this project have decided that cortisol values be evaluated by comparing baseline values to values after a challenge or by repeated measures, versus comparing to previously published values.

Other biochemical markers of health, inflammation, and chronic stress include the acute phase proteins. Acute phase proteins are released from the liver under inflammatory conditions. Haptoglobin (Hp) is used for clinical evaluation of inflammation, infection, and stress. Haptoglobin is produced primarily by hepatocytes but also by skin, liver, lung, and kidney. In some species, including humans, mice, and cows, it is produced in adipose tissue as well. Haptoglobin binds free hemoglobin when red blood cells are lysed. The
binding of Hp inhibits its oxidative activity and also sequesters the iron within hemoglobin, preventing iron-utilizing bacteria from using the iron to proliferate. Because it is a positive acute phase protein, any inflammatory process e.g., infection, chronic stress, injury, or allergy, may cause an increase in haptoglobin levels. Haptoglobin levels can be undetectable in healthy cattle and increase 50 to 100 times during an acute phase response. Although acute phase proteins are traditionally considered to be the markers of inflammation and infection, there is also a link between non-inflammatory stress and the acute phase protein response (Murata et al., 2007). Serum haptoglobin levels in Holstein calves increased from 7.33 µg/mL before weaning to 32.27µg/mL one day post weaning, to 77.76 µg/mL at three days post weaning (Myung-Hoo Kim et al., 2010), showing that this acute-phase protein responds to psychological as well as physical and physiological stresses. Mastitis induced with *Staphylococcus aureus* in Swedish Red and White and Swedish Holstein cows caused a significant increase in serum Hp in the acute phase after infection from 52.5 mg/L ± 6.2 mg/L to 945.5 mg/L ± 651.1 mg/L (Grönlund et al., 2003). Nazifi et al., (2008), found the concentration of serum Hp in healthy cattle to be 0.20 ± 0.03 mg/mL and 0.80 ± 0.12 mg/mL in cows with clinical mastitis.

In conclusion, PUFAs, specifically omega-3 FAs, and CLA, have been found to decrease the physiological response to stress and infection. The question proposed in this study is whether dairy cattle managed on pasture, will exhibit a different health profile than those managed in confinement and fed a TMR. In the following study, clinical markers of health were studied from Jersey dairy cows in both pastured and confined management systems.
CHAPTER II

MATERIALS AND METHODS

Experimental Design

Two groups of 9 Jersey dairy cows matched for age and number of lactation cycles were established at the UNH Organic Dairy in Lee, NH and the conventional Fairchild Teaching and Research Dairy in Durham, NH. The organic group was managed on pasture eating a high forage diet, and the conventional group was managed in confinement eating a TMR with corn silage as the major forage. The two groups were compared for measurements of health and inflammation before, during, and after the pasture season. General markers of health included body weights, BCS, and milk production data (yield, MUN, SCC, and other milk components). Clinical markers of health measured were serum cortisol and haptoglobin. Measurements were compared within groups over time and between groups over time.

Objective

The objective of this study is to investigate whether Jersey dairy cows on pasture, eating a diet possibly higher in omega-3 and lower in omega-6 will differ in general and clinical markers of health than those of Jersey dairy cows raised indoors and fed a TMR.
**Hypothesis**

Dairy cows managed on pasture will demonstrate a healthier condition than dairy cows managed in confinement and fed a TMR.

**Animal Management**

Nine Jersey dairy cows were used from the Jersey herd at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm located at the farm in Lee, NH. Cows were out on pasture from May 16, 2011 to October 19, 2011 where they grazed typical cool season, New England pasture grasses such as orchard grass, reed canary grass, timothy, perennial ryegrass, white and red clover, birdsfoot trefoil, etc. Cows were supplemented twice a day after milking at approximately 0500 hr and 1500 hr, with organic baleage top-dressed with organic grain consisting of: organic corn meal, 5.64 lbs/day DM, organic soy meal, 1.373 lbs/day DM, organic barely, 2.677 lbs/day DM, organic roasted soy, 1.012 lbs/day DM, organic mids, 0.621 lbs/day, and vitamins and minerals, 0.914 lbs/day DM (Morrison’s Custom Feeds, Inc. Barnet, VT). Water was provided in the fields *ad libitum* via a livestock tub with float controlled water from a hose line. The total diet was approximately 75:25, forage to concentrate. When not on pasture, late November through early May, cows were housed in a free-stall, bed pack, open barn with constant access to the outdoors and water and organic feed baleage, provided *ad libitum*. In accordance to USDA organic standards, cows are on pasture a minimum of 120 days out of the year, have access to the outdoors all year except in severe weather, and may not be treated with antibiotics or other non-approved drugs.
Nine Jersey cows were matched to the above organic cows by age, parity and stage of lactation, and housed at the University of New Hampshire Fairchild Dairy Teaching and Research Center in Durham, NH. Cows were managed along with the existing Holstein herd and housed in a free-stall pen with padded stalls, concrete slatted floors, a head-gate feed bunk and water and mineral lick provided ad libitum. Cows were milked at 0530hr and 1730hr approximately and fed twice per day a 55:45 forage to concentrate ratio TMR consisting of corn silage, 17.5 lbs/day DM, energy mix containing, steam flaked corn, ground corn, citrus pulp, beet pulp, soybean hulls, 12.74 lbs/day DM, alfalfa hay, 3.58 lbs/day DM, haylage, 5.5 lbs/day DM, and minerals and vitamins, urea, 9.92 lbs/day DM. Cows were treated for infection per standard practices of animal health with antibiotics given as needed.

**Sample Collection**

The following table shows the respective dates corresponding with sample number when data is displayed by sample number and not by date.

<table>
<thead>
<tr>
<th>Blood Sampling Dates</th>
<th>Milk Sampling Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample#</strong></td>
<td><strong>Organic</strong></td>
</tr>
</tbody>
</table>
Animal weights were taken once per month starting one week before the pasture season started at Burley-Demeritt farm, following afternoon milking. The UNH Organic Dairy Research Farm used a SmartScale500 (Gallagher, USA) and the Fairchild Dairy used a Cardinal Scale (Cardinal Scale Manufacturing Co., Webb City, MO). Body condition scores were taken monthly by three UNH faculty or staff using a score of 1-5 following the method of Edmonson et al., (1989) and averaged for that month.

Milk samples were collected starting one week before the cows went out to pasture and then every two weeks at regular milking times, 0430 hr and 1500 hr at the Burley-Demeritt organic farm and 0530 hr and 1730 hr at the Fairchild dairy. Milk was sampled by automatic sampling cups (Westphalia Separator, Northvale, NY) over the entire milking and after agitation, aliquots of 40mL were placed into milk collection vials containing Broad Spectrum Microtabs II (D&F Control Systems, Norwood, MA) to be sent to Dairy One, Inc. (Ithaca, NY) for analysis of percent fat, percent of true protein, SCC, MUN, lactose, and total solids. Fat and energy corrected milk (FCM) and (ECM), were calculated and adjusts the pounds of milk to a standard fat test, usually to 3.5% milk fat. The formula to calculate FCM is: 3.5% FCM = (milk lbs x .432) + (Fat lbs x 16.216). The formula for ECM is = (0.327 x milk lbs) + (12.95 x fat lbs) + (7.65 x protein lbs) and determines the amount of energy in the milk based upon milk yield, fat, and protein. It adjusts yield to 3.5% fat and 3.2% protein.

An equal volume of milk sample was placed into sterile polypropylene 50 mL plastic centrifuge tubes (Fisherbrand, Fisher Healthcare, Houston, TX) to be lyophilized. Milk from AM and PM was composited in proportions determined by milk yield of that milking. Milk samples for were frozen by shell freezing in glass vacuum bottles in dry ice...
and a 10:1 ratio of ethanol/acetone bath then lyophilized using a Labconco Freeze Dryer 5 (Labconco, Kansas City, MO) and stored at -80°C for future analysis.

Blood samples were taken every two weeks, on alternate weeks from milk collection, at approximately 1600 hrs. Blood was taken from the coccygeal (tail) vein using 1”, 21 gauge needles (Kendall Monoject) and collected into 10ml Vacutainer™ tubes with clot activator for serum and also into 10ml Vacutainer™ tubes with K2 EDTA (BD, Franklin, NJ) for plasma. Whole blood from EDTA tubes was used to make smears on frosted slides which were stained with Wright’s stain for differential white blood cell counts. Both clot and EDTA tubes were centrifuged at 3300 rpm for 20 minutes. Plasma and serum were collected into 2 mL Microtubes (Sarstedt, Newton, NC) in aliquots of 3, and stored at -80°C until analyzed. Red blood cells from clot tubes with serum removed were also stored in the original vacutainer at -80°C for future work. All experimental procedures were reviewed and approved by the UNH Institutional Animal Care and Use Committee (IACUC #120506).

**Blood Analyses**

All serum samples were analyzed in duplicate and results averaged. Serum haptoglobin was measured using a commercial bovine haptoglobin ELISA (Life Diagnostics, Inc., West Chester, PA) with samples diluted 2000 fold per manufacturer’s recommendation. Serum cortisol was detected using a human cortisol ELISA (BioVendor LLC, Candler, NC). All ELISA kits were used according to the manufacturer’s instructions. Standard curves were established by running provided standards in duplicate. Absorbance was read using a EL 800 Plate Reader (Biotek Instruments, Inc.,
Winooski, VT) and collected by Gen5 Microplate Data Collection & Analysis software (Biotek, Inc.).

**Statistical Analyses**

Changes within one farm over time were analyzed using repeated measures with time as the repeat variable (SAS Inst. Inc., Cary, NC). Between-farm comparisons were analyzed using a non-Metric Multidimensional Scaling (nMDS) plot. The nMDS plot is an ordination technique in which cells, i.e. points, are plotted relative to their similarity to one another. Somatic cell counts, cortisol and haptoglobin levels between conventional and organic farms were plotted using a Bray-Curtis similarity index and a 1-way analysis of similarities (ANOSIM) was used to examine differences between farms. Analyses and graphs were generated using Primer 6.0 (Primer-E Ltd. Lutton, Ivybridge, UK). Effects were considered significant at p< 0.05. Three time points during the pasture season were selected and a 1-way ANOSIM performed. These points were chosen for an ANOSIM because they had an equal number of samples for each group and represented the early, mid, and late summer.

**Weather Data**

The follow weather data was compiled from Pease International Trade port at Pease Air Force Base in Portsmouth, NH and accessed from the National Oceanic and Atmospheric Administration (NOAA) website. The temperature-humidity index (THI) is shown below. The THI is given in degrees Fahrenheit and is a measure of how hot it feels when relative humidity is factored with the actual, dry air temperature (US National Weather Service).
CHAPTER III

RESULTS

Body Weights and Condition Scores

Over the course of the study, the average weight for the organic study cows was 902 lbs and the average weight of the conventional study cows was 1050 lbs. On average, the organic group had a 13.2% difference between their maximum and minimum weights and the conventional group had an 8.5% difference.

Body condition scores (BCS) did not differ between farms, with an average over the entire study period from April to November, of 2.9 for the organic and 3.0 for the
conventional. Average herd BCS for any time point over the study ranged from 2.8 to 3.2. See discussion on BCS.

Graph 2
Conventional Cow Weights

Graph 3
Organic Cow Weights
Milk Production

Average milk production over the course of the study was 44.7 lbs/day for the conventional farm and 30.8 lbs/day for the organic farm. Milk yield changed significantly over time within both farms, p<.01.

Milk production calculated as fat corrected milk had means of 59.9 lbs/day and 37.4 lbs/day for the conventional and organic farms, respectively. Fat corrected milk changed significantly over time within both farms, p<.01.

Milk production calculated as energy corrected milk had means of 60.8 lbs/day and 37.1 lbs/day for conventional and organic farms, respectively. Energy corrected milk changed significantly over time within both farms, p<.01. Energy and fat corrected milk equations were sourced by Dairy Records Management Systems (Dairy Records Management Systems Online, www.drms.org/PDF/materials/glossary.pdf).
Milk true protein represented as a percent of milk weight for the conventional farm was 4.1% versus the organic farm at 3.6%. Milk true protein shown as lbs/day was 1.8 lbs/day for the conventional farm, whereas the organic farm had a mean of 1.1
lbs/day. Milk protein as both a percent of milk and lbs/day, changed significantly over time within farms, p<.01.

Graph 7
Percent Milk Protein

Graph 8
Milk True Protein

Milk fat represented as a percent of milk weight for the conventional farm had a mean of 5.8% versus the organic farm at 4.9%. Milk fat as lbs/day for the conventional
farm was a mean of 2.5 lbs/day, whereas the organic farm had a mean of 1.5 lbs/day. Milk fat as percent of milk and lbs/day, changed significantly over time within both farms, p<.01.

Graph 9
Percent Milk Fat

Graph 10
Milk Fat Pounds per Day
Milk lactose as a percent of milk yield was a mean of 4.6% for the conventional farm and 4.7% for the organic farm. Milk lactose in pounds per day yielded means of 2.04 lbs/day for the conventional and 1.45 lbs/day for the organic. Milk lactose as a percent of milk and lbs/day, changed significantly over time within both farms, p<.01.

Graph 11
Percent Milk Lactose

Graph 12
Milk Lactose Pounds per Day
Milk total solids as a percent of yield gave mean percentages of 15.3% at the conventional farm and 14.1% at the organic farm. Milk total solids as lbs/day gave means of 6.4lbs and 4.3lbs for the conventional and organic farms, respectively. Milk solids as a percent of milk and as lbs/day, changed significantly over time within both farms, p<.01.

**Graph 13**
Percent Total Milk Solids

**Graph 14**
Total Milk Solids Pounds per Day
Milk urea nitrogen measured in mg/dL, averaged 11.7 and 11.8 mg/dL at the conventional and organic, respectively. Measured in pounds per day, the means were 5.4 and 3.8 lbs/day at the conventional and organic, respectively. Milk urea nitrogen changed significantly over time within both farms, p<.01.

Graph 15
MUN (mg/dl)

Graph 16
MUN Pounds per Day
Table 3

Conventional Group Milk Components

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<tr>
<th>Week</th>
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<th>SED</th>
<th>Fat lbs/d</th>
<th>SED</th>
<th>Prot %</th>
<th>SED</th>
<th>Prot lbs/d</th>
<th>SED</th>
<th>MUN mg/dl</th>
<th>SED</th>
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*Means within columns with common superscripts are not significantly different (P<.05).
Table 3: Conventional Group Milk Components Over Time Con’t

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*Means within columns with common superscripts are not significantly different (P<.05).
Table 4
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*Means within columns with common superscripts are not significantly different (P<.05).
Table 4: Organic Group Milk Components Over Time Con’t

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*Means within columns with common superscripts are not significantly different (P<.05).
Average milk SCC of the conventional group exceeded the threshold characterizing normal milk of 200,000 cells/mL on September 1 and September 30, 2011. A 1-way ANOVA found SCC did not change significantly over time in either the conventional group (p=.5) or the organic group (p=.98). The following graphs show individual milk SCC from the conventional and pasture group cows over the course of the study. The dashed line denotes the cell count limit of normal milk. The conventional group had a mean milk SCC over the entire study of 113.8 x 10^3 cells/mL. The organic group had mean milk SCC over the entire study of 57.7 x 10^3 cells/mL.
Graph 18
Milk Somatic Cell Counts Conventional Group

Graph 19
Milk Somatic Cell Counts Pasture Group
Somatic cell counts on both farms over the course of the study were analyzed in a Bray Curtis Similarity Matrix. The following graph spatially shows the similarity of the data points between farms. A One-Way Analysis of Similarities (ANOSIM) was performed and found SCC between farms over the course of the study were not significantly different, $p=.864$ and $r=0$.

**Graph 20**

**SCC Between Farm Similarity Distributions**
**Blood Markers**

Serum cortisol levels in the conventional group had a mean value of 93.64 ng/mL, p<.01.

The organic group had a mean of 126.78 ng/mL, p<.01.

**Graph 21**
Serum Cortisol - Conventional

![Graph 21](image)

**Graph 22**
Serum Cortisol - Organic

![Graph 22](image)
Serum Haptoglobin levels in the conventional group had a mean value of 0.008 mg/mL, p<.01, and organic group had a mean value of 0.006 mg/mL, p<.01.

Graph 23
Serum Haptoglobin-Conventional

Graph 24
Serum Haptoglobin-Organic
The following graphs display data points for cortisol and haptoglobin in a Bray Curtis Similarity Matrix. It shows the similarity of the data points between farms at a single time point. A One-Way Analysis of Similarities (ANOSIM) was performed with farm as a factor. For cortisol, farm was a significant factor at time 1, June 15, 2011, \( p = 0.032 \) and \( r = 0.183 \).

**Graph 25**

*Cortisol Between Farm Similarity Distributions*

*June 15, 2011 (time 1)*
Farm was not a significant factor for cortisol levels at time 2, August 9, 2011, p=.299 and r=.02.
Farm was a significant factor for cortisol levels at time 3, September 6, 2011, p=.001 and r=.527.
Farm was a significant factor for cortisol levels across all three time points, $p=.001$ and $r=.219$. 
Farm was a not a significant factor for haptoglobin levels at time 1, June 15, 2011, p=.158 and r=.056.
Farm was not a significant factor for haptoglobin levels at time 2, August 9, 2011, $p=.114$ and $r=.076$. 
Farm was not a significant factor for haptoglobin levels at time 3, September 6, 2011, p=.859 and r=0.
Farm was not a significant factor for haptoglobin levels across all three time points with $p=.191$ and $r=.016$. 
CHAPTER IV

DISCUSSION

Cortisol

Cortisol is a quick responding hormone that is activated under stress to restore homeostasis and to conserve resources during a stressful situation. Levels usually return to their baseline values after the stressor is removed or after a period of acclimation if the stressor is not extreme. However, prolonged cortisol secretion due to persistent stress may result in physiological and behavioral changes that affect both production and animal health.

The stress hormone cortisol was used as one indicator of overall health as it is a well-established marker of stress in numerous other studies involving both domestic and feral animals. Various forms of stress, such as heat, social status, crowding, disease, malaise, etc., can raise cortisol levels and if the stress is chronic, the levels of cortisol can remain elevated. Cortisol levels changed significantly over time at each farm and were found to be significantly different between farms over time (graph 28). The conventional group had a lower average cortisol over the course of the study than the pastured group (graphs 21 and 22). Cortisol levels found in the pastured group were higher at every time point except the first time point, on May 17, 2011. Cortisol levels in the organic group trended with the THI, rising in accordance with THI, an indication of possible heat stress. Acute heat stress has been shown to significantly increase plasma cortisol levels from baseline with a subsequent decrease in cortisol levels after the heat stress was alleviated.
(Abilay et al., 1975). It is generally regarded that dairy cattle will start to experience stress at a THI of 72°F with higher producing cows more affected because of their higher feed intake (Jones and Stallings, 1999). Cortisol levels in the organic group trended with the THI yet did not decrease as rapidly when the THI decreased throughout late July and on into November. The higher cortisol levels regardless of lower THI may have also been attributed to stress caused by flies. Cows on pasture were observed to have numerous flies on them, especially on hot, windless days. Cattle bothered by insects will exhibit head tossing, foot stomping, skin twitching, and tail switching behaviors and spend less time grazing. Dry matter intake may not be affected directly by decreased grazing time as animals are eating faster, not necessarily less. However, fly stress may also cause bunching of the herd, which increases heat stress as animals stand closer together and lie down less, compounding the stress from both the heat and the flies (Taylor et al., 2012). Physiological stress from heat, as well as from insects, and the possible compounding effect of the two, may be factors in the higher cortisol levels measured in the organic group.

The conventional group was never in direct sunlight, being housed indoors, and had fans constantly moving air and restricting flies when temperatures exceeded 75°F. The cortisol levels at the conventional farm did not trend as closely with THI as the organic group likely due to the better controlled climate at the conventional farm. Increases in cortisol levels during the months of October and November at both farms are probably not due to THI.

The organic group had consistently higher average cortisol levels than the conventional group and trended closely with THI most likely due to exposure to the
outdoors and insects. The increases in cortisol seen at both farms in the early fall cannot be directly attributed to any one factor but could be correlated with the start of the academic year (August 29, 2011) when a sudden increase in new personnel, visitors, and on-farm traffic may have caused some stress as cows are sensitive to changes in routine and environment. With regards to cortisol levels, the organic group showed a chronically stressed state compared to the conventional group over most of the study period and cortisol levels did not decrease as rapidly once the stress of a high THI was removed as was seen in other studies (Abilay et al., 1975). Heat and insect stress are likely contributors to chronically higher stress levels in pastured versus confined dairy cows.

**Haptoglobin**

Serum haptoglobin levels changed significantly within both farms over time. The conventional group had a slightly higher mean than the organic group, .008 mg/mL versus .006 mg/mL, yet both farms had values indicative of healthy cattle. Haptoglobin levels can be undetectable in healthy cattle and increase 50 to 100 times during an acute response to infection or injury. There was no significant difference in haptoglobin levels between farms, p=.191. The same three time points that were used to compare cortisol levels were used to compare haptoglobin levels. There was no significant difference in haptoglobin levels between farms at any of the three time points, June 15\(^{th}\), August 9\(^{th}\), or September 6, 2011. Because haptoglobin has been known to increase with stressful events such as weaning (Myung-Hoo Kim, 2010), the higher levels at both farms during the months of June and July may have been influenced by heat stress, although the values were still indicative of healthy cattle. One individual in the conventional group had
values 2, 3 and 17 times higher than the other animals in that group at five different time points (graph 23). This animal had a hock abscess that would regress and flare again over time. While this animal may be an outlier for the group, her values show the effects of a chronic injury on haptoglobin levels. If this animal is removed from the data as an outlier, the mean for the conventional group over the course of the study decreases from .008 mg/mL to .007 mg/mL. Because the removal of this individual did not change the significance, she was left in the data set to show the effects of a chronic injury on haptoglobin levels.

Neither pasture nor confinement seemed to have affected haptoglobin levels significantly and both groups exhibited values indicative of healthy cattle, although minor increases were seen to trend with the THI during June and July.

**Milk Somatic Cell Counts**

Milk SCCs are indicative of sanitary practices in both the immediate living environment of the animals and in the milking equipment. However, there are many factors that may cause individuals to have higher levels than others under normal, infection-free conditions. Stress may also affect the chances of infection because of the effects of cortisol on the immune system. Milk SSCs from this study show the conventional group with a higher average count over the study compared to the organic group. The conventional group had 4 individual cows that had counts greater than 200 x 10^3 cells/mL at 6 different time points (graph 18). The organic group had 2 individual cows that had counts greater than 200 x 10^3 cells/mL on 10 different time points (graph 19). The conventional group had greater variation although some individuals did remain with a low count over the course of the study. Despite higher cortisol levels from heat...
and insect stress, as well as being outside where exposure to dirt, mud, and rain usually contribute to higher SCCs, the organic group had half the count of the conventional group. High cell counts decrease the quality of the milk and well as subsequent milk yields, so SCC is extremely important for producers. The data show that regardless of higher chronic stress, the organic group did not have significantly different milk SCC and had numerically lower means at every time point except three.

**Milk Data**

The conventional dairy group produced 37% more milk (lb/day) than the organic cows over the course of the study. This difference increases to 47% when looking at fat and energy corrected milk. Percent milk protein and fat were 13% and 17% higher respectively in the conventional group while percent milk lactose was 2% higher in the organic group. Milk fat synthesis can be depressed when cows eat a diet high in PUFAs, which may a reason for the low milk fat in the pastured group. Lactose is the most consistent component in milk so a small difference was not unexpected. One likely reason that milk yield was higher in the conventional group is that the individuals in the group were larger. The conventional group had an average weight of 1050 lbs while the organic group had an average weight of 902 lbs. The largest weight difference was on August 31, when the conventional group averaged 202 lbs more in body weight than the organic group (graphs 2 and 3). Another reason for the production differences may be dietary. The diet of the conventional group has more rapidly digestible nutrients with higher energy content as well as being precisely formulated and dispensed without large seasonal or managerial variations. Less energy is used by the conventional group in obtaining feed by walking a short distance to the bunk than is used by the organic group.
while grazing. Diets were formulated to meet a net energy of lactation (NEL) of 0.78 Mcal/lb for the conventional group and 0.74 Mcal/lb for the organic group. While this small difference could possibly affect milk production, it is more likely that the organic diet fluctuated around this number since it is harder to control the nutrient intake of an animal on pasture. Also, since intake was not recorded in this study, it may be possible that the organic group’s intake decreased during the periods of high THI and stress, reducing the amount of nutrients available for milk production.

Milk urea nitrogen was more consistent in the conventional group ranging from 9.65 to 13.08 mg/dL while the organic group ranged from 7.04 to 15.83 mg/dL with greater variation noted throughout the study period (graph 15). The greater variability of MUN in the organic group may be attributed to the variable protein content of grasses throughout the spring and summer. Young grasses in spring and early summer, in the vegetative state, are much higher in protein that at maturity. Higher protein diets can produce excess nitrogen that is secreted in the milk (MUN) and blood (BUN). A high MUN can be correlated with BUN and a high BUN is associated with reproductive problems since urea and ammonia are toxic to sperm and embryos. While reproductive fitness was not measured in this study, variability in protein content of pasture is an added management aspect that grass-fed producers must be conscious of while conventional producers do not experience such great variability where diet is formulated and more easily controlled.

**Body Weights**

Body weights fluctuated over the course of the study in both groups though the organic group averaged a 13.2% change in body weight between the highest and lowest
weight, while the conventional group averaged an 8.5% change. This could be a reflection of the consistency of the conventional TMR diet versus the inconsistency of the pasture diet. The effects of smaller and fluctuating body weights could be one reason for the differences in milk production between the two groups. Body weights within groups also were closer in the conventional group with a difference between the heaviest cow average and the lowest cow average of 110 lbs. The same comparison in the organic group yielded a difference of 285.5 lbs. A better experimental design may have matched animals by body weight, as well as by parity and stage of lactation.

CHAPTER V

CONCLUSIONS

In conclusion, pastured cows were not found to be significantly different in markers of health than conventionally raised cows fed a TMR, based on Hp levels and SCCs. Pastured cows also did demonstrate a significantly higher indication of stress than their confined counterparts, based on cortisol levels. This is most likely due to exposure to heat and humidity. Results from this study reinforce the impact of heat, humidity, and insects on stress levels and production. Producers currently, or considering, pasturing dairy cows should be conscious of the impacts of heat and fly stress and plan accordingly for shade and fly control.

All cows in this study had haptoglobin levels within normal, healthy limits which may indicate that a higher stress level does not necessarily leave an animal more susceptible to disease depending on the management system. A study utilizing more
animals is needed to obtain clearer conclusions based on results from a stronger statistical model.

It appears that exposure to the elements, most importantly heat and humidity, and stress from insects was the main cause of the elevated cortisol levels in the pastured group. While cortisol levels were lower in the conventional group, other indicators of health may bring greater insight into the health status of these animals.

The somatic cell counts were consistently lower in the organic group on average yet this may have been a coincidence since cleanliness scores and other measurements of sanitation were not measured.

Chronically high stress can cause nutrients to be partitioned away from tissues responsible for growth and milk production and heat stress in particular has been shown to decrease dry matter intake and decrease milk yield (West, 2003). The difference in production between the two groups, while certainly due in part to the difference in size of individuals within groups, as well as the conventional group receiving more grain, may also have been caused by heat stress experienced by cows out on pasture.

While this study was designed to observe the effects of diet and management systems on health, there are inherently many uncontrolled variables within farm systems. Feeding irregularities, weather, and varying management practices within systems, made it difficult to isolate differences in stress and health to diet and management exclusively. More studies, with a greater number of animals, are necessary to determine a stronger relationship between management system and animal health.
LIST OF REFERENCES


11-Jul-2012

Foxall, Thomas L
Molecular, Cellular & Biomedical Sciences, Kendall Hall Rm 519
Durham, NH 03824

IACUC #: 120506
Project: Markers of Health Status in Pasture-Fed vs. Total Mixed Ration-Fed Dairy Cows
Category: C
Approval Date: 09-Jul-2012

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jill A. McGaughy, Ph.D.
Chair

cc: File