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Effects of drinking water on feed intake, growth performance, health status, nutrient digestibility and composition of gut microbiota in young dairy calves

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Effects of drinking water on feed intake, growth performance, health status, nutrient digestibility and composition of gut microbiota in young dairy calves

by

Handagala Wickramasinghe

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Program of Study Committee:
Ranga Appuhamy Jayasooriya, Major Professor
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Stephan Schmitz-Esser

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2019

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DEDICATION

This work is dedicated to all my teachers who taught me from primary school to graduate school, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.
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Finally, I would like to thank my family and friends for their support and encouragement throughout the past two years. To my loving wife, Thanuja and son, Yethuja, to my parents and in-laws, thank you for encouraging me to follow my dreams. Your support has been unwavering as I have ventured out on my own.
There is a lack of data on water consumption and its role in dairy calves. The water requirements of dairy calves are negligibly defined. Producers pay little attention to whether calves consume enough drinking water (also called free water) as they consider water in milk or milk replacer would satisfy the total water requirement. A recent USDA survey revealed that dairy producers wait on average 17 d to first offer drinking water to newborn calves. Unlike milk or milk replacer directly shunted to the abomasum, drinking water first enters the rumen and then moves to the rest of the gastrointestinal tract. Therefore, drinking water presumably play a unique role in the development of gut and its microbiome in turn affecting nutrient intake, nutrient digestibility, and growth. The present study was conducted 1) to determine if newborn calves consume significant amount of free water separate from the water in milk, and 2) to examine the effects of offering drinking water since birth (W0) vs. 17 days later (W17) on starter intake, health status, growth, nutrient digestibility, and the composition of gut microbiota in young dairy calves. Thirty Holstein heifer calves were randomly assigned to W0 or W17 (n = 15). Calves had free access to drinking water and a starter ration, and bottle-fed with pasteurized waste milk until weaning at 49 d of age. Fresh fecal samples were collected directly from the rectum at 14, 42, and 70 d of age. The DNA were extracted from fecal samples and sequenced using 16S rRNA gene-amplicon sequencing on an Illumina MiSeq system. The sequences were clustered into operational taxonomic units with a 99% similarity threshold. Total fecal output of individual calves was measured over two consecutive days to determine apparent total-tract digestibility (ATTD) of nutrients three weeks post-weaning. Newborn calves consumed a significant amount (0.75±0.05 kg/d) water separate from the water in milk during the first 16 d. Once offered, W17 drank 59% more free water than W0 during the rest of the pre-weaning period. Starter intake of W0 and W17
was similar, but W0 consumed 0.285 kg/d more milk and tended to achieve greater BW and heart girth (HG) compared to W17 during the pre-weaning period. Offering water from birth versus offering it later did not affect the number of days with diarrhea, intensity of diarrhea, and blood hematocrit values. Despite a similar starter intake, W0 had greater hip height, body length, ATTD of ADF and NDF, and gain: feed ratio than W17 post-weaning. At 14 d of age, feces from W0 had a greater number of bacterial species (5908 vs. 4698, \( P = 0.033 \)) and species richness (Chao1 index, \( P = 0.042 \)) than feces from W17. The number of bacterial species, and Chao1 index increased with age (\( P < 0.001 \)) and became similar between W0 and W17 at 42 d of age. At 42 d of age, the abundance of \textit{Faecalibacterium prausnitzii}, and \textit{Bifidobacterium breve} previously shown to improve gut health and growth were greater in W0 than W17 (\( P < 0.040 \)). Overall, the present study highlighted that offering drinking water since birth has a potential to improve growth, nutrient digestibility, feed conversion efficiency, and the abundance of beneficial bacterial communities in the gut of young dairy heifer calves.
CHAPTER 1. GENERAL INTRODUCTION

Even though water is considered the most essential nutrient for animals to sustain life and production performance, water requirements of livestock are often overlooked relative to those of other nutrients (Appuhamy et al., 2014). When it comes to dairy cattle, the situation is further aggravated for calves compared with mature cows in both research and production settings (Kertz et al., 2017). Dairy farmers are not reasonably concerned about whether calves meet their daily water requirements. Furthermore, the water requirements of calves have been negligibly described in current nutrient requirement models (e.g., NRC, 2001) because of a lack of reliable data (Kertz et al., 2017). Similar to other mammals, calves meet their water requirements using three sources: 1) drinking water (also called free water), 2) water in feed, and 3) the metabolic water. Relative contribution of these sources to total water intake varies significantly between calves and mature animals. For instance, drinking water contributes to 82% of the total water intake of lactating cows (Appuhamy et al., 2014), whereas its contribution is less than 20% in calves as they consume a large amount of water via milk or milk replacer (Thomas et al., 2007). Nonetheless, drinking water has the opportunity to first enter the rumen and then move to the rest of the gut, whereas water in milk or milk replacer is directly shunted to the abomasum by the esophageal groove.

It is well accepted that postnatal nutrition can significantly influence the future performance of animals. This phenomenon is particularly true to mammals as the development of organs is fairly incomplete at birth and continues to grow during the postnatal period. The rumen is a prime example of an underdeveloped organ at birth, introducing a unique opportunity to manipulate early development of it, particularly its microbiome to produce lasting effects into the animal’s adult life (Khan et al., 2007; Yanez-Ruiz et al., 2015; Reddy et al., 2017). Lam et al. (2018) and Paz et al. (2018) demonstrated that growth and feed efficiency were strongly related to
microbial community composition, epithelial histomorphology and pH in the rumen of mature cattle. Moreover, Shanks et al. (2011) demonstrated that feed efficiency was different in cattle with different bacterial community compositions in feces. Drinking water is hypothesized to provide a physical stimulant for structural development and a media for microbial growth in the early stage of rumen development. Once starter feed intake commences at around 3 weeks of age, drinking water is assumed to affect rumen development in several different ways (Leadley, 2010; Earleywine, 2015; Cullens, 2016; Kertz et al., 2017). Drinking water entering the rumen could facilitate feed mixing and fermentation of volatile fatty acids that are powerful stimulants to rumen development. Moreover, depending on some observations made on mature cattle and humans, drinking water is capable of altering pH, digesta passage rates, nutrient absorption rates, and the composition of microbiota in the gastrointestinal tract (Fraley et al., 2015; Sasada et al., 2015; Cremer et al., 2017). It is commonly considered that increased starter feed intake is indicative of improved development of the rumen in young calves. Kertz et al. (1984) found neonatal calves offered drinking water consumed 30% more starter feed than those deprived of drinking water suggesting drinking water potentially enhanced the rumen development. Gottardo et al. (2002) observed improved rumen papillae development in Holstein veal calves having access to drinking water than those deprived of drinking water. On the other hand, Górka et al. (2011) observed that starter intake had a stronger relationship with the development (i.e., mass and length) of the small intestine than that of the reticulorumen in pre-weaned dairy calves.

It has been 35 years since Kertz et al. (1984) examined the importance of offering drinking water to newborn dairy calves in the US. Given the fact that dairy calf management practices have markedly changed over the last couple of decades, the relevance of the importance of drinking water shown by Kertz et al. (1984) to modern dairy calves is questionable. For instance, calves in
Kertz et al. (1984) received only 4.0 L of milk per day, whereas the majority of modern dairy farms feed 5.0 to 8.0 L of milk or milk replacer per day (USDA, 2016). A survey conducted by USDA in 2014 revealed that an average dairy farmer waits for 17 days to first offer drinking water to newborn dairy calves, even though offering drinking water since birth is strongly recommended (USDA, 2016). The majority of farmers assume that water in milk and milk replacer is adequate for neonatal calves to meet their total water requirements. Some farmers also assume that offering drinking water to newborn calves increases the incidence of diarrhea, reduces milk or milk replacer intake, and is a hassle particularly during winter (Leadley, 2010; Earleywine, 2015; Cullens, 2016).

Overall, systematic investigations into the roles and true requirement of drinking water in modern dairy calves appeared to be an eminent need for educating producers more effectively on the importance of provision of drinking water to newborn dairy calves. Therefore, we conducted the present study to 1) determine if newborn calves consume significant amount of free water separate from water in milk, 2) examine the effects of offering drinking water since birth vs. later on feed intake, health status, growth, and feed efficiency, and 3) explore how the composition and abundance of bacterial communities in the gut would respond to drinking water in early postnatal period of modern dairy heifer calves.

**Thesis Organization**

The following chapter, CHAPTER 2, will present a detailed review of literature emphasizing the importance of drinking water for growth, feed intake, health status, and the development of gut microbiome in calves published in peer reviewed journal articles, conference proceedings, and online dairy extension articles. The following chapter, CHAPTER 3 will present a manuscript describing the effect of drinking water intake of newborn dairy calves on feed intake, growth performance, health status, and nutrient digestibility. This manuscript is already published in the Journal of Dairy Science. The CHAPTER 4 includes a manuscript prepared for the Journal
of Dairy Science and is based on a fecal microbiome analysis aiming at exploring how the composition and abundance of bacterial communities in the gut would respond to drinking water in early postnatal period of dairy heifer calves. The concluding chapter, CHAPTER 5 summarizes the most important findings of the present research project and related conclusions.

**Literature Cited**


CHAPTER 2. LITERATURE REVIEW

Pre-Weaned Calves and Significance of Growth

Raising pre-weaned dairy heifer calves is a large expense to a dairy operation with little short-term income opportunities. Together with current milk prices, feed and labor costs, it is necessary to evaluate every component of cost associated with raising heifer calves. For instance, raising replacement dairy heifers accounts for approximately 20% of the total expenses in a dairy operation (Heinrichs, 1993). Pre-weaning calves are the most expensive group within the replacement herd. It typically costs $5.0 to 6.0 per day to raise a dairy calf from birth to weaning (Tranel, 2019). Enhancing growth and thereby weaning calves as early as possible is a promising strategy to reduce the cost of raising pre-weaned calves. Moreover, several studies (Shamay et al., 2005; Moallem et al., 2010; Heinrichs and Heinrichs, 2011; Soberon et al., 2012) have shown that BW, average daily gain (ADG), and withers height of pre-weaned calves could be used to predict the future production performances. A study conducted at Cornell University reported that heifers produced an average of 850 kg more milk in their first lactation for every 1 kg of pre-weaning ADG increase (Soberon et al., 2012). Similarly, Raeth-Knight et al. (2009) reported pre-weaning ADG was significantly associated with age at puberty, age at first calving and first lactation milk yield. A meta-analysis by Van de Stroet et al. (2016) using growth data across various calf nutrition experiments showed that calves which had higher ADG during early life were more likely to remain in the herd and complete the first lactation rather than being dead or culled. Van De Stroet et al. (2016) speculated that pre-weaning ADG may be an indicative of an inherent metabolic efficiency and therefore, it is possible that metabolically efficient calves continue to be metabolically efficient as adults as well. On the other hand, Van Amburgh (2008) considered that calves growing faster are healthier and thus have a longer productive life than those are less healthy
and thus grow slower. Nevertheless, in that meta-analysis, Van de Stroet et al. (2016) categorized pre-weaned heifers based on not only body weight but also hip height, and then compared the performance of first lactation among categories. Interestingly, they were the first to discern that calves with shorter pre-weaning hip height had lower milk production potential than that of the calves with taller hip height. Those authors further reported that an increased likelihood for calves with greater pre-weaning hip height to remain in the herd until first calving as opposed to calves with lower hip height. Henderson et al. (2011) also observed that larger calves at weaning had greater longevity than smaller calves at weaning. Besides the BW, hip height, and ADG, dry matter intake at weaning is positively associated with future milk yield of heifer calves (Heinrichs and Heinrichs, 2011; Van de Stroet et al., 2016). Further, Bach (2014) reported that the average starter intake during the week before weaning was significantly associated with ADG after weaning. For instance, for every 0.45 kg increase of the starter intake, post-weaning ADG increased about 0.41 kg per day. Overall, growth and starter intake of pre-weaned calves influence not only the cost of raising replacement herds but also generating income via future milk production.

Factors Affecting Pre-Weaning Calf Growth

Colostrum

Colostrum is undeniably rich in a variety of nutritive and non-nutritive factors such as protective immunoglobulins (Ig), maternal leukocytes, growth factors, hormones, cytokines, and nonspecific antimicrobial factors. Thus, early and adequate intake of high-quality colostrum is widely recognized as the single most important factor affecting health and growth of neonatal calves (NAHMS, 1993; Davis and Drackley, 1998; Weaver et al., 2000; McGuirk and Collins, 2004). Although the immunologic importance of colostrum is often discussed, the nutritional significance of the first colostrum meal to neonatal calf should not be overlooked (Davis and Drackley, 1998; Quigley and Drewry, 1998). Since, the supplies of endogenous fuels are exhausted
within hours after birth (Rowan, 1992; Okamato et al., 1986), carbohydrates, fat and, proteins in colostrum are essential energy sources for newborn calves. Moreover, most of the essential minerals and vitamins are more concentrated in colostrum than that of in whole milk (Foley and Otterby, 1978). For example, Ca, Mg, Zn, Mn, Fe, Co, Se and vitamins A, E, carotene, riboflavin, B12, folic acid and choline are found in greater concentrations in bovine colostrum than in whole milk (Foley and Otterby, 1978; Przybylska et al., 2007). The insulin like growth factor-I (IGF-I) in colostrum is a key regulator of the development of gastrointestinal tracts of bovine neonates, which intern enhances overall growth of the body (Baumrucker et al., 1994; Bird et al., 1996; Bu’hler et al., 1998). Other benefits of colostrum feeding includes reduced risk of pre-weaning morbidity and mortality, improved feed efficiency, reduced mortality post-weaning, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation (DeNise et al., 1989; Robison et al., 1998; Wells et al., 1999; Faber et al., 2005).

The plane of nutrition

During the first two weeks of life, calves meet most of their nutrient requirements using liquid diets such as milk or milk replacer. However, when calves suckle milk or milk replacer, from either a teat, or a bottle reflexive closure of the esophageal groove occurs and thus those liquids bypass the rumen and directly enter the abomasum (McGeady et al., 2006). Then, milk constituents, fat, lactose, and protein are digested by abomasal enzymes and enzymes in the small intestine (Drackley, 2008). This gastric and intestinal digestion provides small- and medium-chain fatty acids, glucose, galactose, and amino acids for the maintenance of vital body functions and growth during early life (Khan et al., 2011). Several studies have demonstrated that pre-weaning growth is directly proportional to the amount of milk or mil replacer consumed by calves (Khouri and Pickering, 1968; Hodgson, 1971; Huber et al., 1984). National Animal Health Monitoring
System (NAHMS) survey study in 2014 (USDA, 2016) found that more than half of pre-weaned heifers in the US (54.5%) were fed 6 or more liters of milk per day while about 61% of all operations making diet modifications based on age or size of the calf. Thus, it is apparent that dairy operations in the US increasingly adopting high plane of nutrition in order to achieve higher pre-weaning growth in young calves.

Raising calves on a high plane of nutrition leads to many advantages such as achieving greater ADG (Jasper and Weary, 2002; Khan et al., 2007), earlier onset of puberty (Bar-Peled et al., 1997; Bartol et al., 2013), greater milk production in the first lactations (Moallem et al., 2010; Soberon et al., 2012), and fewer behavioral signs of hunger (de Passillé et al., 2011; Miller-Cushon and DeVries, 2015). Several studies have however highlighted an inverse relationship between milk or milk replacer intake and solid feed (also called starter feed) intake eventually leading to a lack of rumen development (Kertz et al., 1979; Terré et al., 2007; Raeth-Knight et al., 2009). This inverse relationship between liquid feed volume and starter intake is assumed to stem from an increased satiety related to chemical (higher blood glucose and insulin) and mechanical cues such as continuous gut-filling by curd formation (Khan et al., 2011). On the other hand, some studies (Shen et al., 2004; Gorka et al., 2011; Schaff et al., 2018) demonstrated that ad libitum milk intake enhances growth of the rumen epithelium, through elevated insulin and IGF-I concentrations in blood and increased IGF-I receptor mRNA abundance in the rumen epithelium.

Solid feeds such as starter feeds are also an important part of a pre-weaned calf’s diet and usually considered as a major driver of rumen development (Harrison et al., 1960; Lesmeister and Heinrichs, 2004; Khan et al., 2011). In majority of dairy calf operations, calves are fed a constant volume of milk or milk replacer (4 to 5 L/d) until weaning (USDA, 2016). However, as calf grows, it needs more nutrients to maintain the body mass and support growth, which is where importance
of starter feed comes in (NRC, 2001). Starter feed fills possible gaps between the nutrient requirement of growing animal and constant nutrient supply from liquid diet. Therefore, availability and intake of calf starter is important to young calves prior to weaning. Bovine Alliance in Management and Nutrition (BAMN) recommends that calves should be offered starter feed by 4 days of age. Many studies (Beharka et al., 1998; Zitnan et al., 1999; Heinrichs, 2005; Kristensen et al., 2007) highlight the importance of offering starter feed as early as from the first days of life to enhance solid feed intake itself and support growth. Solid feed intake has also shown to affect positively the development of rumen microbiota, rumen fermentation capacity, and rumen epithelium. Since, starter feed, unlike liquid feed, are directed to the reticulo-rumen (Church, 1988), starter feed becomes the primary, if not only source of nutrients for the rumen (Govil et al., 2017). It has been shown that calves offered starter feed since 4 week of age had a more developed rumen than those did not have access to starter feed until 12 weeks of age (Govil et al., 2017). Calves could potentially start consuming a measurable amount of solid feed as early as 14 d of age (Williams and Frost, 1992; Khan et al., 2008), and the intake often increases rapidly when daily milk allowance is partially (Khan et al., 2007a, b) or completely cut down (Jasper and Weary, 2002). Even with surplus milk volumes (> 6 L/d), calves begin to chew solid feed within first two weeks of age (Forbes, 1971). There are no guidelines on how much solid feed suckling calves should be eating. A thumb-rule is that calves should be eating about one-half pound by 4 weeks of age (Heinrichs, 2014). Health status of the calf, drinking water availability, starter grain texture, freshness of starter ration (not dusty and moldy), calf housing, weather, and management significantly affect starter intake in suckling calves. Moreover, Malmuthuge et al. (2013) highlighted a strong relationship between the composition of gut microbiota and starter intake
suggesting probiotics supplements would be a promising strategy to improve the performance in pre-weaned calves.

**Probiotics**

Food and Agricultural Organization of the United Nations and World Health Organization (2001) defined probiotics as ‘live microorganism which, when administered in adequate amounts confers a health benefit on the host’. They can regulate the balance of the composition of gut microbiota, promote the growth and development of animals, and improve the host resistance to diseases (Sarowska et al., 2013). During the early life, young calves are highly susceptible to intestinal infectious diseases having marked impact on nutrient intake and thus growth (Cruywagen et al., 1996). A meta-analysis conducted by Frizzo et al. (2011) showed that supplementation of probiotics has beneficial effects on body weight gain and feed efficiency in young calves. As per their findings, dairy calves had gained 228 g/d body weight and better feed efficiency (consumed 814 g less feed to gain 1.0 kg of body weight on daily basis). Similarly, Timmerman et al. (2005) reported a clear increase in body weight gain in 1-week old veal calves supplemented with probiotics. Fuller (1989) and Blum et al. (1999) highlighted that the probiotic effect was more evident during the first few weeks of life, when gut microbiome is still being established and colonized. Most of the probiotics preparations shown to improve health, growth, and feed efficiency of suckling calves include the members of *Lactobacillus, Bifidobacterium,* and *Faecalibacterium,* which are also predominant members in the commensal gut microbiome of pre-weaned calves. Therefore, one might speculate how responsive the establishment and prevalence of those beneficial microbial taxa in the gut of newborn calves to early management practices including colostrum feeding, feeding liquid and solid feeds, and offering drinking water.
Drinking Water to Newborn Calves

Water is one of the essential nutrients such as essential amino acids and essential fatty acids, as the body is unable to produce a sufficient amount to sustain its total requirement (Appuhamy et al., 2014). Water contributes about 70-75% of the body weight of calves and plays an important role as a solvent for nutrients, a thermoregulator and an osmoregulator (Davis and Drackley, 1998). Like other mammals, calves meet their water requirement via three methods: 1) drinking water intake (also called free water intake), 2) from moisture contained in feed, and 3) metabolic water. Relative contribution of given sources to total water intake varies significantly between calves and mature animals. For instance, drinking water contributes to 82% (Appuhamy et al., 2014) of the total water input for lactating cows, whereas its contribution is less than 20% in calves as they receive most of their water via milk or milk replacer (Thomas et al., 2007). Water makes about 80% of the total body weight of the newborn Holstein heifer calves. The body water content dramatically decreases during the postnatal period but remains above 70% until about 40 d of age, which is still 10 percentage units greater than that of mature animals (Sekine and Hirose, 1968). On the other hand, neonate calves lose a great volume of water due to diarrhea, which is seemingly inevitable in many dairy operations. Body water losses of calves increase further during warm weather. Even though calves have a greater water requirement per unit of body weight compared to mature cows, drinking water (also called free water) requirement of calves is more severely overlooked as it is usually assumed that milk or milk replacer is adequate to satisfy the total water requirement (NRC, 2001). For instance, the majority of producers seem to view the drinking water requirement of calves to be insignificant when separated from liquid feed intake. The USDA’s National Animal Health Monitoring System (NAHMS) study survey in 2007 found that an average dairy producer waited 15 d to first offer drinking water to newborn calves. Even though much attempt has been made to promote offering clean and adequate amounts of drinking
water to calves, the same survey conducted in 2014 indicated that an average dairy farmer in the US still waits for 17 d to first offer drinking water to newborn calves. A lack of scientific evidence emphasizing importance of drinking water separately from the water in liquid diets could be a major limitation in educating producers. Thickett et al. (1981) emphasized the importance of examining the effect of age at which newborn dairy calves first have access to drinking water. Consequently, Kertz et al. (1984) conducted an observational study using data from several calf trials conducted by Ralston Purina (St. Louis, MO) and showed more than a 30% increase in starter intake and ADG in calves offered drinking water compared with those deprived of it over the first 28 d of life. Those astounding responses from Kertz et al. (1984) could be related to two aspects of their studies: 1) calves did not receive sufficient water from liquid feed as the daily milk allowance was about 4.0 L and 2) calves were weaned as early as 21 d of age while the water requirement per unit body weight was quite high. Given the fact that the majority of modern dairy calves are fed more than 4.0 L/d of milk or milk replacer and are weaned at a much later age (USDA, 2016), the importance of the drinking water stressed in Kertz et al. (1984) may be unconvincing to producers. Nonetheless, the importance of drinking water stressed in Kertz et al. (1984) has been the only scientific evidence in educating and encouraging producers to offer drinking water to newborn dairy calves over last four decades.

Some dairy producers are reluctant to offer drinking water to newborn calves, assuming it would cause diarrhea. Kertz et al. (1984) reported no link between drinking water intake and the incidence of diarrhea in neonatal dairy calves. On the other hand, Jenny et al. (1978) observed that calves having diarrhea was related to 25 to 50% greater water intake than those without diarrhea. However, it is uncertain whether calves drank more water because of diarrhea or whether increased water intake caused diarrhea. Nevertheless, neonate calves lose a considerable amount of water
accounting for 10-12% of body weight due to diarrhea, which is usually inevitable when calves are 2 to 3 weeks of age. Therefore, having access to clean drinking water could be critical in avoiding neonate calves experiencing extremely negative water balance, which may result in death (NRC, 2001). Some producers appear to assume that offering drinking water would also reduce milk or milk replacer consumption by calves. Gottardo et al. (2002) demonstrated no difference in milk replacer intake between Holstein veal calves with and without access to drinking water. Gottardo et al. (2002) also reported longer rumen papillae in calves with water than calves without water indicating a positive impact of drinking water on the development of rumen mucosa.

**Potential Impacts of Drinking Water on Development of the Rumen**

Development of the rumen can be discussed pertaining to multiple aspects of the rumen including anatomy and morphology, metabolism of volatile fatty acids, and the microbiome. Previous research focused on the effects of solid feed intake on rumen development found that physical stimulation of solid feed in the rumen accounted for a measurable increase in both rumen weight and musculature development (Baldwin et al., 2004; Heinrichs et al., 2005; Khan et al., 2007). However, for papillae to grow the microbial fermentation must take place and volatile fatty acids (VFA) should be present in the lumen (Sander et al., 1959; Hamada et al., 1976). Therefore, both physical and metabolic development of the rumen usually follow the initiation of solid feed intake as it introduces fermentable substrates to the rumen.

As mentioned above, newborn calves begin to consume starter feed during the first two weeks and starter intake markedly increases during the third to fourth weeks of their lives. At the same time, they drink a significant amount of free water, which directly moves to the rumen. Given the fact that the rumen volume is small at birth (25% of the whole stomach, Govil et al., 2017), water entering the rumen could exerts a physical stimulation on particularly the muscular growth of the rumen wall. Even though it has yet to be proven experimentally, it is often hypothesized that...
developing rumen has a specific requirement of water, which could not be satisfied with water in milk or milk replacer as they bypass the rumen and directly shunted to the abomasum via the esophageal groove (Warner et al., 1956; Quigley, 2001). Drinking water would assist in metabolic development of the rumen by 1) increasing fermentation substrate supply via enhancing starter intake, 2) creating an anaerobic and aqueous environment necessary for fermentation to occur and 3) enhancing mixing of digesta, which is important for efficient fermentation (Kertz et al., 1984; Quigley, 2001; Gottardo et al., 2002;). Several studies have highlighted that early alterations in rumen development likely possess long-lasting effects on growth, feed efficiency, and milk production in dairy cattle (Abecia et al., 2014; Mao et al., 2015; Li and Guan, 2017). Manipulation of gut microbiota in early stage of development is assumed a promising way to achieve permanent benefits pertaining to nutrient utilization efficiency in animals (Yanez-Riuz et al., 2015).

**Microbiota in the Developing Gut**

A mature ruminant harbors a complex and diverse microbiome in the gastrointestinal (GI) tract that permits them to convert digested plant-based material into edible high nutritive outputs such as meat and milk (Henderson et al., 2015). However, at birth the GI tract of ruminants, particularly the complex forestomach is sterile and not yet developed to fully functional stage. A rapid microbiota colonization in the developing rumen (and the rest of the digestive tract) of calves begins during (and immediately after) birth (Meale et al., 2016; Yeoman et al., 2018). Introduction, maturation and succession of gut microbiota of neonatal calves is influenced by various external and internal factors (Malmuthuge et al., 2013). The dam plays a crucial role during the establishment of gut microbiota via several sources including the vaginal canal, fecal material, colostrum, skin and saliva (Skillman et al., 2004). Moreover, microorganisms in the environment (Dominguez-Bello et al. 2010), type of colostrum (Malmuthuge et al., 2015), type of liquid feed (Edrington et al., 2012), housing type (Pereira et al., 2014), probiotics, prebiotics and antibiotics
Foditsch et al., 2015; Oultram et al., 2015; Van Vleck Pereira et al., 2016), the plane of nutrition, intestinal pH, and passage rates are found to significantly influence establishment and development of gut microbiota in the rumen and post-rumen compartments of calves (Mackie et al., 1999).

**Development of rumen microbiota**

W. A. Pounden and J. W. Hibbs first examined the development of gut microbiota in pre-ruminants in the late 1940s (Pounden and Hibbs, 1948 and 1949). Fonty et al. (1987) were the first to demonstrate age-dependent changes in the appearance of different microbial populations in the rumen. Fonty et al. (1987) found that the anaerobic bacteria dominated in the rumen of neonatal lambs by the second day of life while the abundance of cellulolytic bacteria stabilized within the first week of life. Further, anaerobic fungi and methanogens appeared in the neonatal rumen between 8-10 days postpartum (Fonty et al., 1987), while protozoa appeared only after 15 days postpartum (Fonty et al., 1988). Type of the diet plays a significant role during the sequential development of rumen microbiota. As described above, the effect of liquid diets on the rumen ecosystem are limited as milk or milk replacer is funneled through the esophageal groove directly to the abomasum (Amaral-Phillips et al., 2006). In contrast, solid feed intake is considered to have a significant impact on establishment of the microbiota in the developing rumen (Govil et al., 2017). Even though, calves may take 3 to 4 weeks to commence a significant amount of solid feed intake, a noted diversity of rumen microbiota have been found as early as 12 days of age (Rey et al., 2014). For instance, *Bacteroides* (21%), *Prevotella* (11%), *Fusobacterium* (5%), and *Streptococcus* (4%) were found to be the dominant genera at about two weeks of age in the rumen (Rey et al., 2014). However, as solid food intake rapidly increased, *Prevotella* became the most dominant (42%) genera and the presence of other genera decreased markedly highlighting the ability of solid feeds to shape up the rumen microbial community composition (Rey et al., 2014).
**Development of microbiota in the distal gut**

Smith (1965) was the first to study bacterial colonization and development in the distal gut and observed colonization of *Escherichia coli* and *Streptococcus* in small intestine and cecum of calves within eight hours after birth. Smith (1965) also observed that *Lactobacillus* colonized one day after birth, and was the most abundant genera across all post-rumen compartments. A considerable abundance of *Bacteroides* was observed only in the cecum and feces. Rada et al. (2006) and Vlkova et al. (2006) later found a higher abundance of *Bifidobacterium* in the distal gut and fecal samples of neonatal dairy calves. Uyeno et al. (2010) found *Faecalibacterium* as one of the most abundant bacteria in one-week-old calves. Then Malmuthuge et al. (2014) reported that the abundance of *Faecalibacterium* was greater in the large intestine than the small intestine of three-week-old calves. Overall, the distal gut of calves appears to be first colonized by facultative anaerobes, such as *Lactobacillus* and *Escherichia coli*, which would then create anaerobic conditions required for the colonization of obligate anaerobic bacteria, such as *Bifidobacterium, Faecalibacterium* and *Bacteroides* (Smith, 1965). Meale et al. (2017) reported that the abundance of several bacterial taxa in the feces of pre-weaned dairy calves had significant correlations ($P < 0.05$) with drinking water intake (L/d). For instance, the abundance of family *Ruminococcaceae* was positively associated with drinking water intake, whereas the abundance of genus *Streptococcus* was negatively associated with drinking water intake.

**Potential Impact of Drinking Water on the Development of Gut Microbiota**

Drinking water is often assumed to have a critical impact on the development of rumen microbiota (Earleywine, 2015; Cullens, 2016; Kertz, 2017). To date, no scientific investigation has focused on the role of drinking water in the establishment, and colonization of microbiota in the rumen or in other compartments of the gastrointestinal tract of dairy calves. However, studies focused on gut microbiota of mature cattle, humans, and other species provide some support to the
idea that drinking water intake would affect microbial community composition in the gut of calves. Cremer et al. (2017) demonstrated that drinking water had significant influence on composition of microbiota in the lower gut of humans by altering the pH via changing digesta passage rate, digesta mixing, and epithelial absorption of water. Sasada et al. (2015) showed that chlorinated water altered the enteric environment and selectively decreased the relative abundance of some bacterial communities. Pinto et al. (2012) indicated the possibility that drinking water could affect the composition of the gut microbiota by inoculation with its own microbiota. Moreover, Faulkner et al. (2017) showed that trace minerals such as copper (Cu), zinc (Zn), and manganese (Mn) significantly affected the relative abundance of some microbial communities in the colon of lactating dairy cows. Therefore, determining the impact of drinking water availability and intake on rumen and/or distal gut microbiota would be a worthwhile goal of any study attempting to understand the roles of water in growth and development of dairy calves.

Methods Available to Study Gut Microbiota

Sampling methods

The accuracy of a gut microbiota analysis depends primarily on methods applied to collect and process the samples (Sarangi et al., 2019). Researchers usually face difficulties when it comes to choose the most appropriate method for sample collection. Several studies in literature have used non-invasive (and cost and time effective) methods such as fecal samples (Michelland et al., 2009; Frey et al., 2010; Romero-Perez et al., 2011; de Oliveira et al., 2013), oral swabs (Tapio et al., 2016) or oral-stomach tube (Shen et al., 2012), as well as invasive methods including rumenocentesis (Duffield et al., 2004) or ruminal cannula (Lodge-Ivey et al., 2009; Shen et al., 2012). Owing to the cost and time effectiveness, oral stomach tubing (also known as oral intubation) is the most frequently used technique for collecting sample from the rumen. However, the representativeness of samples collected from oral tubes is often questioned particularly when
it comes to neonate calves having underdeveloped rumen with small volume of rumen content (personal communication with Dr. Howard Tyler at Iowa State University). Although rumen fistulas allow for a satisfactorily representative sample collection (Martinez-Fernandez et al., 2019), surgery cost and potential challenges of performing successful surgery with calves may limit the number of animals sampled and thus the statistical power.

Fecal sample collection is the most commonly used method to examine the gut microbiota as it is relatively non-invasive, affordable, and allows for repeated sampling of individual animals over time (Ingala et al., 2018). Therefore, many studies of humans and animals including calves have included fecal sampling as a proxy for the gut microbiota analyses. Because the composition of microbiota attached to gut mucosa are significantly different from that in ingesta, fecal samples are considered insufficient in representing true characteristics of gut microbiota particularly the interactions between the host and the microbiota (Malmuthuge et al., 2012; Malmuthuge et al., 2014). Moreover, the original composition of fecal microbiota can easily be compromised if contamination occurs or the samples are exposed to oxygen, moisture, and sunlight before being stored (Ingala et al., 2018). Further, when it comes to ruminants, fecal samples are largely representative of the microbiota in the distal gut particularly the large intestine than the rumen (Song et al., 2017). On the other hand, microbiota in distal gut have more implication for health, growth, and feed efficiency of suckling calves as the rumen is still relatively underdeveloped. Therefore, once being able to collect from the rectum and preserve (e.g., in dry ice) immediately, fecal samples provides effective and feasible option to study the gut microbiota of pre-weaned calves over a given time.
Methods for Determining the Composition of Gut Microbiota

16S ribosomal RNA gene sequencing

Traditional bacterial culture techniques coupled with phenotyping for morphology and biochemical characteristics (i.e. pH tolerance) have long been used to determine the composition of gut microbiota. However, majority of bacteria in the gut are obligate anaerobes. Therefore, the relative abundance of the bacteria deduced using culture-based techniques are heavily biased in favor of aerobic organisms that grow easily in in vitro culture (Sarangi et al., 2019). On the other hand, only less than 10% of the gut microbiota can be studied using available anaerobic culture methods (Yeoman and White, 2014). Thus, to overcome these limitations, several molecular approaches such as culture-independent sequencing techniques were developed. These techniques allow for rapid identification of bacterial species, and are primarily based on the 16S ribosomal RNA (16S rRNA) gene sequence (Tringe and Hugenholtz, 2008; Lagier et al., 2015, Sarangi et al., 2019). Further, it should be noted that the DNA sequences could be classified taxonomically or phylogenetically to describe microbial community structure, dynamics, and how those communities might influence or be influenced by their surroundings (i.e., gut mucosa, Poretsky et al., 2014).

16S rRNA gene is one of the most conserved molecules generated from 30S rRNA precursor molecule (Rajendhran and Gunasekaran, 2011). By far, it is used as the most common housekeeping genetic marker to study bacterial phylogeny and taxonomy in systems ranging from ocean to soil to the human gut (Kent et al., 2004; Costello et al., 2009; Nemergut et al., 2011; Gilbert et al., 2012). Moreover, the 16S rRNA gene contains conserved and hypervariable regions [69-99 (V1), 137-242 (V2), 433-497 (V3), 576-682 (V4), 822-879 (V5), 986-1043 (V6), 1117-1173 (V7), 1243-1294 (V8) and 1435-1465 (V9)] which are used in differentiating bacterial species. For instance, V2 region could be used to distinguish pathogens, except the members of
Enterobacteriaceae family (Chakravorty et al., 2007). The popularity of 16S rRNA gene sequences in phylogenetic and taxonomic studies are attributed to 1) its presence in almost all bacteria; 2) it being a highly conserved gene sequence, and 3) it being a sufficiently large gene (1,500 bp) to support bioinformatics analyses (Patel, 2001). However, a major limitation of 16S rRNA gene sequencing is the high sequence similarity between some bacterial species (Janda and Abbott, 2007). The 16S rRNA-based techniques are also known to be limited by the short-read-lengths sequencing errors (Quince et al., 2009; Quince et al., 2011), differences arising from the different regions chosen (Youssef et al., 2009), and difficulties in assessing operational taxonomic units (OTUs, Huse et al., 2010). Thus, as a means to minimize errors arising from 16S rRNA gene sequence, Rajendhran and Gunasekaran (2011) suggested use of other marker genes with a single gene copy in the bacterial genome such as RNA polymerase beta subunit-encoding gene (rpoB). Mollet et al. (1997) more successfully discriminated Enterobacteriaceae family using rpoB gene than the 16S rRNA gene. Nonetheless, 16S rRNA gene sequencing is able to identify the genus with >90% success rate and the species with 65 to 83% success rate (Drancourt et al., 2000; Woo et al., 2003; Mignard and Flandrois, 2006). Therefore, the 16S rRNA gene targets may still provide a much more sensitive and definitive platform for bacterial species identification (Janda et al., 2007), even though newer and enhanced molecular-based technologies, such as next-generation sequencing (NGS) are emerged as alternatives.

Next-generation sequencing (NGS)

The NGS is an enhanced molecular-based technology which provides an excellent platform to identify both culturable and non-culturable microbes, as well as characterizing their potential functions (McCann et al., 2014). For instance, it is now possible to generate a comprehensive profile of both microbial diversity and functions and explore potential associations between the microbiota and early rumen development due to application of NGS (Malmuthuge, 2016).
Moreover, the use of an NGS approach provides a greater understanding of region- (rumen, small intestine, large intestine) and sample type- (content, mucosa) specific bacteria throughout the GI tract of pre-weaned calves (Malmuthuge et al., 2014). Another major advantage of NGS approach is that ability to generate high throughput data via sequencing large number of samples pooled together, after the addition of identifiers/barcodes (Tringe and Hugenholtz, 2008). In addition, use of NGS approaches not only allow sequencing large number of samples at once, but also drastically decrease the cost involved in sequencing as opposed to 16S rRNA gene sequencing (Tringe and Hugenholtz, 2008).

**Literature Cited**


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CHAPTER 3. DRINKING WATER INTAKE OF NEWBORN DAIRY CALVES AND ITS EFFECTS ON FEED INTAKE, GROWTH PERFORMANCE, HEALTH STATUS, AND NUTRIENT DIGESTIBILITY

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Abstract

Although it is recommended to offer drinking water to newborn calves immediately after birth, producers wait, on average, 17 d to first offer drinking water to newborn dairy calves. The objective of this study was to examine water and feed intake, growth performance, health status, and nutrient digestibility of Holstein heifer calves offered drinking water from birth (W0) as compared to those offered it at 17 d of age (W17), when fed an ad libitum volume of milk. Thirty Holstein heifer calves, balanced for parity of the dam, birth weight, and birth week were randomly assigned (n = 15) to W0 or W17. Calves had free access to drinking water and a starter ration, offered in two separate buckets, until they were 70 d of age. Calves were bottle-fed with pasteurized whole milk 3× per day (2.0 kg/feeding until d 14, and 3.2 kg/feeding thereafter). Calves were partially weaned (33% of the milk allowance 1× per day) at 42 d of age and completely weaned at 49 d of age. Drinking water intake (FWI), starter intake, milk intake, ambient temperature, and the fecal consistency were recorded daily. Body weight, hip height (HH), hip width, heart girth (HG), and body length (BL) were measured weekly. Blood (drawn from jugular vein) was analyzed for hematocrit and haptoglobin concentrations at 14 d of age. On d 69 and 70, total fecal output of individual calves was measured to determine apparent total-tract digestibility (ATTD) of nutrients. When offered from birth, newborn calves consumed 0.75±0.05 kg/d water aside from the water they received from ad libitum milk allowance during the first 16 d. Once
offered, W17 calves drank more water (59%) than W0 calves during the pre-weaning period. Starter intake of W0 and W17 calves was similar, but W0 calves consumed 0.285 kg/d more milk and tended to achieve greater BW and HG compared to W17 calves during the pre-weaning period. Offering water from birth versus offering it later did not affect the number of days with diarrhea, intensity of diarrhea, or blood hematocrit and haptoglobin concentrations in neonate calves. Despite a similar starter intake, W0 calves had greater HH, BL, ATTD of ADF and NDF, and feed efficiency than W17 calves post-weaning (50 to 70 d of age). When followed up to 5 months of age, W0 calves had greater BW than W17 calves. Provision of drinking water immediately after birth could improve growth and development of calves pre- and post-weaning, potentially by stimulating rumen development, thus increasing nutrient availability.

Key words: ambient temperature, drinking water, feed efficiency, hip height

Introduction

Water is an essential nutrient and is second in importance, behind oxygen, to sustain life and performance of a living organism (Beede, 2005). Failure to fulfill the water requirement of animals results in short- and long-term impairments of body functions which are in turn reflected by lowered performance (Gottardo et al., 2002). Nonetheless, water requirements of livestock are often overlooked compared to the requirements of other nutrients. In dairy cattle, the situation is further aggravated for calves compared to mature cows (Beede, 2005; Kertz et al. 2017). Water contributes to about 80% of the BW of newborn Holstein heifer calves. The body water content dramatically decreases as calves grow and deposit more fat displacing body water (Reid et al., 1955). Body water content however remains above 70% until about 40 d of age, which is still 10 percentage units greater than that of mature animals (Sekine and Hirose, 1968). Moreover, neonatal calves lose a great volume of water due to diarrhea, which is seemingly inevitable in many dairy operations. Body water losses further increase during warm weather. Even though
water is a vital nutrient for calves, the requirement has been poorly described in current nutrient requirement models (e.g., NRC, 2001).

Similar to other mammals, calves meet their water requirement via three methods: 1) free drinking water (hereafter called drinking water), 2) moisture from feed, and 3) metabolic water. Relative contribution of these sources to total water intake varies significantly between calves and mature cows. For instance, drinking water contributes to 82% (Appuhamy et al., 2014) of the total water input for lactating cows whereas its contribution is less than 20% for calves as they receive the majority of their water via milk or milk replacer (Thomas et al., 2007). Therefore, the drinking water requirement of calves appears to be insignificant when separated from liquid feed intake. The USDA’s National Animal Health Monitoring System (NAHMS) study in 2014 (USDA, 2016) demonstrates that dairy producers wait for, on average, 17 days to first offer drinking water to newborn calves. Producers also seem to be hesitant to offer water to newborn calves, assuming it would cause diarrhea (Beede, 2005; Kertz et al., 2017). Overall, a lack of scientific evidence on drinking water intake (FWI) of newborn calves and its impact on their performance likely encumbers the effectiveness of efforts promoting the notion of offering drinking water to newborn calves at birth.

Thickett et al. (1981) indicated an importance in examining the impact of the age at which calves are first offered drinking water. Then, Kertz et al. (1984) conducted an observational study using data from several calf trials conducted by Ralston Purina (St. Louis, MO, US) and demonstrated more than 30% increase in starter intake and weight gain in calves receiving drinking water from birth compared to calves deprived of drinking water until they were 28 d of age. Those astounding responses could be related to two aspects of the study: 1) calves did not receive much water from liquid feed as the daily milk replacer allowance was only 3.8 kg/d, and 2) calves were
weaned as early as 28 d of age while the water requirement per unit of body weight was quite high. Given the fact that the majority of modern dairy calves are fed more than 3.8 kg/d of milk or milk replacer daily and weaned at a much later age (USDA, 2016), the relevance of the findings in Kertz et al. (1984) might need to be reevaluated for modern dairy production systems. Depending on the improved starter intake and weight gain in Kertz et al. (1984), it is often hypothesized that drinking water would enhance rumen development, thus increasing nutrient availability for growth and development of calves. However, no systematic attempt has been made to study this link between drinking water intake and nutrient availability to the animal. Therefore, the objectives of this study were 1) to determine drinking water intake (FWI) of newborn calves, and 2) to investigate the impact of first offering drinking water from birth versus 17 d later on milk and starter intake, growth performance, health status, and nutrient digestibility in dairy heifer calves fed ad libitum amounts of liquid feed.

Materials and Methods

All animal procedures in this study were conducted under approval of the Animal Care and Use Committee at Iowa State University (IACUC# 7-17-8570-B). The experiment was conducted from August to December of 2017 at the Dairy Research and Teaching Unit at Iowa State University (ISU Dairy). Thirty Holstein heifer calves born at the ISU Dairy from August 22 to October 8 in 2017 were used. All calves were bottle-fed at least 3.78 kg of thawed colostrum within the first 4 h of life. Colostrum was collected from cows at the ISU Dairy, pooled, aliquoted and frozen in plastic bags (Dairy Tech Inc., CO, US). Immediately after feeding colostrum, calves were matched for week they were born (WB), parity of the dam (PD: primiparous or multiparous), and birth weight (BW0) and randomly allocated to two treatments (15 calves per treatment): 1) provision of drinking water from birth (W0), and 2) provision of drinking water 17 d after birth (W17). All the calves were housed in individual pens bedded with straw (floor area = 1.2 m × 1.8 m).
m) in the indoor calf facility at ISU Dairy until they were 9 wk old. At wk 10 calves were housed individually in a separate set of cages bedded with rubber mattresses (3/4 inch thick) for a digestibility trial involving total fecal collection (described in details below). At the end of the wk 10, calves were moved to the heifer barn at the ISU Dairy, where they were housed and fed in groups.

Once offered, each individual calf had free access to an ad libitum amount of clean drinking water in a plastic bucket throughout the study. Each plastic bucket had a maximum capacity of 7.5 L. Water buckets were filled with clean potable water and the weight (bucket + water) was recorded before offering to calves at 0700 h. The water volume was monitored at 1400 and 2200 h and maintained above 50% of total bucket volume throughout the day. The buckets and leftover water were weighed before offering fresh water the following morning. Drinking water intake (kg/d) of individual calves was calculated using the difference of freshwater weight and leftover water weight. A separate set of water buckets with similar water volumes was used to measure the evaporative water losses, of which the FWI calculations were corrected for. The temperature inside the calf barn was recorded using temperature loggers (Lascar Electronics Inc., Erie, PA) placed along the span of the barn leaving an equal distance between each logger.

All calves were bottle-fed with 2.0 kg of pasteurized liquid milk at each of the three feeding times per day (0600, 1400 and 2200 h) until they were 14 d old. From d 14 to 42, the milk volume was increased to 3.2 kg/calf/feeding. Calves were partially weaned on d 42 and fed 3.2 kg of milk only once per day (2200 h) until they were completely weaned on d 49. Actual weights of milk offered and leftover (kg) were recorded. All calves received milk from the same batch of pasteurized milk at each feeding. All calves had free access to a pelleted starter with whole corn grain (89% DM, 18% CP, 3.5% crude fat, 10% crude fiber, 13% ADF; Vita Plus Corporation,
Madison, WI) in plastic buckets until they were 10 wk old and moved to group pens. Feed buckets were separated from water buckets using a metal plank to reduce contaminations from each other. All calves were fed with the same batch of starter throughout the 10 wk. After recording the leftover starter weight, the buckets were filled with a known amount of fresh starter and offered to the calves at the same time (0700 h) that drinking water was offered. Daily starter intake of individual calves was calculated using the difference of offered feed and leftover feed weights. An ad libitum starter intake was maintained by offering 10% more starter relative to intake from the previous day.

Fecal consistency of neonatal calves was scored on a scale of 1 to 4 (1 = normal, 2 = viscous feces, 3 = runny feces, and 4 = runny feces with splatters). Calves with a fecal score of 2 or greater were offered an electrolyte (5.5–7.0% Sodium, 1.65% Potassium; Land O’Lakes, Inc., MN, US) solution (2 kg/calf) before the morning feeding. Calves with a fecal score of 4 received an extra volume of the electrolyte solution before the afternoon milk feeding until the consistency became normal.

Birth weight was recorded within few hours after birth. Body weight, hip height (HH), body length (BL), heart girth (HG) and hip width (HW) of individual calves were then measured weekly. All parameters were measured by the same person to maintain consistency of measurements across all weeks. Average daily gain (ADG) within each week was calculated by dividing the difference of consecutive weekly BW by 7. Blood was collected 2 to 3 h (0900 h) after morning feedings on the days in which body measurements were recorded. Blood was drawn from the jugular vein into vacutainer tubes containing EDTA and heparin (Dickinson and Company, Buena, NJ) using 18-gauge 1-inch needles. Blood was centrifuged at 2,000 × g at 4 °C for 25 min. Blood plasma was separated and stored in a −20 °C freezer until subsequent analyses.
On d 65, calves were moved to a separate set of individual cages each having a rubber mattress, to collect total daily fecal output. Calves were allowed to be acclimated to new cages with new bedding until starting fecal collection. Fecal collection began immediately after feeding at 0600 h on d 69 and lasted 48 h. Feces from individual calves was scraped from the rubber mattress into a plastic scoop using a large baking spatula immediately after defecation. Feces in the scoop were then transferred to a plastic bag assigned to each calf. Fresh fecal weight from each bag was recorded every 4 h and eventually summed up to obtain total feces output (kg/d). A sample of feces (~200 g) collected within each 4 h interval was retrieved and placed into a –20 °C freezer. Consequently, there were 6 samples representing feces collected from each calf over a period of 24 h. Later, those 6 samples were thawed completely and thoroughly mixed to obtain a composite sample of feces of each calf from each day of total fecal collection. Calves were fed with the same starter during the total fecal collection period. Composite samples of the starter offered and leftover from each calf were obtained and stored at –20 °C until analysis. Once fecal collection was completed in the end of d 70 (the end of the experiment), calves were moved to heifer barn and followed until 5 months of age, at which BW were measured to see any long-term effects of the treatments. One calf from the W0 treatment was diagnosed with aspiration pneumonia and died at wk 7 of age was not replaced and the data were excluded.

Blood samples collected at the end of wk 2 were analyzed for plasma concentrations of haptoglobin (HPT) using a commercially available ELISA kit according to the manufacturer’s instructions (Immunology Consultants Laboratory, Inc. OR, US). Blood samples were also analyzed for hematocrit (Hct), Na, K, and pH using a portable blood analyzer system (Abbott Laboratories, East Windsor, NJ). Approximately 200 g of sample from feces, starter offered, and starter leftover was dried in an oven at 60 °C for 72 h to determine DM. The dried samples were
analyzed (Cumberland Valley Analytical Services, Waynesboro, PA) for CP (N × 6.25; AOAC, 2000; method 990.03), NDF (Van Soest et al., 1991), ADF (AOAC, 2000; method 973.18), starch [(Hall, 2008); with correction for free glucose], and ether extract [EE (AOAC, 2000; method 920.39)].

Statistical analysis

Treatment effects on FWI, milk and starter intake, growth performance (BW, BL, HW, HG and HH), ADG, and fecal scores, for which each calf has multiple measurements at different time points, were determined using the MIXED procedure with REPEATED option of SAS (version 9.4, SAS Institute Inc., NC, US) according to the following model:

\[ Y_{ijkl} = \mu + T_i + P_j + W_k + C_l + \text{TMP}_{ijkl} + \epsilon_{ijkl} \]

where \( Y_{ijkl} \) = the response variable of interest, \( \mu \) = overall mean, \( T_i \) = fixed effect of \( i \)-th treatment (\( i = W0 \) and \( W17 \)), \( P_j \) = fixed effect of \( j \)-th parity of the dam (\( j = \) primiparous and multiparous), \( W_k \) = fixed effect of \( k \)-th week of age, \( C_l \) = random effect of \( l \)-th calf, \( \text{TMP}_{ijkl} \) = fixed covariate effect of daily mean ambient temperature, and \( \epsilon_{ijkl} \) = random error assumed to be independent and identically distributed from a normal distribution with a mean of 0 and a variance of \( \sigma^2 \) \([~N(0, 1\sigma^2)\)]. The treatment effects on nutrient digestibility measured on d 69 and 70 were tested with the above model excluding the week effect. Interactions among treatments, parity, and week were initially tested but were not included in the model as they were not statistically significant. The treatment effects on plasma HPT, blood Hct, blood electrolytes, and pH were tested excluding the week effect and the random calf effect using the GLM procedure of SAS as individual calves provided only a single measurement at wk 2. Pair-wise comparisons of treatments means were carried out with the Tukey-Kramer adjustment test. Statistical differences were declared at \( P < 0.05 \) and a tendency toward significance was considered at \( 0.05 < P < 0.10 \).
Results and Discussion

The main objective of this study was to examine the importance of providing drinking water to newborn dairy calves from birth in terms of water and nutrient intake, nutrient digestibility, growth performance, and health. Offering drinking water from birth was evaluated in comparison to a 17 d delay as dairy producers in the US wait, on average, 17 d to first offer drinking water to newborn dairy calves (USDA, 2016). This delay has been there for several years as the NAHMS study in 2007 also indicated that dairy producers waited, on average, 15 d to first offer drinking water to newborn dairy calves (USDA, 2007). The majority of producers seem to assume that neonatal calves receive adequate amounts of water from milk or milk replacer to fulfill the total water requirement of the body. Given the water requirement of calves are poorly described in our current nutrient requirement models (e.g., NRC, 2001) and few published research articles involve water intake measurements of calves (Kertz et al., 2017), it is difficult to systematically evaluate the rationality of this consideration. In the present study, we measured FWI of calves daily fed a high volume of milk (6.0 to 9.6 kg/d) representing approximately the 70th percentile of liquid feed allowance across US dairy operations (USDA, 2016). Therefore, a significant FWI observed, independent of water in liquid feed intake (milk, in the present study) should be applicable to the majority of dairy operations.

Water intake

Treatment effects on FWI, and total water intake measurements are given in Table 1. Mean FWI in each week of the experiment are shown in Figure 1. Calves from the W0 treatment consumed, on average, 0.75±0.05 kg/d of water aside from the water consumed via milk during the first 16 d (P < 0.01). They had a mean milk intake of 6.25 kg/d during this period. Assuming water comprises 87% of milk (Winchester and Morris, 1956), the mean total water intake during this period was 6.19 kg/d. Given the fact that 45% of dairy calves are fed less than 5.0 kg of milk
or milk replacer daily and producers wait, on average, 17 d to offer drinking water to newborn calves (USDA, 2016), a large number of dairy calves in the US appear to consume less water than their requirement during the first two weeks of their life. Kertz et al. (1984) pioneered the importance of offering drinking water to dairy calves at birth. They found that calves offered water from birth had a higher FWI (1.40 kg/d) compared to that of our calves during the first two weeks. This discrepancy could be primarily due to a lower contribution of milk replacer (11.4% DM) to total water intake as they fed a lower volume of milk replacer compared to that of milk we fed during the first two weeks (3.8 vs. 6.0 kg/d in Table 1). Despite the progressive increase in BW (Figure 2), W0 calves had similar FWI (0.82±0.04 kg/d), even during the remainder of the pre-weaning period (Table 1 and Figure 1). In line with the milk allowance increase (6.0 to 9.6 kg/d) at two weeks of age, W0 calves consumed, nearly 2.0 kg/d more milk between 16 and 42 d of age compared to the milk consumption during first 16 d (8.20 vs. 6.25 kg/d). Consequently, the total daily water intake increased by 1.77 kg/d from the first 16 d to the remainder of the pre-weaning period (7.96 vs. 6.19 kg/d, Table 1). This increment seemed to be adequate enough to fulfill body water requirements of tissue accretion and maintenance of increasing BW (Figure 2). Sekine and Hirose (1968) demonstrated that total body water content of dairy calves decreased from 80 to 70% during the first 40 d. Reid et al. (1955) reported an inverse relationship between body water and body fat content emphasizing the fact that animals deposit fat displacing body water. Overall, the potential increase in body water requirement of growing calves appears to be slower than the rate of body growth. Since we offered ad libitum amounts of drinking water and milk, the total water intake measurements in Table 1 can be considered as voluntary water intake measurements, potentially reflecting the actual total water requirement of the body. Unlike water in milk, which is shunted directly to the abomasum, drinking water enters and becomes a part of the developing
reticulorumen (hereafter called rumen) of pre-weaned calves (Govil et al., 2017). Therefore, voluntary FWI of pre-weaned calves (0.75 to 0.82 kg/d, Table 1) partially represents a water requirement of developing rumen.

Once offered, W17 calves consumed, on average, about 0.48 kg/d (59%) more water ($P < 0.001$) than W0 calves between d 17 and d 42 (Table 1). This increment in drinking water was constant until calves were weaned partially (Figure 1). Therefore, the age at which drinking water is first offered to calves could be an important variable to consider in determining water intake of pre-weaned calves. Moreover, the excessive intake of drinking water might affect microbial fermentation in the rumen and thus rumen development. No study has examined the impact of water on rumen development of calves. Few studies focusing on the relationship between drinking water and rumen functions in mature cows provide some insight on potential effects of drinking water in calves. For instance, Rogers et al. (1982) and Fraley et al. (2015) found lower molar proportions of butyrate and propionate in the rumen, which were associated with lower liquid weight in the rumen and high fractional liquid passage rates in lactating cows with high FWI. Butyrate and propionate are often considered major drivers of rumen development in calves (Lane et al., 2000; Baldwin et al., 2004). Therefore, the increased FWI of W17 calves could be hypothesized to inversely affect the rumen development.

Once completely weaned, FWI of both groups increased from about 2.00 to 5.30 kg/d (Table 1). In terms of total water intake (FWI plus water in milk and starter), calves however consumed more water during the pre-weaning period compared to the post-weaning period (e. g., 6.19 and 7.96 vs. 4.42 and 5.48 kg/d; Table 1). Calves require more water, partially due to increased water losses through diarrhea, which is seemingly inevitable during the pre-weaning period. Also, newborn calves can have an inherently greater water requirement than mature calves.
as their body water content is much higher than calves at 60 d of age (Sekine and Hirose, 1968). It is worth noting that we did not observe any behavior leading to spilling water from buckets during both pre- and post-weaning periods in the present study.

Average daily ambient temperature had a significant impact on FWI. Since calves were born, and thus included in the study, at different time points (late August through early October), there are representative samples of calves at different ages exposed to various ambient temperatures. For instance, calves at 7 and 28 d of age were exposed to average daily ambient temperatures ranging from 7.6 to 26.5 °C, and from 5.6 to 24.4 °C, respectively. When adjusted for the treatment and age effects, FWI of pre- and post-weaning calves increased by similar amounts (0.068 and 0.055 kg, respectively) per unit (°C) increase in average daily ambient temperature (Table 3). The increment during weaning, in which milk allowance was cut down by 67%, was twice as much as that during the other periods (0.141 vs. 0.068 and 0.055 kg per 1.0 °C) emphasizing the importance of providing sufficient drinking water to calves being weaned under hot weather conditions.

**Milk and starter intake**

Mean milk intake and starter intake (kg/d) are given in Table 1. Regardless of the treatment, calves left, on average, 0.62±0.09 kg/d and 1.27±0.09 kg/d of milk in the bottles during the first two weeks, and the rest of the pre-weaning period, respectively (P < 0.01; data not shown) indicating that the milk allowances were ad libitum. The W0 calves consumed, on average, 360 g/d (6.25 vs. 5.89 kg/d) and 240 g/d (8.20 vs. 7.96 kg/d) more milk than W17 calves during the first 16 d and thereafter, respectively (Table 1). Gottardo et al. (2002) also showed that provision of drinking water reduced milk refusals in veal calves. On the other hand, based on findings in Kertz et al. (1984), it is often considered that neonatal calves offered drinking water from birth consume more starter feed than those without access to drinking water. However, in the present
study, we did not observe such an effect on starter intake (Table 1). Starter intake of our calves (0.02 to 0.06 kg/d) was much lower than the calves (0.23 to 0.95 kg/d) in Kertz et al. (1984) during the pre-weaning period. This could be due to a larger contribution of liquid feed (6.0 to 9.6 vs. 3.8 kg/d) to total DMI of calves in our study compared to that in Kertz et al. (1984). Through a meta-analysis, Gelsinger et al. (2016b) demonstrated a significantly inverse relationship between milk or milk replacer intake and starter intake in pre-weaned calves. The drinking water restriction and its effects on starter intake in Kertz et al. (1984) also may have been more severe due to their water restriction being longer (28 vs. 16 d) and the calves were weaned at a much earlier age (<28 d vs. 42 d) compared to our study. As observed with FWI, starter intake increased sharply once calves were partially weaned and exceeded 2.0 kg/d a couple of weeks after they were completely weaned (Table 1). Starter intake was positively associated with FWI throughout the study (Figure 1). However, the relationship was much stronger during the post-weaning period than the pre-weaning period ($R = 0.85$ vs. 0.14; Table 2) suggesting that the commonly-accepted strong positive relationship between drinking water intake and starter intake (Quigley et al., 2006) is reasonably applicable to calves fed lower milk or milk replacer allowances, which is the case in the majority of commercial dairy operations (USDA, 2016).

**Growth performance**

Mean BW, BL, HG, HH, and HW during the pre- and post-weaning periods are given in Table 4. Figure 2 shows how they changed over the first 10 wk. Since BW0 was a main factor considered in the random assignment of calves to treatments, the mean BW0 of W0 and W17 were similar at 38.0 kg ($P = 0.785$, Table 4). Calves offered drinking water from birth tended to achieve greater BW during the pre-weaning period than W17 calves ($P = 0.085$, Table 4). The differences became more pronounced toward the end of the pre-weaning period (Figure 2). Average daily gain was not significantly different between W0 and W17 but W0 calves had numerically greater ADG
than W17 calves during the pre-weaning period (0.66±0.03 vs. 0.61±0.03, \( P = 0.227 \)). Body weight was significantly correlated with HG \((R = 0.95;\) data not shown) across the treatments, indicating that HG is a sound proxy in determining BW of pre-weaned calves in commercial operations (Heinrichs et al., 2017). Consequently, W0 calves also tend to have greater HG \((P = 0.091;\) Table 4) than W17 calves. Age at which calves were first offered drinking water did not impact BL \((P = 0.656)\), HH \((P = 0.458)\), or HW \((P = 0.794)\) of pre-weaned calves. Hip height and BL were positively correlated with BW \((R = 0.88\) and 0.81, respectively; data not shown), but the relationships were not as strong as that of HG \((R = 0.95,\) data not shown) as shown previously by Wilson et al. (1997). Regardless of the treatments, ADG during the pre-weaning period had a significantly positive relationship \((P < 0.001)\) with average daily ambient temperature (Table 3). Average daily gain increased by 20.0 g for each unit \(^\circ\text{C}\) increase in ambient temperature, but did not change starter intake. Daily mean ambient temperatures that pre-weaned calves experienced ranged from 6.1 to 24.8 \(^\circ\text{C}\), respectively. Thus, increasing ambient temperature within the thermoneutral zone appears to enhance tissue accretion in calves independent of DMI. Little information is available on the effects of ambient temperature on the growth of cattle. Kang et al. (2016) indicated that lipolysis that occurs during cold ambient temperatures (to generate heat) could result in decreased feed efficiency. Partial weaning with a restricted (to 33\%) milk allowance decreased ADG from about 0.60 to less than 0.40 kg/d (Table 4). Calves exhibiting better growth performance during the pre-weaning period appear to be more negatively affected by the milk allowance restriction. Regardless of the treatments, ADG during weaning (d 43 to 49) was negatively associated with ADG during the week immediately before weaning \((R = -0.39\) and \(P = 0.039;\) data not shown). Low starter intake that primarily stem from high milk allowances (Gelsinger et al., 2016b) during pre-weaning period likely caused calves to be ill-prepared for
forthcoming weaning in the present study.

In line with the markedly increasing starter intake (Figure 1), BW increased significantly after calves were completely weaned (Figure 2). During the first three weeks post-weaning, W0 and W17 calves had similar ADG and starter intake of 1.06 kg/d and 2.32 kg/d, respectively. Rosenberger et al. (2017) also observed similar ADG and starter intake post-weaning for calves fed 8 to 10 kg of whole milk daily. Hip height and BL of W0 calves were greater than that of W17 during the post-weaning period ($P < 0.05$, Table 4). Overall, calves having access to drinking water from birth had better structural growth compared to those first receiving drinking water at d 17. Structural growth of calves has been shown to be positively related to future BW and milk yield during the first lactation (Van De Stroet et al., 2016). In the present study, W0 calves with greater HH and BL at 10 wk of age achieved greater BW at 5 months of age (199.9 vs. 186.9 kg, $P = 0.048$).

**Nutrient digestibility post-weaning**

Nutrient intake, ATTD of nutrients, and FCE of calves at 10 wk of age are given in Table 5. Intake of individual nutrients was similar between W0 and W17 calves, primarily due to similar starter intake during the final two days of the experiment (d 69 and 70) when total fecal output of individual animals was collected and sampled to determine ATTD of nutrients. The mean ATTD of DM, starch, CP, EE (fat), NDF, and ADF (Table 5) are consistent with the ranges previously reported (Terré et al., 2007; Hill et al., 2010; Castells et al., 2012; Montoro et al., 2013; Daneshvar et al., 2015; Chapman et al., 2016). The digestibility of starch and CP were not affected by the age at which calves were first offered drinking water ($P > 0.550$). Compared to W17 calves, W0 calves had greater ATTD of ADF ($P = 0.047$) and tended to have greater ATTD of NDF ($P = 0.078$). Nonetheless, the discrepancies of ADF and NDF digestibility had no impact on DM digestibility (Table 5). Dennis et al. (2018) saw similar ATTD of DM post-weaning despite the significantly
changing ADF and NDF digestibility between calf groups fed different amounts DM from milk replacer. Opposed to the effects on ADF and NDF digestibility, calves offered drinking water from birth tended to have lower ATTD of fat ($P = 0.094$). Quigley et al. (2017) and Dennis et al. (2018) observed that ATTD of ADF and NDF were inversely related to ATTD of fat in weaned heifer calves fed different liquid feed allowances. The increased ADF and NDF digestibility were related to improved FCE post-weaning ($P = 0.057$, Table 5) supporting the fact that increasing fiber digestibility greatly improves feed utilization efficiency of ruminants (Beauchemin et al., 2003).

Unlike milk, which is shunted directly to the abomasum via the esophageal groove, drinking water is capable of entering the rumen and becoming an integral part of its development (Govil et al., 2017). Drinking water can affect rumen development and establishment of its microbiome by altering the pH, digesta mixing and passage, and volatile fatty acid composition similar to what has been shown with the microbiome of mature cattle and humans (Rogers et al. 1982; Fraley et al., 2015; Sasada et al., 2015; Cremer et al., 2017). Given the fact that the contribution of the rumen to total-tract digestibility of fiber is substantially greater than the contribution from the other parts of the gut (Gressley et al., 2011), the improved NDF and ADF digestibility suggests an improved rumen function in calves offered drinking water from birth. Even though it has long been hypothesized that drinking water facilitates rumen development in calves, no systematic attempt has been made to explore those effects. Such an attempt will improve current understanding of development of the rumen and its microbiome and their relevance to growth and production performance of dairy heifers. Moreover, the new knowledge will assist in effectively educating producers on the importance of providing drinking water to dairy calves.

**Health status**

Severity of diarrhea (scores of fecal consistency) and the number of days with diarrhea for W0 and W17 calves are given in Table 6. Some important blood parameters related to diarrhea
are also shown in the Table 6. Age at which drinking water was offered did not have an impact on the severity of diarrhea \((P = 0.209)\) or the number of days that calves had diarrhea \((P = 0.890)\). Kertz et al. (1984) also reported an insignificant impact of offering drinking water to newborn dairy calves on diarrhea. Disturbed electrolyte homeostasis in the blood, dehydration (as indicated by high blood Hct), and reduced blood pH (indicative of metabolic acidosis) are the most significant consequences of diarrhea in calves (Sayers et al., 2016). Blood HPT concentration has been identified as a useful tool in evaluating health status of dairy calves (Ganheim et al., 2007; Balikci et al., 2014). Mean Hct, pH, Na and K concentrations, and HPT concentration in blood at two weeks of age, when the incidence of diarrhea was most prevalent (Table 6), were consistent with the values previously reported for neonatal calves (Reece, 1980; Bouda and Jagos, 1984; Ramin et al., 2012; Murray et al., 2014). Unaffected blood parameters, except Na concentration, further reinforce the observation that offering drinking water to newborn calves does not impact diarrhea or the general health status of calves. Blood Na concentration of W17 calves were greater than that of W0 calves \((136.6 \text{ vs. } 135.2 \text{ mmol/L, } P = 0.035)\). However, none of those concentrations were representative of severe consequences associated with diarrhea. Trefz et al. (2017) saw that diarrhea significantly increased calf mortality when blood Na concentrations were greater than 151 mmol/L. It is worth reminding here that the calves in the present study were fed a relatively large amount of milk \((6.0 \text{ to } 9.6 \text{ kg/d})\), greatly contributing to their total water intake during the period \((6 \text{ to } 21 \text{ d of age})\) in which more than 90 percent of the diarrhea incidences occurred (data not shown). More prominent effects of water deprivation could be expected among calves having lower milk or milk replacer allowances (e. g., half of the dairy operations in the US offering less than 5.0 kg/calf/d) and are raised under hot weather conditions associated with significant water losses from the body.
Conclusions

Holstein heifer calves drank 0.75 kg of drinking water daily aside from the water they receive from an ad libitum milk intake during the first 2 wk of life. Calves first offered drinking water at 17 d of age consumed 59% more water during the rest of the pre-weaning period. Calves having access to drinking water from birth drank about 300g more milk and tended to achieve greater body weights and heart girths pre-weaning and had greater apparent total-tract digestibility of NDF and ADF, feed efficiency, hip height, and body length post-weaning. The age first offered free drinking water did not have an impact on starter intake, severity and duration of diarrhea, and general health status of calves. Regardless of the age first offered drinking water, calves had low starter intake during pre-weaning period and experienced a significant decline in average daily gain during weaning. When offered from birth, neonatal calves consumed a significant amount of drinking water and had improved growth, which could be potentially linked to a positive effect of drinking water on gut development, and thus nutrient utilization efficiency.

Literature Cited


Tables and Figures

Table 1. Free drinking water intake (FWI), pasteurized whole milk intake, starter intake, and total water intake of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Least Squares Means</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W0</td>
<td>W17</td>
<td></td>
</tr>
<tr>
<td>FWI, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 16 d</td>
<td>0.75</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>17 to 42 d</td>
<td>0.82</td>
<td>1.30</td>
<td>0.04</td>
</tr>
<tr>
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<td>1.88</td>
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</tr>
<tr>
<td>50 to 70 d</td>
<td>5.26</td>
<td>5.32</td>
<td>0.09</td>
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</tr>
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<td>0.10</td>
</tr>
<tr>
<td>17 to 42 d</td>
<td>8.20</td>
<td>7.96</td>
<td>0.07</td>
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<tr>
<td>43 to 49 d</td>
<td>2.85</td>
<td>2.75</td>
<td>0.08</td>
</tr>
<tr>
<td>50 to 70 d</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Starter Intake, kg/d</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 to 16 d</td>
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<td>0.02</td>
<td>0.01</td>
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<tr>
<td>17 to 42 d</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
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<tr>
<td>43 to 49 d</td>
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<td>0.70</td>
<td>0.04</td>
</tr>
<tr>
<td>50 to 70 d</td>
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<td>2.27</td>
<td>0.04</td>
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<td>Total Water Intake*, kg/d</td>
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<tr>
<td>0 to 16 d</td>
<td>6.19</td>
<td>5.16</td>
<td>0.11</td>
</tr>
<tr>
<td>17 to 42 d</td>
<td>7.96</td>
<td>8.23</td>
<td>0.07</td>
</tr>
<tr>
<td>43 to 49 d</td>
<td>4.42</td>
<td>4.48</td>
<td>0.10</td>
</tr>
<tr>
<td>50 to 70 d</td>
<td>5.48</td>
<td>5.59</td>
<td>0.09</td>
</tr>
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</table>

*= FWI + (Milk Intake × 0.87) + (Starter Intake × Moisture content of starter).
Table 2. The Pearson’s correlation coefficients (R) and the corresponding $P$-values for relationships among drinking water intake (FWI), starter intake, and milk intake of Holstein heifer calves offered drinking water from birth and weaned at 42 d of age.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>R</th>
<th>$P$ - value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 42 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FWI &amp; starter intake</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>FWI &amp; milk intake</td>
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<td>0.245</td>
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<tr>
<td>Milk intake &amp; starter intake</td>
<td>0.04</td>
<td>0.298</td>
</tr>
<tr>
<td>43 to 70 d</td>
<td></td>
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</tr>
<tr>
<td>FWI &amp; starter intake</td>
<td>0.85</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3. Effects (regression coefficients) of mean daily ambient temperature on free drinking water intake (FWI), starter intake, and average daily gain (ADG) of Holstein heifer calves.

<table>
<thead>
<tr>
<th>Variable (all in kg/d)</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Weaning (0 to 42 d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FWI</td>
<td>0.068</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Starter intake</td>
<td>-0.0002</td>
<td>0.736</td>
</tr>
<tr>
<td>ADG</td>
<td>0.020</td>
<td>&lt;0.001</td>
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<tr>
<td>During Weaning (43 to 49 d)</td>
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<td></td>
</tr>
<tr>
<td>FWI</td>
<td>0.141</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Starter intake</td>
<td>0.002</td>
<td>0.816</td>
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<tr>
<td>ADG</td>
<td>-0.019</td>
<td>0.352</td>
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<tr>
<td>Post-Weaning (50 to 70 d)</td>
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<td></td>
</tr>
<tr>
<td>FWI</td>
<td>0.055</td>
<td>0.012</td>
</tr>
<tr>
<td>Starter intake</td>
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<td>0.110</td>
</tr>
<tr>
<td>ADG</td>
<td>-0.027</td>
<td>0.153</td>
</tr>
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</table>
Table 4. Growth performances of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17). Calves were partially and completely weaned at 42 and 49 d, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Least Squares Means</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W0</td>
<td>W17</td>
<td></td>
</tr>
<tr>
<td>Birth Weight, kg</td>
<td>37.5</td>
<td>37.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Pre-Weaning (0 to 42 d)</td>
<td></td>
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<tr>
<td>Body Weight, kg</td>
<td>53.8</td>
<td>53.1</td>
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<tr>
<td>Hip Height, cm</td>
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<td>0.2</td>
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<tr>
<td>Hip Width, cm</td>
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<td>20.6</td>
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<td>Body Length, cm</td>
<td>71.2</td>
<td>71.0</td>
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<td>Heart Girth, cm</td>
<td>86.9</td>
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<td>ADG, kg/d</td>
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</tr>
<tr>
<td>During Weaning (43 to 49 d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.27</td>
<td>0.37</td>
<td>0.09</td>
</tr>
<tr>
<td>Post-Weaning (50 to 70 d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>81.9</td>
<td>80.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Hip Height, cm</td>
<td>93.3</td>
<td>92.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Hip Width, cm</td>
<td>24.0</td>
<td>23.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Body Length, cm</td>
<td>86.8</td>
<td>85.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Heart Girth, cm</td>
<td>100.3</td>
<td>99.6</td>
<td>0.5</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.09</td>
<td>1.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Follow-up (at 5 mon of age)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>199.9</td>
<td>186.9</td>
<td>4.31</td>
</tr>
</tbody>
</table>
Table 5. Nutrient intake, apparent total-tract digestibility (ATTD, %) of nutrients, and feed conversion efficiency (FCE) of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17) three weeks after weaning (at 10 wk of age).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Least Squares Means</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W0</td>
<td>W17</td>
<td></td>
</tr>
<tr>
<td>Intake, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>2.80</td>
<td>2.71</td>
<td>0.13</td>
</tr>
<tr>
<td>CP</td>
<td>0.60</td>
<td>0.58</td>
<td>0.03</td>
</tr>
<tr>
<td>Starch</td>
<td>0.95</td>
<td>0.91</td>
<td>0.05</td>
</tr>
<tr>
<td>EE</td>
<td>0.12</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>ADF</td>
<td>0.57</td>
<td>0.57</td>
<td>0.03</td>
</tr>
<tr>
<td>NDF</td>
<td>0.76</td>
<td>0.75</td>
<td>0.04</td>
</tr>
<tr>
<td>Apparent Total-tract Digestibility, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>74.3</td>
<td>74.2</td>
<td>0.7</td>
</tr>
<tr>
<td>CP</td>
<td>73.4</td>
<td>73.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Starch</td>
<td>97.6</td>
<td>98.1</td>
<td>0.5</td>
</tr>
<tr>
<td>EE</td>
<td>86.3</td>
<td>89.0</td>
<td>1.0</td>
</tr>
<tr>
<td>ADF</td>
<td>55.1</td>
<td>50.2</td>
<td>1.6</td>
</tr>
<tr>
<td>NDF</td>
<td>51.2</td>
<td>47.2</td>
<td>1.5</td>
</tr>
<tr>
<td>FCE*</td>
<td>0.49</td>
<td>0.34</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* = (ADG during wk 10/Average DMI during wk 10).
Table 6. Attributes of diarrhea and some blood parameters of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Least Squares Means</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W0</td>
<td>W17</td>
<td></td>
</tr>
<tr>
<td>Attributes of Diarrhea (0 to 28 d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days with diarrhea</td>
<td>6.8</td>
<td>7.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Fecal consistency score</td>
<td>2.00</td>
<td>2.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Blood Parameters (14 d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, % PCU</td>
<td>23.51</td>
<td>24.18</td>
<td>1.35</td>
</tr>
<tr>
<td>Na, mmol/L</td>
<td>135.24</td>
<td>136.58</td>
<td>0.41</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>4.33</td>
<td>4.25</td>
<td>0.10</td>
</tr>
<tr>
<td>pH</td>
<td>7.43</td>
<td>7.41</td>
<td>0.01</td>
</tr>
<tr>
<td>Haptoglobin, ug/mL</td>
<td>65.88</td>
<td>128.26</td>
<td>51.12</td>
</tr>
</tbody>
</table>
Figure 1. Weekly Mean (±SEM) drinking water intake (FWI) and starter intake of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17) during the first 10 wk of their life. All calves were partially weaned by reducing the milk allowance by 2/3 at 42 d of age and completely weaned at 49 d of age.
Figure 2. Weekly Mean (±SEM) body weight, body length, hip width, and hip height of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17) during the first 10 wk of their life. All calves were partially weaned by reducing the milk allowance by 2/3 at 42 d of age and completely weaned at 49 d of age.
CHAPTER 4. OFFERING DRINKING WATER FROM BIRTH INCREASES THE ABUNDANCE OF *FAECALIBACTERIUM* AND *BIFIDOBACTERIUM* IN THE GI TRACT OF PRE-WEANED DAIRY HEIFER CALVES

A paper to be submitted to the Journal of Dairy Science

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Abstract

We previously demonstrated that dairy calves having access to drinking water from birth (W0) achieved greater body weight, fiber digestibility, and feed efficiency than those that first received drinking water at 17 d of age (W17). The degree of bacterial diversity in the gut has been shown to be related to those traits in other species. The objective of this study was to examine the impact of drinking water intake on the diversity and the abundance of bacterial taxa in the gut of dairy calves using a fecal microbiota analysis. Fresh feces were collected directly from the rectum of calves in both W0 (n = 14) and W17 treatment groups (n = 15) at 2, 6, and 10 wk of age. All the calves were fed with pasteurized waste milk and weaned completely at 7 wk of age. The DNA was sequenced using 16S rRNA gene-amplicon sequencing on an Illumina MiSeq system. The sequences were clustered into operational taxonomic units (OTU) with a 99% similarity threshold. Treatment effects on species richness, diversity, and the relative abundance of the 25 most abundant OTUs were analyzed using GLIMMIX and GENMOD procedure in SAS. At 2 wk of age, W0 had a greater number of observed bacterial species (5908 vs. 4698, \(P = 0.033\)) and species richness (Chao1 index, \(P = 0.042\)) than W17. The number of observed species, and Chao1 index
increased with age \((P < 0.001)\) and became similar between W0 and W17 at 10 wk of age \((P > 0.050)\). The evenness of the abundance of bacterial species (Shannon and inverse-Simpson indices) were similar \((P > 0.050)\) between W0 and W17 but increased with age \((P < 0.001)\). At 2 wk of age, the abundance of an OTU with high similarity to \textit{Bacteroides fragilis} was greater \((P=0.047)\) in W0 than W17, whereas an OTU of genus \textit{Lachnospiraceae} \((P = 0.037)\) and an OTU related to \textit{Streptococcus gallolyticus} \((P = 0.002)\) increased in W17 than W0. At 6 wk of age, the abundance of OTUs related to \textit{Streptococcus gallolyticus}, \textit{Faecalibacterium prausnitzii}, and \textit{Bifidobacterium breve} were greater in W0 than W17 \((P < 0.040)\), whereas an OTU related to \textit{Bacteroides sartorii} was greater in W17 than W0. At 10 wk of age, the abundance of two OTUs in genera \textit{Succinivibrio} and \textit{Alloprevotella} increased in W0 than W17. Offering drinking water from birth was related to increased species richness in the gut of neonate calves. Higher abundance of some potentially beneficial OTUs (\textit{Bifidobacterium}, \textit{Faecalibacterium}, \textit{Succinivibrio}) could partly contribute to the improved performance of calves who were first receiving drinking water from birth (W0).

Key words: drinking water, \textit{Faecalibacterium}, \textit{Bifidobacterium}

**Introduction**

At birth, the gastrointestinal tract in ruminants is sterile and not yet fully functional. However, it is rapidly colonized by diverse communities of microbiota during and immediately after birth (Meale et al., 2016; Alipour et al., 2018; Yeoman et al., 2018). Several factors including pH, digesta passage rate, and oxygen gradient in the gut greatly affect the composition of microbial communities in the digestive tract of calves (Malmuthuge et al., 2015). Several studies have demonstrated that bacterial communities quickly established in the rumen and distal gut of calves as early as two weeks of age (Rey et al., 2014; Meale et al., 2016; Dill-McFarland et al., 2017). Dairy calves consume a significant amount of drinking water at this age, even though the grain
intake could still be insignificant (Kertz et al., 1984; Wickramasinghe et al., 2019). Water is often offered to calves in individual buckets, drinking from which does not stimulate formation of the esophageal groove (REF). Unlike milk or milk replacer bypassing the rumen, drinking water receives a chance to contribute to the ecology of the entire gastrointestinal tract. Meale et al. (2017) reported significant relationships between drinking water intake of calves and the abundance of several bacterial communities in the rumen and feces. Studies using other species also suggest that drinking water could affect the composition of gut microbiota by changing some physiochemical properties of the gut. For instance, Cremer et al. (2017) reported that drinking water altered digesta passage rate, digesta mixing and epithelial absorption of water, and thereby changing the composition of gut microbiota in humans. Sasada et al. (2015) showed that chlorinated water selectively decreased the abundance of some bacterial communities in human gut. Sofi et al. (2014) suggested that pH of drinking water affects the composition of gut microflora in mice. Moreover, Faulkner et al. (2017) indicate that trace minerals in drinking water could affect the relative abundance of some gut microbial communities. Nonetheless, no controlled study has explored the impact of drinking water on physiochemical properties or the composition of microbiota in the gut of calves or any other group of cattle.

Even though water is recognized as the most essential nutrient to sustain life and performance of animals, water requirements of livestock species are often overlooked. The situation appears to be even worse for young calves than mature cattle (Beede, 2005; Kertz et al. 2017). The majority of dairy producers tend to refrain from offering drinking water to newborn dairy calves assuming that water in milk or milk replacers are adequate to fulfil the total water requirement. The USDA’s National Animal Health Monitoring System (NAHMS) study in 2014 found that dairy producers wait for, on average, 17 days to first offer drinking water to newborn
dairy calves (USDA, 2016). In a controlled study, we examined the impact of offering drinking water to newborn dairy calves at birth (W0) vs at 17 d of age (W17) on drinking water intake and performance pre- and post-weaning (Wickramasinghe et al., 2019). The W0 group consumed a significant amount of water from buckets (0.75 kg/calf/d) in addition to the water they received via a large volume of milk (~ 6.0 L/calf/d) during the first 16 d of their life. The W17 group however consumed 59% more drinking water than W0 once they got the access to it. Nonetheless, W0 achieved greater BW pre-weaning, and had better fiber digestibility and feed efficiency than W17 post-weaning (Wickramasinghe et al., 2019).

Efficient growth during pre-weaning period has positive impact on future milk production in dairy heifers (Soberon et al., 2012; Van De Stroet et al., 2016). Oikonomou et al. (2013) demonstrated that pre-weaned calves with increased average daily gain (ADG) had a greater bacterial species richness (Chao 1 index) in the gut than those with low ADG as determined with a fecal microbiota analysis. Meale et al. (2016; 2017) observed significant relationships between BW and the abundance of several bacterial taxa in fecal microbiota of dairy calves. Moreover, Shanks et al. (2011) and Paz et al. (2018) demonstrated that feed efficiency and nutrient concentrations in feces were different in cattle with different bacterial community compositions in the rumen and feces. We hypothesized that drinking water intake during the post-natal period would affect the bacterial community composition in the gut of pre-weaned dairy calves. Objectives of the present study were 1) to investigate the impact of offering drinking water since birth vs. a couple of weeks later on the species richness, abundance, and diversity of bacterial communities in the feces of pre-weaned calves and 2) to examine if those effects are carried over to the post-weaning.
Materials and Methods

Animals, treatments and measurements

All animal procedures in this study were conducted under approval of the Animal Care and Use Committee at Iowa State University (ISU, IACUC# 7-17-8570-B). The fecal samples used in the present study were obtained in a dairy calf trial published in Wickramasinghe et al. (2019). Additional details of the materials and methods are available in Wickramasinghe et al. (2019). Thirty Holstein heifer calves born in the ISU-Dairy from August 22 to October 8 in 2017 were allotted to two treatments (15 calves per treatment); 1) provision of drinking water at birth (W0), and 2) provision of drinking water 17 d after birth (W17). However, one calf in W0 died before the end of the study. Therefore, it was excluded from the analyses leaving 14 replicates for W0 (n = 14). All the calves were housed in individual pens in an indoor calf facility until they were 10 wk old. Calves were bottle-fed with pasteurized whole milk 3× per day (2.0 kg/feeding until 14 d, and 3.2 kg/feeding thereafter). All the calves were weaned completely at 49 d of age. Once offered, each individual calf had free access to clean drinking water and a grain-based solid feed in separate plastic buckets throughout the study (from 0 to 70 d of age). Approximately 5.0 g of fresh feces was collected directly from the rectum to sterile bags at 2 and 6 wk of age (pre-weaning), and at 10 wk of age (post-weaning). Fecal samples were flash frozen in dry ice immediately after collection and then transferred to a freezer at –80 °C for storage until further processing.

DNA extraction

Fecal samples stored at –80 °C were thawed at room temperature and kept on ice during the extraction process. Approximately 2.5 g fecal sample (wet weight) was used for DNA extraction using DNeasy PowerLyzer PowerSoil Kit (Qiagen Sciences Inc, Germantown, MD) following the manufacturer’s protocol that included a bead-beating step (Fisherbrand™ Bead Mill
Homogenizer, Fisher Scientific, Portsmouth, NH) for mechanical disruption of microbial cells. DNA was eluted from the column with an elution buffer (provided with DNeasy PowerLyzer PowerSoil Kit), and DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). The samples were adjusted to contain 25 to 50 ng of DNA/μL, and loaded to a 96-Well microtitre plate (Microtiter™ Microplate, ThermoFisher Scientific, Waltham, MA), and sealed (Thermo Scientific™ Nunc™ Sealing Tapes, ThermoFisher Scientific, Waltham, MA) and then stored at – 80 °C until shipping to the DNA sequencing facility (Iowa State University DNA facility, Ames, IA) for 16S rRNA gene-amplicon sequencing on an Illumina MiSeq system using 150bp paired-end sequencing technology.

**Microbiota sequencing, sequence processing, and analysis**

The microbiota sequencing was conducted using a protocol designed to amplify bacteria and archaea (The Earth Microbiome Project; http://www.earthmicrobiome.org/) in the DNA facility at Iowa State University (Ames, IA). Briefly, the genomic DNA from each sample was amplified using Platinum™ Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA) with one replicate per sample using universal 16S rRNA gene bacterial primers [515F (5′-GTGYCAGCMGCGGGTAA-3′; Parada et al., 2016), and 806R (5′-GGACTACNVGGGTWTCTAAT-3′; Apprill et al., 2015)] for the variable region V4, as previously described (Kozich et al., 2013). All the samples underwent PCR with an initial denaturation step at 94°C for 3 min, followed by 45 s of denaturing at 94°C, 20 s of annealing at 50°C, and 90 s of extension at 72°C. This was repeated for 35 total PCR cycles and finished with a 10 min extension at 72°C. All the PCR products were then purified with the QIAquick 96 PCR Purification Kit (Qiagen Sciences Inc, Germantown, MD) according to the manufacturer’s recommendations. PCR bar-coded amplicons were mixed at equal molar ratios and used for
Illumina MiSeq paired-end sequencing with 150 bp read length and cluster generation with 10% PhiX control DNA on an Illumina MiSeq platform (Illumina Inc., San Diego, CA). After sequencing, corresponding overlapping paired-end reads were stitched to get an approximate amplicon size of 255 bp.

Raw sequence data in fastq format were analyzed using mothur v.1.40.4 (http://www.mothur.org/; Kozich et al., 2013). The number of raw reads per sample varied from 41,076 to 239,819. Briefly, paired-end reads were combined into contigs and the sequences were then subsampled randomly to obtain 41,000 sequences per sample and screened for quality with the `screen.seqs` command excluding sequences with any ambiguities, the length of allowed homopolymers was set to eight. A total of 3,177,001 sequences (89.2%) passed the quality control. These sequences were clustered into operational taxonomic units (OTU) with a 99% similarity cutoff (0.01 distance). The SILVA SSU reference database (version 132, Pruesse et al., 2007) was used as taxonomic reference. Chimeras were removed using the `chimera.uchime` command. The nonparametric species richness estimates [Chao 1 and abundance-based coverage estimator (ACE)], the diversity indices (Shannon and Non-parametric Shannon), and the evenness and coverage-based index (Simpson) were calculated using the “`summary.single`” command. Analysis of beta diversity was achieved by using the Analysis of Molecular Variance (AMOVA) and Analysis of Similarity (ANOSIM) commands in mothur.

**Statistical analysis**

Treatment effects on alpha diversity measures were analyzed using GLIMMIX procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC) using following model.

\[ Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + C_k + e_{ijk} \]
where $Y_{ijk} = \text{the diversity measure of interest}$, $\mu = \text{overall mean}$, $T_i = \text{fixed effect of ith treatment}$ ($i = \text{W0 and W17}$), $W_j = \text{fixed effect of jth week of age}$, $(T\times W)_{ij} = \text{fixed effect of interaction between ith treatment and jth week of age}$, $C_k = \text{random effect of kth calf}$, and $e_{ijk} = \text{random error assumed to be independent and identically distributed from a normal distribution with a mean of 0 and a variance of } \sigma^2 \text{ [~ N (0, I\sigma^2)].}$ Relative abundance of OTU were ranked highest to lowest and only the 25 most abundant OTU at each time point (2, 6, and 10 wk) were chosen separately. Composition of the top 25 OTU were markedly different across the time points (discussed below). Therefore, the treatment effects on the relative abundance of each of 25 most abundant OTUs at each time point (2, 6, and 10 wk) were analyzed separately with negative binomial models using GENMOD procedure of SAS.

**Accession numbers**

Sequencing data are available in the BioProject SRA database under the accession number PRJNA526931.

**Results and Discussion**

Pertaining to the fact that dairy producers wait for, on average, 17 d to first offer drinking water to newborn dairy calves in US (USDA, 2016), we studied the impact of first offering drinking water to heifer calves from birth (W0) vs. 17 d after (W17) on growth, health, and nutrient digestibility, feed efficiency and fecal microbiota community composition. The effects on all response variables except the composition of the fecal microbiota were published in Wickramasinghe et al. (2019). The present paper includes the effects on fecal microbiota as measured at 2, 6, (pre-weaning) and 10 wk (post-weaning) of age. At each time point, fecal microbiota of 14 calves in the W0 and 15 calves in the W17 group were analyzed. These sample sizes can be assumed to be related to adequate statistical power. Kelly et al. (2015) demonstrated
that a sample size of 10 was related to a sufficient statistical power to detect differences of treatment effects in microbiota analyses. All calves were born and housed in the same production environment, and managed the same from calving and colostrum feeding to the last day of fecal sampling. Therefore, bias from microbial load in the environment and delivery mode (Dominguez-Bello et al. 2010), type of colostrum (Malmuthuge et al., 2015), type of liquid feed (Edrington et al., 2012), and housing (Pereira et al., 2014) were minimal in the present study. In line with several previous studies (Callaway et al., 2010; McGarvey et al., 2010; Mao et al., 2012; Oikonomou et al., 2013; Kim et al., 2014; Klein-Jöbstl et al., 2014), we chose to study the fecal microbiota of calves as fecal samples are easy to collect in a noninvasive manner. One major limitation of using fecal samples to study the gut microbiota is the fact that the fecal microbiota primarily reflects luminal microbiota, which is significantly different from gut mucosal microbiota (Malmuthuge et al., 2012 and 2014). Moreover, fecal samples are highly representative of the digesta in the lower gut and do not reveal important host–microbial interactions specific to different regions of the gut (Malmuthuge et al., 2014). However, this won’t be a significant issue in young calves as the rumen is yet to be fully developed. Van Vleck Pereira et al. (2016) showed that the fecal microbiota adequately reflected the changes in gut microbiota of pre-weaned calves in response to very low concentrations of antimicrobials. Moreover, fecal samples make periodic examination of gut microbiota over time feasible and effective. While acknowledging those limitations and strengths, we assume that the present fecal microbiota analysis is capable of adequately describe the true effects of drinking water on bacterial community composition in the gut of dairy calves.

**Effects on the alpha diversity**

Treatment effects on alpha diversity parameters including the total number of bacterial species observed, and Chao1, ACE, Shannon, and Simpson indices are given in Table 1. The
majority of alpha diversity indices of W0 and W17 calves varied differently with age as indicated by significant interactions between treatment and age. At 2 wk of age, W0 had a greater number of observed bacterial species as opposed to W17 calves. \((P = 0.033)\). However, no differences were found at the age of wk 6 or 10. The number of observed species continued to increase with age in both groups \((P < 0.001)\), but the increment from wk 2 to 6 in W17 was more prominent than the increment in W0 (53% vs. 13%). In line with the observed species number, W0 calves also had greater Chao1 and ACE indices indicating improved species richness \((P = 0.042)\) at 2 wk of age. Chao1 and ACE continued to increase with age \((P < 0.001)\) but became similar between W0 and W17 at 6 and 10 wk of age. Again, the increment from wk 2 to 6 was more pronounced in W17 (52 and 62%, respectively) than in W0 (8 and 11%, respectively). The diversity of gut microbial communities is often associated with diet diversity and thus the number of available nutritional niches. Variability in nutrients reaching the lower gut (e.g., fiber and starch) resulting from increasing starter intake would considerably explain the increases in the diversity and richness of gut microbiota with age. However, at 2 wk of age, milk was the only contributor to the nutrients in the gut as starter intake was virtually zero in both W0 and W17 (Wickramasinghe et al., 2019). Perhaps, the bacterial communities present in drinking water themselves had a significant contribution to the diversity and richness of gut microbiota in calves. Ling et al. (2018) examined bacterial community composition of a chlorine-treated tap water system and found a significant diversity at phyla level. Previous studies with mice (Sofi et al., 2014; Sasada et al., 2015) and humans (Cremer et al., 2017) showed that physiochemical properties of drinking water such as pH and concentrations of chlorine residues had significant impact on bacterial community composition in the gut. Once offered on d 17, W17 calves consumed 59% more drinking water than W0 calves until they were 6 wk of age (Figure 1). The average starter intake however
remained unchanged between W0 and W17 during this period (Wickramasinghe et al., 2019). Therefore, drinking water intake appeared to be the primary cause of the sharp increase in species number and the richness indices from wk 2 to 6 in W17.

The Shannon’s index of diversity accounts for the number and evenness of the species present. Evenness describes whether the microbiome is dominated by many (high evenness) versus few (low evenness) and is positively related to the Shannon’s index. Calves receiving drinking water since birth and W17 had similar Shannon’s indices (4.3 vs. 4.2, Table 1) at wk 2. The Shannon index of both W0 and W17 increased significantly from wk 2 to 6. Klein-Jöbstl et al. (2014) and Yeoman et al., (2018) also demonstrated continuously increasing Shannon indices in pre-weaned calves. However, W17 drinking 59% more water than W0 during the period from wk 2 to 6 had a significantly greater Shannon’s index than W0 (5.5 vs. 5.1, \( P = 0.029 \)) at wk 6. The Shannon’s index markedly increased from pre-weaning period to post-weaning period (wk 2 and 6 vs. wk 10, \( P < 0.001 \)) and became similar between W0 and W17 at 10 wk of age. Meale et al. (2016) demonstrated that fecal microbiota of weaned calves had greater richness and evenness than suckling calves. The Simpson’s index is another widely used marker of the alpha diversity and calculated to be inversely proportional to the Shannon index. In the present study, we calculated the inverse of the Simpson’s index (Table 1) for convenience of interpreting it. In line with the Shannon’s index, the inverse of the Simpson index was similar between W0 and W17 at wk 2 (\( P = 0.747 \)) but increased markedly in W17 compared to W0 at wk 6 (\( P = 0.055 \)). As observed with the Shannon’s index, the inverse of Simpson’s index increased significantly, as calves were weaned and thus the diet was changed from milk to a starter ration, regardless of the treatment. Khafipour et al. (2011) and Meale et al. (2016) concluded that reduced variability in pH and improved availability of nutrients in more developed and stable hind gut could promote greater
bacterial diversity and evenness post-weaning than pre-weaning. Malmuthuge et al. (2013) also highlighted supplementation of a milk replacer diet with calf starter during weaning tended to increase the number of bacterial phylotypes.

**Effects on the beta diversity**

When comparing the fecal communities as a whole, no significant differences were found between W0 and W17 using AMOVA ($P \geq 0.31$) and ANOSIM ($P \geq 0.25$).

**Effects on the most abundant OTUs**

Overall, 4,961 OTUs were generated after quality control, subsampling and removal of OTUs representing less than 10 sequences from the original 12.6 million sequence reads, of which 3.2 million reads (25%) remained after quality control and subsampling. Most of the reads were bacterial, 0.5% of all reads were classified as Archaea. The 25 most abundant OTUs across all three-time points (2, 6, and 10 week of age) are presented in Figure 2. The statistical significance ($P$-values) of offering drinking water since birth vs. 17 d later on the relative abundance of the 25 most abundant OTUs at each time point are given in Table 2. As reported previously (Jami et al., 2013; Meale et al., 2016), the fecal microbiota was dominated by OTUs belonging to the phyla *Bacteroidetes, Firmicutes* and *Proteobacteria* (Figure 3) across all the time points. Composition of the highly abundant OTUs at wk 2 was more similar to that of wk 6 than wk 10. Eleven OTUs were common at both time points representing the pre-weaning period. Only three of those OTUs were found among the highest abundant OTU list (Table 2) post-weaning. For instance, *Lactobacillus* (OTU1) was the most abundant OTU during pre-weaning period but was not ranked even within the top 100 OTUs post-weaning. Overall, these observations indicate a significant role of diet in shaping gut bacterial community composition and confirm other reports highlighting the dynamic development of calf microbiota during the first weeks of life.
At 2 wk of age

*Bacteroides* (OTU5) closely related to *Bacteroides fragilis* was the most abundant OTU at wk 2. The dominance of *Bacteroides fragilis* even over *Lactobacillus* (OTU1) was not a surprise as it is often found to be highly prevalent in feces from calves with diarrhea (Border et al., 1985). The prevalence of diarrhea usually peaks at two weeks of age in most calf herds (Cho et al., 2014), including the present one (data not shown). Calves having access to drinking water had greater relative abundance of *Bacteroides* (OTU5) than W17 calves at wk 2 \( (P = 0.047, \text{ Table 2}) \) suggesting W0 calves might be affected more severely by diarrhea than W17 calves. We however did not observe a difference in the intensity and duration of diarrhea between W0 and W17 calves in the present study (Wickramasinghe et al., 2019). As Border et al. (1985) reported, some *Bacteroides fragilis* strains do not have enterotoxin-like activities in calves. Overall, the relative abundance of phylum *Bacteroidetes* in W0 calves was greater than W17 calves (26.8 vs. 18.1%, Figure 3) at wk 2. Being a predominant phylum in pre-weaned calves (Jami et al., 2013; Rey et al., 2013; Meale et al., 2016), *Bacteroidetes* plays a fundamental role in digesting polysaccharides in the host intestine (Wexler, 2007; Henderson et al., 2015). *Bacteroidetes* possess the highest number of genes for glycoside hydrolases in their genome compared to any other bacterial phyla in the gut (El Kaoutari et al., 2013). In the present study, neonate calves received a large volume of milk (6 to 9 kg/d) and thus consumed insignificant amount of starter feed. Therefore, the high abundance of *Bacteroidetes* in W0 might not provide a significant advantage to them in terms of digestibility of polysaccharides such as starch.

The other OTUs, the abundance of which was affected by drinking water was *Streptococcus* (OTU12) and *Lachnospitaceae* (OTU09). The relative abundance of *Streptococcus* (OTU12) was more than 7 times greater in W17 calves than W0 calves at 2 wk of age \( (P = 0.002, \text{ Table 2}) \).
Table 2) and found to be closely related to *Streptococcus galloyticus* (formerly known as *Streptococcus bovis*). *Streptococcus galloyticus* has been shown to be strongly associated with colorectal cancer in human. About two thirds of patients diagnosed with an infection of it had a concomitant colorectal neoplasia (Pasquereau-Kotula et al., 2018). Romero-Hernandez et al. (2015) isolated some strains of *Streptococcus galloyticus* in feces from calves that belong to lineages different from those infecting humans. Nonetheless, our results suggest that drinking water intake could be inversely related to the abundance of *Streptococcus galloyticus* in the gut.

The relative abundance of *Lachnospiraceae* (OTU09) was about 2.5 times greater in W17 than W0. Overall, OTUs of *Lachnospiraceae* were more abundant in pre-weaned calves (wk 2 and 6) than post-weaned calves (Table 2). Song et al. (2017) found *Lachnospiraceae* to be one of three highly abundant group of bacteria in the hindgut of newborn dairy calves. They also found *Lachnospiraceae* among highly abundant bacterial genera in both mucosa-attached and digesta-associated microbiota in the hindgut of dairy calves at 21 and 42 d of age. All the members of genus *Lachnospiraceae* are strictly anaerobic and reside mainly within the digestive tract of mammals. Some species of *Lachnospiraceae* produce butyric acid that prevents growth of some microbes within the digestive tract and provides a source of energy for other microbes and epithelial cells lining the gut (Meehan and Beiko, 2014). Some members of *Lachnospiraceae* have also shown to be associated with improved gut health in human (Mancabelli et al., 2017). Nonetheless, Gomez et al. (2017) found *Lachnospiraceae* in 10 most abundant genera in feces from both healthy and diarrheic calves. The *Lachnospiraceae* (OTU09) was not matched with any cultured species of *Lachnospiraceae* in our library searches. Therefore, it was difficult to conclude on a potential impact of the increased abundance of *Lachnospiraceae* (OTU09) in W17.
At 6 wk of age

In contrast to what observed at wk 2, the abundance of *Streptococcus* (OTU12) was lower in W17 than W0 at wk 6 ($P = 0.030$, Table 2). Given the fact that W17 consumed more (59%) water than W0 during the period from 2 to 6 week of age (Figure 1), the lower abundance of *Streptococcus* (OTU12) in W17 calves further support the idea that drinking water intake could be inversely related to the abundance of *Streptococcus gallolyticus* in the gut. The abundance of *Streptococcus* (OTU12) significantly declined with age and it was not found even within the top 100 OTU at wk 10 (data not shown). In general, the genus *Streptococcus* can be highly abundant in the gut of newborn calves (Smith, 1965; Jami et al., 2013) but the abundance significantly declines with age (Uyeno et al., 2010). The abundances of *Bacteroides* (OTU47), *Bifidobacterium* (OTU14), and *Faecalibacterium* (OTU8) were also significantly different between W0 and W17 at wk 6. *Bacteroides* (OTU47) was found to be closely affiliated with *Bacteroides sartorii* and more abundant in W17 than W0 ($P = 0.030$). *Bacteroides sartorii* is a relatively novel species in the genus *Bacteroides* (Clavel et al., 2010). So, only little is known about its role in the gut. Schaubek et al. (2015) reported a positive relationship between the abundance of *B. sartorii* and severity of inflammation in the gut of mice. *Faecalibacterium* (OTU8) was found to be closely related to *Faecalibacterium prausnitzii* and about seven times more abundant in W0 than W17 ($P = 0.008$). *F. prausnitzii* OTU8 and OTU11 were also numerically more abundant in the W0 animals than the W17 at week 2. *F. prausnitzii* belongs to the phylum *Firmicutes* and is an obligate anaerobic, Gram-positive, and butyrate-producing bacterium in the gut (Foditsch et al., 2015). Increased abundance of *F. prausnitzii* has been previously shown to improve health and growth performance in calves. Oikonomou et al. (2013) reported that high prevalence of an OTU closely affiliated to *F. prausnitzii* in the feces which was related to high weight gain and a lower incidence
of diarrhea in pre-weaned calves. Moreover, Foditsch et al. (2015) showed that feeding *F. prausnitzii* as a probiotic improved gastrointestinal health and growth of pre-weaned calves. Consistently, we observed pre-weaned calves in W0 group achieved greater body weight and heart girth than the W17 calves, when adjusted for the differences in birth weight (Wickramasinghe et al., 2019). In line with Uyeno and Kamagata (2010), the abundance of *Faecalibacterium* (OTU8) declined markedly as calves were weaned (data not shown).

*Bifidobacterium* (OTU14), the abundance of which was numerically greater in W0 at 2 wk of age (*P* = 0.523), became nearly 5 times greater in W0 than W17 at 6 wk of age (*P* = 0.019, Table 2). The abundance of *Bifidobacterium* (OTU14) markedly decreased with age. It was not found among the top 25 OTUs post-weaning. This inverse relationship between *Bifidobacterium* (OTU14) and weaning is not surprising as *Bifidobacterium* species thrive on milk sugars (Wickramasinghe et al., 2015). Vlkova et al. (2008) showed a significant reduction in the abundance of *Bifidobacterium* in fecal microbiota of calves, when milk intake decreased. *Bifidobacterium* species in the gut provide a number of health benefits including antagonistic effects against pathogenic bacteria, maintenance of the intestinal mucosal barrier function, and improvements in ADG and feed conversion efficiency (Abe et al., 1995; Simmering and Blaut 2001; Vaughan et al., 2002; Chierici et al., 2003; Bujnáková et al., 2004; Paggi and Fay 2004; Vlková et al., 2004). *Bifidobacterium* (OTU14) was closely related to *Bifidobacterium breve*. Among different species of the genus *Bifidobacterium*, *B. breve* is the most dominant in suckling animals. The species *B. breve* was first isolated from breast-fed infant feces (Reuter, 1963). Kelly et al. (2016) isolated *B. breve* from the gastrointestinal tract of calves. The W0 calves drank 300g more milk than W17 calves throughout the pre-weaning period (Wickramasinghe et al., 2019). Therefore, it can be assumed that the increased milk sugar supply might have partly enhanced the
colonization of \textit{B. breve} in W0 compared to W17 during suckling. However, the 5-fold increase in the abundance specifically at 6 wk of age is not completely clear. Owusu-Asiedu et al. (2006) observed that \textit{Bifidobacteria} counts were negatively related to digesta passage rate in the ileum of growing pigs. Perhaps, a potential increase in digesta passage rate due to the surplus drinking water intake (Fraley et al., 2015) might have negatively affected the abundance of \textit{B. breve} in W17 at wk 6.

**At 10 wk of age**

In agreement with previous observations (Meale et al., 2017), \textit{Prevotella} (OTU06), \textit{Alloprevotella} (OTU02), \textit{Bacteroides} (OTU19), \textit{Succinovibrio} (OTU22), and \textit{Treponema} (OTU27) were the most dominant OTUs at wk 10 representing an early post-weaning stage. None of those OTUs were however significantly different between W0 and W17. In addition, the abundance of any of the OTUs showing treatment effects at wk 2 or 6 was not different between W0 and W17 at wk 10 (data not shown). Among the top 25 OTUs, only \textit{Succinivibrio} (OTU61) and \textit{Alloprevotella} (OTU73) were different between W0 and W17 post-weaning. Two \textit{Succinivibrio} taxa, \textit{Succinovibrio} (OTU22) and \textit{Succinovibrio} (OTU61) were ranked within the 25 most abundant OTU at wk 10. On the contrary, no \textit{Succinivibrio} OTU was prominent in the microbiota at wk 2 and 6. Meale et al. (2016) observed \textit{Succinivibrio} to be more abundant in feces from weaned calves than suckling calves, whereas the abundance in the rumen showed an opposite trend as they found more \textit{Succinovibrio} in the rumen of suckling calves than weaned calves.

The abundance of \textit{Succinovibrio} (OTU61) in W0 was about 5 times greater than W17 (Table 2). Rey et al. (2013) showed strong positive correlations of genus \textit{Succinovibrio} with total volatile fatty acid concentration in the rumen and concentrate intake in dairy calves. Moreover, Hernandez-Sanabria et al. (2012) observed a positive correlation between the abundance of
**Conclusions**

Feces from W0 were more rich in the number of observed bacterial species than feces from W17 at 2 wk of age. Calves first receiving drinking water 17 d later had improved evenness in the abundance of individual bacterial species at 6 wk of age, where they consumed significantly more (59%) drinking water compared to W0. The species richness and evenness of bacterial communities became similar between both groups once the calves were weaned. The abundance of several OTUs including those closely related to beneficial bacterial species such as *Faecalibacterium prausnitzii*, and *Bifidobacterium breve* were greater in W0 than W17 during the pre-weaning period. The abundance of those OTUs however became insignificant after weaning and was not different between W0 and W17. The age that newborn dairy calves first receive drinking water and the amount of drinking water they consume could significantly affect the
composition of gut bacterial communities during pre-weaning periods. Those effects however did not appear to be carried over to the post-weaning period.

**Literature Cited**


Henderson, G. F. Cox, S. Ganesh, A. Jonker, W. Young, Global Rumen Census Collaborators, and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep. 5:14567. DOI: 10.1038/srep14567.


### Tables and Figures

**Table 1.** Alpha diversity indices of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17).

<table>
<thead>
<tr>
<th>Variable</th>
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<th>W17</th>
<th>SEM</th>
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<th>Week</th>
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<td>Week 6</td>
<td>Week 10</td>
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<td></td>
<td>5908.4</td>
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<td>7638.1</td>
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<td>55752.0</td>
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Figure 1. Weekly Mean (±SEM) drinking water intake of Holstein heifer calves first receiving free drinking water from birth (W0) or 17 d later (W17) during the first 10 wk of their life. All calves were partially weaned by reducing the milk allowance by 2/3 at 6 wk of age and completely weaned at 7 wk of age.
Figure 2. The most abundant operational taxonomic units (OUT) across all three time points (2, 6, and 10 week of age) and the relative abundance (%) of each OUT in calves receiving water from birth (W0) or 17 d of age (W17) at each time point.
Figure 3. Distribution of bacterial phyla of each calf across treatments in fecal calf samples. Columns represent the relative abundance of phyla with more than 2% of total relative abundance for each calf for treatments W0 and W17 during wk 2, 6, and 10.
CHAPTER 5. GENERAL CONCLUSIONS

This research stemmed from a lack of data on the roles of drinking water in young dairy calves (Kertz et al., 2017). It has been 35 years since Kertz et al. (1984) systematically studied the importance of offering drinking water to newborn dairy calves on feed intake and growth. Since, raising dairy calves has changed markedly over the recent decades; the findings in Kertz et al. (1984) might not be reasonably relevant to modern calves. This deficit of relevant data appears to be a major limitation in educating farmers on the importance of drinking water to calves. An average dairy farmer waits for 17 days to begin offering drinking water to newborn calves in the US (USDA, 2016). The primary reason behind this delay is that producers usually assume water in milk or milk replacer would be enough to satisfy the total water requirements. In the present study, we showed that newborn calves consumed a significant amount (0.75±0.05 kg/d) drinking water besides the water they consumed in 6.25kg/d of milk during the first 16 days. Assuming 87% of milk is water, the total water intake and perhaps the total water requirement of those calves were at 6.20±0.11 kg/d. Given the fact that 45% of dairy calves in the US are fed less than 5.0 kg/d of milk or milk replacer (USDA, 2016), a large number of dairy calves appear to consume less water than their requirement during the first 2 weeks. Nonetheless, the hematocrit values at 2 weeks of age were similar between calves with and without drinking water suggesting water in milk (6.0 kg/d) was adequate to keep animals hydrated.

Some producers also believe that calves drinking more water would consume less milk (Leadley et al., 2010). On the contrary, calves offered drinking water since birth consumed more milk during the first 16 days (6.25±0.10 vs. 5.89±0.10 kg/d) as well as the rest of the pre-weaning period (8.23±0.07 vs. 7.96±0.07 kg/d) than the calves offered drinking water later in the present study. Moreover, the intensity of scours and the number of days with scours were similar between
both groups opposing the concern of some producers that drinking water would increase the incidence of diarrhea. On the other hand, when measured at 14 days of age, calves with drinking water had greater sodium concentration in blood than calves without drinking water suggesting drinking water could enhance the electrolyte balance in the body. Pre-weaning calves offered drinking water since birth tended to achieve greater body weight and heart girth than those first offered drinking water at 17 d of age. When measured over three weeks post-weaning, calves offered drinking water since birth had greater hip height and body length than those first offered drinking water at 17 d of age. Van De Stroet et al. (2016) showed positive relationships of body weight and hip height of young calves with body weight and milk yield in the first lactation, respectively. In partial agreement with those findings, we observed that calves drinking free water since birth and having greater body weights during pre-weaning period had greater body weight at five months of age as well.

At two weeks of age, calves with drinking water had greater number of bacterial species in feces than calves without drinking water suggesting a favorable impact of drinking water on diversity of microbiota in the developing gut. Nonetheless, as calves grew and weaned, the bacterial species number and the diversity became similar between calves having drinking water since birth and later. Interestingly, the calves deprived of drinking water consumed 59% more of it, once offered. Rogers et al. (1982) and Fraley et al. (2015) found cows drinking more water had lower liquid weight in the rumen, and higher liquid passage rate from the rumen than cows drinking less water. They also reported lower molar proportions of butyrate and propionate in the rumen. Butyrate and propionate are well known stimulants for development of the rumen and the small intestine in calves. Therefore, it could be assumed that the elevated drinking water intake of those calves might have negatively affected the development of the rumen and/or the distal gut. In line
with this idea, apparent total-tract digestibility of ADF and NDF were lower in calves first receiving drinking water at 17 days of age compared to those receiving it since birth. Moreover, increased drinking water consumption would be negatively related to the abundance of some bacterial taxa in the gut. For instance, Maele et al. (2017) reported significantly negative correlations between drinking water consumption of pre-weaned calves and the abundance of genera *Bifidobacterium* and *Ruminococcus* in feces. Consistently, the calves first offered drinking water since 17 days of age had lower abundance of beneficial bacteria *Faecalibacterium prausnitzii*, and *Bifidobacterium breve* in feces, in the present study. Overall, the present study highlighted that offering drinking water since birth vs. later at 17 d of age has a potential to improve growth, nutrient digestibility, feed conversion efficiency, and the abundance of beneficial bacterial communities in the gut of young dairy heifer calves. Investigations into implications of those improvements in young calves for their future performances such as age and body weight at puberty, and milk production in the first lactation could be warranted.

**Literature Cited**


