METHODS OF PROGRAMMING INCREASED MILK PRODUCTION AND ITS RELATIONSHIP WITH SUSTAINABILITY OF THE DAIRY INDUSTRY

by

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B.S., Michigan State University, 2010
M.S., University of Minnesota, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences & Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

High levels of milk production has been and will continue to be a priority for the global dairy industry. Non-steroidal antiinflammatory drugs administered to dairy cattle following calving can be an effective way of programming higher milk production for the entirety of lactation. When dairy cattle on a commercial dairy received either sodium salicylate or meloxicam following calving, they responded with increased whole-lactation milk production, which was driven by higher daily milk yields following the seventh week of lactation. When dairy cattle at a research dairy received sodium salicylate following calving, they did not show the same increase in milk production but feed intake, feeding behavior, and blood parameters were altered for an extended period of time. The response to treatment was largely dependent on the parity of the animal. In an effort to determine whether re-programming of the rumen environment could explain these findings, sodium salicylate was administered to batch cultures of rumen fluid, and as a result, fermentation was inhibited. When substrate was fermented in rumen fluid from heifers who had been dosed with sodium salicylate, fermentation was inhibited for an extended period of time following sodium salicylate administration. Beyond the use of compounds such as these, other factors can program lactation for higher milk production, including the gender of the calf. Analysis of lactation records from the US has indicated that cows produce more milk following the birth of a heifer calf compared to a bull. With further research, findings such as these can provide farmers with more tools for improving productivity and lead to the sustainability of the dairy industry as a whole.

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Dr. Barry Bradford

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"Not that we are sufficient in ourselves to claim anything as coming from us, but our sufficiency is from God." (II Corinthians 3:5-6)

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My students have been my great joy and my great challenge through my career at this university, but I wouldn't trade you for anything. (Most of you, anyway.)

Dedication

Dedicated in loving memory to Tom, Ron, and Hayes (“Carl”) Hobolth

“Man’s chief end is to glorify God and to enjoy Him forever.”

Chapter 1 - Review of the literature: The relationship between increased milk production and sustainability of the dairy industry

A. J. Carpenter

ABSTRACT

The human race faces the challenge of feeding a growing population while maintaining the limited resources that sustain them. As dairy producers push progressively for higher levels of production, it comes at a cost of resources. This review will address whether progressively increasing levels of milk production harms, enhances, or has no effect on the sustainability of dairy production on a global scale. The three particular facets of sustainability analyzed are satisfaction of human food needs, enhancement of environmental quality, and sustainment of economic viability of farm operations. Even now, with the current population at approximately 7 billion and not the 9 billion that is projected by 2050, a large proportion of the world's population is at some level hungry, undernourished, or both. With its high nutritional quality, milk and products made from milk are important players in the prevention or treatment of malnutrition. Increasing the output of milk produced can help to sustain the nutritional needs of the world. Despite this, the dairy industry has come under fire for its contributions to climate change, particularly due to production of high levels of enteric gasses such as methane. It is true that on a basis of total emissions, dairy cattle are producing more greenhouse gasses than they were at the beginning of the twentieth century; however, as milk production increases within a set period of time, the cost of maintenance per unit of milk produced is decreased. This concept is known as "the dilution of maintenance," and several authors have demonstrated that as milk production per cow increases, the environmental burden per unit of milk produced is mitigated, contributing to the overall sustainability of the dairy

industry. Finally, increased milk production in general also contributes to the economic viability of farm operations, particularly in economic models where producers are paid on a volume basis. However, as the economics of milk pricing is highly complex and varies widely worldwide, some economies are more protected than others from market forces. Evidence from this review indicates that in general, increasing milk production will have an overall positive impact on the sustainability of the dairy industry. There are countless management strategies for increasing milk production per cow, including improved genetics and use of recombinant bovine somatotropin, monensin, sexed semen, and non-steroidal antiinflammatory drugs.

INTRODUCTION

The world's population is projected to exceed 9 billion by 2050, and with this growth, there is an increased demand for food, including dairy products. In 2010, per capita consumption of dairy products excluding butter was 89.1 kg. In 2050, this is estimated to increase to 116.55 kg per capita. This translates to a projected demand for dairy products in 2050 of 1093.1 million metric tonnes, in comparison to the 606.9 million metric tonnes consumed in 2010 (Knapp and Cady, 2015). This increased demand becomes even more critical when it is taken into consideration that globally, a large proportion of the current population does not achieve the recommended daily intake for dairy products, particularly in Africa and Asia; milk consumption is low (<30 kg/capita/year) in Vietnam, Senegal, and most of Central Africa as well as East and Southeast Asia (Gerosa and Skoet, 2012). The world's food producers are therefore facing the challenge of feeding the poorest of our population and meeting the increased demand for food production in an environmentally and socially sustainable way (Godfray et al., 2010). At the same time, the human race is faced with the reality of limited natural resources such as land, energy, and water. While

intensification of animal agriculture has been touted by its advocates as a way to maximize production of animal products with high efficiency, those who oppose intensification call its sustainability into question.

The term "sustainability" means many things to many different people. For the purpose of this review, sustainability will be defined by the current United States legal definition (US Code Title 7, Section 3103), which is "an integrated system of plant and animal production practices having a site-specific application that will over the long-term (A) satisfy human food and fiber needs; (B) enhance environmental quality and the natural resource base upon which the agriculture economy depends; (C) make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls; (D) sustain the economic viability of farm operations; and (E) enhance the quality of life for farmers and society as a whole." The 3 pillars of sustainability—environment, economic, and social—model this definition (Figure 1.1; von Keyserlingk et al., 2013).

This definition will serve as the outline for this review, dividing the paper into the following components: (1) satisfaction of human food needs; (2) enhancement of environmental quality and natural resources (including efficient use of nonrenewable resources); and (3) sustaining economic viability of farm operations. Finally, the review will conclude with examples of current technologies and strategies used commonly on dairy farms in the US and how they fit with the picture of sustainability described herein.

Several excellent reviews on the topic of the dairy industry and sustainability have been published, and readers are encouraged to refer to these publications, particularly Capper and Bauman (2013), Von Keyserlingk et al. (2013), and Knapp et al. (2014). This review differs from those published previously in that the focus is solely on the level of milk production and will take

a global view of the dairy industry worldwide rather than focusing on a specific country or region. While increased milk production is a hallmark characteristic of the US dairy industry, this still provides a unique perspective from previously published reviews because of its focus on this characteristic. It is often portrayed that the current push for extremely high per-cow levels of milk production in the US needs to be balanced with a sustainable system. But are these two goals mutually exclusive or at odds? Or is the question more complex than that?

It should be noted in the following discussion that the focus of this review is on cow's milk rather than milk from other mammals. It is true that other species may be more suited for different economic, social, and environmental situations worldwide. There are differences across domesticated mammals that have been utilized for milk production in the composition of their milk; however, most of the following discussion can be broadly applied to milk procured from other species.

SATISFACTION OF HUMAN FOOD NEEDS

Poverty and the needs of a growing world population

Currently, an estimated 795 million people worldwide—approximately 1 in every 9 people—do not have enough food to lead a healthy lifestyle (FAO, 2015), and the number of people experiencing micronutrient inadequacies is even greater. Fortunately, the increase in food production experienced in the past several decades has outpaced the growing population, resulting in overall decreased numbers of hungry people; however, the need to feed the remaining hungry still remains (Godfray et al., 2010).

In 1996, food security was defined at the World Food Summit as "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life," and it

encompasses both physical and economic access to food. The 3 pillars of food security are availability (adequate quantity of food on a consistent basis), access (adequate resources to obtain food), and use (nutrition and food safety knowledge, including sanitation; see http://www.who.int/trade/glossary/story028/en/). Smith et al. (2013) argued that livestock such as milk producing animals contribute to food security of impoverished people in developed countries because of their high quality nutrition; however, livestock provide food security mostly indirectly because many poor families will sell their animal source products rather than consume them, providing income to spend on farm inputs and food purchases.

At the turn of the century, leaders at the United Nations developed Millennium Development Goals as an action plan against world poverty. The first of these 8 goals was the eradication of extreme poverty and hunger (see http://www.unmillenniumproject.org/goals/). This goal included 3 sub-targets, including "halve, between 1990 and 2015, the proportion of people who suffer from hunger." The indicators for this goal were the prevalence of children under 5 who are underweight and the proportion of people in the population who consume below the minimum energy requirement. In 2015, these goals have expired, and this goal has been almost met on a global level, with 72 of the 129 countries being monitored having reached that secondary target (FAO, 2015)

The Sustainable Development Goals (SDG) are set to pick up after the expiration of the Millennium Development Goals (see http://www.un.org/sustainabledevelopment/sustainable-development-goals/). This includes as its second of 17 goals to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture." Included in the sub-targets for this goal is to "by 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round,"

and to "by 2030, end all forms of malnutrition, including achieving, by 2025, the internationally agreed targets on stunting and wasting in children under 5 years of age, and address the nutritional needs of adolescent girls, pregnant and lactating women and older persons." Clearly, the objectives of sustainability and adequate nutrition go hand in hand.

A common thread among low income populations worldwide is a lower intake of animal protein. Van Vliet et al. (2015) summarized some numbers that reflect this issues. Low income populations have lower protein intake (63 g/d on average) than high-middle (83 g/d) and high income (101 g/d) populations. Low income populations also have lower energy intake (2393 kcal/d) than high-middle (2907 kcal/d) and high income (3296 kcal/d) populations. As a percent of their total protein intake, low income populations consume about 21% animal protein, significantly lower than high-middle and high income populations, which on average consume 46% and 58% of their protein from animal sources.

A lack of adequate nutrition often results in a sort of positive feedback loop for disadvantaged groups. A link between adequate nutrition and cognitive function has been shown repeatedly (Black, 2003). If access to high quality nutrition and/or nutritional education are limiting, cognitive and physical function can be impaired, making it difficult for these groups to maintain employment and rise out of poverty. Micronutrients such as iodine, iron, zinc, and vitamin B_{12} have been implicated in this link. Milk and dairy products are particularly good sources of vitamin B_{12}. This will be discussed in further detail below.

Dairy products can either be produced in the area where they are consumed, or they can be imported from another region or country. More discussion of this will be addressed in the economic viability section below. For the moment, our discussion will focus on how higher production of milk and subsequent manufactured dairy products meet the goal of sustainability to satisfy human

food and nutritional needs. The evidence detailed below on the health effects of dairy products would seem to indicate that in general, more milk production is beneficial for global human nutrition and health. The economic and environmental challenges to this goal will be expounded on in their own sections.

Dairy products as a source of high-quality nutrition

Animal source foods are important sources of high quality nutrition. In fact, it has been postulated that the consumption of protein and energy from animal sources was an important factor in the evolutionary development of *Homo sapiens*. Milton (2003) concluded that "without routine access to [animal source foods], it is highly unlikely that evolving humans could have achieved their unusually large and complex brain while simultaneously continuing their evolutionary trajectory as large, active, and highly social primates." It is estimated that in developing countries, the average person only consumes 25% of the daily recommended intake of dairy products (Blaskó, 2011). It is common knowledge that milk is a good source of calcium. This is particularly critical for the nutritional requirements of the population targeted in the SDG: children, adolescent girls, pregnant and lactating women, and the elderly. However, there are several other positive nutritional aspects of milk that are often overlooked. In the following section, these will be broken down into macronutrients, specifically energy and protein, and micronutrients, including several minerals and vitamins.

One cup of whole milk contains approximately 149 kilocalories (USDA National Nutrient Database for Standard Release 27, Basic Report 01077). In undernourished populations, the lactose and milk fat in dairy products can be a valuable source of energy (excluding, of course, those who suffer from intolerance to lactose). The World Health Organization reported that in 2014, the

global wasting rate was 7.5%, and 50 million children under the age of 5 experienced wasting (Levels and trends in child malnutrition, 2015). While childhood stunting is decreasing worldwide, stunting prevalence was still 23.8% in the same year. Conversely, in developing countries, the prevalence of obesity is reaching epidemic proportions, and the caloric content of milk is a concern for many people who are concerned about being overweight. The relationship between dairy products and weight management will be discussed in the following section.

Milk fat contains both saturated and unsaturated fatty acids, including conjugated linoleic acids (CLA). The fatty acid composition of milk is largely influenced by the diet that the cow receives and its interactions with rumen microorganisms during the process of biohydrogenation, wherein the rumen bacteria utilize unsaturated fatty acids as "sinks" for hydrogen resulting in the formation of saturated fats (Jenkins et al., 2008). The CLA in milk are a result of this process in the rumen. Health benefits of CLA include favorable effects on plasma lipid status and potentially even anticarcinogenic effects (Haug et al., 2007). The most abundant unsaturated fatty acid in milk is oleic acid, which is considered to have several beneficial health effects, including membrane stabilization and the prevention of coronary heart disease (Haug et al., 2007). Although saturated fats have been implicated in cardiovascular disease, the link between consumption of saturated fats from milk and cardiovascular disease has been weak (Siri-Tarino et al., 2010).

Milk products are an excellent source of a balanced amino acid profile, including branched-chain amino acids which are required for protein synthesis and can be glucogenic (Harper et al., 1984). Van Vliet et al. (2015) illustrated this in their review when they summarized the amino acid content of a variety of plant and animal sources (Table 1.1). It is important, particularly in non-ruminants, to consider both the quantity and the quality of protein supplied in the diet. If high levels of protein are consumed, but this protein is deficient in a critical amino acid, the remainder

of the protein source cannot be maximally utilized, and that amino acid is considered to be limiting. The two most common limiting amino acids are lysine and methionine, and occasionally leucine. As illustrated in Table 1.1, many plant foods that are good sources of protein are low in leucine content, and those that are sufficient in lysine content tend to be low in methionine (van Vliet et al., 2015). Thus, to achieve a balanced amino acid intake with only plant proteins, it would be necessary to incorporate a variety of plant source foods into the diet as opposed to a single animal source protein such as milk. This is easily achieved in first world countries, but in developing nations, this can still remain a challenge due to limited economic resource and food availability.

In their 2004 report estimating the precedence of low birth weight infants globally, the World Health Organization states, "Children can be ensured a healthy start in life if women start pregnancy healthy and well nourished, and go through pregnancy and childbirth safely (WHO, 2004)." Protein is a critical component of development in young children. Early protein intake is critical for very low birth weight infants for proper growth and development. The World Health Organization estimated that approximately 15% of all births worldwide (more than 20 million births per year) are low birth weight, often as a result of poor health care and nutrition, and this estimate rises as high as 18% in Asia (WHO, 2004). In a study of very low birth weight infants born at Helsinki University Central Hospital (Finland) from January 1978-December 1985, adequate protein intake during the first 3 weeks after birth was associated with lean body mass, and a tendency for an association between protein intake during this time and height and body mass index was also reported (Matinolli et al., 2015). Conversely, there was no association found between early life protein intake and body fat or waist circumference. For infants weighing < 1 kg, the current recommendation for protein intake is 4.0-4.4 g/kg daily, and for those weighing between 1.0-1.8 kg, the daily recommendation is 3.5-4.0 g/kg. This must often be supplemented

in addition to breast milk, as human milk on average supplies < 2 g/kg daily (Matinolli et al., 2015). Formula ingredients used for supplementation almost without exception include milk components. Even in healthy full-term babies and toddlers, the source of protein after weaning can be critical for growth and development. Two case studies were reported in Pediatrics in 2001 where toddlers were given milk alternatives after weaning and developed conditions seen more commonly in developing nations—kwashiorkor and rickets (Carvalho et al., 2001). Vegetarian diets can have disastrous effects on growing children. Because of their small body size, they have limited gut volume, and the low energy density combined with the bulkiness of plant-based foods can limit their energy intake and subsequent growth if not properly balanced (Sanders and Reddy, 1994).

In addition to these macronutrients, milk and dairy products are good sources of several minerals. The most well-known is calcium, but phosphorus, magnesium, zinc, and selenium are also found in high levels in milk. Calcium is probably most recognizable for being critical for the development and maintenance of bones and teeth; however, it has a plethora of other functions, as it plays a role in blood pressure, muscle contraction, and blood clotting, and it is a cofactor of enzymatic systems (Gaucheron, 2011). Approximately 70% of calcium in the human diet is from dairy product; children and elderly adults have the highest requirements for calcium (1200 mg/d), and the recommended intake for pregnant and lactating women is also higher than for other adults (1000 mg/d; Gaucheron, 2011). Calcium and vitamin D are critical to the prevention of rickets, and low intake of milk has been implicated as a partial cause for the resurgence of rickets in Kenyan children (Bwibo and Neumann, 2003). Another causative factor for rickets is phytic acid, which interferes with calcium absorption. High intake of unrefined cereal grains contributes phytic acid to the diet, so diets high in these grains and low in dairy products, such as those in rural Asian

populations, are particularly susceptible to the development of rickets (Sanders and Reddy, 1994). Additionally, calcium plays an important role in energy partitioning and weight regulation in human adults. This will be discussed in further detail in the following section. It is important to note that the level of calcium in dairy products is effected by processing. Fresh milk and cheeses provide the most calcium, while hard cheeses provide the lowest amount of calcium (Gaucheron, 2011).

Iodine is essential due to its critical role as a component of thyroid hormones (Haug et al., 2007) and prenatal development (Black, 2003). Unfortunately, although milk can be a good source of iodine, there is large seasonal variation in the amount of iodine that is found in milk (Haug et al., 2007). Some regions have iodine-deficient soil, and in those places, milk is not likely to be a good source of iodine (Black, 2003). Global efforts in improving iodine status have focused on the supplementation of iodine through ocean products (Neumann et al., 2002). However, considering the prevalence of iodine deficiency and its impact on development and cognition, the iodine consumed with dairy products should not be discounted. It was estimated in 2003 that 30% of the world's population lives in iodine-deficient areas, and iodine deficiency has been called the most preventable cause of mental impairment in the world (Black, 2003).

Phosphorus has important roles in bone and teeth growth and maintenance, metabolism, and blood pH; and it is an important component of biological molecules such as DNA, RNA, ATP, and phospholipids (Gaucheron, 2011). Deficiency of phosphorus has been implicated in the etiology of rickets (Bwibo and Neumann, 2003), as well as kwashiorkor (Carvalho et al., 2001) and other disorders. Milk is an excellent source of phosphorus, with a concentration of total phosphorus of approximately 950 mg/L (Gaucheron, 2011). Phosphorus exists in both organic and inorganic forms, both of which are present in milk.

Magnesium is a component of more than 300 reactions in the body (Haug et al., 2007). It is an enzyme cofactor for many reactions and is involved with DNA transcription, protein synthesis, neuromuscular transmission, and phosphorylation (Gaucheron, 2011). Deficiency of dietary magnesium is associated with greater risk of cardiovascular disease (Del Gobbo et al., 2013), decreased insulin sensitivity (Nadler et al., 1993), and the development of atherosclerosis (Maier, 2003). The concentration of magnesium in milk is fairly low relative to other minerals (approximately 120 mg/L), but dairy products are still considered to be important sources of magnesium, considering that 600 mL of milk provides about 16% of the recommended daily allowance for this mineral (Gaucheron, 2011).

Zinc is involved with gene expression, cell division and differentiation, and DNA and RNA synthesis, and it is critical on a biochemical level for the maternal, infant, and child survival (Neumann et al., 2002). Because of their higher requirements, these groups are particularly susceptible to zinc deficiencies, and even mild deficiencies can cause depressed growth in children and may have negative effects on activity and cognition in children (Neumann et al., 2002). Zinc bioavailability is decreased by the phytate content of cereal grains, thus negating the reasonable levels of zinc in these foods (Neumann et al., 2002). Zinc deficiency is often a result of low intake of animal source foods, and 600 mL of milk provides approximately 20% of the recommended daily intake of zinc (Gaucheron, 2011).

Selenium plays an important antioxidant role in the body, and milk can be particularly good source of selenium (Gaucheron, 2011). Selenium plays a role in DNA synthesis and repair, and it has some anti-carcinogenic properties (Haug et al., 2007). Some evidence indicates that there is a negative association between selenium intake and cardiac disease, although this relationship is still weakly defined (Flores-Mateo et al., 2006). Importantly, the health effects of selenium

supplementation show a somewhat quadratic effect, wherein excessively high and excessively low selenium status can both have adverse consequences (Rayman, 2012). The contribution of dairy product consumption to selenium intake varies across countries, ranging from 8-39% of selenium in the diet (Gaucheron, 2011).

Animal source foods from ruminant animals such as dairy products are an important source of vitamin B_{12}. Normal blood formation as well as neurological development and function is dependent on adequate B_{12} (Neumann et al., 2002). This vitamin is produced by bacterial synthesis, and it is not found in plant foods with the exception of algae. For nursing infants, intake of B_{12} depends on the B_{12} intake of the mother, and mothers who consume low amounts of animal protein are more likely to become deficient in this vitamin (Allen, 1994). Dutch children receiving a macrobiotic diet based on whole-grain cereals, legumes, and vegetables with limited animal protein consumption were shown to be prone to B_{12} deficiency (Dagnelie and van Staveren, 1994). A common assumption in the past has been that small amounts of animal source foods will provide adequate amounts of B_{12}; however, high prevalence of B_{12} deficiency has been demonstrated in several less developed countries, including Kenya, India, Guatemala, and Mexico (Murphy and Allen, 2003). Vitamin B_{12} has been implicated in the link between cognition and nutrition (Tangney et al., 2005). For example, the Dutch children receiving macrobiotic diets had delayed motor and language development compared to matched omnivorous children (Dagnelie and van Staveren, 1994). Among the oldest participants in a prospective study of community-dwelling older persons, high intake of vitamin B_{12} was associated with slower cognitive decline (Morris et al., 2005).

The fat-soluble vitamin A is an important antioxidant, and it plays an important role in skin health, reproduction, immunity, growth, and gene regulation (Gaucheron, 2011). Deficiency of

this vitamin is associated with rickets (Carvalho et al., 2001). Perhaps the role that vitamin A is most well-known for, however, is vision, particularly in developing countries. While green leafy vegetables are also good sources of vitamin A, increasing their consumption is not always correlated with higher levels of serum retinol because the conversion of β-carotene to retinol is not as efficient as previously believed (Bwibo and Neumann, 2003). Therefore, the best solution for preventing vitamin A deficiency is increasing dietary diversification and quality (Bwibo and Neumann, 2003). Whole cow's milk contains approximately 126 IU of vitamin A per serving (Gaucheron, 2011). The characteristic "golden" color of milk from the Guernsey breed is due to its high concentration of β-carotene (Baumann et al., 1934).

Dairy products are an important source of another fat-soluble vitamin, vitamin D. Calcium transport across the duodenum is dependent on vitamin D (Caroli et al., 2011). Therefore, the positive effects of calcium are impossible without adequate vitamin D consumption. Whole milk is fortified with added vitamin D in some countries is an effort to meet the requirements; however, whole milk does naturally contain some vitamin D. Values for vitamin D naturally present in milk range from 0.3-1.0 μg/kg, while average fortified values are 7.05 and 9.9 μg/kg for fortified milk in the US and Canada, respectively (Schmid and Walther, 2013).

Nutritional role of dairy products in developed countries

Poor nutrition is characterized not by the failure to receive enough nutrients but rather the failure to receive the right balance of nutrients to meet but not overly exceed requirements. Dairy products have come under scrutiny by some groups in the US and other developed countries. High fat and high sugar dairy products such as ice cream are incriminated in the obesity epidemic that the US currently faces.

Counterintuitive to conventional opinion, epidemiological evidence indicates that consumption of the recommended minimum of 3 servings a day of dairy products has a positive effect on weight management in obese individuals, despite their fat content and relatively high caloric value. Calcium is a regulator of lipid metabolism in adipocytes, and high-calcium diets can attenuate weight gain through these mechanisms, and this effect is particularly pronounced when calcium intake is largely derived from dairy products (Zemel, 2004). This is most likely due to the bioactive properties of some compounds found in dairy products.

There has been some disagreement in the literature on the effect of dairy product consumption on health and chronic disease. In a recent meta-analysis, Chen et al. (2015) reported that consumption of dairy products is negatively associated with metabolic syndrome in a dose-response manner. These authors stated that "our novel findings…in combination with recent evidence on [Type 2 diabetes], [cardiovascular disease], and specific cancers, provide further supports for public health recommendations to increase dairy consumption to prevent series of chronic diseases." Historically, dietary recommendations have been to lower fat consumption, resulting in the endorsement of low-fat dairy products by nutritionists; however, a 2012 review of the literature comparing high and low-fat dairy products found little evidence that high fat dairy consumption is linked to increased cardiovascular disease risk (Huth and Park, 2012). Indeed, observational evidence by other authors indicates that within normal dietary patterns, high-dairy product consumption may have an inverse relationship with obesity (Kratz et al., 2013).

The protein found in milk products is popular among athletes. Chocolate milk is commonly recommended as a recovery drink for athletes after a workout, and with good reason. Skeletal muscle responds differently to animal-based protein compared to plant-based. This has been postulated to be due to a number of reasons, as discussed by van Vliet et al. (2015). First, sources

of animal protein such as milk have a more balanced amino acid profile than plant proteins, and contain higher levels of the essential amino acids. In particular, animal proteins contain high levels of leucine, which is the most potent amino acid for stimulating muscle protein synthesis. In addition to their function when incorporated into body protein, essential amino acids can act as signaling molecules for muscle protein synthesis. Secondly, the absorption kinetics of amino acids from animal source foods are generally more efficient than those from plants. It has been estimated that 50-70% of the amino acids from beef or dairy products are absorbed within 5-6 h of consumption. Milk and isolated milk proteins (whey and casein) have Protein Digestibility Corrected Amino Acid Scores (PDCAAS) of 1.00, as opposed to soy (the plant with" the highest PDCAAS), which has a score of 0.91, although isolated soy protein also has a score of 1.00. The PDCAAS is used to estimate the ability of a protein to support skeletal muscle anabolism.

ENHANCEMENT OF ENVIRONMENTAL QUALITY AND NATURAL RESOURCES

In 2006, the FAO released a publication titled "Livestock's Long Shadow," which highlighted—and according to some, exaggerated—the detrimental effects of livestock production on the environment (Steinfeld et al., 2006). This report concluded that livestock are responsible for over 8% of human water use, contribute 18% of greenhouse gas (GHG) emissions, and account for 30% of land surface on Earth. The dairy industry is a significant contributor to the environmental impact of the livestock industry as a whole. As discussed in the previous section, milk and dairy products play an important role in meeting the dietary and protein needs of the human race, but in order for the production of milk to remain sustainable, the industry must not

deplete the natural resources that it depends on. In this section, the role of increased milk production on the environment and natural resources will be explored.

It is obvious that on a global scale, there is an increasing volume of milk being produced. As a result, there is an increased demand for milk processing facilities and it might be assumed that there is thus greater energy utilization in the form of fossil fuels. However, the majority of environmental impact still occurs on-farm. Thoma et al. (2013) reported that 72% of the total GHG emissions associated with milk production are accumulated before the farm gate. Based on these conclusions, the following discussion will focus largely on mitigating negative environmental impacts on-farm rather than address the additional environmental burdens associated with greater milk volume in the processing stage.

Mechanisms of the dairy industry's environmental impact

The focus of a large body of media emphasizing agriculture's effect on the environment has been on its contribution to climate change through GHG production. The importance of GHG in climate change and the influence of the dairy industry will be discussed in further detail below, but considering the high percentage of GHG from the dairy industry that is attributed to enteric methane production, an overview of the mechanisms in the rumen that produce methane—one of the most potent GHG—is an appropriate preamble to the discussion of the dairy industry's environmental impact and the importance of increased milk production. Thoma et al. (2013) estimated that enteric methane contributes 25% of the total burden of GHG emissions associated with the entire lifecycle of milk production and processing. O'Brien et al. (2011) reported that enteric fermentation was the largest contributor to GHG emissions from dairy farms regardless of the methodology used, varying from 45-65%. Mc Geough et al. (2012) concluded based on their

case study in Eastern Canada that mitigating enteric methane would result in the greatest reductions in methane emissions from dairy farms. Improving productivity and production efficiency per cow is an effective way of mitigating the emissions released per unit of milk produced (Capper et al., 2008), consistent with the concept of dilution of maintenance.

Greenhouse gases contribute to the "greenhouse effect" that has been implicated in climate change. The greenhouse effect can be defined as "the infrared radiation energy trapped by atmospheric gases and clouds" (Raval and Ramanathan, 1989). In other words, the greenhouse effect is the difference between energy emitted from the Earth's surface and the energy released into space. The natural atmosphere includes many GHG. Clouds themselves are an important greenhouse substance; however, human activities also result in climate "forcings." The major GHG include carbon dioxide (CO_2), methane, nitrous oxide, water vapor, ozone, and chlorofluorocarbons (Climate change science: An analysis of some key questions, 2001). Each GHG has a different potency for its contribution to the greenhouse effect; for example, CO_2 has a relatively low contribution to the greenhouse effect on a per mol or per kg basis, but it is still the largest contributor because of its high concentration in the atmosphere (Rohde, 1990). To simplify this concept and standardize GHG across different efficacies, many publications will express GHG production on a basis of CO_2-equivalents (CO_2e), defined as an estimate of the concentration of CO_2 required to result in a given change in radiative forcing, a measure of change in the radiation balance (Gohar and Shine, 2007). After CO_2, methane is the next largest contributor to the greenhouse effect because of both its quantity and efficacy (Rohde, 1990). Although natural sources of methane emissions do exist—the largest of these sources being wetlands—the majority of anthropogenic methane is a result of enteric fermentation from livestock at 17% of total methane

produced globally (Knapp et al., 2014). Of the global sources of methane, agriculture as a whole contributes approximately 29%.

As reviewed by Knapp et al. (2014), anaerobic metabolism of carbohydrates in the rumen and the production of the VFA acetate result in the production of the reducing equivalent hydrogen (Equation 1 & 2). Hydrogenase-expressing bacterial species convert this hydrogen to H¬2, and through negative feedback pathways, this H2 has an inhibitory effect on rumen fermentation pathways. Therefore, so-called "hydrogen sinks" are a necessary component of optimum rumen function. Common hydrogen sinks include the VFA propionate and butyrate (Equation 3 & 4), unsaturated fatty acids (Equation 5), and methane (Equation 6).

$$\text{Glucose} \rightarrow 2 \text{ pyruvate} + 4H; [1]$$

$$\text{Pyruvate} + H_2O \rightarrow \text{acetate} + CO_2 + 2H; [2]$$

$$\text{Pyruvate} + 4H \rightarrow \text{propionate} + H_2O; [3]$$

$$2 \text{ acetate} + 4H \rightarrow \text{butyrate} + 2H_2O; [4]$$

$$C_{18:2} \text{ (linoleic acid)} + 4H \rightarrow C_{18:0} \text{ (stearic acid)}; [5]$$

$$CO_2 + 8H \rightarrow CH_4 + 2H_2O; [6]$$

It is clear from these equations that methane production by methanogens is an efficient way of disposing of a large amount of hydrogen. However, aside from the environmental effects of excessive methane production, methane represents a loss of energy (Moe and Tyrrell, 1979). Managing nutrition on dairy farms to decrease the production of acetate (a hydrogen-producing reaction; Equation 2) and increase the proportion of propionate production (a hydrogen sink; Equation 3) can have the dual positive effects of increasing efficiency and production and decreasing the amount of methane produced per kg of milk (Knapp et al., 2014).

Ultimately, the root cause of methane production is inherent nutrient loss that occurs in any metabolic process. In addition to GHG, nitrogen in the form of nitrate (NO_3^-), phosphorus, and potassium are pollutants of concern that are excreted due to incomplete utilization in the animal (Tamminga, 2003). Nitrogen losses occur in the rumen, feces, and urine, although these losses can be mitigated by maximizing milk protein output (Tamminga, 1992). Although increasing output of phosphorus and potassium into milk can at least partially mitigate their loss into the environment, a more effective method of reducing the waste of these minerals is to decrease their consumption (Tamminga, 2003).

The concept of dilution of maintenance is key component to the discussion of livestock production systems and nutrient loss to the environment, and it summarized the relationship between higher production and the amount of waste products produced. As described by Capper and Bauman (2013), a cow's maintenance requirement is considered to be a fixed cost of milk production. Therefore, as milk production is increased within a set period of time, the cost of maintenance per unit of milk produced is decreased (Figure 1.2). Since waste production and use of resources are functions of maintenance costs, increased productivity will decrease the amount of waste produced or resources used per unit of milk produced.

Greenhouse gas emissions

Before the European settlement of the United States, bison were the largest ruminant contributor to methane emissions. While estimating methane production by these animals during this period are challenging due to the inability to measure population size and feed consumption, Hristov (2012) conservatively estimated that methane production from this period was 86% of that from farmed ruminants in the modern US; however, if the largest estimations of bison population

size are used to estimate methane production during that time, methane emissions could have been as much as 23% higher than the modern livestock industry. While these animals were hunted as a source of meat and pelts by Native Americans, if methane emissions are considered as a proportion of animal products harvested, the numerical differences would be even greater than total methane alone. Globally, the dairy industry is evolving to meet the needs of a growing demand for dairy products, and this is particularly characteristic in the United States (Von Keyserlingk et al., 2013). Much of this evolution has involved the intensification of the industry, a concept which goes hand-in-hand with increased milk production. Although nutritional and management practices play a large role in this increase in production, it has been estimated that 55% of the 3,500 kg increase in average lactation yield between 1980 and 2006 is due to genetic improvements (Shook, 2006).

The evolution of the dairy industry has altered the profile of GHG emissions across the past several decades. In 2007, 84.2 billion kg of milk were produced in the US compared to 53.1 billion kg in 1944, and this was achieved with 936,000 dairy cattle in 2007 as opposed to 4,148,000 in 1944 (Capper et al., 2009). Therefore, although average CO_2 emissions produced per cow has increased from 13.5 to 27.8 kg during that time period, total methane emissions decreased from 61,800,000 to 26,800,000 kg, and a similar trend was observed in reduction of CO_2 emissions because of decreased cow numbers. Furthermore, CO_2e produced per kg of milk have decreased from 3.66 kg in 1944 to 1.35 kg in 2007. Similarly, in Ontario between 1991 and 2011, kg of CO_2e per kg of fat and protein corrected milk decreased by 22% (Jayasundara and Wagner-Riddle, 2014). This decrease was partially due to decreased contribution of enteric fermentation although a larger contributor to this decrease was changes in feed production.

To further compliment the trends in milk production as intensification of the dairy industry increases, there has been a shift in the dairy breeds utilized in the US from 1944 to 2007 (Capper

et al., 2009). Currently, Holsteins are the overwhelming majority of cattle at approximately 90% of cows in the US, while in 1944, Holsteins and Brown Swiss combined made up 46% of the dairy cattle population. Holsteins are a large breed and are characterized by large milk volume and low total milk solids on a percentage basis, although their greater milk output results on average in greater output of milk solids (Capper and Cady, 2012). Following Holsteins, Jerseys are the next most common breed of dairy cattle in the US. As opposed to Holsteins, this breed is known for their small size and high milk component percentages. Capper and Cady (2012) reported that producing 500,000 tons of cheese from Jersey milk would decrease the carbon footprint of production compared to using Holstein milk, as only 3.99 billion kg of Jersey milk would be required as opposed to 4.94 billion kg of Holstein milk due to the higher levels of milk solids in Jersey milk. Furthermore, it is perceived that because of their small body size and corresponding lower maintenance energy requirement that Jerseys are more efficient milk producers; however, published evidence for this belief is limited (Knapp et al., 2014). Mature Jerseys and Holsteins did not show any differences in maintenance or production requirements when adjusted for metabolic body weight (Tyrrell et al., 1991), as well as methane emissions as a proportion of DMI (Munger & Kreuzer, 2006) and methane emissions as a proportion of milk production (Tyrell et al., 1991; Munger & Kreuzer, 2006). Based on the current research, it seems likely that Jersey milk is more environmentally sustainable for cheese production and other processes that utilize the solid components of milk (although possibly not for fluid milk production), while Holstein milk is more economically sustainable for farmers when they are paid for milk on a volume basis, as they are in the US. This will be addressed again in the following section discussing economic sustainability.

There are multiple approaches to increasing milk production and efficiency that are effective in mitigating enteric methane production, and some will be discussed in further detail in

a later section. Yan et al. (2010) utilized a data set of 579 lactating dairy cows used in 20 energy metabolism studies to analyze the relationship between efficiency of energy utilization and productivity. These authors reported that methane energy as a proportion of energy intake decreased as milk yield and efficiency increased. Knapp et al. (2014) reviewed the use of multiple strategies, including nutrition, genetics, and management. Similarly, Place and Mitloehner (2010) included cow comfort and herd health as methods of improving production efficiency for methane mitigation in their review. Given these observations, it is not surprising that Buddle et al. (2011) concluded that "there are currently no robust, reproducible and economically viable methods for reducing methane emissions from ruminants grazing on pasture." This is in agreement with the findings by Bell et al. (2011) that nongrazing, intensive feeding systems result in the lowest GHG emissions as a proportion of energy-corrected milk (ECM) production.

In addition to enteric methane emissions, manure management makes a significant contribution to GHG emissions by the dairy industry. In addition to methane, manure application results in the emission of ammonia and nitrous oxide (Guerci et al., 2013). In a cradle-to-grave assessment of GHG emissions by the dairy industry, Thoma et al. (2013) estimated that of the 35.4 million metric tonnes of CO_2e generated in 2007 from the production of fluid milk that 8.8 and 8.0 million metric tonnes of CO_2e resulted from enteric fermentation and manure management, respectively. The amount of GHG produced varies between manure management methods. For example, anaerobic lagoons and deep bedding produce significantly more GHG than dry lot and solid storage systems; however, even the process of shifting systems of nutrient management can increase a farm's environmental burden (Thoma et al., 2013). In agreement with the concept of dilution of maintenance, the manure output of the dairy industry per unit of milk production has decreased with increasing intensification and higher milk production, being reduced by 24%

between 1944 and 2007 (Capper et al., 2009). In a case study of different dairy systems in Georgia, Belflower et al. (2012) demonstrated that long-term manure storage in confinement dairies significantly increases methane emissions on a per cow basis (70% greater), although per kg of milk grazing dairies produce approximately 30% more methane per unit of ECM.

Water

Over the course of the twentieth century, human water use has increased at twice the rate of population growth (Ridoutt et al., 2010). Fresh water sources are generally categorized into 2 classes, "green" and "blue." Natural rainfall over agricultural lands is defined as green water, while blue water is sourced from surface water and groundwater. Use of these different categories of water are not equivalent in their water footprint (Ridoutt et al., 2010).

As a whole, animal agriculture has been estimated to account for approximately one-third of the global water footprint (Medonnen and Hoekstra, 2012). The dairy industry in particular is a heavy user of water, being responsible for approximately 19% of animal agriculture's water footprint. Some examples of water usage in dairy production systems are drinking water for cattle, feed production, and heat stress mitigation. Milk is 87% water, so high levels of milk production increase the water requirement of dairy cattle. Cattle meet their requirement for water through free water intake, water in their feed, and metabolic water. Of these, free water intake and water in feed are the two most biologically significant sources of water (NRC, 2001). Furthermore, as milk production increases, the amount of feed needed to sustain this level of production must also increase, and this has implications for the industry's environmental impact. The use of these feeds increases the water requirement of the dairy industry, potentially decreasing sustainability. In the year 2000, approximately 4.7 billion tons of feed were consumed by livestock, 3.7 billion tons of

which was eaten by ruminants (Herrero et al., 2006). Approximately 48% of this biomass was comprised of grasses. Some of this demand for water can be mitigated by utilizing more drought-tolerant crops. Often, producers utilize water as a method of reducing heat stress—a particularly important use for high-producing animals—for washing equipment on the farm, and on some farms for flushing manure. Once the product is off the farm, increased milk volume results in increased water usage for the processing of milk products. It should be noted that the water footprint of dairy products depends on the product in question. For example, skim milk powder can have a very low water impact (Ridoutt et al., 2010).

Capper et al. (2009) demonstrated that the dairy practices used in 2007 to produce 1 billion kg of milk used approximately 35% of the water than industry practices used to produce the same amount of milk in 1944. Looking forward to continued modernization and development, it was estimated that in 2010, the global dairy industry (including both cattle and buffalo) utilized approximately 4,931 billion L of water (Knapp and Cady, 2015). Knapp and Cady (2015) calculated that in 2050, if milk production per animal was frozen to 2010 levels and animal numbers increased to meet demands that 7,941 billion L of water would be needed for global milk production. Alternatively, these authors calculated that with continued innovation, if animal numbers were maintained at 2010 levels and milk production per animal increased to meet the population demands in 2050, only 5,707 billion L of water would be needed for global milk production. Medonnen and Hoekstra (2012) argued that decreasing consumption of animal products would have a beneficial effect on water usage, since the water footprint of animal products are larger than the water footprint of nutritionally equivalent crops (per calorie or per g of protein). However, they did not account for the quality of nutrition, particularly protein, in animal food products compared to plants. As discussed in the previous section, animal products

are a more high quality food source, providing a more balanced source of protein. It was demonstrated that grazing systems have the lowest impact on water footprint (Medonnen and Hoekstra, 2012). This conclusion highlights the importance of maximizing the utilization of human inedible feedstuffs in ruminant systems. Reducing the environmental impact of milk production depends on reducing competition for sources of nutrition between animals and humans, and ruminants are particularly well-suited for this because of their ability to gain energy from cellulose. Furthermore, utilization of pasture increases the percentage of green water used for milk production as opposed to blue. While it has been demonstrated that increasing the intensity of milk production enhances the environmental stewardship of the dairy industry through the dilution of maintenance (Capper and Bauman, 2013), it could be that utilizing grazing systems and byproduct feeds may ultimately decrease milk production yet still increase the environmental sustainability of the industry as a whole. Based on this discussion, a balance of multiple farm types may be the best way to maximize sustainability and responsible resource use.

SUSTAIN THE ECONOMIC VIABILITY OF FARM OPERATIONS

It is important to note from the overall picture of sustainability of the dairy industry that the 3 pillars of sustainability are interconnected. For example, it is clear from previous discussions that milk and dairy products play important roles in satisfying human food needs globally. It will be shown in the following section that, at least in the US, higher levels of milk production increase the income of dairy farmers, thereby sustaining the economic viability of farm operations. This is largely a consequence of the relatively high levels of price regulation that the US dairy industry experiences. However, large families have a higher per capita expenditure on milk than childless families (Dhar and Foltz, 2005), meaning that families with children and lower income families

bear a greater burden due to regulation from the milk marketing orders than higher income and/or childless families (Chouinard et al., 2010). Charity initiatives such as the "Pour It Forward" campaign and well as government assisted programs have been used in an effort to balance these two pillars of sustainability that can be at odds with each other under certain scenarios.

Influence of the global economy

In the following sections, the economics of milk production will be assessed based on the specific country or region where milk is being produced. This can vary based on the culture and as well as the local economy and milk pricing scheme. Before that discussion, it is important to realize that none of these economies are completely autonomous. Supply and demand from different countries can affect the price of milk in a different country. For example, at the same time that milk pricing in Europe switched away from the quota system and became more susceptible to market forces, China experienced a collapse in demand for dairy products and Russia placed a ban on imports, resulting in a dramatic drop in milk prices due to decreased global demand (http://www.bbc.com/news/uk-33953963).

The United States produces more milk than any other developed country. It is second to India for total milk produced as of 2013 (http://faostat3.fao.org/browse/Q/QL/E), and its production share is steadily growing. Milk is the second most produced animal commodity in the US, and the country is a major exporter of milk and milk products, although the growth of the dairy industry in other countries such as China—a major importer—may shift the global landscape of milk production and pricing (FAO Food Outlook, 2015). Border measures are in place to insulate the price of dairy products in the US from global market forces, both in terms of imports and exports. Exported dairy products are directly subsidized by the federal government. In fact,

this is often how the government is able to dispose of excess milk that is purchased under price support. Imported dairy products are subject to tariff-rate quotes, whereby low tariffs are imposed up to a certain quantity (or "quota") of dairy imports, after which high tariffs are imposed. This has effectively insulated US milk prices from foreign supplies of dairy products and allowed domestic prices to remain higher than global prices (Sumner and Balagtas, 2002).

Influence of region

The region in which milk is being produced is critical to the economic sustainability of the farm operations, largely because pricing is often regionally driven. Globally, there is an imbalance across regions where the areas that the population is growing the fastest is not being met by concurrent increases in food production, resulting in a growing yield gap in these areas (Godfray et al., 2010). Regional specialization in food products such as milk may increase global efficiency and help to balance supply and demand in areas where milk production is limited; however, this may increase the negative environmental impact of milk production due to increasing emissions from transportation of dairy products (Pretty et al., 2005), although the relative contribution of transportation costs is small compared to on-farm costs.

Despite the benefits of regional specialization in food products on global efficiency of food production, this strategy does have its trade-offs. While it contributes to the sustainability of the dairy industry by satisfying human food and fiber needs, the increased market competition could have a negative effect on families in developing nations who produce milk as part of their income (Steinfeld, 2003). Conversely, it has been shown in India, Bangladesh, and Brazil that small dairy producers have been able to successfully compete with larger, more intensive systems because they do not have the same high cost for labor (Tarawali et al., 2011). Women in particular are

empowered in these economies as more of them are responsible for food production in these systems (Staal et al., 2009).

It is common for producers in developing nations to utilize mixed crop livestock systems for food production rather than the highly specialized intensive systems common in more developed countries. Tarawali et al. (2011) reported that 75% of the world's milk was produced in these mixed systems. There is great value to these types of systems in these economies, as livestock—and ruminants in particular—can convert low-value foods and inedible crop residues into high quality animal source foods (Smith et al., 2013). Tarawali et al. (2011) argued that there is greater value in these systems to having a smaller number of animals with higher production per animal rather than gaining the same level of production by utilizing more animals. This is a sustainable practice from the standpoint of environmental impact, as discussed above, as well as from an economic efficiency perspective. However, even while making a case for increasing intensification, these authors admit that this will increase the spatial separation between livestock and crop production, similar to the model seen in intensive agriculture in developed countries (Tarawali et al., 2011). While this may be a positive development from a purely economic standpoint, it does not take into consideration the social and cultural aspects of animal ownership that may create barriers for animal production (Steinfeld, 2003).

Smith et al. (2013) reported that 13% of the human race's energy intake is provided by livestock, but that livestock consume a disproportionately high amount of the world's grain at approximately 50%. From values like these, it would be easy to conclude that animal products are an inefficient way to meet both the nutritional and economic needs of developing nations, and that livestock production is an inefficient method of using natural resources. However, as discussed in previous sections, this viewpoint does not account for the higher nutritional quality of animal

source foods, and it does not take into consideration the dilution of maintenance effect for environmental impact. Furthermore, animal product production is an efficient way of generating income for poor families in developing countries, since livestock utilize unexploited resources that would otherwise be wasted (Randolph et al., 2007). Poor people tend to sell rather than consume their animal products, meaning that animal production may contribute to their income while not necessarily contributing to the nutritional security of their family (Smith et al., 2013). Steinfeld (2003) outlined some potential barriers for livestock production in developing countries. These include financial and asset barriers, such as investment costs and land availability; technical barriers, such as food preservation technologies; social and cultural barriers; production and transaction costs; and high market and production risks.

The dairy industry in developed nations is obviously different than the industry in developing countries. Furthermore, the pricing of dairy products is vastly different between developed and developing countries, and even between developed countries. Pricing of dairy products has historically been highly regulated in developed nations. On one extreme end of the spectrum is the quota system that has been utilized by the European Union and Canada. The European Union has de-regulated milk pricing recently, with disastrous results in the short-term.

The European Union introduced milk quotas in 1984 (Guyomard et al., 1996). Under the quota system, increasing milk production does not in turn increase the profitability of the dairy farm. On April 1, 2015, European milk quotas were abolished, resulting in a financial crisis for dairy farmers. Without the constraints of the quota system, farmers increased their milk output in an attempt to increase their income, resulting in an imbalance of supply and demand and a severe drop in the price that they received for milk. In August of 2015, farmers were actually losing an average of 7p per liter of milk. In the UK, tensions culminated in a protest in August 2015, with

farmers herding cattle through supermarkets in an attempt to raise awareness of the financial crisis. In response, several supermarket chains in the UK agreed to set minimum prices for milk sold in the store (http://www.bbc.com/news/uk-33953963). During the crisis, increasing milk production was not actually economically viable for many farms, as they were actually losing money by producing milk. Additionally, due to the milk pricing structure at the time, elevated milk production actually decreased the sustainability of the dairy industry in the UK as a whole, as the glut of supply resulted in a further decrease in milk price.

Milk pricing in the US became more highly regulated during the Great Depression as a result of the New Deal (Chouinard et al., 2010). Currently, 85% of milk in the US is marketed through 11 Federal Milk Marketing Orders, which are organized based on location. There are 2 key components of milk pricing under the Federal Milk Marketing Orders. These components are classified pricing and pooling. Classified pricing refers to the concept that minimum prices are set based on what the milk and milk components will be ultimately used for (i.e., fluid milk or manufactured products), while pooling guarantees that farmers are paid a uniform price for their product based on volume and components, regardless of what end-product the fluid milk is used for. In addition to these regulations, there are other measures in place that safeguard the economic viability of American dairy farmers. One of these measures is federal price support, which sets a minimum price, under which the federal government will buy dairy products in order to increase demand and thereby increase price. Although this price has been dropping in the latter half of the twentieth century and beyond, it is still in place as a safety net for dairy producers (Jesse and Cropp, 2008).

High levels of nationwide milk production do not upset the balance of supply and demand in terms of milk price as they would in a de-regulated system. Dairy farmers in the US are typically

paid by volume of milk produced, with premiums available for high quality and components. Therefore, in a simplistic view, increasing the volume of milk production increases revenue for an individual dairy producer. An example of this is the case of the Holstein vs. the Jersey breed, the two most popular breeds in the US. Holsteins are known for high volumes of milk production with relatively low percentages of milk components, while Jerseys are known for lower production and relatively high percentages of milk components. Bailey et al. (2005) simulated variety of scenarios under multiple component pricing comparing herds of Holsteins vs. Jerseys. They reported that across all scenarios, the income over feed cost (IOFC, a common metric used to benchmark profitability of dairy farms) was most affected by the volume of fat and protein produced by the farm rather than the percentage of these components. Therefore, Holsteins were more profitable than Jerseys under these situations, because although Jersey milk is more valuable per pound produced, Holsteins produce more total milk pounds, offsetting the Jersey's advantage.

Strategies for increasing economic viability of milk production

Under the US pricing system, the major sources of economic risk for dairy farmers is milk and feed price volatility, and although these variables are not the sole indicators of a dairy farm's profitability, they are two very key components. While income from milk production will make up the majority of a typical dairy farm's income, the cost of feed can be as high as 50% of variable costs on a dairy (Hardie et al., 2014). Income over feed cost is a commonly used metric to measure the income of milk relative to the cost of feed, and it is simply defined as the cost of feed subtracted from the income from milk produced and sold. Therefore, from a simplistic view, maximum profitability from a dairy farm will maximize IOFC by maximizing milk income and minimizing feed cost; however, dairy farming is inherently complex, and this simple view may not fully

characterize all scenarios and management structures. This is demonstrated in a model scenario published by Liang and Cabrera (2015). These authors reported that as target milk production increased, ECM increased to point, where it continued to increase but at a slower rate until it flattened. Net return to management showed a similar trend. The authors described increased milk production as a "win-win" in relation to GHG emissions as well as net return to management.

Obviously, when discussing economic viability, there are balances to be considered. Some expensive technologies may be cost-prohibitive for small farm operations because the increase in milk production does not cover the cost of the technology. Alternatively, decreased milk production may be economically viable under certain management structures. For example, least cost rations or grazing systems may not maximize milk production, but they enhance profitability because of their low cost. These types of systems may also contribute to overall environmental sustainability of the industry by utilizing otherwise untapped resources for productive use. A diverse range of management styles may be important for maximizing the sustainability of the dairy industry as a whole.

Theoretically, decreased feed costs might increase IOFC if milk production did not decrease proportionally. In contrast, Hardie et al. (2014) reported that organic dairies in Wisconsin that had the lowest IOFC were those that relied the most heavily—even exclusively—on pasture compared to other organic dairies that used more concentrates in their ration; however, the authors indicated that IOFC could not be isolated to one single driving factor. It is important to note that a large proportion of these farms utilized less efficient dairy breeds or crossbred animals.

Most milk produced in the US is from conventionally managed cows; however, niche markets exist to appeal to certain groups of consumers. Examples of these include organic dairying, grazing dairies, and A2 casein milk. The economic viability of these markets may be

driven by lower input costs (such as grazing) or by consumer willingness to pay a higher price for the product (such as A2 or organic milk as well as grazing). Labeling these other processes of milk production has the effect of increasing competition between fluid milk products, decreasing the price that consumers pay and farmers receive for their milk (Dhar and Foltz, 2005). Consumer perception as a driver of income from milk sales will be discussed in further detail in a following section.

Consumer perception plays a large role in the development of niche markets. The influence of consumer perception on prices of dairy products can be viewed through the concept of elasticity. In economics, this is defined as the sensitivity of one variable to a change in price to another variable. In dairy products, a change in the price of one often results in a consumer's switch to another replacement product. For example, Andreyeva et al. (2010) reported that consumers may respond to an increase in the price for whole milk by switching to low fat or skim milk, not by cutting out milk altogether. Recombinant bovine somatotropin is an excellent example of the effect of consumer willingness to pay higher prices for a perceived benefit; it is discussed in the following section.

STRATEGIES FOR INCREASING MILK PRODUCTION AND THEIR EFFECT ON SUSTAINABILITY

In the following section, specific examples of strategies used by dairy producers to increase milk production will be analyzed in the context of sustainability. This list includes the use of rbST, monensin, sexed semen, and non-steroidal antiinflammatory drugs (NSAID) on dairy farms. The discussion will focus on the use of these technologies in the US and should not be viewed as exhaustive.

Genetic selection for increased milk production

Genetic selection for increased milk production is common in the US dairy industry, although certainly not exclusive to this country. Selection for high levels of milk production has been reported to be associated with some negative management outcomes such as exacerbated negative energy balance in early lactation and reduced reproductive performance (reviewed by Rauw et al. (1998)). These authors suggested that "artificial selection for a particular trait may lead to the situation in which resources are used to the maximum, i.e. no buffer is left to respond adequately to unexpected stresses and challenges." However, Bello et al. (2012) challenged this generally held belief, especially in regards to reproduction, claiming that various statistical pitfalls have led to the erroneous conclusion that these two traits are homogenously antagonistic, and called for further research to determine whether such a relationship exists. If these negative associations are truly existent, they may decrease the overall profitability of intensive genetic selection for milk production on a farm level. Despite this, 55% of the gains in production observed between 1980 and 2006 can be accounted for by genetic improvement. As evidenced previously in this review, the large increase in milk production experienced by the dairy industry in recent decades is a large driver of the overall sustainability of the industry.

Exogenous recombinant bovine somatotropin

Perhaps the most well-characterized example of a common technology used in the dairy industry to increase milk production is rbST. Administration of rbST results in differences in nutrient partitioning, allowing more nutrients to be available to the mammary gland for milk production (Bauman and Currie, 1980). In the short term, rbST injections do not influence feed intake, and

milk production is increased through an “an exquisite coordination of metabolism to meet nutrient needs for increased synthesis of milk components (Bauman et al., 1988, Tyrrell et al., 1988).

The use of rbST has been researched from the 3 pillars of sustainability addressed in this review. It meets the goal of satisfying human nutritional needs through a direct effect on increasing milk production—averaging 4.5 kg/cow/d (Capper et al., 2008)—but beyond that, it has been studied for its environmental and economic effects. Through dilution of maintenance, administration of rbST has a striking effect on the environmental burden of milk production per unit of milk, reducing the amount of land required by 9.2%, water by 10.4%, and the carbon footprint by 9.1%. Supplementation of 1 million cows with rbST would have a similar environmental effect as removing approximately 400,000 cars (Capper and Bauman, 2013).

The effect of rbST on the economic viability of farm operations is somewhat less clear cut. It is true that it has a high return on investment in comparison to other technologies used on the modern dairy farm; however, consumer perception plays an important role in its economic viability. Despite the clearly demonstrated safety of rbST, use of this technology met with resistance from consumers when it was implemented. Grobe and Douthitt (1995) concluded that consumers overestimate the risk of rbST in milk, and that this was not necessarily due to a lack of education and information. They demonstrated that price reductions of up to 10% could not compensate for risk perceptions. These authors reported that risk perceptions were positively influenced by quantity of milk purchased and gender, while income had a negative impact on risk perception. Dhar and Foltz (2005) reported that the substitution of unlabeled (conventional) milk for rbST-free or organic milk is asymmetric. In other words, consumers are likely to switch from conventional milk to rbST-free or organic milk in response to a change in price in conventional

milk, but once they have switched to another process, they are less likely to switch back to conventional milk, even if there are significant price changes in rbST-free or organic milk.

Monensin

Monensin is a gold standard of sorts in the manipulation of rumen fermentation for improved efficiency. Since its acceptance in 1975, it has been widely utilized by both the beef and dairy industries because of its remarkable positive impact on the economic viability of farm operations (Goodrich et al., 1984). Elanco Animal Health (Greenfield, IN) markets monensin as Rumensin©, and the company boasts that this product averages a 5:1 return on investment (http://www.elanco.us/pdfs/optimizing-response.pdf).

Monensin is an ionophore, which translates to "ion bearer." It creates channels in the cell walls of Gram-positive bacteria, resulting in so-called "futile cycling," where energy is expended while growth is stagnated (Russell and Strobel, 1989). This results in changes in the fermentation profile that lend themselves to increased efficiency, such as a decreased ratio of acetate to propionate and decreased methane production.

Obviously, monensin is an example of a technology that meets all of the aspects of sustainability discussed in this review, particularly environmental and economic. However, like rbST, this technology has met with some resistance at the consumer level. Although it does not cause classic antibiotic resistance in the form of mutations (Russell and Houlihan, 2003), some groups of consumers are concerned about its widespread use due to its antibiotic activity. It remains to be seen if this effect of consumer perception will influence the use of monensin on dairy farms, but for now it is apparent that monensin is an effective way of increasing milk production and the overall sustainability of the industry.

Sexed semen

At times, the use of sexed semen has gained popularity in the dairy industry. A high percentage of female calves translates into more replacements available for the milking herd, and heifer calves are generally worth more than bull calves when they are sold. However, sexed semen is more expensive than non-sexed semen, and the conception rate when using sexed semen is often lower than conventional semen (DeJarnette et al., 2009).

Until recently, the utilization of sexed semen on dairy farms has been approached from the standpoint of increasing the number of replacements and the trade-offs with semen prices and conception rates. However, Hinde et al. (2014) demonstrated that the sex of the calf is associated with differences in 305-d milk production in the following lactation, and that this effect compounds over time. Ettema and Østergaard (2015) calculated that this effect on milk production increased milk production and net return per cow per year.

Since the initial publication of Hinde et al. (2014), several authors have attempted to replicate these results in various regions. Evidence that Holstein cattle produce more milk after giving birth to a heifer calf has been demonstrated in data sets from Canada (Beavers and Van Doormaal, 2014), Iran (Chegini et al., 2015), New Zealand (Hayr, 2014), and Poland (Sawa et al., 2014). On a study of dairy cattle performed in Colombia, it was reported that cows who gave birth to heifers had higher colostrum production than those that gave birth to bulls (Angulo et al., 2015). Alternatively, Græsbøll et al. (2015) reported that in Danish Holsteins, cows produced more milk when they gave birth to bull calves.

While little research has been done mechanistically to determine the cause of this sex-bias towards heifer calves—assuming that it is a real effect—one might speculate that following the

concept of dilution of maintenance, this effect on milk production would have a positive impact on the environmental burden of the dairy industry in addition to the beneficial economic effects for dairy farms. Therefore, this technology appears to be in line with the pillars of sustainability outlined in this review.

Non-steroidal antiinflammatory drugs

The transition period is defined as 3 weeks prior to and 3 weeks following parturition. As a result of various metabolic, dietary, and social pressures, early lactation dairy cattle are prone to various health and metabolic disorder, which can lead to continuous reproductive and production issues throughout the remainder of the lactation. Linked to many of these transition disorders is systemic metabolic inflammation (Bradford et al., 2015), which has been linked by multiple authors to negative productive outcomes (Bionaz et al., 2007, Bertoni et al., 2008).

While the therapeutic use of NSAID in the dairy industry is relatively common, some studies have suggested that the blanket use of NSAID in older cows after calving can have a positive impact on milk production in the ensuing lactation. Bertoni et al. (2004) demonstrated that treatment with lysine acetyl-salicylate post-calving increased daily milk production. Farney et al. (2013) and Carpenter et al. (2016) demonstrated that the administration of sodium salicylate or meloxicam following parturition to cows in their second or third parity and greater was associated with increased 305-d milk production. However, several other published reports have not seen the same positive response to NSAID (Shwartz et al., 2009, Priest et al., 2013, Mainau et al., 2014, Meier et al., 2014). This may be due to differences in administration or differences in the time periods measured between studies. Carpenter et al. (2016) showed that there was no difference in daily milk production until 7 weeks in lactation, so studies that evaluate milk production for less

than this may not detect a difference in milk production due to treatment. Further details can be found in Chapter 2 of this dissertation, where this paper is presented.

Further research is needed to determine the effect of NSAID use on the economic viability of farms and the sustainability of the industry. It is unknown what the economic impact of the use of NSAID is at this juncture. In the US, some NSAID need veterinary approval for their use and are not approved for general administration, so the cost of using these products is difficult to determine. It also remains to be determined how NSAID administration influences feed intake. Unpublished results showed that sodium salicylate did not influence DMI, but in the same study, no differences in milk production were observed (Chapter 3). Although the exact mechanism by which NSAID treatment influences milk production is unknown, there is evidence that some NSAID manipulate rumen function (Chapter 4).

CONCLUSION

The ultimate objective of the dairy industry is to produce a high-quality food product to provide for the nutritional needs of the world while at the same time providing a livelihood for its producers. To sustain this industry, these goals must be met while avoiding the depletion of the natural resources that the industry depends on. Under most circumstances, high levels of milk production promotes the sustainability of the dairy industry by meeting all of these conditions.

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Table 1.1 Amino acid concentrations of various common dietary protein sources. Adapted from van Vliet et al. (2015).

Source	Essential amino acids, % total protein	Leucine, % total protein	Lysine, % total protein	Methionine, % total protein
Plant sources				
Spirulina	41	8.5	5.2	2.0
Mycoprotein	41	6.2	6.7	1.5
Lentil	40	7.9	7.6	0.9
Quinoa	39	7.2	6.5	2.6
Black bean	39	8.4	7.3	1.6
Corn	38	12.2	2.8	2.1
Soy	38	8.0	6.2	1.3
Pea	37	7.8	6.3	1.6
Rice	37	8.2	3.8	2.2
Oat	36	7.7	4.2	1.9
Hemp	34	6.9	4.1	2.3
Potato	33	5.2	5.7	1.7
Wheat	30	6.8	2.8	1.9
Animal sources				
Whey	52	13.6	10.6	2.3
Milk	49	10.9	8.6	2.7
Casein	48	10.2	8.1	2.7
Beef	44	8.8	8.9	2.5
Egg	44	8.5	7.1	3.0
Cod	40	8.1	8.8	3.0

Figure 1.1 The 3 pillars of sustainability. Adapted from von Keyserlingk et al., 2013.

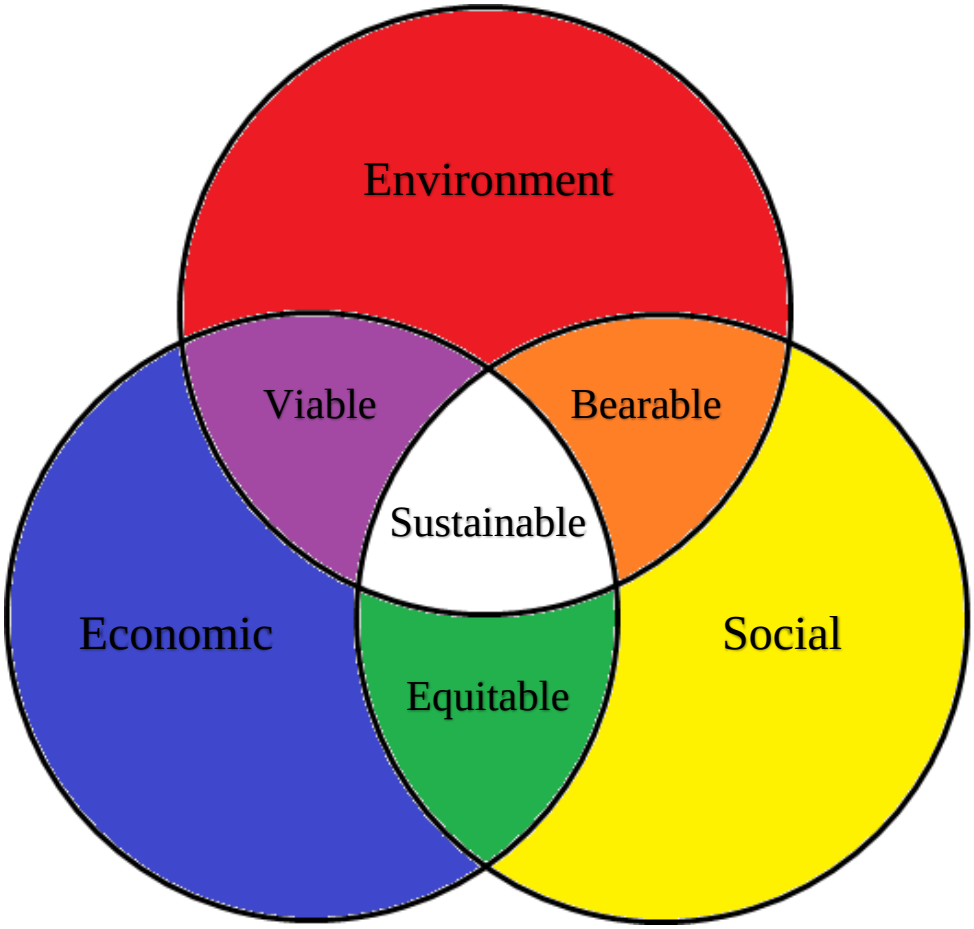
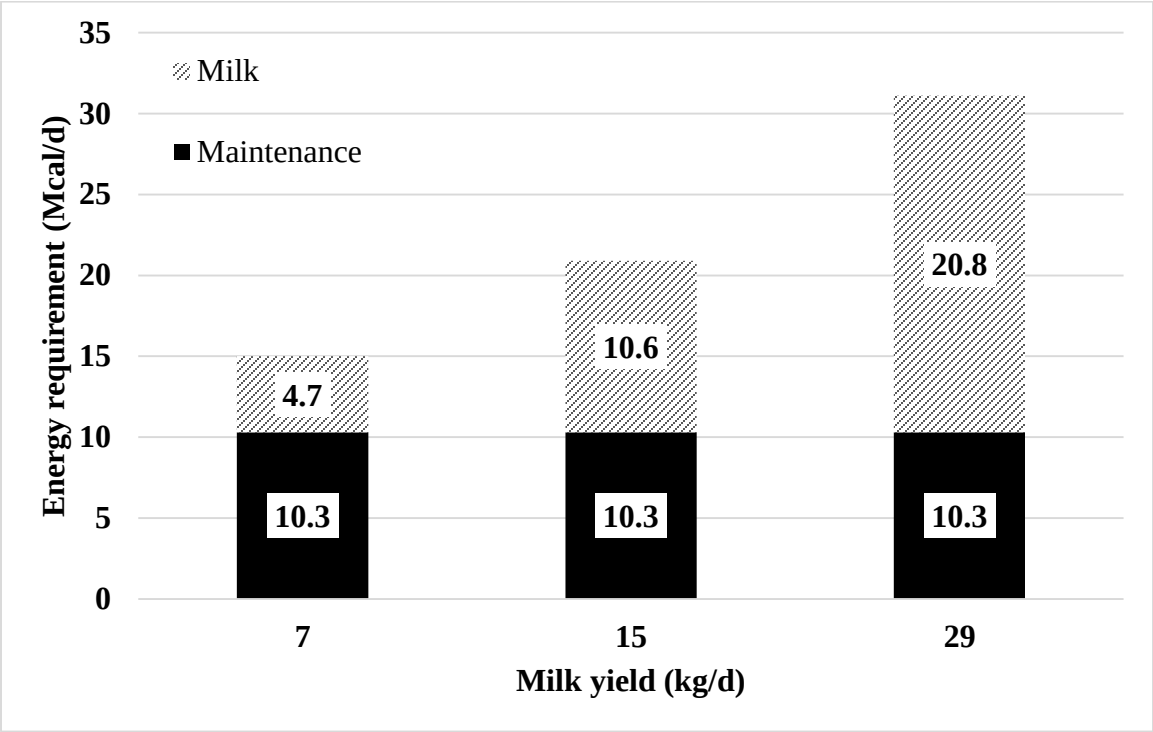


Figure 1.2 Increasing milk production in a lactating dairy cow results in dilution of maintenance. Adapted from Capper et al. (2009).



Assuming a body weight of 650 kg and a milk fat percentage of 3.69%, the energy requirement for maintenance will account for 69, 49, and 33% of the total energy requirement at 7, 15, and 29 kg of daily milk production, respectively.

Chapter 2 - *Hot topic*: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield

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ABSTRACT

Previous research has shown that postpartum administration of the nonsteroidal antiinflammatory drug (NSAID) sodium salicylate can increase 305-d milk yield in older dairy cattle (parity 3 and greater). However, in this prior work, sodium salicylate was delivered to cows via the drinking water, a method that does not align well with current grouping strategies on commercial dairy farms. The objective of the current study was to replicate these results on a commercial dairy farm with a simplified treatment protocol and to compare sodium salicylate with another NSAID, meloxicam. Dairy cattle in their second lactation and greater (n = 51/treatment) were alternately assigned to 1 of 3 treatments at parturition, with treatments lasting for 3 d. Experimental treatments began 12 to 36 h after parturition and were (1) 1 placebo bolus on the first day and 3 consecutive daily drenches of sodium salicylate (125 g/cow per day; SAL); (2) 1 bolus of meloxicam (675 mg/cow) and 3 drenches of an equal volume of water (MEL); or (3) 1 placebo bolus and 3 drenches of water (CON). Blood samples were collected on the first day of treatment, immediately following the last day of treatment, and 7 d after the last day of treatment; plasma was analyzed for glucose, β-hydroxybutyrate (BHB), free fatty acids, haptoglobin, and

paraoxonase. Milk production, body condition score, reproductive status, and retention in the herd were monitored for 365 d posttreatment, and effects of treatment, parity, days in milk, and interactions were evaluated in mixed effects models. Significance was declared at $P < 0.05$. Whole-lactation milk and protein yields were greater in NSAID-treated cows, although 305-d fat production was not affected. There was a significant interaction of treatment and parity for plasma glucose concentration; MEL increased plasma glucose concentrations compared with CON and SAL in older cows. Sodium salicylate decreased plasma BHB concentration compared with MEL at 7 d posttreatment, although no difference was detected immediately following treatment. Haptoglobin concentrations were elevated in SAL cows compared with CON. There was a tendency for CON cows to be removed from the herd more quickly than MEL cows (42 vs. 26% at 365 d posttreatment). Body condition score, concentrations of plasma free fatty acids and paraoxonase, and time to pregnancy were not affected by treatment. These results indicate that NSAID administration in postpartum cows has the potential to be a viable way to improve productivity and potentially longevity in commercial dairies, although further research is necessary to optimize recommendations for producers.

HOT TOPIC

Despite ongoing research, the transition period remains a high-risk period for dairy cattle. A growing body of research indicates that systemic metabolic inflammation occurs in dairy cows following parturition and that this inflammation may be linked to negative production outcomes. Bionaz et al. (2007) demonstrated that reduced levels of the liver hydrolase paraoxonase are associated with increased markers of inflammation such as haptoglobin and globulin in early-lactation dairy cattle. Those authors reported that animals in the experiment with the greatest

plasma paraoxonase activity during the first 30 d of lactation produced 10,090 ± 1,504 kg of milk in a 305-d lactation, whereas those with the least activity produced 8,119 ± 2,042 kg, a 1,971-kg difference. Bertoni et al. (2008) reported that cows in the highest quartile of an inflammatory index had decreased milk production in the first month of lactation compared with their counterparts with the lowest inflammatory markers (24.4 vs. 30.9 ± 2.11 kg/d). Farney et al. (2013b) administered dairy cattle with the nonsteroidal antiinflammatory drug (NSAID) sodium salicylate via drinking water in the week following calving in an attempt to suppress inflammation. Cows in their third lactation and greater that received sodium salicylate produced 21% more milk over a 305-d lactation than did parity-matched controls. Interestingly, although the initial hypothesis was that inflammation was linked to suboptimal metabolism, this productivity response occurred even though both the control and treatment groups had low incidence of clinical metabolic disorders.

Meloxicam is another drug in the NSAID class that has high oral bioavailability and a long plasma elimination half-life in cattle compared with sodium salicylate (Coetzee et al., 2009; Malreddy et al., 2013). Although no published studies have demonstrated that meloxicam affects milk production, research in lactating cattle has focused on its use during clinical mastitis (McDougall et al., 2009) and following assisted parturition (Newby et al., 2013). Considering the effect of sodium salicylate on production, it is likely that meloxicam, with a longer elimination half-life, may also have beneficial effects in lactation after a single dose. Therefore, the objective of this study was to determine if NSAID treatment in the first days following parturition would positively affect milk production and health of cows on a commercial dairy farm.

Multiparous cows from a commercial dairy (n = 51/treatment) were enrolled in the study 12 to 36 h after calving. Animals were managed similarly throughout the dry period and early lactation. Cows assigned to sodium salicylate treatment (SAL) received a placebo bolus on d 1 of

treatment and an oral drench containing 125 g/d of sodium salicylate (estimated to be approximately 185 mg/kg of BW; Wintersun Chemical, Ontario, CA) in 375 mL of water for 3 consecutive days beginning on d 1 of treatment. Cows assigned to meloxicam treatment (MEL) received 675 mg of meloxicam (estimated to be approximately 1 mg/kg of BW; Unichem Pharmaceuticals, Rochelle Park, NJ) as a bolus on d 1 of treatment as well as 3 consecutive daily drenches of 375 mL of water. Control cows (CON) received a placebo bolus on d 1 and water drenches (375 mL) for 3 d. The placebo and meloxicam boluses both contained casein as a filler. Only cows entering their second lactation and greater were enrolled in the study (CON = 18 cows in parity 2 and 33 cows in parity ≥3; MEL = 27 cows in parity 2 and 24 cows in parity ≥3; SAL = 20 cows in parity 2 and 31 cows in parity ≥3). Cows were blocked by mastitis at parturition (CON = 1, MEL = 2, SAL = 2), breed (CON = 6, MEL = 6, SAL = 4 crossbred; all others were Holstein), dystocia (calving difficulty score ≥3; CON = 5, MEL = 5, SAL = 6), and twin births (CON = 4, MEL = 4, SAL = 3) and were sequentially assigned to treatment within block between July 15 and September 2, 2013. Mastitis was determined by farm staff for blocking purposes, and was defined as clinical mastitis with abnormal appearance of milk, such as clots. Milk from treated cows was discarded for 10 d after the start of treatment to ensure that no drug residue entered the saleable milk stream, particularly for meloxicam (Malreddy et al., 2013).

Blood samples were collected via the coccygeal vein on the first and last day of treatment and 7 d after the completion of treatment. Plasma was collected and stored at −20°C until analyzed for glucose by a colorimetric kit (kit #439-90901; Wako Chemicals USA Inc.), free fatty acids using an enzymatic colorimetric procedure (NEFA-HR; Wako Chemicals USA Inc., Richmond, VA), and BHB using a commercial kit (kit #H7587-58; Pointe Scientific Inc., Canton, MI). Haptoglobin was measured by the method of Cooke and Arthington (2013), a colorimetric

technique that uses differences in peroxidase activity to measure haptoglobin-hemoglobin complexing. Absorbance was measured with a spectrophotometer (PowerWave XS; BioTek Instruments Inc., Winooski, VT) and calculations were performed using Gen5 software (BioTek Instruments Inc.). Paraoxonase was measured by the method of Ferré et al. (2002).

Reproduction and culling data were recorded in PC-Dart (Dairy Records Management Services, Raleigh, NC) by the farm staff, who were blinded to treatments. Reasons for culling were grouped into the following 7 categories: injury, lameness, low milk, mastitis, SCC, unknown, and other disease. Milk weights were collected electronically at each milking and stored in PC-Dart. Milk composition (including SCC) and yield were tested for individual cows at approximately 6-wk intervals by DHIA technicians, and 305-d mature-equivalent lactation yields were calculated by DHIA for animals that remained in the herd for at least 90 d. Body condition score was recorded as the average of responses from at least 3 independent observers on the last day of treatment (3 d after enrollment) and approximately 2, 5, and 8 mo following enrollment.

Statistical analyses were carried out using SAS (version 9.3; SAS Institute Inc., Cary, NC) and JMP (version 10; SAS Institute Inc.). Plasma variables were analyzed using d-0 values as a covariate along with fixed effects of block, parity (2 or 3+), treatment, sample day, and treatment by day interaction, and the random effect of cow. Milk data were analyzed with fixed effects of block, parity, treatment, week of lactation, and treatment × week interaction, along with the random effects of cow and week of the year. Both models accounted for repeated measures over time with autoregressive covariance structures. This covariance structure was selected based on Bayesian information criterion (BIC) values. Blocking factors and treatment × block interactions were tested and removed from models when $P > 0.10$. Additionally, d-0 covariate values (plasma analytes and BCS) and their interactions with treatment were tested in models for milk yield, but

all were removed because they were not significant predictors. To assess treatment effects on SCS, cows with mastitis at enrollment (n = 5) were excluded, and then individual test-day values between 4 and 305 DIM were modeled with linear and quadratic DIM terms (the cubic term was not significant), treatment, and treatment × DIM interaction, as well as the random effect of cow. Mastitis at enrollment was included in the statistical model for all responses other than SCS.

In addition to milk responses by week, 305-d mature-equivalent lactation yields were evaluated as described by Farney et al. (2013b), using the PTA for the applicable component as a covariate to account for genetic differences; 122 cows had the requisite data and were used for this analysis. Survival analysis was used to assess treatment effects on retention in the herd and time to pregnancy. Pregnancy date was determined based on breeding dates when pregnancy was confirmed by ultrasound 65 d postinsemination, and cows that left the herd before 365 d postcalving were censored from pregnancy analysis on that date. Wilcoxon Chi-squared tests were used to assess differences between treatments for survival curves. Incidence of specific disorders and pregnancy on first service were evaluated by pairwise Fisher's exact tests. Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

Both MEL and SAL increased daily milk production compared with CON ($P < 0.05$; 36.8, 36.3, and 32.8 ± 2.2 kg/d, respectively). We found no evidence of treatment interactions with time ($P = 0.56$), although the contrasts between NSAID treatments and CON did not become significant until 7 wk in milk (Figure 2.1A). Analysis of 305-d mature-equivalent milk yield resulted in similar findings, with MEL and SAL increasing yields compared with CON (both $P < 0.03$; 11,205, 11,411, and 10,472 ± 486 kg, respectively). Similar results were observed when daily and 305-d data were analyzed after removing cows with mastitis at parturition ($P < 0.05$ for all contrasts with CON, except SAL vs. CON for daily yield: $P = 0.06$). Figure 2.1B shows 305-d mature-equivalents

yields for milk fat and milk protein. No differences were observed for milk fat yield ($P = 0.13$) but protein yields were increased by each NSAID treatment ($P < 0.05$). We observed an interaction between treatment and DIM for SCS ($P = 0.02$, Figure 2.1C), with the results suggesting that NSAID treatments decreased SCS in the first several months of lactation. However, when the data set was limited to values measured in the first 50 DIM, a simple NSAID treatment contrast (CON vs. MEL + SAL) was not significant ($P = 0.13$).

The increase in milk production is consistent with the response observed by Farney et al. (2013b). A few other experiments have assessed blanket treatment with NSAID in postpartum cows. Bertoni et al. (2004) gave acetyl-salicylate to cows for the first 5 DIM and saw a tendency for increased peak milk yield. Priest et al. (2013) gave carprofen as a blanket NSAID treatment to multiparous cows 21 to 31 d after calving and saw no effect on milk production. The authors hypothesized that this was due to the delay in treatment after calving. In a follow-up study, Meier et al. (2014) reported that whether NSAID treatment was administered beginning 1 d following calving or 19 d, there was no difference between control and NSAID groups for milk production. In an experiment reported by Shwartz et al. (2009), 26 cows received either intravenous flunixin meglumine or saline for the first 3 DIM. In the first 7 d of lactation, milk yield was decreased for cows receiving NSAID treatment, although there was no overall effect on milk yield up to 35 DIM. One study that administered injectable meloxicam as a blanket treatment postcalving showed no effect on milk production (Mainau et al., 2014).

There are several possible explanations for variability in the reported milk responses to NSAID treatments. Meier et al. (2014) attributed the difference in results between their experiment and those of Bertoni et al. (2004) and Farney et al. (2013b) to the differences in mode of action between different NSAID treatments. This is a valid differentiation between their results and

responses to salicylates; however, it does not explain the differences between our results and those of Mainau et al. (2014), in which meloxicam was also used as a blanket treatment in early-lactation dairy cattle. This could be attributed to differences in sample size (n = 51 vs. n = 30), although there are other differences between these experiments. The method of meloxicam administration differed; in our experiment, meloxicam was given orally, whereas Mainau et al. (2014) injected meloxicam. Although BW was not measured in our experiment, if it is assumed that the average BW of cows on this study was approximately 680 kg, our dose of 675 mg would be approximately double the dosage of 0.5 mg/kg used by Mainau et al. (2014). Another difference between the current experiment and that of Mainau et al. is the timing of treatment administration (12–36 h postcalving vs. a maximum of 6 h postcalving).

Perhaps the most important confounding effect on results of NSAID trials is the duration of milk production measurement. In the current experiment, statistical tendencies were detected beginning at 4 wk in milk (P = 0.07), and significance was not detected until 7 wk in milk (P = 0.02). Priest et al. (2013) and Meier et al. (2014) measured milk production for 6 wk. Milk production was monitored for 5 wk by Shwartz et al. (2009). Mainau et al. (2014) reported milk production over 1 mo. It is possible that some of the discrepancy between the current experiment and these previously published reports is because milk production was not monitored long enough to detect any differences in previous studies.

It is important to note that both NSAID treatments necessitated the discarding of milk to prevent the sale of milk contaminated by drug residues. There are no official guidelines in the United States for disposal of milk following salicylate or meloxicam treatment in lactating cows. However, based on the short half-life of salicylate, a milk withdrawal time of 24 h is considered adequate to avoid residues (US Pharmacopeia, 2004). In contrast, the recently approved use of

injectable meloxicam at 0.5 mg/kg of BW for mastitis in Canada requires a 4-d withdrawal, and oral meloxicam at 1 mg/kg of BW (approximately the dose used herein) resulted in undetectable residues in milk by 80 h posttreatment (Malreddy et al., 2013). Although these data suggest that a 4- or 5-d withdrawal is likely adequate for avoiding residues following meloxicam treatment, we discarded milk for 10 d to remove any uncertainty about residue avoidance. The increased 305-d milk production that we observed can compensate for loss of milk revenue during the withdrawal period, but the inconvenience of segregating milk from these cows could limit adoption of postpartum NSAID treatment on dairies. On the other hand, several commonly used dry cow antibiotic therapies require that milk be discarded for the first 3 to 4 DIM (Royster and Wagner, 2015), and the gland is transitioning from colostrum to mature milk secretion during this time, making the product of poor quality for dairy products anyway. Considering this, the SAL protocol would add only 1 to 2 d and MEL only 2 to 3 d of additional milk discarding.

We found no effect of treatment on blood glucose concentration in second-parity cows, but MEL increased plasma glucose concentrations in older cows compared with CON and SAL ($P < 0.05$; treatment × parity: $P < 0.05$; Figure 2.2A). Although plasma BHB concentrations were similar across treatments at the end of the treatment period ($P > 0.1$), SAL decreased plasma BHB concentration compared with MEL at 7 d posttreatment ($P = 0.02$; treatment × day: $P < 0.05$; Figure 2.2B). Plasma free fatty acid concentrations were not affected by treatment ($P = 0.8$; Figure 2.2C). No other interactions were significant for free fatty acids, glucose, or BHB ($P > 0.1$). Neither plasma variables nor BCS measured on d 1 was a significant predictor of any of the outcomes measured ($P > 0.1$).

The time points for blood sample collection were chosen based on the results of Farney et al. (2013a), which showed that glucose, BHB, and some signaling molecules were significantly

altered by NSAID treatment on either the final day of treatment or 7 d posttreatment. They reported that cows in third parity and greater experienced hypoglycemia at the end of 7 d of sodium salicylate treatment, even though they also had increased 305-d milk production. The SAL treatment regimen in the current experiment did not induce the same response, possibly because of the shorter treatment window. The elevation in blood glucose after meloxicam treatment in older cows in the current study is unique in the literature. Blood glucose was not measured in every experiment, and Newby et al. (2013) observed no changes in glucose levels. Unlike in the current experiment, BHB levels increased after treatment with sodium salicylate in Farney et al. (2013a), consistent with the hypoglycemia reported in that paper. Although there were no differences between CON and either NSAID treatment in the current experiment, SAL and MEL groups differed in BHB concentration, suggesting subtle differences in responses to different NSAID treatments. The failure to demonstrate a consistent pattern of blood metabolites between MEL and SAL would seem to indicate that the differences observed in milk production are due to something beyond simple transition health, perhaps a programming effect. Paraoxonase did not differ between treatments (P = 0.15; Figure 2.2D), although surprisingly, haptoglobin levels were elevated in SAL cows compared with CON (P = 0.02), with levels in MEL being intermediate (Figure 2.2C). Proinflammatory eicosanoids were elevated 7 d following the cessation of NSAID treatment in the study reported by Farney et al. (2013a). Those authors attributed this to a "rebound" effect of the inflammatory response after the antiinflammatory agent was removed. Though there was no interaction between treatment and time for haptoglobin or paraoxonase in the current study, it is possible that the "homeostatic target" for the inflammatory response postulated by these authors contributed to the differences in haptoglobin levels. It is important to recognize that not all metabolic changes may have been captured in the sampling time selected for

the current experiment, and it may be advisable in future studies to monitor blood metabolites for longer than the 7-d posttreatment period chosen in the current experiment.

We detected a tendency for CON cows to leave the herd more quickly than MEL cows over the first 365 d postenrollment ($P = 0.06$; 21 vs. 13 gone by 365 d, Figure 2.3A). There was a tendency for CON to differ from MEL in "other disease" incidence ($P = 0.09$), a category that includes periparturient metabolic disorders, as only 2 cows in MEL were removed for this reason, compared with 8 cows in CON and 6 cows in SAL. No other statistical differences or tendencies were detected for other culling categories ($P > 0.1$; data not shown). Interestingly, no SAL cows were culled for low milk production, although 4 and 3 cows left the herd for this reason from CON and MEL groups, respectively. The incidence of culling due to mastitis was similar across treatment groups, with 2, 1, and 4 cows culled for mastitis in CON, MEL, and SAL treatments, respectively, suggesting that observed treatment effects on SCS dynamics (Figure 2.1C) did not translate into different clinical mastitis outcomes. We observed no difference between treatments for the time to pregnancy ($P = 0.68$; Figure 2.3B), and no differences in first-service pregnancy rate ($P > 0.78$; 21.3% overall). No differences were observed in BCS ($P = 0.93$, Figure 2.3C), and there was no interaction between treatment and time (treatment × time: $P = 0.76$).

Few studies have directly analyzed the long-term effects of NSAID administration in the transition period on reproductive performance. Those that have focused on the transition period have generally investigated the use of NSAID as a therapeutic treatment for metritis. One concern regarding NSAID treatment too soon after calving is that the incidence of retained fetal membranes could be elevated; however, evidence suggests that its use does not increase the percentage of cows with retained placenta following meloxicam administration, even immediately after calving (Newby, 2014). Amiridis et al. (2001) demonstrated that cows treated for metritis with flunixin at

5 to 8 DIM had shorter intervals to first estrous, and uterine involution occurred more rapidly in these cows. Priest et al. (2013) reported that cows with subclinical metritis responded to treatment with the NSAID carprofen at 21 to 31 DIM with increased pregnancy rates 4 wk after the planned start of mating, whereas cows with normal to moderate uterine pathology did not respond in this way to NSAID. Alternatively, Drillich et al. (2007) reported no difference in first-service conception rate in cows with metritis that received flunixin plus an antibiotic compared with an antibiotic alone. When carprofen was administered after a voluntary waiting period, other researchers have reported no effect of carprofen on reproduction when administered 14 to 16 d after insemination (von Krueger and Heuwieser, 2010) or both before and after insemination (Heuwieser et al., 2011). Other researchers have shown no benefit of flunixin in combination with timed insemination (Rabaglino, 2010). When meloxicam was delivered intramuscularly at various time points surrounding breeding and throughout pregnancy, no differences in reproductive performance were observed (Hirsch and Philipp, 2009). Considering the positive effects of NSAID treatment on reproduction that other authors have observed, it is possible, if an NSAID effect is more potent in cows with metritis, that the number of cows with uterine infections was inadequate to detect a treatment effect in the current experiment.

In conclusion, early-lactation treatment with NSAID from 2 different classes increased whole-lactation milk yield by 7 to 9%, with only a 3-d treatment window. When possible, NSAID effects should be recorded throughout the entire lactation, as treatment differences may be delayed and not immediately apparent following administration, such as in the current experiment. Furthermore, the tendency for MEL to delay the mean time to removal from the herd points to a fruitful area of investigation for future research.

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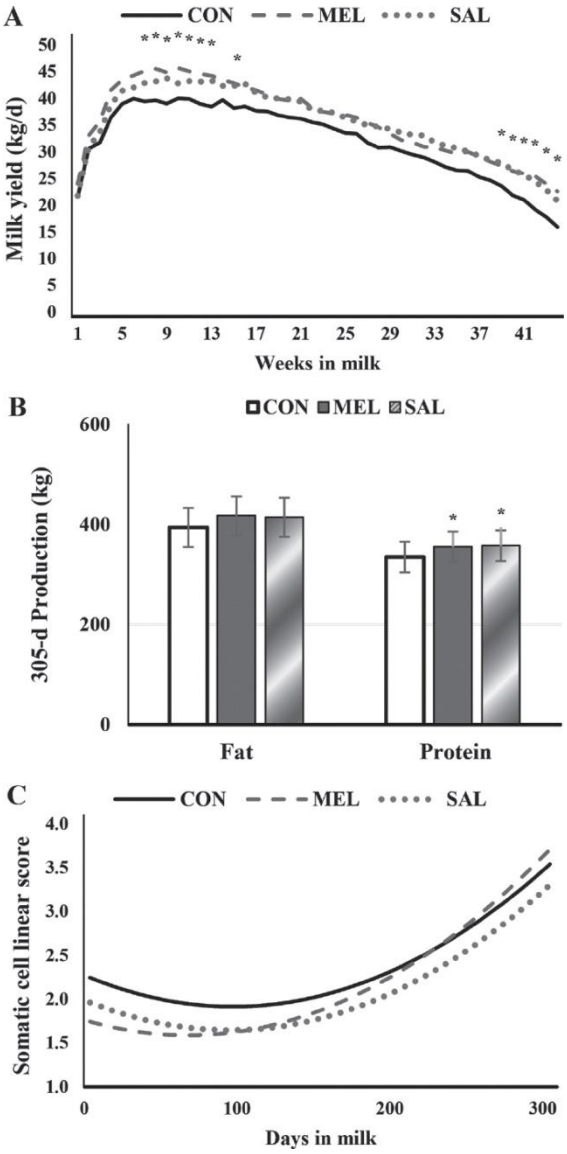
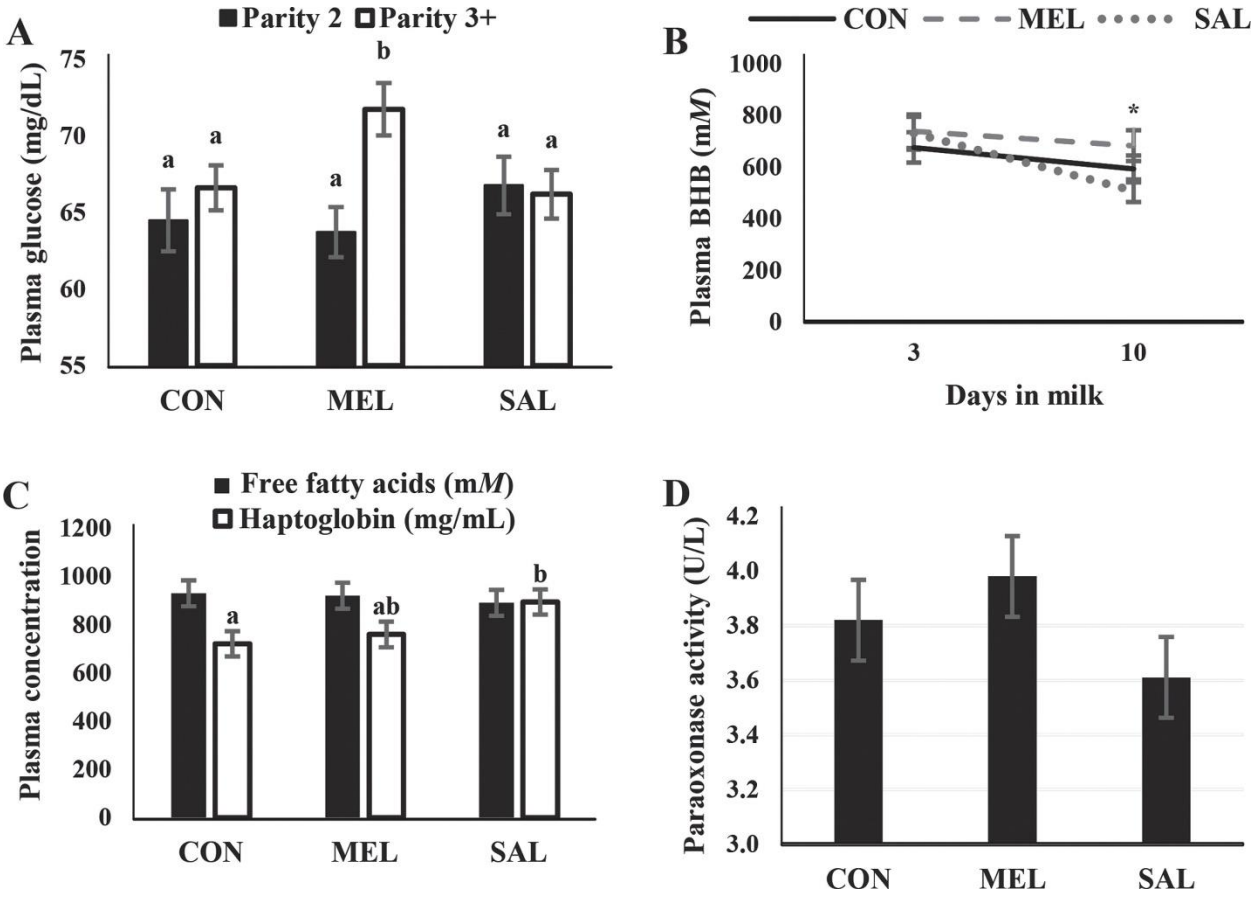
Figure 2.1 Whole-lactation milk responses following early-lactation treatment with placebo (CON), meloxicam (MEL), or sodium salicylate (SAL).



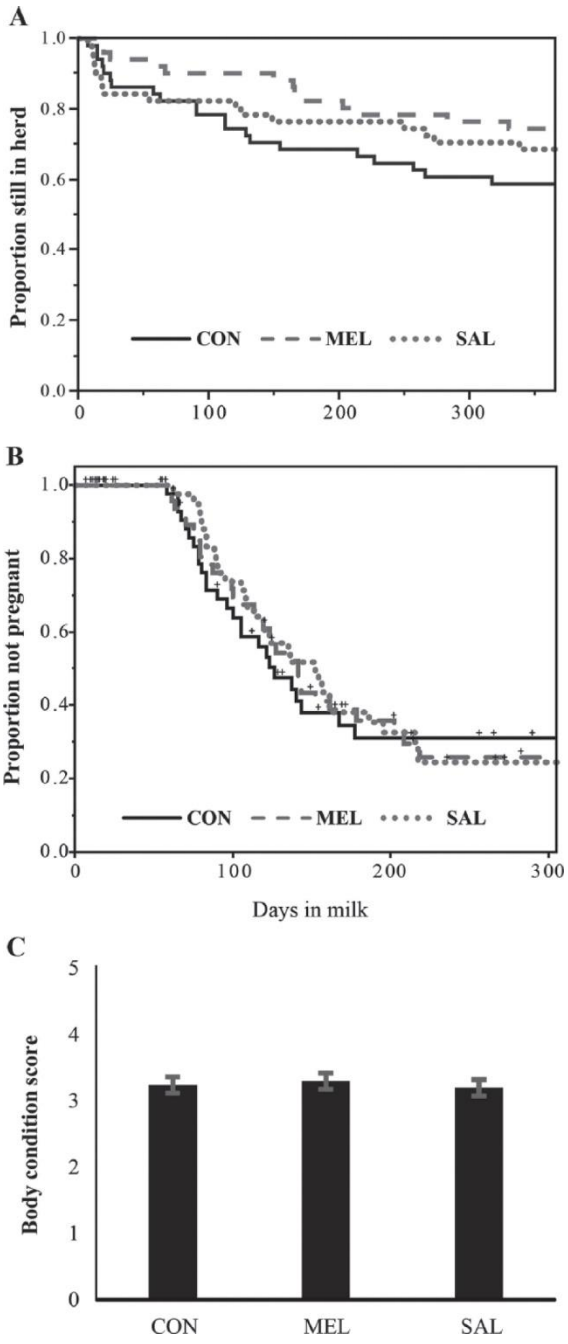
Treatments were administered for 3 d beginning 12 to 36 h postpartum, and values are means ± SEM. (A) Daily milk yield data were compiled by week and analyzed with repeated measures (pooled SEM = 2.4 kg/d). *NSAID treatments differ from CON ($P < 0.05$, n = 51). (B) 305-d mature-equivalent component yields were analyzed in a model that accounted for genetic effects. *Differs from CON ($P < 0.05$, n = 39–42). (C) Individual test-day SCS data were modeled to account for DIM and cow (n = 49–50). Treatment interacted with DIM to influence SCS, due to a different treatment × DIM coefficient for MEL versus SAL ($P = 0.02$). As an example, the equation for SAL was SCS = 1.27 + 0.0032 × DIM + 0.0000378 × $(\text{DIM} - 137.9)^2$.

Figure 2.2 Plasma constituent responses following early-lactation treatment with placebo (CON), meloxicam (MEL), or sodium salicylate (SAL).



Treatments were administered for 3 d beginning 12 to 36 h postpartum. Unless otherwise noted, values represent the overall mean (±SEM, n = 50–51) across samples collected at 3 and 10 DIM, and means with different letters (a, b) differ ($P < 0.05$). (A) Plasma glucose concentration was increased by MEL in parity 3+ cows. (B) Treatment interacted with time to alter plasma BHB concentration. *SAL differs from MEL at d 7 post-treatment ($P = 0.02$). (C) Plasma free fatty acids concentration was not altered by treatment, but SAL increased haptoglobin concentration compared with CON. (D) Plasma paraoxonase activity was unaltered by treatment.

Figure 2.3 Survival analysis and BCS responses following early-lactation treatment with placebo (CON), meloxicam (MEL), or sodium salicylate (SAL).



Treatments were administered for 3 d beginning 12 to 36 h postpartum (n = 51). (A) Retention in the herd by treatment; 30, 38, and 35 cows remained in the herd at 365 d posttreatment for CON, MEL, and SAL, respectively. MEL tended to delay removal from the herd relative to CON (P = 0.06, Wilcoxon chi-squared test). (B) Pregnancy survival analysis showed no effect of treatment on mean time to pregnancy (P > 0.78). +Cows removed from the herd were censored from analysis. (C) BCS was evaluated on d 3 of treatment and approximately 2, 5, and 8 mo posttreatment. No treatment × time interaction was detected (P = 0.76), and values represent the overall means (±SEM, n = 50–51). No treatment effect was detected (P = 0.93).

Chapter 3 - Effect of early postpartum treatment of dairy cows with sodium salicylate on long-term milk, intake and blood parameters

A. J. Carpenter, C. M. Ylioja, L. K. Mamedova, B. J. Bradford

ABSTRACT

Previous research has shown that cows who receive treatment with non-steroidal antiinflammatory drugs (NSAID) after calving may have increased milk yield after early lactation, resulting in greater 305-d milk production. It has not been demonstrated whether this increased production is associated with greater feed intake following the first 3 weeks of lactation. In this experiment, daily feed intake and milk yield were measured for 56 cows over the first 120 days in milk. Cows in their second parity and greater were blocked by parity and alternately enrolled into 1 of 2 treatments 12-36 h after calving, either 3 daily drenches of water (CON) or 3 daily drenches of similar volume of water containing 125 g of sodium salicylate (SAL). Cows were housed in individual stalls to monitor DMI. Blood samples were collected before calving and on the last day of treatment, as well as at 7, 11, 14, 18, 21, 35, 49, 63, 77, 91, 105, and 120 DIM. Treatment with SAL did not affect 305-d milk, fat, or protein yields, daily milk yield or components, ECM, FCM, or DMI ($P > 0.10$); however, a significant interaction between parity and DMI was observed ($P = 0.03$), where second parity cows had decreased intake while no differences were observed in older cows. This resulted in a tendency for a parity by treatment interaction on milk yield:DMI ($P = 0.08$). Similary, no main effects of treatment were observed for glucose, BHBA, or FFA ($P > 0.10$), but there were significant interactions between treatment and parity for glucose, BHBA, and insulin ($P < 0.05$). Older cows had greater plasma glucose and insulin concentrations and decreased plasma BHBA following SAL but no differences were observed in second parity

animals. There was a tendency for SAL to increase insulin across all parities ($P = 0.08$). These alterations in glucose and insulin resulted in a tendency for a treatment interaction with the Revised Insulin Sensitivity Check Index and time ($P = 0.08$). Feeding behavior was also altered following SAL administration, with treatment resulting in a greater number of daily meals and greater average meal weight ($P = 0.03$), as well as a tendency for a longer meal length for SAL ($P = 0.10$). A tendency for treatment by week interaction for inter-meal interval was observed ($P = 0.06$), as was a significant parity by treatment interaction for average meal weight ($P = 0.04$). Overall, SAL had a prolonged programming effect on the first 120 DIM, with responses being largely dependent on parity.

INTRODUCTION

The transition period—defined as 3 weeks prior to and 3 weeks following parturition in dairy cattle—is notorious for its potential pitfalls and challenges to dairy farm management. In addition to the event of parturition itself, milk production requires an enormous amount of energy, resulting in a vast shift in metabolism (Bell, 1995). As a result of numerous metabolic, dietary, and social pressures, early lactation dairy cattle are prone to a plethora of metabolic disorders. Metritis, retained placenta, and excessive negative energy balance (Staples et al., 1990) can lead to reproductive failures in the ensuing lactation. Therefore, a successful lactation is dependent on a successful transition period.

A growing body of research indicates that systemic metabolic inflammation is elevated in dairy cows at parturition and that this inflammation may play a role in the development of metabolic disorders during the transition period (Bradford et al., 2015). Furthermore, inflammation has been linked to negative production outcomes (Bionaz et al., 2007; Bertoni et al., 2008; Yuan

et al., 2013). However, it is not fully understood what role inflammation plays in the early lactation dairy cow.

Farney et al. (2013b) demonstrated that administration of the nonsteroidal antiinflammatory drug (NSAID) sodium salicylate (SS) increased 305-d milk production. Similarly, Carpenter et al. (2016) showed that not only was 305-d milk yield increased when cows received NSAID treatment, but that weekly milk yields were also elevated in these cows. Interestingly, Farney et al. did not detect a difference in milk production during the first 21 days of milk production, and Carpenter et al. demonstrated that elevated milk production due to NSAID treatment did not occur until 7 weeks in milk, with a tendency for treatment effects by 4 weeks. Thus, the fact that Farney et al. did not detect a difference in feed intake in the 2 weeks following SS administration is not surprising, since this milk production response appears to be delayed. Carpenter et al. performed their experiment on a commercial dairy, and so it is unknown whether feed intake was also effected by NSAID treatment and to what extent.

The objective of the current experiment was to monitor feed intake, production efficiency, and blood parameters on dairy cows who received SS treatment following calving. Sodium salicylate was administered according to the protocol of Carpenter et al. (2016), and cows were monitored up to 120 days following calving.

MATERIALS AND METHODS

Animals and treatments

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Multiparous Holstein cows (n = 28/treatment) were enrolled on this study between January 24, 2014 and December 24, 2014. Farney et al. (2013b) demonstrated

that SS did not increase 305-d milk production in cows less than their third parity, so no cows in their first lactation were enrolled. A total of 30 cows were in their second parity, whereas 16 cows were in their third parity and greater. Cows were blocked according to parity and sequentially assigned to either SS drench (SAL) or water drench (CON) at calving. Treatment drenches were given according to the procedure of Carpenter et al. (2016). In short, oral drenches were given once daily for 3 consecutive days between 1400 and 1600 h, beginning 12-36 h after parturition. Cows assigned to SAL treatment received 125 g/d of SS dissolved in approximately 375 mL of water, while CON animals received an equal volume of water without SS.

All animals were housed at the tie-stall facility at the Kansas State University Dairy Teaching and Research Center from calving to 120 DIM. Some cows were removed from the study due to injury or health events; if they were on study for at least 90 d, they were included in analyses. A total of 4 cows, all in their second parity, were excluded. Of these, 2 were suspected to have ruptured colonic ulcers (n = 1/treatment), and 2 experienced injury (n = 1/treatment). In total, 52 cows remained on study for the full 120 d.

Feed delivered and refusals were measured daily to determine feed intake. Cows were fed twice daily at 0630 and 0530 h. Samples of TMR were collected weekly. Ingredient composition of the ration is presented in Table 3.1. Cows were milked 3 times daily (0000, 1000, and 1700 h), and milk weights were recorded at each milking. Milk samples were collected twice weekly. Feeding behavior was measured on all cows in their third parity and greater (parity 3+) and 26 second parity cows by feed bunks suspended from load cells for continuous monitoring of bunk weight. Feeding behavior variables (meal weight, meal length, number of meals/d, and intermeal interval) were determined as described by Yuan et al. (2015).

Sampling and analysis

Blood samples were collected via the coccygeal vein on the first and last day of treatment as well as day 7, 11, 14, 18, 21, 35, 49, 63, 77, 91, 105, and 120 following calving prior to the morning feeding. Two tubes (approximately 14 mL each) were used for each blood sample time point; one tube contained potassium EDTA and the other containined potassium oxalate with sodium fluoride as a glycolytic inhibitor (Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). Plasma was collected and stored at -20°C until analyzed for glucose by a colorimetric kit (kit #439–90901; Wako Chemicals USA Inc.), NEFA using an enzymatic colorimetric procedure (NEFA-HR; Wako Chemicals USA Inc., Richmond, VA), insulin using a bovine-specific sandwich ELISA (no. 10-1201-01; Mercodia AB, Uppsala, Sweden) with a detection limit of 0.025 pg/µl, and BHBA using a commercial kit (kit #H7587–58; Pointe Scientific Inc., Canton, MI). Haptoglobin was measured by the method of Cooke and Arthington (2012), a colorimetric technique that uses differences in peroxidase activity to measure haptoglobin-haemoglobin complexing. Absorbance was measured with a spectrophotometer (PowerWave XS; BioTek Instruments Inc., Winooski, VT) and calculations were performed using Gen5 software (BioTek Instruments Inc.). Insulin sensitivity was estimated using the Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) as described by Holtenius and Holtenius (2007) using the equation RQUICKI = 1/[log(Gb) + log(Ib) + log(FFAb)], where Gb is the blood plasma concentration of glucose, Ib is the blood plasma concentration of insulin, and FFAb is the blood plasma concentration of NEFA.

Milk samples were analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN), urea nitrogen (MUN spectrophotometer; Bentley Instruments Inc.), and somatic cells (SCC 500, Bentley Instruments

Inc.; Heart of America DHIA, Manhattan, KS). Samples of TMR were pooled in two month intervals and analyzed for chemical composition with near infrared reflectance spectroscopy by Dairy One (Ithaca, NY). Average chemical composition is reported in Table 3.1.

Data analysis

Daily milk yield, DMI, and feeding behavior variables were averaged by week for analysis. Somatic cell linear score (SCLS) was determined through the following equation: $\log_2(SCC/100) + 3$ (Shook, 1993). Energy corrected milk yield (ECM) was calculated as (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield) (Dairy Records Management Systems, 2014), and fat corrected milk yield (FCM) was calculated as (0.432 × milk yield) + (16.216 × fat yield) (NRC, 2001). Insulin and BHBA data were log transformed for analysis, and reported means are back-transformed. The BOXPLOT procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) was used to identify extreme outliers for milk, DMI, and feeding behavior variables. Outliers were identified by the procedure as being greater than 1.5 times the interquartile range above the 75th percentile, or less than 1.5 times the interquartile range below the 25th percentile.

Data were analyzed using PROC MIXED in SAS with the main effects of treatment, parity, time (week or day), the interaction between treatment and time, and the interaction between treatment and parity. Cow was included as a random effect, and repeated measures were used with an autoregressive covariance structure, selected based on the Bayesian information criterion value. Plasma variables were analyzed using the pre-treatment value as a covariate. Daily milk yield and DMI were analyzed with pre-treatment haptoglobin as a covariate for lactation milk, fat, and protein yields, effects of being enrolled on other research trials following the current experiment

were tested and removed if $P > 0.20$. Significance was declared at $P \leq 0.05$, and tendensies were identified at $0.05 < P \leq 0.10$.

RESULTS

Milk production and feed intake

Data for daily milk and feed intake are presented in Table 3.2. Whole-lactation milk, fat, and protein yields were not affected by treatment ($P \geq 0.39$) or the interaction between treatment and parity ($P \geq 0.20$). The main effect of week was significant for daily milk yield, ECM, FCM, DMI, milk yield:DMI, ECM:DMI, SCC, and all components ($P < 0.01$) with the exception of MUN concentration which only showed a tendency ($P = 0.07$). Daily milk production was not affected by treatment ($P = 0.79$), and no treatment by parity or treatment by week interactions were detected ($P \geq 0.82$). Similarly, milk fat, protein, lactose, and MUN percentage was not affected by treatment ($P \geq 0.18$) or by treatment interactions with parity or week ($P \geq 0.16$). As a result, there were no effects observed due to SAL ($P \geq 0.63$) or interactions with SAL and week or parity ($P \geq 0.35$) on fat, protein, or lactose yield; ECM and FCM were also unaffected (treatment: $P \geq 0.79$; treatment by parity: $P \geq 0.93$; treatment by parity: $P \geq 0.76$). No effects of treatment were observed for SCC or somatic cell linear score (treatment: $P = 0.63$; treatment by week: $P = 0.65$; treatment by parity: $P = 0.63$). Dry matter intake was not affected by SAL ($P = 0.70$), but there was a significant interaction of parity and treatment ($P = 0.03$)—with no DMI response observed in older cows but a decrease in feed intake in second parity cows (Table 3.2)—although the interaction between treatment and week was not significant ($P = 0.75$). Due to the lack of any observable effect on daily milk production, this change in DMI resulted in a tendency for an interaction between treatment and parity for milk yield:DMI ($P = 0.08$) and a significant interaction between

treatment and parity for ECM:DMI ($P = 0.02$), although treatment was not significant for either of these parameters ($P \geq 0.33$; treatment by week: $P \geq 0.46$). Second-parity cows had numerically greater milk yield:DMI after SAL administration, while older cows receiving SAL had numerically decreased milk yield:DMI. Meanwhile, cows receiving SAL tended to have decreased ECM:DMI than CON, but only in older cows ($P = 0.06$).

Blood parameters

Results of plasma analysis are shown in Table 3.3. Predictably, the effect of time was significant for plasma glucose, FFA, BHBA, insulin, and haptoglobin ($P < 0.01$); however, it is interesting to note that RQUICKI only showed a tendency to change due to time ($P = 0.10$). Pre-treatment plasma concentrations were tested as covariates for all plasma variables and were found to be significant predictors of plasma insulin, and haptoglobin ($P < 0.01$), while pre-treatment concentrations of glucose, FFA, and BHBA were not significant ($P \geq 0.11$). Glucose was not affected by treatment ($P = 0.11$), and there was no interaction between treatment and day ($P = 0.90$), although there was a tendency for an interaction between treatment and parity ($P = 0.06$), with glucose concentrations being elevated due to SAL but only in older cows. A similar pattern was observed for BHBA, with lower plasma concentrations observed due to SAL treatment, but again only in parity 3+ (treatment: $P = 0.31$; treatment by day: $P = 0.94$; treatment by parity: $P = 0.02$). Insulin tended to differ between treatments ($P = 0.08$), with a significant interaction between insulin and parity ($P = 0.04$), but no interaction observed between treatment and time ($P = 0.73$). Once again, this treatment and parity interaction resulted from the fact that second parity animals were not affected by SAL while older cows had elevated insulin concentrations when treated with SAL. There was no effect of treatment or treatment interactions on FFA (treatment: $P = 0.70$;

treatment by day: $P = 0.81$; treatment by parity: $P = 0.28$). Interestingly, although RQUICKI did not differ due to treatment ($P = 0.14$) or its interaction with parity ($P = 0.21$), a significant treatment by day interaction was observed ($P = 0.05$; Figure 3.1). Haptoglobin was measured from samples collected up to 21 days on study; no differences due to treatment were observed (treatment: $P = 0.54$; treatment by day: $P = 0.80$; treatment by parity: $P = 0.78$).

Feeding behavior

Results for the main effect of treatment on feeding behavior measurements are reported in Table 3.4. The number of meals in a day was lower for cows receiving SAL treatment ($P = 0.03$), and there was a tendency for a longer meal length as well as higher meal weight for SAL ($P = 0.10$). Inter-meal interval (IMI) did not differ between groups ($P = 0.18$). The main effect of week was significant for all measurements ($P < 0.01$), and the main effect of parity was significant for meal count and IMI ($P \leq 0.04$), but not meal weight and length ($P \geq 0.14$; Table 3.4). There was a tendency for treatment by week interaction for IMI ($P = 0.06$; Figure 3.2), and a significant parity by treatment interaction for meal weight ($P = 0.04$; Table 3.4).

DISCUSSION

Comparison of milk production response with previous NSAID research

The treatment protocol for SAL administration was based on the procedure of Carpenter et al. (2016) as milk production was significantly increased after SS treatment following calving in that study. It was considered to be a superior treatment method to that used by Farney et al. (2013b) due to its ease of administration; in the Farney experiment SS was administered via the drinking water for the first 7 DIM, whereas in the Carpenter experiment, SS was given once daily for 3 d.

Despite both experiments reporting a response in milk production, Farney et al. (2013b) showed a greater increase in 305-d milk production than Carpenter et al. (2016); however, if the response of second and third parity cows are averaged from the Farney experiment, it appears numerically similar to Carpenter. Farney reported that there was only a response in milk production in cows in their third parity and greater (n = 15). In the current experiment, 16 cows in their third parity and greater were enrolled. It is possible that there was not a large enough cohort of older cows to detect a statistical difference in daily milk production when the protocol of Carpenter et al. was used.

Another potential difference between the current study and that of previous experiments is the level of inflammation that cattle experienced at calving. Haptoglobin levels were measured to estimate the level of inflammation up to 21 DIM (Table 3.3), and these levels reported are less than those reported by Carpenter et al. (2016), although measurements in that experiment were taken closer to the time of calving. On the last day of treatment and d 7 in the current experiment—approximately equivalent to the time points monitored by Carpenter—haptoglobin levels averaged 292 ± 42.6 and 209 ± 43.3 μg/mL, respectively, across treatment. This is much lower numerically than the levels of haptoglobin exceeding 600 μg/mL in the study published by Carpenter. The concentrations observed in the current study fall within the range of the lowest quartile reported by McCarthy et al. (2016), and the concentrations reported by Carpenter fall within the second lowest quartile. McCarthy et al. reported that cows in the second lowest quartile of inflammation had different production responses following calving that those in the lowest quartile, having depressed DMI and a treatment by time interaction indicating a tendency for decreased milk yield in early lactation. Farney et al. (2013b) did not report a similar metric of inflammation for comparison across these studies. It is possible that the level of response observed by Carpenter was greater because baseline inflammation was higher in these cows. To investigate this

hypothesis, milk yield and DMI were analyzed with pre-treatment haptoglobin as a covariate in an attempt to see if differences in these variables could be detected when inflammatory response was used to account for some variation in the model; however, treatment remained an insignificant predictor ($P \geq 0.91$). Pre-treatment haptoglobin concentration was tested as a predictor of daily milk yield and DMI response to SAL but was not significant ($P \geq 0.19$); similarly, the interaction between pre-treatment haptoglobin and treatment did not affect milk yield or DMI ($P \geq 0.68$). Numerically, haptoglobin concentrations levels were lower in SAL than CON (194.6 ± 27.83 vs. 171.7 ± 24.58 μg/mL for CON vs. SAL, respectively; Table 3.3). Carpenter et al. reported that haptoglobin levels were actually greater in cows receiving SS treatment than control.

Carpenter et al. (2016) speculated that differences in milk production response to NSAID treatment observed between experiments was due at least in part to differences in the time period that milk production was measured. They reported that no difference in milk production response due to NSAID treatment was detected in their experiment until 7 weeks into lactation. Despite the extended sampling time period that daily milk production was measured in the current experiment, no differences in milk production responses were detected. Other authors have reported that daily milk production did not differ following NSAID treatment (Priest et al., 2013; Mainau et al., 2014; Meier et al., 2014). Among these reports, the current experiment stands out in the length of time that daily milk production was monitored.

Differences in milk components following SAL administration in previous studies have been reported in terms of component yield, not percentages. Farney et al. (2013b) demonstrated that 305-d fat and protein yields were increased or tended to increase in cows in their third lactation and higher following SS treatment, largely driven through increased 305-d milk production. Milk fat yield—but not protein or lactose yield—was increased in the third week of lactation, although

differences in component percentages were not reported (Farney et al., 2013a). Conversely, Carpenter et al. (2016) reported that 305-d fat yield did not differ following SS treatment in early lactation, although protein yield was elevated compared to control. It is not surprising in the current experiment that there were no differences observed in component yield, considering that the same 305-d milk response reported by previous authors was not observed here. Although there was a significant interaction between treatment and DIM for meloxicam vs. SS treatment reported by Carpenter, no differences or interactions in SCC due to treatment were detected between SS and control animals in that study. This is consistent with the results of the current experiment. No information regarding SCC was reported by Farney et al. (2013a, b).

Differences in response to SAL as a function of parity

Overall in the current experiment, SAL appeared to have a positive effect on metabolism in older cows, with increased plasma glucose and insulin concentrations, and decreased BHBA and RQUICKI in this group. This is in contrast with Farney et al. (2013a). These authors reported hypoglycemia in cows in their second parity and higher on day 7 of lactation, which corresponded to the last day of SS in their treatment protocol, whereas those in their first parity did not experience the same drop in blood glucose concentration in response to SS; notably, first parity cows also tended to have a decrease in 305-d milk production (Farney et al., 2013b). This corresponded with a tendency for decreased plasma insulin on day 7 in the SS treatment group, as well as significantly increased plasma BHBA on day 14 and 21, higher FFA on day 21, and increased RQUICKI on day 7. Carpenter et al. (2016) also failed to observe any parity interactions or the same negative effects on metabolism reported by Farney et al. (2013a) when they utilized a treatment protocol similar to that used in the current experiment. In their experiment, SS did not result in any changes

in glucose, BHBA, or FFA compared to control animals, and no parity interactions between SS and control were detected. Nevertheless, these authors did observe a similar increase in milk production as Farney et al. (2013b). The adverse metabolic effects observed by Farney et al. (2013a) may have at least in part been a result of the extended treatment period (7 d) compared to Carpenter et al. and the current study (3 d).

Our results indicate that use of NSAID such as SS may help to mitigate the negative effects of age on metabolism reported by Lee and Kim (2006) and van Dorland et al. (2009) in the absence of a milk production response. Elevated plasma glucose might indicate increased glucose entry into the blood, decreased removal from the blood into tissues, or some combination of both, so it is difficult to determine the exact cause of higher plasma glucose in older cows without speculation. However, with the corresponding decrease in BHBA, it would appear that these cows were in a more favorable metabolic state. Decreased insulin sensitivity in SAL may have caused the observed increase in plasma glucose by slowing its uptake into tissue, promoting gluconeogenesis in the liver, or a combination of these (Aschenbach et al., 2010); however, inflammation is often linked to blunted insulin sensitivity (Odegaard and Chawla, 2013), and it follows that antiinflammatory treatment would result in higher RQUICKI as observed in Farney et al. (2013a). Therefore, the mechanism wherein SAL would decrease insulin sensitivity is unclear. A depressed responsiveness to insulin is often observed in non-mammary tissues in early lactation as a mechanism of increasing nutrient partitioning toward milk production (De Koster and Opsomer, 2013). Bjerre-Harpøth et al. (2012) showed that early lactation dairy cattle have increased RQUICKI in response to nutrient restriction, while cows in mid and late lactation do not demonstrate the same coping mechanism. The logical inverse of this finding is that in the absence of a difference in milk production, a depressed responsiveness to insulin in early lactation could

possibly be an indication of more available nutrients. Therefore, the decreased insulin sensitivity observed in response to SAL in the current experiment may be due at least in part to favorable metabolic conditions. These alterations in blood metabolites in parity 3+ occurred in spite of the fact that no differences in DMI were reported for this group ($P = 0.13$). Yet it should still be noted that average DMI for cows in party 3+ was numerically greater after SAL administration, which may ultimately be the cause for differences in plasma metabolite concentrations (Table 3.2).

Despite the lack of milk production response in the current experiment, it is possible that decreased efficiency in older cows may have positive health benefits, particularly regarding transition disorders. Lee and Kim (2006) and van Dorland et al. (2009) have reported that higher parity cattle often have a less favorable metabolic status in early lactation, so decreased efficiency could potentially help prevent disorders associated with negative energy balance. The number of animals experiencing metabolic disorders or health incidents was not analyzed in this study due to the relatively small number of animals. Administration of the NSAID meloxicam decreased the likelihood of leaving the herd in commercial dairy cattle compared to control (Carpenter et al., 2016); however, in that experiment, SS did not alter the risk of leaving the herd. In the current experiment, SS increased plasma glucose concentration and decreased plasma BHBA in older cows (see below and Table 3.3), indicating potentially positive effects on metabolism; however, plasma FFA was not affected. Alterations in metabolism following SAL may have resulted in differences in feed efficiency in second lactation cows (Table 3.2), while in older cows these alterations manifested as differences in blood metabolites, resulting in healthier metabolic profiles. For example, it could be speculated that some mechanism that resulted in increased plasma glucose concentration in older animals may have also occurred in second parity cows, but no differences

were detected between treatment groups in younger cows because of inadequate glucose precursors due to lesser DMI.

In the current experiment, parity was a significant predictor of daily meal count and IMI (Table 3.4). In general, second parity cows consumed fewer meals and had a longer IMI than cows in parity 3+. Differences in feeding behavior due to parity have been reported previously (Dado and Allen, 1994, Azizi et al., 2009); however, there is a lack of examples in the literature where second parity cows were compared to older cows. Rather, previous research has compared primiparous and multiparous animals. Dado and Allen (1994) did not observe statistical differences between primiparous and multiparous cattle for any feeding behavior responses measured. In comparision, Azizi et al. (2009) reported that multiparous cattle had a decrease in meal frequency and time spent eating, and an increase in meal size compared to primiparous cows, with significant day by parity interaction for meal frequency and size from 2-5 weeks in lactation. Differences between the two experiments are likely due at least in part to differences in sample size, as Dado and Allen (1994) utilized 6 primiparous and 6 multiparous cows, while Azizi et al. (2009) enrolled 23 primiparous and 47 multiparous cows in their experiment.

Some of the variable responses to SAL between second parity cows and older cows may be due to differences in gut fill. Additionally, van Dorland et al. (2009, 2014) reported differences in metabolism between older and younger groups of multiparous cows. Younger cows had lower BHBA and free fatty acid levels than cows in their fourth parity and greater (van Dorland et al., 2009), and in another study, cows in their fourth parity and greater had lower mRNA abundance for genes related to fatty acid synthesis (ATP citrate lyase and glycerol-3-phosphate actyltransferase) combined with higher abundance for hydroxybutyrate dehydrogenase 2, which is involved with ketone body synthesis (van Dorland et al., 2014). This may indicate greater rates of

oxidation occurring in the older cows. Lower rates of propionate production due to SAL may help older cows compensate for this increased oxidation, which can act as a satiety signal (Allen et al., 2009), and allow them to still increase their feed intake and alter feeding behavior (see below).

Feed intake and feeding behavior

These changes in feeding behavior may be related to alterations in rumen function due to SAL. In humans, NSAID use has been associated with differences in the gut microbiome (Rogers and Aronoff, 2015). Previous research in our lab has indicated that SS affects rumen microorganisms when administered directly (Carpenter et al., 2015b) and has a sustained effect on the fermentative abilities of ruminal microbes following administration to heifers (Carpenter et al., 2015a). When administered directly to batch cultures (Carpenter et al., 2015b), SS decreased dry matter disappearance and increased final pH, indicating negative effects on ruminal fermentation. When batch cultures were performed with rumen fluid from heifers who did or did not receive oral drenches of SS (Carpenter et al., 2015a), dry matter disappearance was decreased 13 and 35 d following administration. This result was seen despite the fact that no treatments were administered *in vitro* to the cultures; the only difference was from treatment administration to the animals themselves. Furthermore, the rate of *in situ* dry matter disappearance was lower in heifers who had received SS treatment in this experiment. These results are in agreement with other reports of salicylate administration to rumen microbes. Ruiz-Moreno et al. (2015) and Fessenden (2013) utilized bismuth subsalicylate in batch and continuous cultures in an attempt to mitigate production of hydrogen sulfide. Both authors reported negative effects on rumen fermentation when bismuth subsalicylate was administered to ruminal microbes *in vitro*. To date, no one has investigated the effects of salicylates on rumen function *in vivo* to our knowledge; however, these reports would

indicate that an effect of SS on the ruminal microbes *in vivo* is likely. It also seems likely that the alterations in feeding behavior and even feed intake observed in the current experiment may be partially explained by changes in the rumen.

Even in the absence of a milk production response, feed intake tended to be decreased in cows in their second parity receiving SAL treatment ($P = 0.07$; treatment by parity: $P = 0.03$). This was accompanied by alterations in feeding behavior in these cows, as well as in higher parity cows on SAL (Table 3.3). There was only a tendency for an effect of SAL on meal weight ($P = 0.10$), although a treatment by parity interaction was apparent, where SAL only affected meal weight in older cows, similar to the response observed for DMI. Further research is necessary to fully elucidate the *in vivo* relationship between feeding behavior, feed intake, and the effects of SS on the rumen, if these relationships exist. In the current experiment, it is possible that the lower intake observed in second parity cows in response to SAL is at least in part due to differences in gut fill. If fermentation was inhibited in these animals, their rumens may not have emptied as quickly, resulting in increased satiety. While this may explain the differences in intake, it does not account for how these cows were still able to maintain the same level of milk production as their CON peers.

While it is possible that SS directly affects feed intake and behavior by altering rumen fermentation, it is also possible that this is confounded by secondary effects due to other effects of SS. In other words, instead of alterations to the rumen changing feeding behavior, one could predict that SS alters feeding behavior, which changes the rumen via differences the rumen environment (i.e., salivary buffering, rate of passage, steady state, etc.). This may explain the extended effect of SS on the ability of rumen fluid to digest dry matter *in vitro* observed by Carpenter et al. (2015a). It has been speculated that signals from the liver to the brain indicating energy status can alter feed

intake and behavior, and one of these proposed signals is AMP-activated protein kinase (AMPK; Allen et al., 2005). Salicylates have been shown to directly activate AMPK (Hawley et al., 2012). This may be enough to stimulate greater intake and alter feeding behavior, which may influence the rumen environment and microbial population for an extended period of time following treatment with SS; therefore, the long-term programming effects on ruminal fermentation observed by Carpenter et al. (2015a) could be due to alterations in the liver or the brain or both, or even some other organ or tissue. Indeed Weimer et al. (2010) demonstrated the importance of the animal itself in determining the microbial population of its rumen; even following near-total exchange of the contents of the rumen, animal factors eventually guided the microbial population to its original state. The microbial population of the rumen and responses of the ruminant itself have an inherent "which came first" relationship that is difficult to fully illuminate, and further research is necessary to determine the cause of alterations in feed intake and behavior in cows receiving SAL treatment in the current experiment.

It is worth noting that the second bout of decreased insulin sensitivity that SAL cows experienced corresponded approximately with the time points of statistical difference between SAL and CON on various feeding behavior measurements (Figure 3.1 and Figure 3.2). If heightened insulin sensitivity speeds the clearance of fuels from the blood, it would be expected that hunger would occur sooner (Allen et al., 2009); therefore, if responsiveness to insulin is depressed as it was in SAL cows, it is not surprising that the time between meals would be lengthened as was observed here, which would at least in part explain the decrease in daily meal count during that time.

While several authors have compared feeding behavior responses to treatment at various time points in lactation, to our knowledge, this is the longest continuously monitored time frame

of feeding behavior beginning within 24 h of parturition. Huzzey et al. (2005) monitored feed intake of cows from 10 d before to 10 d after calving, reporting that following calving, time spent feeding increased by approximately 3.3 min/d for the 10 d. DeVries et al. (2003) monitored feeding behavior from early to peak lactation; however, these authors collected data over 3 periods of 8 d each (approximately 35, 57, and 94 DIM). The data from the current experiment encompasses these time points. Effect of stage of lactation on feeding behavior was also investigated by Friggens et al. (1998). Similar to DeVries, these investigators collected feeding behavior in isolated periods throughout lactation (4 periods at approximately 6-9, 18-21, 23-26, and 35-38 weeks postpartum). The only period in this study overlapping the time frame of the current experiment is the first period. DeVries et al. (2003) reported that there were significant differences in daily mealtime, meal frequency, and meal duration between the first and second period on their experiment, although there were no differences in these measurements between the second and third period. This indicates a stabilization or plateau between the second and third periods, which is consistent with the data illustrated in Figure 3.2. In contrast, Friggens et al. (1998) did not see any effect on feeding behavior due to stage of lactation with the exception of a small but significant decrease in time spent feeding in period 4. This could be due to differences in period time points. The first period in Friggens' experiment took place between the first and second period of DeVries, when presumably cows may have begun to stabilize in feeding behavior. Furthermore, only 20 cows were utilized across 4 treatments by Friggens et al., and these authors noted a high variation in feeding behavior, even between animals on the same treatment; therefore, the lack of adequate sample size may have masked any differences due to stage of lactation.

Other authors have investigated the effect of stage of lactation in combination with other experimental factors. Several authors have found no effect of stage of lactation on feeding behavior

in combination with various other experimental factors (Cassida and Stokes, 1986, Benson et al., 2001, Bradford and Allen, 2007), possibly due to low numbers of replicates or the choice of DIM for comparison or both. Alternatively, Abrahamse et al. (2008) reported that late lactation cows (approximately 245 DIM) ate more meals and spent less time eating per day than the same cows in early lactation (approximately 61 DIM), with a more pronounced effect in cows on high roughage diets. Oba and Allen (2003) also reported that although early and mid-lactation cows (approximately 9 vs. 192 DIM, respectively) had the same decreased response to propionate infusion, IMI increased in mid-lactation cows but not for those in early lactation. These authors hypothesized that IMI was not influenced in early lactation because the high demand for glucose by the mammary gland for milk production decreased the proportion of propionate that was oxidized in the liver to stimulate satiety, while in mid-lactation the mammary gland has lower glucose demand, allowing more propionate to be oxidized. In contrast, in the current experiment, SAL increased IMI in mid-lactation, although IMI was lower in early lactation across treatment (Figure 3.2), contrary to our hypothesis that SAL decreased propionate production for an extended period of lactation. In theory, if DMI was increased in these cows, passage rate would also increase, making gut fill less limiting; however, with the corresponding decrease in fermentation, it could be that particle size of feed did not decrease fast enough to allow rate of passage to increase as much as it would have otherwise. Again, further research is necessary to fully understand these interactions, since multiple competing mechanisms following SAL treatment may negate each other and mask a nuanced effect of treatment.

CONCLUSION

This experiment was conducted to determine whether the long-term milk production response to early lactation treatment with SS was achieved through differences in DMI. Unfortunately, in this experiment, a replication of previous results showing a response in milk production in older cows was not achieved (Farney et al., 2013b; Carpenter et al., 2016). Despite the failure to demonstrate a change in milk production, subtle differences in feed intake, efficiency, metabolism, and feeding behavior were reported. Second lactation cows achieved similar levels of milk production following SAL administration as their CON counterparts while consuming less feed, resulting in a greater efficiency of milk production in these animals. At the same time, older cows in their third parity and greater consumed similar amounts of feed and produced similar amounts of milk across treatment groups; however, older cows receiving SAL treatment appeared to have a healthier metabolic profile. Alterations in feeding behavior were also observed. These may be a result of long-term effects of SAL on the rumen, or it is possible that long-term ruminal effects reported previously are a result of these differences in feeding behavior (Carpenter et al., 2015a). While it remains unclear why no differences in milk production were observed in this experiment, further research is necessary to elucidate the mechanisms promoting the differences observed in metabolism in response to SAL.

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Table 3.1 Average composition of diet fed to cows up to 120 DIM.[1]

Item	Amount		
	% of diet DM		
Alfalfa hay	19.6		
Corn silage	20.9		
Wet corn gluten feed[2]	29.3		
Cottonseed	3.8		
Fine rolled corn	15.9		
Expeller soybean meal[3]	4.9		
Straw	1.5		
Vitamin & mineral mix	4.3		
	%		
	High	Mean	Low
Dry matter	53.9	51.2	49.7
	% of DM		
	High	Mean	Low
Crude protein	18.7	17.8	16.9
ADF	23.2	21.2	18.7
aNDF	36.1	33.2	30.6
Crude fat	5.3	4.8	4.1
TDN	73.0	71.3	70.0
	(Mcal/kg)		
	High	Mean	Low
NE_l	1.63	1.68	1.72
NE_m	1.63	1.70	1.74
NE_g	1.04	1.08	1.12

[1]Samples of TMR were collected once per week throughout the experiment and pooled into 2-month intervals for analysis. The highest and lowest observations are reported, as well as the mean of all values across time.

[2]Sweet Bran; Cargill, Inc., Blair, NE

[3]Soybest; Grain States Soya, West Point, NE

Table 3.2 Milk and feed responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d).

	Treatment							
	CON		SAL			*P-value*		
Item	Parity 2	Parity 3+	Parity 2	Parity 3+	Pooled SE	Treatment	Parity	Treatment × parity
305-d milk yield (kg)	14,809	14,124	14,589	13,635	426.0	0.39	0.28	0.76
305-d fat yield (kg)	518	488	497	522	20.5	0.78	0.91	0.20
305-d protein yield (kg)	429	400	416	399	10.3	0.60	0.09	0.65
Milk yield (kg/d)	52.6	55.3	52.6	54.6	1.37	0.79	0.12	0.12
Fat %	3.69	3.69	3.69	3.60	0.118	0.66	0.80	0.77
Protein %	2.82	2.68	2.81	2.77	0.048	0.42	0.09	0.37
Lactose %	4.96	4.86	4.88	4.89	0.038	0.60	0.26	0.16
MUN (mg/dL)	15.2	14.6	14.9	13.6	0.44	0.18	0.03	0.50
Fat yield (kg/d)	1.9	2.0	1.9	2.0	0.06	0.76	0.37	0.84
Protein yield (kg/d)	1.5	1.5	1.5	1.5	0.04	0.89	0.55	0.35
Lactose yield (kg/d)	2.6	2.7	2.5	2.8	0.08	0.99	0.03	0.35
Energy-corrected milk yield (kg/d)	53.6	55.5	52.7	55.6	1.53	0.79	0.16	0.76
Fat-corrected milk yield (kg/d)	54.2	56.6	53.3	56.0	1.62	0.65	0.14	0.93
DMI (kg/d)	26.2	25.7	25.1*	27.3	0.57	0.70	0.16	0.03
Milk yield:DMI	2.0	2.2	2.1	2.1	0.06	0.85	0.03	0.08
ECM:DMI	2.1	2.3	2.1	2.1*	0.05	0.33	0.03	0.02
SCC	17.2	44.5	22.6	44.3	0.1	0.65	0.01	0.63
SCLS	0.7	1.2	0.8	1.3	0.1	0.64	0.03	0.91

*Tended to differ from CON within the same parity ($0.05 < P \leq 0.10$).

Table 3.3 Blood parameter responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d).

	Treatment							
	CON		SAL			*P-value*		
Item[a]	Parity 2	Parity 3+	Parity 2	Parity 3+	Pooled SE	Treatment	Parity	Treatment × parity
Glucose (mg/dL)	49.2	42.5	48.4	47.4‡	1.47	0.12	0.01	0.06
FFA (μ*M*)	400.3	417.6	418.4	380.7	25.17	0.72	0.70	0.29
BHBA (μ*M*)	590.5	755.4	635.5	628.3‡	1.05	0.32	0.04	0.02
Insulin (ng/mL)	0.25	0.19	0.24	0.26*	0.034	0.08	0.16	0.03
Haptoglobin (μg/mL)	200.5	184.3	192.8	148.9	38.03	0.59	0.54	0.72

[a]No significant interactions between treatment and time were detected ($P \geq 0.10$).

*Differs from CON within the same parity ($P \leq 0.05$).

‡Tends to differ from CON within the same parity ($0.05 < P \leq 0.10$).

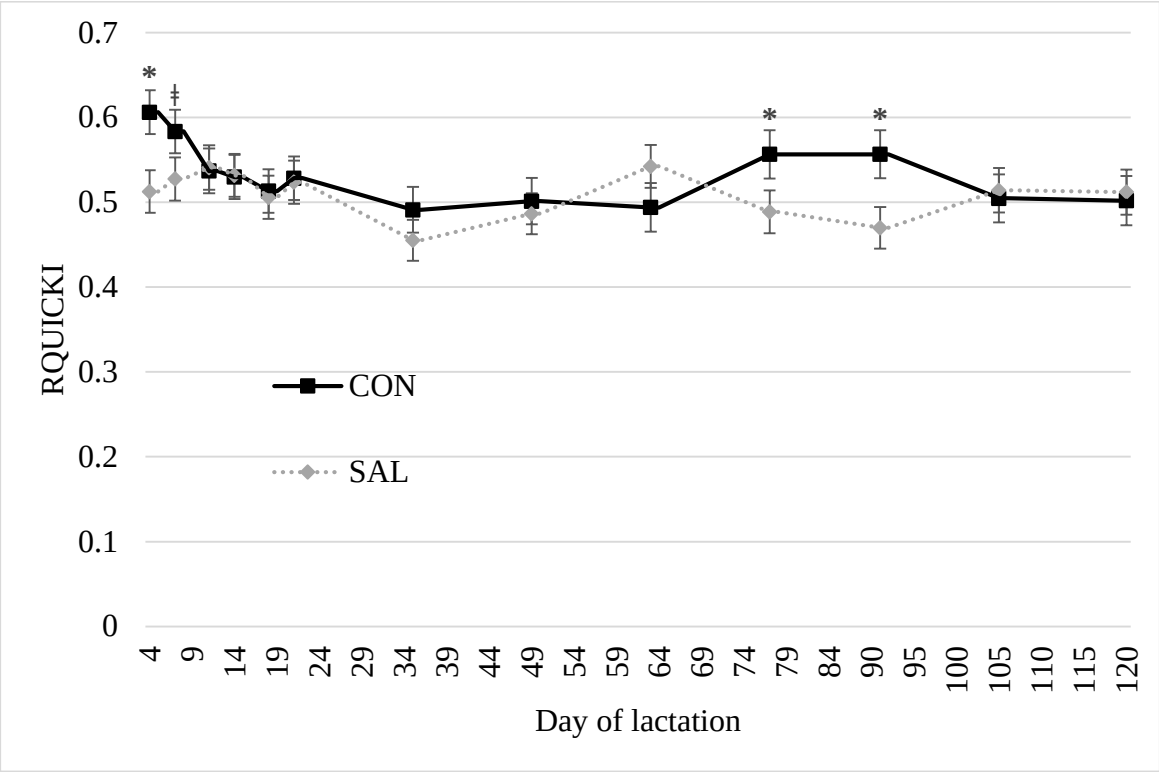
Table 3.4 Feeding behavior responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d).

	Treatment							
	CON		SAL			*P*-value		
Item[a]	Parity 2	Parity 3+	Parity 2	Parity 3+	Pooled SE	Treatment	Parity	Treatment × parity
Meal count (d^{-1})	12.3	14.3	11.8	12.5	0.504	0.03	0.01	0.18
Meal weight (kg)	4.45	3.78	4.36	4.59*	0.467	0.10	0.30	0.04
Meal length (min)	19.9	17.9	20.9	20.5	0.807	0.03	0.15	0.34
Inter-meal interval (h)	1.70	1.45	1.72	1.64	0.078	0.18	0.04	0.29

[a]For effects of time and interactions between treatment and time, refer to Figure 3.2.

*Differs from CON within the same parity ($P \leq 0.05$).

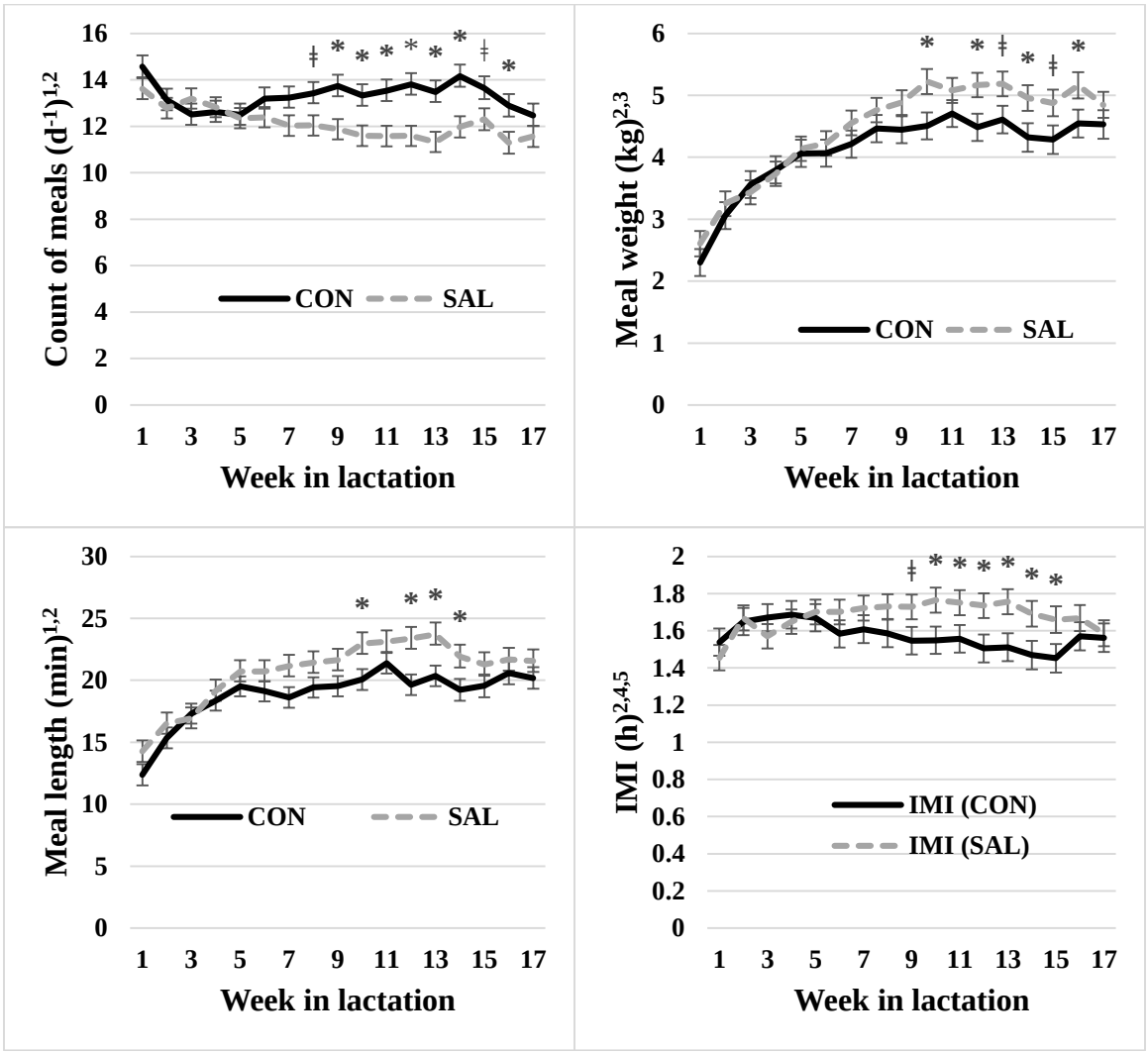
Figure 3.1 RQUICKI response over time in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d).



*Means differ due to treatment ($P \leq 0.05$).

‡Means tend to differ due to treatment ($0.05 \leq P < 0.10$).

Figure 3.2 Feeding behavior responses over time in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d).



[1]SAL differs from CON ($P \leq 0.05$).

[2]Significant effect of week ($P \leq 0.05$).

[3]SAL tends to differ from CON ($0.05 < P \leq 0.10$).

[4]Tendency for treatment × week interaction ($0.05 < P \leq 0.10$).

[5]IMI = inter-meal interval

*Means differ due to treatment ($P \leq 0.05$).

‡Means tend to differ due to treatment ($0.05 \leq P < 0.10$).

Chapter 4 - Sodium salicylate negatively impacts rumen fermentation *in vitro* and *in situ*

A. J. Carpenter, C. F. Vargas Rodriguez, J. A. B. Jantz, and B. J. Bradford

ABSTRACT

Administration of sodium salicylate (SS) to cows in early lactation has been shown to have a positive impact on whole-lactation milk production but a negative effect on metabolism in some cases. The objective of this trial was to determine if this effect was mediated through action on rumen microorganisms. The first experiment was designed to investigate the effects of direct inclusion of SS into a 24-h batch culture, and the objective of the second experiment was to test the fermentative ability of rumen fluid from heifers who had received SS. In the first experiment, strained and pooled rumen fluid from 3 heifers was combined in a 2:1 ratio with McDougall's buffer, and 150 mL of the inoculum was added to each flask (n = 5/treatment) with 2.5 g of fermentation substrate. Premixed treatment mixtures (1 mL volume) were added to achieve the desired final amount of SS (CON1 = 0 mg, LOW = 125 mg, MED = 250 mg, HI = 375 mg). In the second experiment, 6 heifers were drenched daily for 3 d, either with 62.5 g of SS (SAL) dissolved in water or an equal volume of water (CON2; n = 3/treatment). Rumen fluid was collected from each heifer and was not pooled. After being mixed 2:1 with McDougall's buffer, 150 mL of inoculum was added to the fermentation flasks (n = 4/heifer) with 2.5 g of fermentation substrate. This experiment was performed the day before SS treatment began and repeated 1, 12, and 35 d following the end of the treatment period. An *in situ* experiment was also performed at each of these time points. In the first experiment, inclusion of SS resulted in a decrease in DM disappearance (DMD) over 24 h ($P < 0.05$), as well as an increase in final pH ($P < 0.05$). There

was no difference detected between treatments for gas production in volume, rate, or lag ($P \geq 0.28$). In the second experiment, a significant treatment by day interaction was detected for DMD ($P = 0.01$), where there was no difference in DMD during a 24-h batch culture on the day following treatment, although SAL resulted in lower DMD on d 12 and d 35 ($P < 0.05$). There was no treatment effect on final pH of batch culture ($P = 0.71$) or on any gas production parameters ($P > 0.60$). There was a tendency for SAL to result in lower DMD rate *in situ* on the day following treatment ($P = 0.07$). These results indicate SS administration has a negative effect on rumen microorganisms.

SHORT COMMUNICATION

During the transition into lactation after calving, dairy cattle experience elevated systemic inflammation. The administration of the anti-inflammatory medication sodium salicylate (SS) after calving has been shown to increase whole-lactation milk production in cows in their third lactation and greater; however, treatment with SS is associated with hypoglycemia following its administration in some circumstances. Farney et al. (2013a) reported that cows had decreased blood glucose after receiving SS in the drinking water for 7 days following calving, but 305-d milk was greater in older cows receiving SS compared to control (Farney et al., 2013b). This effect was only observed in cows in their third parity and greater. Conversely, in a follow-up study, Carpenter et al. (2016) demonstrated that giving 3 daily doses of SS after calving in multiparous cows did not result in the same hypoglycemia, although 305-d milk was still increased .

Salicylic acid is a compound that functions as a hormone in plants to combat pathogens. It has antiinflammatory properties in mammals through its interactions with the NF-κB pathway (Kopp and Ghosh, 1994). To investigate the effects of blocking inflammation in transition dairy

cattle, the first experiment to investigate the effect of SS in transition cattle was published by Farney et al. (2013b). In this study, SS (1.95 g/L) was administered through the drinking water to cows housed in tie stalls resulting in an average SS intake of 123.3 ± 5.5 g/d for 7 days following parturition. The authors reported that 305-d milk production was greater in cows receiving SS compared to controls by 21.1% in animals in their third parity and greater; however, primiparous cows tended to have decreased milk production. An experiment was performed to replicate these results on a commercial dairy farm (Carpenter et al., 2016). In this experiment, SS (125 g/d) was administered to multiparous cows via a drench gun once daily for 3 days. Similar to previous results, 305-d milk production was greater in cows receiving SS compared to control animals, and SS did not affect an animal's risk of leaving the herd.

Other forms of salicylate have been shown to be antimicrobial with the ability to depress rumen microbial function. Ruiz-Moreno et al. (2015) administered bismuth subsalicylate (BSS) to rumen microbes in a batch culture in an attempt to reduce hydrogen sulfide production resulting from the fermentation of distiller's grains. When BSS was included at 2% and 4% of DM, final pH was increased, and total VFA concentration was decreased at 4% of DM. When BSS was added during continuous culture fermentation at 1% of DM, total VFA concentrations were also decreased, while pH and digestion of OM, NDF, and ADF were increased. Similarly, Fessenden (2013) reported that when BSS was administered at 0% or 0.5% of diet DM (at 2 different levels of sulfur), total VFA concentrations were decreased and pH was increased, and OM digestion was also decreased.

Experiments with human colonic bacteria have also shown an antimicrobial effect of BSS. In the stomach, BSS is hydrolyzed to form salicylic acid (Bierer, 1990). Salicylate is believed to be partially responsible for the antibacterial effects of BSS. Cornick et al. (1990) reported that

although SS was as active as BSS against aerobic bacteria, it was not as active as BSS against anaerobic bacteria such as those found in the rumen, although inhibitory activity was still observed. León-Barúa et al. (1990) demonstrated that BSS reduced gas production by colonic bacteria *in vitro* to a greater extent than other bismuth containing compounds. Additionally, Manhart (1990) showed a dose-dependent response of various strains of bacteria to SS.

The objective of these experiments was to determine the effect of SS on rumen microorganisms. In the first experiment, SS was directly included in a dose-dependent manner on batch cultures of rumen bacteria. In the second experiment, SS was administered to heifers, and the rumen fluid collected from these animals was tested for its fermentative ability in batch culture.

Procedures and analysis

In the first experiment, SS was added directly to batch cultures of rumen fluid at different amounts (CON1 = 0 mg, LOW = 125 mg, MED = 250 mg, HI = 375 mg). Rumen fluid was collected from 3 heifers, strained through 8 layers of cheesecloth immediately following collection and 4 layers of cheesecloth immediately before flocculation, and allowed to flocculate in the lab for 15 minutes to remove feed particles and the protozoal fraction prior to pooling. Pooled fluid was combined in a 2:1 ratio with McDougall's buffer, and 150 mL of the inoculum was added to each 250-mL flask (n = 5/treatment). Five blank flasks contained inoculum alone, while each treated and control flask contained 2.5 g of fermentation substrate (Table 4.1). Before inoculum was added to the flasks, 1 mL of premixed treatment mixtures were added to achieve the desired final amount of SS. Cumulative gas pressure was measured using the ANKOM^{RF} Gas Production System (ANKOM Technology, Macedon, NY). Vessel pressure was recorded at 5 min intervals.

In the second experiment, 6 heifers (n = 3/treatment) were either drenched daily for 3 days with either 62.5 g SS in water (SAL) or an equal volume of water (CON2). Each heifer received the same high forage ration (Table 4.2). Four batch cultures were performed as described above with the exception that rumen fluid was not pooled and SS was not added to the inoculum such that heifer was the experimental unit. Inoculum from each heifer was replicated into 4 flasks with 2.5 g substrate added, and 2 flasks without substrate functioned as blanks for each heifer. Batch cultures were performed the day before the start of treatment, the day following treatment, 7 d after the end of treatment, and 3 weeks following the end of treatment. During each batch culture, heifers were handled in pairs containing one CON and one SAL animal in an attempt to standardize variation due to handling between treatments.

An *in situ* experiment was performed in parallel to the second batch culture experiment. Immediately following each rumen fluid collection, 2 Dacron bags containing approximately 1 g of substrate DM (Table 4.1) were inserted into the rumen of each heifer for each time point. Time points before removal of bags from the rumen were 2, 8, 16, 24, and 48 hr. Additionally, 12 bags were rinsed under running water and washed with other bags upon removal from the rumen to estimate solubility. The 48-hr time point was used to estimate indigestible substrate, while the remaining time points were used to estimate rate of substrate degradation in the rumen.

In both experiments, gas variables—volume, lag, and rate—were estimated using the NLIN procedure of SAS, which obtains estimates using nonlinear least squares. The following model was used to obtain estimates:

$$x = \frac{\mathrm{v}}{1 + e^{(2-4k(t-l))}}$$

In the above equation v = total volume of gas produced, k = rate of gas production, t = time in minutes, and l = lag in gas production. After obtaining estimates, gas production variables were analyzed for each experiment as described above.

Data from experiment 1 were analyzed using the GLM procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) with the amount of SS as the predictive variable and dry matter disappearance (DMD), final pH, and the change in pH as dependent variables. Data from experiment 2 were analyzed using the MIXED procedure of SAS. Each dependent variable was analyzed with its own value on the first day of the experiment (baseline) as a covariate. Besides these covariates, the model included treatment with day and treatment by day interactions. The random statement contained heifer and replicate within heifer, and repeated measures were utilized across days within heifer.

Outcomes and implications

In experiment 1, DMD was significantly depressed by inclusion of SS ($P < 0.05$), with HI having a lower DMD than LOW ($P < 0.05$), and MED intermediate (Table 4.3). Final pH was not different between LOW and CON, but MED and HI had higher final pH than CON ($P < 0.05$; Table 4.3). No differences were observed in gas production for volume, rate, or lag ($P \geq 0.28$; Table 4.3).

Results for experiment 2 are reported in Table 4.4. There was no influence of SAL onfinal pH of batch cultures at any time point ($P = 0.71$). Final pH of the batch culture performed before treatment administration was a significant predictor of final pH at all time points following treatment ($P = 0.03$). Immediately following treatment, SAL had no effect on DMD ($P = 0.67$); however, treatment reduced ($P < 0.01$) DMD in batch culture 7 d following treatment. Three weeks

after the end of the treatment period, DMD was still reduced ($P = 0.01$) by SAL (treatment by day: $P = 0.01$). For the *in situ* portion of the second experiment, no differences due to treatment were detected for rate of DMD ($P = 0.21$). There was a significant effect of treatment and day ($P \leq 0.01$) on DM disappearance at 48 h (treatment by day: $P = 0.31$). Based on these results, it would appear that there is a long-term relationship between SS treatment and rumen fermentation or digestion.

As discussed above, other salicylate compounds—specifically BSS—have been shown to have negative effects on rumen fermentation. Our findings are similar to those of Ruiz-Moreno et al. (2015), who showed that BSS inclusion at 2 and 4% of DM in 24-hr batch cultures increased final pH. In continuous culture, inclusion of BSS at 1% of DM in continuous culture increased average pH, although digestion of OM was also increased (Ruiz-Moreno et al., 2015). Like the current study, however, Fessenden (2013) reported that inclusion of BSS at 0.5% of DM in continuous culture decreased true and apparent DM and OM digestion, with a corresponding increase in average pH. For comparison, when expressed as a % of DM, the current experiment included SS at approximately 5, 9, and 13% for LOW, MED, and HI, respectively. It should be noted, however, that BSS is a much bulkier molecule than SS, at a molecular weight of 362.093 g/mol compared to 160.10 g/mol for SS. Salicylic acid itself has a molecular weight of 138.12 g/mol. Therefore, the salicylate component of BSS is roughly 38% of its molecular mass, while it composes approximately 85% of the SS molecule. Based on these calculations, when BSS was included at 2 or 4% of DM, this is approximately equivalent to inclusion of salicylate at 0.8 or 1.5% of DM, respectively, while SS levels at 5, 9, and 13% of DM approximates to salicylate inclusion at 4, 8, and 11% of DM, respectively. These levels of inclusion are much higher than would be recommended for administration *in vivo*. Nevertheless, the results of the second

experiment indicate that there is an effect of SS administration on the ruminal environment, even at physiologically relevant levels.

Unlike the results presented here, Ruiz-Moreno et al. (2015) reported a significant decrease in gas production during 24-hr batch culture with increasing levels of BSS inclusion. This is likely due to differences in measurements of gas production. Ruiz Moreno measured gas production by displacement of water when batch cultures were performed in serum bottles, while in the current study, the ANKOM^{RF} system was used to measure gas production. It is possible that the simplistic measurement utilized by Ruiz Moreno reduced the measurement variation compared to the current experiment, increasing the statistical power of gas production measurement.

While these results appear to be counterintuitive to previous accounts that showed an increase in milk production in response to SS treatment in early lactation, they may help to explain metabolic outcomes observed in these studies. Despite the positive effects on milk production observed in older cows, cows in their second parity and greater experienced hypoglycemia in early lactation under certain experimental conditions (Farney et al., 2013a). This coincided with a higher value in the Revised Quantitiative Insulin Sensitivity Check (RQUICKI; an estimate of relative insulin sensitivity (Holtenius and Holtenius, 2007)) on d 7 of treatment without any differences in expression of the rate-determining hepatic gluconeogenic enzymes glucose-6-phosphate, phosphoenolpyruvate carboxykinase, or pyruvate carboxylase. In a follow-up study in which a glucose tolerance test was administered to experimental animals receiving SS in early lactation, these cows had enhanced hepatic insulin sensitivity compared to controls, although differences in plasma glucose were not observed in this experiment (Montgomery, 2014). However, this does not rule out the possibility that the hypoglycemia observed by Farney et al. is a result of decreased production of glucogenic compounds by the rumen in addition to the effects of SS on insulin

sensitivity. In fact, it is possible that decreased substrate availability in the liver may serve to enhance insulin sensitivity in these animals. Bjerre-Harpøth et al. (2012) reported that cows in early lactation who underwent feed restriction experienced a significant change in RQUICKI values and had a higher ratio of glucagon to insulin, indicating enhanced insulin sensitivity. It is possible that lower amounts of propionate from ruminal fermentation due to depression in microbial activity or changes in bacterial community composition would have the same result.

There are precedents in the literature of sustained effects following modification of the microbial population, but to our knowledge, this experiment is unique in the length of time that a difference was observed following treatment administration. Weimer et al. (2010b) noted that bacterial community composition did not completely return to its original state up to 4 wk after monensin was removed in combination with a milk fat inducing diet. Although exceptions such as this exist, it has been shown to be difficult to force shifts in the rumen microbial population for an extended period of time (Weimer, 1998). Even following nearly complete exchange of rumen contents, microbial populations in the rumen demonstrate a specificity for the host that is difficult to overcome by non-host forces (Weimer et al., 2010a). This is why experiments utilizing a Latin square design to study various rumen modifiers are able to successfully implement a wash-out period in order to minimize carry over effects. Considering this information, the results of the current experiment are surprising.

Other research with SS has shown long-term alterations in feeding behavior in response to its administration in lactating cows (Carpenter and Bradford, unpublished). It is likely that these observations and the findings of the current experiments are related, but further research is necessary to determine the cause and effect relationship between ruminal fermentation and feeding behavior following SS treatment. While it is possible that some programming effect of the rumen

microbial population resulted in a long-term shift that changed the fermentative ability of the rumen, it is also possible that forces outside of the rumen (such as a neurological effect) altered feeding behavior, which in turn changed the rumen environment and resulted in a population shift.

Despite the relative simplicity of these experiments, they clearly demonstrate the antimicrobial effects of SS on rumen microorganisms. Not only was an immediate and dose-dependent effect of this compound observed *in vitro*, a sustained negative effect on the ability of rumen microorganisms to degrade DM was clearly shown. There are still several questions that remain to be answered. There was no analysis performed on VFA production or profile *in vitro* or *in vivo*. There was no measurement of microbial populations following SAL in the second experiment. Future research should focus on these questions, as well as the effects on the rumen *in vivo*. It is unclear why a sustained positive response on milk production has been observed following SS administration in early lactation when the evidence herein indicates that rumen function is hampered. Future research to explore this relationship as well as the relationship between SS and feeding behavior is clearly warranted.

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Table 4.1 Ingredient and chemical composition of fermentation substrate for *in vitro* rumen fermentation experiments.

Item	% of DM
Corn silage	22.0
Alfalfa hay	21.0
Corn grain	25.0
Cottonseed	4.0
Dried distillers grains	14.0
Soybean meal	14.0
CP	21.5
NDF	24.8
ADF	18.9
EE	3.9

Table 4.2 Chemical composition of rations delivered to heifers donating rumen fluid for *in vitro* and *in situ* fermentation experiments.

Item	% of DM
CP	11.9
NDF	48.5
ADF	32.3
EE	2.5

Table 4.3 Effects of adding sodium salicylate at different amounts on fermentation by rumen microbes *in vitro* for 24 h.

Item	CON1[1]	LOW[1]	MED[1]	HI[1]	Pooled SEM
Final pH	6.31[a]	6.36[ab]	6.42[b]	6.45[b]	0.01
24-h disappearance (% of DM)	48.8[a]	37.08[b]	29.59[bc]	22.78[c]	1.96
Gas production measurements					
Asymptotic volume (mL)	249.8	297.9	276.2	296.0	26.41
Rate (mL/min)	0.0010	0.0010	0.0008	0.0010	0.0002
Lag (min)	137.0	122.2	79.1	127.8	55.09

[abc]Means with uncommon superscripts differ within row ($P < 0.05$).

[1]Sodium salicylate was added to rumen fluid inoculum at the beginning of a 24-h batch culture of mixed rumen microbes in buffer (CON1 = 0 mg, LOW = 125 mg, MED = 250 mg, HI = 375 mg of sodium salicylate; $n = 5$ flasks/treatment).

Table 4.4 Effects drenching heifers with sodium salicylate on the fermentation capacity of rumen microbes *in vitro* for 24 h and *in situ*.

Item	CON2[1]			SAL[1]			Pooled SEM
	d1	d12	d35	d1	d12	d35	
Final pH	6.34	6.34	6.25	6.32	6.34	6.29	0.018
24-h disappearance (% of DM)[2]	46.5[a]	44.9[a]	43.9[a]	46.0[a]	38.9[b]	40.3[b]	0.93
Gas production							
Asymptotic volume (mL)	317.1	365.6	276.3	311.3	364.3	281.2	3.69
Rate (mL/min)	0.0011	0.0012	0.0015	0.0010	0.0012	0.0016	0.0003
Lag (min)	87.5	180.7	125.6	97.1	168.7	124.0	8.18
In situ measurements							
DM disappearance rate ($\% \cdot h^{-1}$)	11.6	12.0	11.7	8.6	9.6	10.7	1.18
48-h disappearance (% of initial DM)[3]	91.3	91.2	92.0	90.4	90.8	91.7	0.33

[ab]Means with uncommon superscripts differ within row ($P < 0.05$).[1]Heifers were drenched with 62.5 g sodium salicylate (SAL) or water (CON2) for 3 d and *in vitro* and *in situ* experiments were conducted at 1, 12, and 35 d following treatment administration to test the ability of rumen microorganisms to ferment substrate.

[2]Means differ due to treatment ($P < 0.01$), day ($P < 0.01$), and the interaction between treatment and day ($P = 0.01$).

[3]Means differ due to treatment ($P = 0.01$) and day ($P < 0.01$; treatment × day: $P = 0.31$).

Chapter 5 - Holsteins favor heifers, not bulls: Biased milk production programmed during pregnancy as a function of fetal sex

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ABSTRACT

Mammalian females pay high energetic costs for reproduction, the greatest of which is imposed by lactation. The synthesis of milk requires, in part, the mobilization of bodily reserves to nourish developing young. Numerous hypotheses have been advanced to predict how mothers will differentially invest in sons and daughters, however few studies have addressed sex-biased milk synthesis. Here we leverage the dairy cow model to investigate such phenomena. Using 2.39 million lactation records from 1.49 million dairy cows, we demonstrate that the sex of the fetus influences the capacity of the mammary gland to synthesize milk during lactation. Cows favor daughters, producing significantly more milk for daughters than for sons across lactation. Using a sub-sample of this dataset (n=113,750 subjects) we further demonstrate that the effects of fetal sex interact dynamically across parities, whereby the sex of the fetus being gestated can enhance or diminish the production of milk during an established lactation. Moreover the sex of the fetus gestated on the first parity has persistent consequences for milk synthesis on the subsequent parity. Specifically, gestation of a daughter on the first parity increases milk production by ~445 kg over the first two lactations. Our results identify a dramatic and sustained programming of mammary function by offspring *in utero*. Nutritional and endocrine conditions *in utero* are known to have pronounced and long-term effects on progeny, but the ways in which the progeny has sustained physiological effects on the dam have received little attention to date.

INTRODUCTION

Since the 1970s, biologists have directed substantial research effort to understanding adaptive sex-biased allocation of maternal resources in animals and plants. Biologists have proposed numerous hypotheses for sex-biases, including local resource competition (Clark, 1978; Silk, 1983), "advantaged daughters" (Simpson and Simpson, 1982), local resource enhancement (Emlen et al., 1986; Silk and Brown, 2008), the "safe bet"/reproductive value (Shibata and Kawamichi, 2009; Leimar, 1996) and sex-differentiated sources of mortality (Smith, 1980). The most well-known and investigated, though, remains the Trivers-Willard hypothesis (Trivers and Willard, 1973). Trivers and Willard hypothesized that a female, as a function of her condition, is expected to preferentially allocate resources to the sex that provides greater marginal return on that investment. In polygynous mating systems characterized by male-male competition, they predicted that good condition females would bias resource allocation in favor of sons because males profit more from additional investment than do females. Collectively, the hypotheses proposed in the literature can be loosely grouped according to the extent that the directionality of the sex-bias is contingent on maternal condition; however, the predictions deriving from these hypotheses are not always mutually exclusive, complicating interpretation of empirical results (Cockburn et al., 2002). Large-bodied ungulates are frequently used for investigating sex-biased maternal allocation because male body size contributes substantially to success in competitive access to mating opportunities, but evidence for systematic sex-biases has been equivocal (Cockburn et al., 2002; Cameron, 2004; Hewison and Gaillard, 1999; Sheldon and West, 2004; Pélabon et al., 1995).

Although sex-ratio at birth has been the primary outcome investigated, post-natal maternal physiological transfer and behavioral care afford females substantial flexibility in sex-biased

resource allocation (Hewison and Gaillard, 1999). Sex-biased nursing behavior has been investigated as a possible proxy for sex-biased milk production in numerous mammalian taxa (Cameron, 1998; Hogg et al., 1992; Byers and Moodie, 1990; Stěhulová et al., 2013; Bercovitch, 2002; Brown, 2001; Clutton-Brock et al., 1981). Suckling behavior, however, is not useful for estimating milk energy transfer as verified by experimental use of radio-labeled isotopes in Equus caballus (Cameron et al., 1999). Direct evidence for sex-biased milk synthesis among non-domesticated species has now been reported in ungulates (*Cervus elaphus hispanicus*, Landete-Castillejos et al., 2005), rodents (*Myodes glareolus*, Koskela et al., 2009), primates (*Macaca mulatta*, Hinde, 2007, 2009; *Homo sapiens*, Fujita et al., 2012, Powe et al., 2010, Thakkar et al., 2013, but see also Quinn, 2013 for exception), and marsupials (*Macropus eugenii*, Robert and Braun, 2012). Drawing systematic conclusions from the studies to date, however, is challenging in part because most have been limited by relatively small sample sizes or report milk composition without accounting for milk yield. The most comprehensive data derive from Iberian red deer (*Cervus elaphus hispanicus*) and rhesus macaques (*Macaca mulatta*). Landete-Castillejos and colleagues (2005) showed that hinds favored sons by producing more milk with higher protein content for them. This bias did not vary as a function of maternal mass or age. Among rhesus macaques, mothers produced higher milk energy density [kcal/g] for sons (Hinde, 2009) due to higher fat content (Hinde, 2007). There was additionally an interaction with maternal life-history; smaller, younger mothers produced even higher fat and protein concentrations for sons and lower concentrations for daughters than did multiparous mothers. However, at peak lactation, mothers of daughters, across parities, produced greater milk volume that offset the reduced energetic density of milk for daughters (Hinde, 2009). These studies failed to support sex-bias hypotheses that predict mothers in better condition will preferentially allocate resources to a particular sex,

suggesting instead that there may be systematic sex-biases that are independent of maternal condition.

Mother's milk, however, is particularly difficult to evaluate when investigating adaptive allocation of maternal resources. Milk synthesis is unlikely to be at the maternal optimum because of parent-offspring conflict (Trivers, 1974; Godfray, 1995). Rather milk reflects a complex physiological and behavioral negotiation between the mother and offspring (Hinde and Milligan, 2011; Neville et al., 2012). Functional development of the mammary gland initially occurs during pregnancy and is orchestrated by maternal and placental hormones, particularly placental lactogen, estrogen, and progesterone (Akers, 2002; Rudolph et al., 2003; Sternlicht et al., 2006). Post-natally, local regulation of milk synthesis is maintained by milk removal via offspring suckling (Akers, 2002; Daly and Hartmann, 1995) but maternal rejection can prevent or limit milk intake (Stěhulová et al., 2013). As a result, sex-biased milk synthesis may reflect differential cellular capacity in the mammary gland, programmed via hormonal signals from the fetal-placental unit, or post-natally through sex-biased nursing behavior (Hinde, 2009). There has been only one study that has investigated mechanisms underlying sex-biased milk synthesis. Koskela and colleagues (2009) used an elegant cross-fostering design in bank voles (*Myodes glareolus*) to demonstrate that all-female litters received significantly greater milk yield than did all-male litters, regardless of litter size or maternal condition. The manipulation was conducted after females gave birth, and the extent to which pre-natal mammary gland development may have been sensitive to litter sex-ratio was not reported. Litter size during gestation has been shown to influence mammary gland development in sheep (Rattray et al., 1974) and milk volume in goats (Hayden et al., 1979), but the effect of fetal sex on milk synthesis has not been investigated.

We investigated the magnitude and direction of sex-biased milk synthesis in the Holstein breed of Bos taurus. Although intensive artificial selection has shaped cattle during recent centuries, domesticated cattle are derived from large-bodied, sexually-dimorphic aurochs (*Bos primigenius*; Ajmone-Marsan et al., 2010, Grigson, 1978). Among beef cattle, several small studies have revealed sex-biased milk production that favors sons (Minick et al., 2001), favors daughters (Rutledge et al., 1971), or no sex-biases (Christian et al., 1965). In contrast, standardized husbandry practices, systematic milking procedures, detailed record-keeping, and large samples sizes make the dairy cow a powerful model for the exploration of maternal milk synthesis from both functional and mechanistic perspectives (Neville et al., 2012; Loor et al., 2011; Rowson et al., 2012). Birth sex-ratio in dairy cows is male-biased (Silva del Río et al., 2007) suggesting that mechanisms for sex-biases are operating in this taxon. Moreover the basic architecture for lactation is more highly conserved than other components of the genome, even for an animal artificially selected for milk yield (Lemay et al., 2009). Notably, because calves are removed from the dam within hours of parturition, this model system allowed us to investigate pre-natal mechanisms of sex-biased milk synthesis independent of post-natal maternal care and infant suckling behavior. Importantly, dairy cows are concurrently pregnant during lactation, typically 200+ days of the 305-day lactation (González-Recio et al., 2012). We therefore predicted that milk synthesis on the first lactation could be affected not only by the sex of the calf produced, but also by the sex of the fetus gestated during lactation. We also predicted that mammary gland programming in response to fetal sex would persist into the subsequent lactation because the capacity to synthesize milk is, to some extent, cumulative across parities (Lang et al., 2012; Anderson and Sheffield, 1983; Miller et al., 2006). These complex predictions are clarified by schematic representation (Figure 5.1).

METHODS

To investigate sex-biased milk synthesis, we acquired all lactation records from 1995 to 1999 in the database managed by Dairy Records Management Systems (http://www.drms.org). Whole-lactation milk yield and composition data were derived from monthly yield and composition data collected on commercial dairy farms across the United States. Standardized lactation curves, characterized over 5 decades of research, were then used to predict production between the monthly data points. Production is adjusted for breed, region, season and parity during the calculation of whole-lactation milk and component production, which was standardized to a 305-day lactation. These records are used daily by most of the 50,000 dairy farmers in the US to make management decisions. Detailed discussions of the program and data analysis have been published elsewhere (Wiggens, 2001; VanRaden, 1997). Data from the late 1990's were used to avoid the influence of sex-selected semen in artificial breeding programs in the commercial dairy industry, which became common in the mid-2000's (Norman et al., 2010; Garnel and Seidel, 2008). Additionally, this period of time allowed for analysis of the effects of recombinant bovine somatotropin (bST; Chilliard, 1998), approved in 1993 for commercial use in the US. The DRMS database includes a field for reporting administration of bST that was introduced into their software (PCDart) from the start of the commercial availability of bST.

Several steps were taken to clean the data prior to analysis. Only records from Holstein cattle were retained, and lactations that began with either twin births or abortions were excluded. Lactations with missing or corrupt lactation number, year, or calf sex designations were deleted. Duplicate records for a single lactation within cow were eliminated, and records for lactation ≥ 6 (representing 3.02% of lactations in the database) were excluded to enable repeated measures analysis of lactations with adequate representation in the database. If at least 1 of the first 5 test

days, typically conducted monthly, were flagged for bST administration, then the lactation was considered bST-positive (n=100,478; 3.9% of lactations). The final database consisted of 2.39 million lactation records, representing 1.49 million individual Holstein cows, however due to missing data in certain fields, some analyses included fewer lactations and final analysis sample sizes are reported for each analysis. Mixed models were used to evaluate the fixed effects of calf sex, parity, bST, and interactions and the random effect of year according to the following model:

$$Y_{ijkl} = \mu + S_i + P_j + B_k + Y_l + SP_{ij} + SB_{ik} + SPB_{ijk} + e_{ijkl}$$

where Y_{ijkl} is a dependent variable, μ is the overall mean, S_i is the fixed effect of calf sex (i=1 to 2), P_j is the fixed effect of parity (j=1 to 5), B_k is the fixed effect of bST (k=1 to 2), Y_l is the random effect of year (l=1 to 5), SP_{ij} is the interaction of calf sex and parity, SB_{ik} is the interaction of calf sex and bST, SPB_{ijk} is the interaction of calf sex, parity, and bST, and e_{ijkl} is the residual error. Repeated lactations within cow were fit to a heterogeneous autoregressive (ARH [1]) covariance structure. Analyses were completed using the Mixed Procedure of SAS (version 9.3; SAS Institute, Cary, NC). Significant interactions were investigated using the SLICE option and means were separated using the PDIFF option of SAS, with significance declared at P <0.05.

To exclude potentially confounding effects of dystocia and bST treatment on results and to evaluate carryover effects of calf sex on multiple lactations, a more conservative data set was generated. All bST-positive lactations were deleted, and only those beginning with a calving difficulty score of 1 or 2 (no or minimal difficulty) were retained. Finally, the data were narrowed to only those cows with both lactations 1 and 2 represented, leaving 113,750 cows. Data for 305-day milk yield in lactations 1 and 2 were modeled with the fixed effects of calf sex1, calf sex2, calf sex1 × calf sex2, and year. Analyses were completed using the Mixed Procedure of SAS (SAS

Institute) and means were separated using the PDIFF option of SAS, with significance declared at $P < 0.05$.

RESULTS

Sex-biased milk synthesis: Full dataset

Holsteins biased milk production in favor of daughters, producing significantly more milk over the 305 days of standard lactation after gestating a daughter (Figure 5.2). These findings are based on 2.39 million lactation records from approximately 1.49 million female cows. First-parity cows giving birth to a daughter produced 142±5.4 kg more milk over the 305-day lactation period than did those giving birth to a son (7,612 vs. 7,470±69 kg, $P < 0.001$). Similar, though marginally smaller, effects were observed in parities 2–5 (Figure 5.2A). The overall effect amounted to a 1.3% increase in whole-lactation milk production for cows bearing daughters (Table 5.1). Extrapolation from total lactation production values revealed that milk composition was similar after gestation of a son or daughter. Fat concentration was 3.61% after gestation of a daughter and 3.62% after gestation of a son; protein concentrations were the same (3.17%).

The disparity between milk produced following birth of a son vs. a daughter was largely eliminated by the use of bST. A recombinant, exogenous form of the growth hormone somatotropin, bST promotes endocrine alterations to partition a greater proportion of nutrient supply to the mammary gland, thereby increasing milk production (Bauman and Vernon, 1993). Recombinant bST is approved for exogenous administration to dairy cows beginning at week 9 of lactation. In our sample, bST accounted for a 12% increase in whole-lactation milk yield (Table 5.1). On first parity, cows administered bST still produced significantly higher milk yield if they

had a daughter (8,681 vs. 8,631±71 kg, $P < 0.05$), but sex-biased milk synthesis was not observed in parities 2–5 (Figure 5.2B).

Sex-biased milk synthesis: Conservative sample

Male calves are typically larger than females, and pose a greater risk of dystocia (Gianola and Tyler, 1974; Dematawena and Berger, 1997). Dystocia is associated with decreases in whole-lactation milk production (Rutherford, 2013), and we hypothesized that the milk yield advantage conferred by a daughter might have been at least partly due to decreased incidence of dystocia compared to delivery of sons. Indeed, in our data, the odds of a son inducing dystocia (calving difficulty score ≥3 on a scale of 1 to 5) were significantly greater than for daughters (5.6 vs. 4.2% incidence, $P < 0.001$, odds ratio 95% CI: 1.32–1.35). Nevertheless, sex-biased milk synthesis remained when analysis was restricted to a subset of the dataset (n=113,750) that excluded cases of bST and dystocia, and included information on individual cows across the first and second parity. On first parity, cows producing daughters had significantly greater 305-day milk yield, with an advantage of 1.6% relative to cows producing sons (7,947 vs. 7,818±9.6 kg, $P < 0.001$). The daughter advantage was also observed in parity 2, although the magnitude of the difference was reduced (0.83%; 8,515 vs. 8,445±37 kg, $P < 0.001$). These results indicate that the milk production advantage associated with birth of a daughter is not attributable to prevention of dystocia.

Inter-parity consequences of fetal sex

Milk production on first lactation was associated with the sex of the fetus on the second pregnancy because the two overlapped temporally (Figure 5.3A). Across the first two parities in the subset that excluded cases of bST and dystocia, birth combinations could be son_1son_2,

$son_1daughter_2$, $daughter_1son_2$, and $daughter_1daughter_2$. Cows that had first produced a son and were gestating a son for their second pregnancy synthesized significantly less milk over 305 days than did all other groups ($P < 0.001$; son_1son_2=7,768±11.4 kg, N=32,294). Gestation of a daughter on the second pregnancy could partially "rescue" milk synthesis on the first lactation if a son had been produced previously ($P < 0.001$; $son_1daughter_2$=7,876±12.2 kg, N=27,807), but remained significantly less than cows that had produced a daughter on their first pregnancy ($P < 0.001$). Fetal sex on the second pregnancy didn't have any effect for cows that produced a daughter on pregnancy 1 ($daughter_1son_2$ and $daughter_1daughter_2$ were 7,940±12.3 kg, N=27,834 and 7,954±12.6 kg, N=25,815, respectively; $P = 0.36$).

Fetal sex on the first parity had persistent effects on milk production during the second lactation (Figure 5.3B). Cows that produced a son on their first parity were handicapped in their milk production on their second lactation ($P < 0.001$), particularly if they gestated a son on the second pregnancy as well (son_1son_2=8,345±18.9 kg). Production of a daughter on the second parity partially increased milk production on second lactation ($P < 0.001$; $son_1daughter_2$=8,539±19.4 kg). Cows that produced a daughter on their first parity produced significantly more milk on their second lactation ($P < 0.001$), regardless of the sex of the calf on the second parity ($daughter_1son_2$ and $daughter_1daughter_2$ were 8,614±19.6 kg and 8,605±19.8 kg, respectively; P=0.19).

DISCUSSION

Holstein dairy cows demonstrate a significant biological effect of sex-biased milk production in favor of daughters. In dairying, calves are removed on the day of birth and standardized mechanical procedures are used for milking, therefore post-natal sex-bias does not

explain the results presented here. Instead milk production varied as a function of fetal sex, indicating that functional development of the mammary gland is influenced pre-natally. Importantly, lower milk yield for sons was not compensated by higher protein and fat production; total production of milk energy was greater in cows that gestated daughters. Among rhesus monkeys, mothers rearing daughters produce more milk, but of significantly lower milk energy density- the aggregated calories derived from fat, protein, and sugar- than do mothers of sons (Hinde, 2009). To our knowledge, the results reported here are the first to document that fetal sex influences milk production. Moreover the effects on milk production were dynamic and persistent across parities. Importantly, gestation of a daughter on the first parity increased milk production across the first two lactations and was protective against the negative effects of male gestation on the second parity. In contrast, gestating a son on the first parity suppressed milk production on the first two lactations, but the conception of a daughter on the second parity partially improved milk production. Nutritional and endocrine conditions in utero are known to have pronounced and long-term effects on progeny (Rutherford, 2013), but the ways in which the progeny has sustained physiological effects on the dam have been less studied.

Sex-differentiated programming of the mammary gland is further substantiated by the greater effect of bST administration in cows gestating sons than cows gestating daughters. Postnatal administration of recombinant bovine somatotropin (bST) in multiparous cows overwhelmed the prenatal effects of offspring sex, but had a greater effect in cows gestating sons. Somatotropin, or growth hormone (GH), is produced in the anterior pituitary, stimulated by GH-releasing hormone. Most notably, GH influences metabolism in hepatic and adipose tissues, shunting more maternal bodily reserves to milk synthesis (Akers, 2006). Insulin-like growth factors are believed to be the major mediators of the effect of GH on the mammary gland (Bauman

and Vernon, 1993), however GH also directly affects the mammary gland and increases milk synthesis (Plath-Gabler et al., 2001; Johnson et al., 2013). While the mean production parameters increased with the administration of bST for cows producing both daughters and sons, the proportional increase in milk production was greater for multiparous cows gestating sons. Rose and colleagues reported that cows that had low milk yield responses to bST treatment within a herd had greater milk yields before bST treatment compared to cows with a high response in milk yield (Rose et al., 2004). This is consistent with our results that cows birthing daughters had elevated milk production and a lower response to exogenous bST administration compared to their counterparts bearing sons. We posit that mechanisms underlying lower initial milk production and greater individual response to bST administration are likely responsible for the greater response to bST in cows with sons. Administration of bST in many ways represents an "experimental" manipulation of mammary gland programming and reveals possible mechanistic pathways through which sex-biases are operating. Although bST was able to overwhelm sex-biased milk synthesis among multiparous cows, significant sex-bias remained among primiparous cows whose mammary glands had functionally developed for the first time in the context of the fetal sex of the first gestation. The magnitude of sex bias is strongest among first parity rhesus monkeys (Hinde 2007, 2009) and possibly humans (Powe et al., 2010; Thakkar et al., 2013) and Tamar wallabies (Robert and Braun, 2012) in which primiparous females have been disproportionately represented in published studies. The effect of fetal sex may diminish to some extent among multiparous females due to the aggregate effects on mammary gland architecture of sequential gestations of different fetal sexes. Alternatively, maternal investment tactics may change as a function of residual reproductive value (Williams, 1966) or targeted effort during critical developmental windows (Cameron et al., 2000).

These biological findings may have economic impact for the modern dairy industry. With the widespread availability of sexed-selected semen for use in artificial breeding programs, dairy managers have the option of achieving approximately 90% female pregnancies rather than a natural rate near 47% (Silva del Río et al., 2007). There are many factors for managers to consider when evaluating the profitability of sexed semen use, including decreased conception rate (Norman et al., 2010) and increased semen cost. Some published analyses have been skeptical of the economic merit of using sexed semen on dairy operations (Olynk and Wolf, 207), although the cost of the cell sorting technology continues to drop, making recent analyses more favorable (Riberio et al., 2012). Accounting for the impact of a female calf on lactation productivity revealed by our analysis, however, further improves the expected profitability of sexed semen use. It is common to use sexed semen for breeding nulliparous heifers only, and given the long-term impact of a first-parity daughter, the production benefits of this management strategy are substantial. The cumulative increase in milk yield over two lactations for a cow giving birth to a daughter on the first parity rather than consecutive bulls is ~445 kg (Figure 5.3). The impact of sexed semen on the structure of the dairy industry has been a complex question already (De Vries et al., 2008), but these results highlight a key factor that has not previously been considered.

The precise mechanistic pathways through which fetal sex influences mammary gland development remain unknown. Fetal-origin hormones may translocate via maternal circulation to bind directly to receptors in the dam's mammary gland influencing functional development and subsequent milk synthesis. Among ungulates, ruminants may be especially valuable for understanding mammary gland development during pregnancy as a function of fetal sex because of their cotyledonary placenta. Klisch and Mess posited that for ruminants, an evolutionary "arms race" between the mother and fetus (Moore, 2012) for glucose transport, necessitated by the lack

of gastrointestinal glucose supply (Aschenbach et al., 2010), resulted in selective pressure that favored an "inefficient" placenta (Klisch and Mess, 2007). For example, the placenta of the domestic cow has ~5 times the surface area as the horse placenta even though the two species produce similarly sized neonates (Baur, 1981). As a byproduct of the greater placental surface area, fetal steroidal hormones can readily diffuse into maternal circulation (Klisch and Mess, 2007). Concentrations of estrogens and androgens differ between male and female fetuses and, if in maternal circulation, potentially enhance or inhibit mammary gland development and consequently milk synthesis during lactation. In dairy cows, fetal steroid hormones are present from the first trimester and are critical for the development of fetal sex organs (Yang and Fortune, 2008; Nilsson and Skinner, 2009). Insulin-like peptide 3 (INSL3), another fetal-origin bioactive, increases in maternal circulation across pregnancy in dairy cows gestating sons and decreases in cows gestating daughters (Anand-Ivell et al., 2011) but the influences of fetal-origin INSL3 on the mammary gland are not known. Functional development of the mammary gland in taxa characterized by highly invasive hemochorial placentas may also be susceptible to fetal hormones; indeed the majority of reports of sex-biased milk synthesis in the literature are from taxa that have greater placental invasion and/or placental surface area (Rutherford, 2013; Baur, 1981; Capellini, 2012). Suggestively, human mothers with higher concentrations of circulating androgens during the 2nd trimester had a lower probability of sustaining breastfeeding to three months post-partum (Carlsen et al., 2010). The higher circulating androgens may have originated from fetal sons, but the effect of fetal sex was not directly analyzed in that study, nor was milk synthesis measured. Indirectly, fetal sex may influence the production of placental lactogen, a primary hormonal driver of mammary gland development during pregnancy (Akers, 2002; Rudolph et al., 2003; Sternlicht

et al., 2006) but as of yet differences in placental lactogen as a function of fetal sex have not been reported.

Daughter-biased milk synthesis may reflect adaptive maternal allocation in response to fetal sex, adaptive fetal manipulation of maternal physiology, or may be a by-product of the maternal-fetal interface. Importantly, uniformly biased milk production in favor of daughters across maternal conditions does not support the Trivers-Willard hypothesis (Trivers and Willard, 1973), or other hypotheses positing facultative sex-biased allocation of resources as a function of maternal condition (Cockburn et al., 2002). Dairy cows have a male-biased birth ratio; in the absence of sex-specific artificial insemination, between 50–54% of calves born are male (Silva del Río et al., 2007; Foote, 1977). The mediating effect of maternal condition on birth-sex ratio has been inconsistent (Meier et al., 2010) as has been the directionality of birth sex-ratio bias. Better-condition cows may produce more sons (Roche et al., 2006) or daughters (Hohenbrink and Meinecke-Tillmann, 2012). Integrating the results presented here, dairy cows produce more sons, but seemingly favor daughters with more milk. Mammalian mothers in polygynous taxa may preferentially allocate physiological resources to daughters so that they are able to initiate reproduction at relatively younger ages than do sons (Hinde, 2009; Hinde et al., 2013). For female mammals, because of the temporal constraints of pregnancy and lactation, lifetime reproductive success of daughters will be contingent on the length of their reproductive careers, a function of age at first birth and longevity (Blomquist, 2009; Martin and Festa-Bianchet, 2012). Among sexually dimorphic polygynous taxa, the temporal constraints are relaxed for males, who benefit from growing bigger and stronger (Willisch et al., 2012; Festa-Bianchet, 2012), allowing males more time to compensate for deficits in early life maternal investment before becoming reproductively active (Bercovitch et al., 2000). Daughter-biased milk production may involve life-

history tradeoffs for both cows and their daughters. High milk production in dairy cows is generally associated with reduced fertility, health, and survival depending on environmental conditions (Windig et al., 2006). Moreover daughters gestated during lactation have moderately reduced survival and milk production in their own adulthood (González-Recio et al., 2012). Although we do not know whether the magnitude of the effects presented here is correlated with such consequences, future research should investigate the fitness effects of daughter-biased milk synthesis both in the short-term (i.e. inter-birth interval), across the lifetime, and inter-generationally.

The question remains though, under natural conditions how do bull calves grow faster during the post-natal period if their dams are producing less milk, and therefore lower total protein and fat production? One explanation may be that females bias nursing behavior such that milk production is up-regulated for sons, a tactic we could not evaluate in conventional dairying as calves are removed after birth. Landete-Castillejos and colleagues (2005) revealed that among captive Iberian red deer, dams rearing sons had greater total milk production and total protein production, possibly due to post-natal hind-calf behavioral dynamics. However in the one study to date of cow maternal behavior, cows do not show any sex biases in nursing behavior (Stěhulová et al., 2013). In beef cattle that are reared by their dam, sons are born bigger and have better post-natal growth than do daughters, but only one out of three studies has shown any evidence of male-biased milk synthesis (Minick et al., 2001; Rutledge et al., 1971; Christian et al., 1965). In the absence of post-natal behavioral modifications of prenatal mammary gland programming, the presence and concentration of other milk bioactives such as immunofactors and hormones that influence offspring development (Neville et al., 2012) may differ in milk produced for sons and daughters. Notably, investigations of sexually dimorphic developmental trajectories, however,

overwhelmingly essentialize the role of the mother and sex-biased allocation of maternal resources. More often overlooked are sexually differentiated mechanisms within offspring that influence utilization and assimilation of early life nutrition and environmental signals (Hinde, 2009; Badyaev, 2002; Aiken and Ozanne, 2013). Consideration of progeny-specific adaptations as well as biased maternal effort will contribute to a better understanding of the ontogeny of sexual dimorphism.

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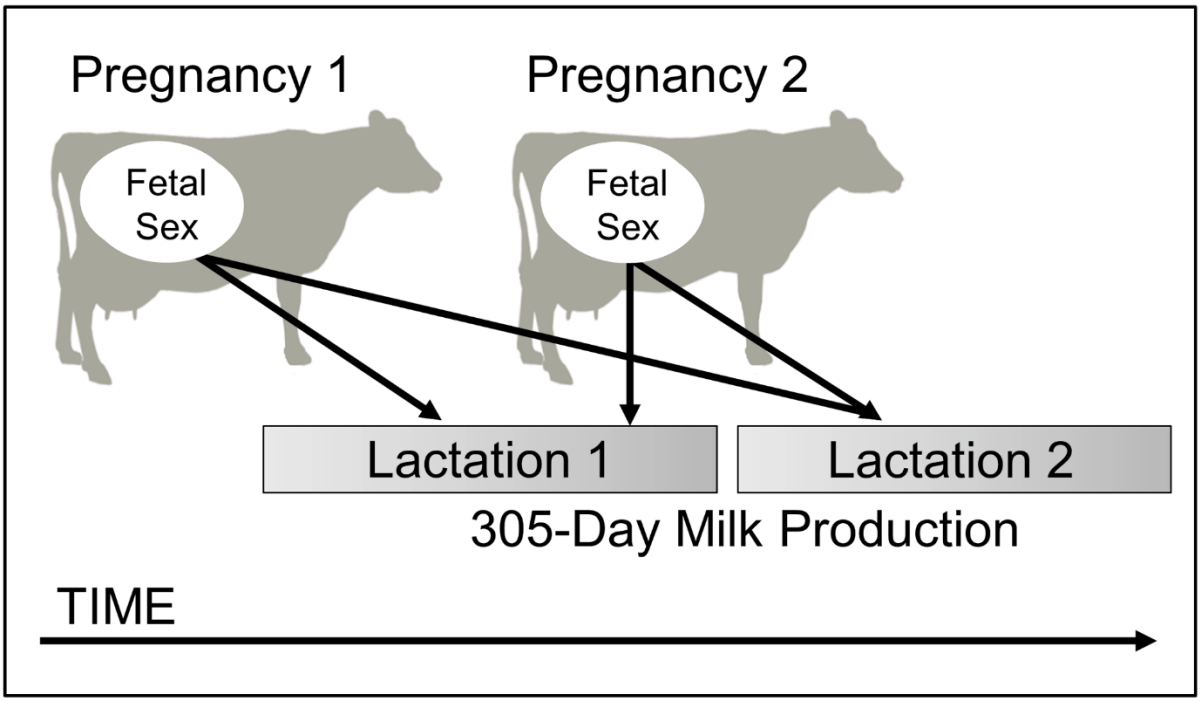
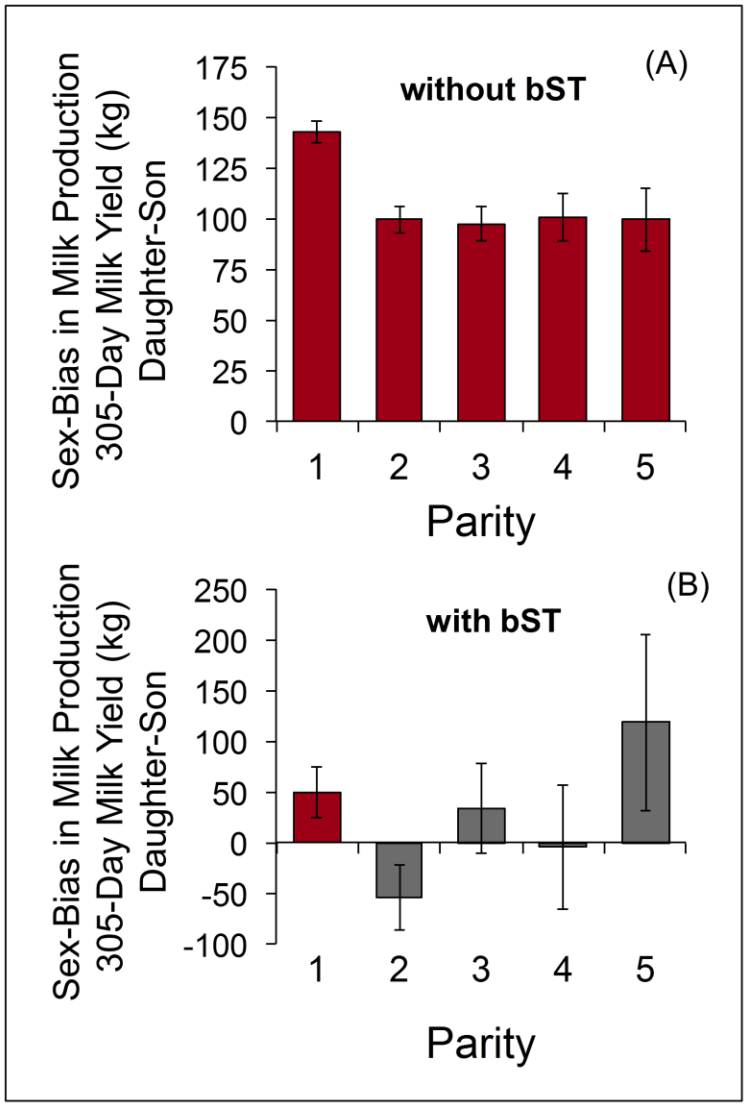
Figure 5.1 Hypothesis: milk production is influenced by fetal sex across lactations.



Fetal sex in pregnancy 1 may alter milk production across multiple lactations because of the critical steps in mammary development that occur during the first pregnancy. In the cow, pregnancy 2 typically overlaps with lactation 1, providing opportunity for calf sex in parity 2 to impact milk production in the first lactation.

Figure 5.2 Daughters result in greater lactation productivity, and this effect is altered by exogenous somatotropin (bST) administration.



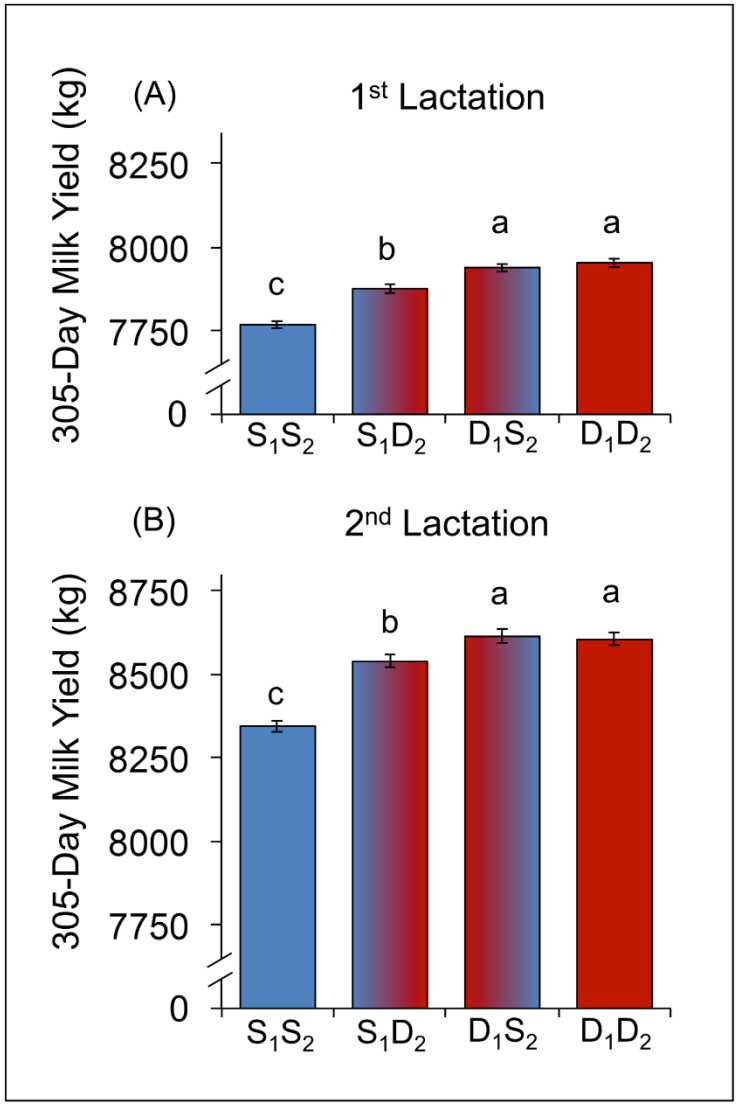
Lactation records from Holstein cows (n=2.39 million lactations) were analyzed to determine effects of calf sex, parity, use of bST, and their interactions on 305-day milk production. Calf sex influence on milk production was dependent on bST use (interaction $P < 0.01$). A) In the absence of bST, daughters resulted in significantly greater milk production compared to sons across all parities (all $P < 0.001$). B) Lactations influenced by bST administration failed to consistently demonstrate the daughter bias. Daughters still conferred an advantage in first-parity cows administered bST ($P < 0.05$), but did not significantly influence milk yield in parity 2–5 cows. Values are differences of LS means ± SED.

Table 5.1 Influence of calf sex, in the presence and absence of exogenous somatotropin (bST), on lactation productivity.

Cow milk production[1]	No bST			bST			N (lactations)
	Daughter	Son	SEM	Daughter	Son	SEM	
305-d milk yield (kg)	8,172.8	8,064.9	68.6	9,123.4	9,094.8	70.8	2,391,300
305-d fat yield (kg)	295.56	291.46	3.25	329.50	328.11	3.40	2,125,643
305-d protein yield (kg)	258.78	255.61	2.06	291.05	290.26	2.16	2,108,796
Peak milk (kg/d)	36.97	36.36	0.34	40.52	40.38	0.35	825,175

[1]For all variables, there was a significant effect of fetal sex ($P < 0.001$), bST ($P < 0.001$), and the interaction between fetal sex and bST ($P < 0.01$).

Figure 5.3 Daughters confer milk production advantages post-natally, during gestation, and across multiple lactations.



Cows (n=113,750) with both first and second parity lactation records, with no reports of dystocia or bST administration, were used to assess effects of calf sex on milk production in the first 2 lactations. Groups are labeled by calf sex (S=son, D=daughter), with the pregnancy denoted by subscript. Values are LS means ± SEM. Means labeled with different letters differ (P < 0.001), and those with common labels do not (P > 0.10). A) First-parity cows having a daughter produced significantly more milk than those having a son, but gestating a daughter in pregnancy 2 increased milk production in cows that had a son first. B) Second-parity milk production is greatest in cows that had a daughter in pregnancy 1. Additionally, cows with a son in pregnancy 1 showed increased milk production if they had a daughter in pregnancy 2.