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# Simulated and Applied Precision Feeding System of High and Low Forage Diets with Different Fat Sources and Sequences of Dietary Fat Concentration in In-Vitro and In-Vivo Studies

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# SIMULATED AND APPLIED PRECISION FEEDING SYSTEM OF HIGH AND LOW FORAGE DIETS WITH DIFFERENT FAT SOURCES AND SEQUENCES OF DIETARY FAT CONCENTRATION IN IN-VITRO AND IN-VIVO STUDIES

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## ABSTRACT

Controlling dry matter intake (**DMI**) is one strategy to meet the animal's requirements while reducing feed costs and increasing feed efficiency. Controlling intake through precision-feeding provides a more nutrient-dense diet, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss. The literature about precision feeding has provided information regarding optimal N intake and different dietary fiber proportions, but more information needs to be addressed. This is one of the first attempts to further our knowledge through the use of fat inclusion. In the present dissertation, a total of 4 in-vitro and in-vivo experiments were conducted. Simulated and applied precision feeding with different forage to concentrate (**F:C**) ratios and fat sources inclusion were used to determine the effect on Holstein and Jersey dairy heifer's digestibility and fermentation.

An introduction to the importance of investigating strategies to fat supplementation in precision feeding for dairy heifers is presented in Chapter 1. Background information and justification of the current dissertation is presented in the systematic Literature Review in Chapter 2. The objective of the first experiment presented in Chapter 3 was to screen the effects of including different types of fat to different F:C ratio on digestibility and in-vitro gas production (**GP**). Treatments included either low forage (**LF**; 35%) or high forage (**HF**; 70%) with 2 dietary fat concentrations (6 or 9% DM) screening for 6 different fat sources plus control (**CON**). The CON diet had a basal fat concentration in the diet [3% fat (0% fat inclusion); and fat sources were added to attain 6% or 9% fat and consisted of Coconut oil, **CO**; Poultry fat, **PF**; Palm oil,

**PO**; Palm kernel oil, **PKO**; Ca Salts, **MEG**; Soybean oil, **SOY**]. Modules were randomly assigned to treatments in a 2×2×7 factorial design and incubated for four 24 h runs. The CO-fed module had the highest DM apparent digestibility (**AD**), followed by SOY and PF. The true DM digestibility (**IVTDMD**) and OM AD were the highest in CO than the other types of fat. The AD for DM, OM, NDF, ADF, and IVTDMD was higher in LF. Total VFA was lower in modules fed different fat types than the CON and acetate, while propionate was the lowest for the CON, which increased the A:P ratio. These results suggested that LF diets with high fat concentration can be used under a precision feeding system, and different types of fat sources may improve DM and fiber digestibility.

The second experiment's objective presented in Chapter 4 was to evaluate the effects of fermentation and digestion of including different fat sources when high concentrate diets with high-fat inclusion are used to simulate precision feeding in continuous culture. Four treatments were randomly assigned to 8 continuous cultures in a randomized complete block design and ran for 2 periods of 10 d. Diets included high concentrate (**HC**; 65%) with high fat inclusion starting with a basal level of fat as CON [3% fat (0% fat inclusion); 9% fat (6% PF; CO; SO inclusion)]. The DM, OM, NDF, ADF, and hemicellulose digestibility coefficients (**dC**) were higher for PF-fed fermenter, and CO followed by SO and then CON. Total VFA was higher for CON, and there was a reduction in acetate and propionate with different fat treatments. These results suggest that simulated precision feeding with HC and high fat supplementation can improve digestibility.

Chapter 5 presents the third experiment to determine the effects of simulated precision feeding of different PF levels at different F:C ratios on digestibility and fermentation in continuous culture. Treatments included 2 forage combinations, low (LF; 35% forage), and high (HF; 70% forage) and 4 levels of PF starting with a basal level of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments were randomly assigned to 8 fermenters in a 2×4 factorial design and ran for 4, 10 d periods. The LF-fed fermenter had higher DM, OM, N, starch, and NFC dC than HF. Nutrients digestibility increased linearly with PF inclusion. Bacterial efficiency was decreased with PF inclusion. Total VFA was higher for LF, and there was a reduction in acetate with LF. The PF inclusion had a linear increase in total VFA, a linear reduction in acetate, and a linear increase in propionate. The A:P ratio decreased linearly in both LF and HF as PF increased. These results suggest that increasing PF in precision fed LF or HF can alter rumen fermentation and improve digestibility.

Finally, the last experiment's objective in Chapter 6 was to evaluate the effects on nutrient digestion and rumen fermentation of including different levels of PF in precision fed Holstein and Jersey dairy heifers. Four Holstein and 4 Jersey cannulated heifers were randomly assigned to 4 treatments, included a 55% forage diet with 4 increasing PF inclusion starting with a basal concentration of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments were administered according to a split-plot, 4×4 Latin square design for 4 periods of 21 d. Holstein-group had a lower DM, OM, NDF, ADF, and NFC AD than Jersey-group. The inclusion of PF did not affect AD. However, starch AD increased linearly as PF increased, whereas NFC AD decreased

linearly. Manure output was higher for Holstein, and the PF inclusion showed a linear decrease in manure output. Total VFA, acetate decreased linearly as PF increased. Concurrently there was a linear increase in propionate, resulting in a linear reduction in the A:P ratio. These results suggest that Jerseys utilized nutrients more efficiently than Holsteins. Dietary PF inclusion up to 6% in the rations can further reduce DMI in precision feeding programs without compromising total-tract digestibility.

Overall, these studie's results indicate that PF can be used as a replacement for corn in precision-fed Holstein and Jersey dairy heifer diets up to 6% DM. Other fat sources with different characteristics can be utilized with relative success, but further research is needed. Incorporation of supplemental fat to controlled intake strategies such as precision-feeding can reduce feed intake for optimal growth, promising impacts on costs. Furthermore, nutrient digestibility, rumen fermentation, and animal performance can be enhanced with positive effects on environmental impact.

## DEDICATION

I would like to dedicate this work to my supportive and loving parents.

## ACKNOWLEDGMENTS

I owe a great deal of gratitude to my advisor, Dr. Lascano, for taking me on as a graduate student. I have enjoyed the opportunities you have provided for me and appreciate your support. I hope that I helped to develop the program you envisioned for Clemson AVS with the Ruminant Nutrition Research Team. Thank you to my other committee members, Dr. Jenkins, Dr. Aguerre, and Dr. Bridges, for your advice and expertise throughout my time at Clemson. It was an honor to work with you all, and I could not have asked for a better committee.

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# CHAPTER ONE

# GENERAL INTRODUCTION

Raising dairy heifers is one of the highest routine costs on a dairy farm because dairy heifers are fed, bred, housed, and cared for over a resource-draining period before they start generating revenue. Therefore, one of the essential intangible goals in a dairy farm is to find an efficient system to raise dairy heifers economically. Even though dairy heifers represent the second greatest contributor to the whole dairy farm expenses (Heinrichs, 1993; Tozer and Heinrichs, 2001), most of the research focuses on lactating dairy cattle growing animals are not a priority. Notably, dairy heifers represent the operation's future and necessary for the dairy farm enterprise (Heinrichs et al., 2013).

In recent years, research in dairy heifer nutrition has been one of the most increasing areas of interest. That is basically because the feed costs represent the most considerable expense in raising dairy heifers representing over 60% of the total cost (Gabler et al., 2000; Harsh et al., 2001; Heinrichs, 2013). Wild ruminants can select a diet that is appropriate to their nutrient requirements. As an innate antipredator, they have adapted to intermittent feeding cycles to avoid grazing at night; therefore, forages consumed result in slower passage rate and more efficient digestion (Jensen, 2017). Consequently, in the last decade, the research has focused on alternative, more efficient feed management practices and less expensive by-products to reduce feed expenses. Precision-feeding dairy heifers have proven to substantially reduce feed intake by feeding a more energy-dense diet to meet the nutritional requirements while nutrient losses are minimized (Zanton and Heinrichs, 2009; Anderson et al., 2015). Accordingly, precision

feeding improves feed efficiency through a reduction in DMI. It provides the heifer with an adequate amount of nutrients to reach the targeted average daily gain (ADG) thus, controlling heifer growth to maximize milk production in subsequent lactations (Hoffman et al., 2007; Zanton and Heinrichs, 2008). Controlled intake programs are a classical physiological based method to reduce feed expenses that have been reported in beef cattle (Koch et al., 1963; Loerch, 1990; Galyean et al., 1999). High forage diets are rich in fiber and inherently inefficient in energy and protein utilization (Moody et al., 2007; Zanton and Heinrichs, 2007). That can be enhanced by incorporating energy-dense sources such as concentrates that provide readily available nutrients that allow reduced intakes to precisely meet heifer requirements on a feeding system (Hoffman et al., 2007; Lascano et al., 2016). However, high-level addition of concentrates can reduce fiber intake and rumen acidosis incidence (Palmquist and Jenkins 1980). Also, the food competition between humans and livestock, even though about 86% of livestock feed is not fit for human consumption, but grains still account for about 13% of the global livestock DMI (FAO, 2018). Modifying the forage to concentrate ratio (F:C) and manipulating nutrient fractions allowed precision-fed dairy heifers to achieve adequate nourishment, improved N and OM digestibility (Zanton and Heinrichs, 2009), and resulted in similar effects on rumen fermentation (Lascano and Heinrichs, 2009; Lascano et al., 2009).

Feeding supplemental fat has gained interest in the last few decades. Adding fat to dairy diets became standard practice for its potential to increase energy density in diets, improve palatability, and reduce dietary dustiness (Azain, 2004). Also, cost-effective by-products from numerous industries can be utilized by ruminants. Several studies

conducted on dairy heifers fed dietary fat up to 5% and 7% DM from traditional high or low-fat distillers grains (DDGS; Anderson et al., 2009, 2015; Schroer et al., 2014; Suarez-Mena et al., 2015). They observed similar total-tract digestibility compared with control diets and a DMI reduction by increasing dietary fat content with no adverse effects on nutrient utilization while maintaining ADG and overall growth performance.

Moreover, different fat sources have shown different effects on nutrient digestibility and rumen fermentation. In a study conducted by Elliott et al. (1997) on the impact of saturation of fat sources in steers, they reported that increasing fat saturation tended to increase the NDF and ADF digestibility in the rumen. Other studies have reported no differences in ruminal or total tract digestibility of OM or fiber in lactating cows fed diets with increasing amounts of dietary fat or different sources of fat (Palmquist, 1991; Drackley and Elliott, 1993). Oldick and Firkins (2000) reported that acetate responded quadratically as the fat sources' unsaturation degree increased. Also, Elliott et al. (1997) reported decreased acetate's molar proportion when different saturation fat was fed and increased linearly as saturation increased. However, there is limited research regarding the effects of feeding fat on growing dairy heifers, and to what extent can be strategically incorporated into precision feeding is unknown.

On the other hand, due to lack of research, current guidelines for feeding dairy cows in the U.S. do not make a specific recommendation for Jerseys (NRC, 2001). Based on the Council of Dairy Cattle Breeding (CDCB; 2015), Jersey is the second breed most popular in the U.S., and its percentage of cow population increased from 4.9 to 6.4 % from 2009 to 2014, while the Holstein population decreased from 89.6 to 83.9%. Also,

there are indications that Jerseys have higher total tract digestibility of nutrients than Holsteins. Olijhoek et al. (2018) reported that Jersey had a higher DM and OM digestibility than Holstein cows fed diets with different two F:C ratios.

Therefore, this research will examine the effect of increasing fat inclusion in simulated and applied precision feeding systems with different F:C ratios on Holstein and Jersey dairy heifers. The overall objective is to determine how various fat sources can be incorporated into precision feeding rations to optimize nutrient utilization, fermentation, and digestibility without impacting animal performance. Overall, the hypothesis is that replacing non-fiber carbohydrates with fat in a precision feeding system will further reduce intake without compromising nutrient digestibility, rumen fermentation.

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# CHAPTER TWO

# LITERATURE REVIEW

## HISTORY OF ANIMAL FEEDING

It is essential to study animal feeding history and understand how and why particular practices have advanced. Animal feeding systems were developed before the advent of writing. Farm management and animal breeding techniques developed spontaneously around 10,000 years, b.c. ago in several ancient areas of early human civilization (Coffey et al., 2015). A crescent-shaped area of fertile land in the Middle East that extends between the valley of the Tigris and Euphrates rivers (Mesopotamia) across to the northeast of the Nile valley was the center of the Neolithic development of agriculture, and the cradle of the Assyrian, Sumerian, and Babylonian civilizations (Clay, 1924). The development of technology and innovation allowed animal agriculture to be more productive, efficient and organized as the world's population grew. With increased demand, it was necessary to provide animals with a nutritionally balanced diet. All these changes in agriculture in general and animal feeding practices began around the beginning of the 19th century (Pederson, 2000).

During the industrial revolution, there was an increase in grain milling waste. The benefits of a balanced diet in animal production and the need to redirect by-products from human food were being realized (Freket and Stark, 2011). The modern feed industry was initiated when the chemical analysis proved the nutritional value of these grain by-products. Nowadays, co-products of grain and animal processing are the appropriate name for these by-products (Schoeff et al., 2005). At the beginning of the 20th century,

feed manufacturing advanced rapidly in all aspects and saw a rapid mill evolution. Companies were involved in the feed industry to utilize grain processing by-products instead of disposing of them. In 1950, diet formulation became more complicated with antibiotics, identifying essential trace minerals, and developing synthetic vitamins. In 1975, the first automated feed mill began to operate, which helped maximize feed production efficiency and minimize the cost of feed per animal produced. Technology and software improved many-sided manufacturing, such as particle size characteristics, mixing and batching, pellet processing, feed delivery logistics, and others (Ferket and Stark, 2011). In 1990, more advanced equipment, including liquid applicators, NIR for nutrient analysis, data collection in the feed mill, and the overall computerization of operations.

In 2050, it is estimated that the global population will count 9.6 billion (FAO, 2018). To feed them, research must continue to increase our knowledge and understanding of nutrient balance and digestion. New nutritional strategies such as feed additives must be involved to achieve higher animal performance while decreasing feed costs. In the future, advanced technology will become further involved in feeding systems. That will include analytical techniques such as genetic selection, nutrigenomics, and bioinformatics, improve the recycling of by-products into livestock feed, and feed consumption for improving animal production efficiency while reducing waste. In this way, by involving these technologies in feed formulation, the nutritional value will become a more precise science. Also, dairy products will increase and fill the increasing population demands (Coffey et al., 2015; FAO, 2018).

## FEEDING SYSTEMS

Management of the growing dairy heifer is a balance between what is biologically reasonable and economically efficient (Bewley, 2010). Raising dairy heifers at a low economic and environmental cost is the goal of each dairy heifer program taking into account the future production, health, and welfare of these animals (Sejrsen and Purup, 1997; Hoffman et al., 2007; Kitts et al., 2011). Usually, producers do not emphasize dairy heifer management properly. That is because heifers are nonproductive and provide no immediate economic benefits until calving and the onset of lactation (Zanton and Heinrichs, 2009a; Kitts et al., 2011). To optimize heifer's body weight gain before calving and develop the mammary gland properly for future milk production, adequate heifer nutrition is the key. Many farmers do not know that dairy cows' future production is based on the impact of heifer nutrition. Even though dairy heifers represent the second greatest contributor to the whole dairy farm expenses, most dairy farmers are focused on lactating dairy cattle, and dairy heifers are not their priority (Tozer and Heinrichs, 2001; Harsh et al., 2001). Very little research has been done on dairy heifer nutrition compared to dairy cow nutrition in the past 50 years, whereas most of the research on dairy heifers focused on calf nutrition and colostrum (Eastridge, 2006). Great attention must be provided to dairy heifer rearing programs because they are the future of milk production (Heinrichs et al., 2013). The farm profitability and productivity can be affected by any dairy heifer’s management (Hutjens, 2004; Zanton and Heinrichs, 2005). In the last few years, there has been tremendous progress toward optimizing heifer’s growth rates, reducing AFC, nutrition, and management. Also, producers have become more

progressive in using some practices based on heifer's nutrients requirements to help them economically (Heinrichs et al., 2017). Researchers have the opportunity to better understand an ideal feeding regime that should be used for animals to provide nutrients in amounts that maximize ruminal fermentation, growth of rumen microbes. At the same time, minimize nutrients losses to the environment (NRC, 2001). In this literature review, we will go through some feeding systems that have been used in dairy heifers programs, such as ad-libitum feeding and restricted feeding.

*Ad-libitum Feeding*

Traditionally, dairy heifers are fed ad-libitum where the diets are mostly high in forage but low in energy and formulated to not necessarily meet their requirements (Pino et al., 2018). In U.S. ad-libitum systems, heifer's energy intake requirements are determine based on the NRC (2001) with a strategy of targeting 1 kg/d as an ADG. Ad-libitum heifers are usually fed with high-forage, low-energy diets at 110% of the expected intake to meet growing animals' nutrient requirements (Greter et al., 2013). However, the dry matter intake could be limited based on the high amount of fiber consumed by dairy heifers under the ad-libitum feeding system. These heifers are physiologically inefficient regarding the forage digestion, and utilization meets the animal's requirements (Pino et al., 2018). Ad-libitum feeding system can result in intake discrepancy between individual heifers, possibly affecting the rumen health through TMR sorting habits (Hoffman et al., 2006; Greter et al., 2008). Thus, heifers can potentially consume a ration that is not balanced for their needs and might increase the risk of metabolic disorders, difficulties to target actual growth rate, and decrease feed

efficiency (DeVries, 2010). Also, feeding ad-libitum diets reduces nutrient digestibility, increasing waste, which could negatively affect the environment.

Another ad-libitum feeding strategy is formulating a nutrient-dense diet and then diluting it with a low-nutritive feedstuff such as straw to limit the feed's nutrient density (Hoffman et al., 1996). Also, the passage rate will decrease, and rumination time will increase by these low-nutritive feedstuffs, which increase the production of the saliva and the buffering capacity of the rumen. Greter et al. (2008) observed that feeding TMR ad-libitum with increasing straw levels resulted in decreased daily DMI, rate of feeding, size, and meal frequency, whereas the time of feeding time and duration of a meal increased. Also, heifers in this study achieved an ADG of 0.9 kg/d on 20% straw dilution and 1.0 kg/d on 10% straw dilution. That indicates that when the ration is appropriately balanced, it may enable producers to effectively target growth rate while reducing DMI while providing a foraging substrate, which helps the heifers to fill their natural forage requirements and behaviors. Reducing the first calving age to 22 to 23 mo reduces the expenses during the nonproductive phase of a dairy animal (Heinrichs, 1993). To do this, improving growth performance and feed efficiency is necessary (Hoffman et al., 2007). It has been shown that increasing dietary energy density can reduce the first calving age from 25 to 21 mo, and the cost of raising dairy heifers as well by 18% (Tozer and Heinrichs, 2001). However, several studies showed that when heifers offered a higher energy diet, their pre-puberty growth rate increased while reducing first lactation milk yield (Little and Kay, 1979; Foldager and Sejrsen, 1991). A study was conducted to compare ad-libitum feeding and limit feeding on behavior patterns and feeding

motivation of dairy heifers. It has been recommended that a low nutritive feedstuff is very important to be provided with limit-fed TMR to allow dairy heifers to practice a "normal" feeding behavior and diurnal feeding patterns (Greter et al., 2015).

Without controlling dry matter intake, dairy heifers were fed high energy diets to increase their average daily gain in a study conducted by Little and Kay (1979). They reported that the milk yield in the first lactation heifers was decreased between 15 to 48%. Tremere et al. (1968) switched heifer diets to high concentrate from high forage by using ground wheat as readily digestible carbohydrate and observed lactic acid accumulation in the rumen; rumen pH declined under 5.0, a reduction in fiber digestion and VFA concentration. Also, depression in the abundance of cellulolytic bacteria was observed when heifers were fed high concentrate diets (Tajima et al., 2001). Calsamiglia et al. (2008) also stated that high concentrate diets reduced rumen pH, acetate, and butyrate concentration. Furthermore, it reduced the digestibility of OM and NDF and reduced nutrient utilization efficiency. However, as a reduction in the calving age is desired, researchers have been studying how energy and DMI affect heifer growth without affecting their production, health, and welfare (Hoffman et al., 2007; Moody et al., 2007; Lascano and Heinrichs, 2009; Zanton and Heinrichs, 2009b; Pino and Heinrichs, 2016). In a recent study, Pino et al. (2018) compared ad-libitum versus precision-fed diets on rumen fermentation, nutrient digestibility, feed efficiency, and Holstein heifers' passage rate. They reported that ad-libitum diets showed lower feed efficiency and rumen pH. In contrast, total VFA concentrations were higher, and the

passage rate was faster, also showed a higher digestion rate with shorter retention time in the rumen than precision diets.

*Restricted Feeding*

In general, restricted feeding refers to feeding the diets in a limited amount or offering ad-libitum access to a diet for a limited time (Greter et al., 2013). Sometimes feed is restricted, but ME and N are similar, known as limit feeding. The feed is restricted for several reasons: avoiding obesity, improving feed efficiency, decreasing feed costs, improving growth and reproductive efficiency, and decreasing nutrient excretion (D'Eath et al., 2009). Sometimes feed allowance appears to be ad-libitum, but the diet quality is reduced, which is still considered restricted feeding because it is consuming a low-quality diet containing less energy and nutrients. These rations are usually diluted through bulky feedstuffs addition (D'Eath et al., 2009). Over the years, research has shown that restricting-feeding has proven successful in many domesticated species such as growing and gestation sows in the swine industry, finishing cattle in the beef industry, and dairy heifers and dry cows in the dairy industry (Loerch, 1990; Susin et al., 1995; Loerch, 1996; Wertz et al., 2001).

Gestating sows are typically fed a nutrient-dense ration in an amount that is approximately 60% of their ad-libitum intake to restrict their feed (Kyriazakis and Savory, 1997). Restricted-fed sows showed increased feeding rate and decreased time spent feeding when sows were restricted-fed compared to ad-libitum-fed animals (Terlouw et al., 1991; Bergeron et al., 2000). Feed restriction in beef cattle is usually done to increase feed efficiency, decrease nutrients excretion, and feed costs (Murphy

and Loerch, 1994; Galyean, 1999). The concept of compensatory growth is typically utilized in growing beef cattle where animals are given ad-libitum access to feed and then a period of feed restriction, resulting in a more efficient deposition of muscle mass (Galyean, 1999). Sainz et al. (1995) reported that calves during the growing period were more efficient during the subsequent finishing phase and showed more significant compensatory growth when restricted-fed than calves ad-libitum-fed high-forage diet. They attributed that to the changes in feed intake and the higher energy requirements that high concentrated in restricted feed can offer. Tamminga et al. (1979) conducted a study examining the effect of feed intake level on the quantity of protein entering the small intestine. Two methods were used to estimate the protein degradation, the first one was based on diaminopimelic as a marker, and the second was based on regression. They observed N's greater flow to the small intestine was a portion of the N ingested when intakes were high compared to low intakes. Due to the increase in the passage rate, N's ruminal degradation was lower at a higher level of intake. Wertz et al. (2001) evaluated the intake restriction on beef heifers' performance and carcass merit during the finishing phase limit-fed or ad-libitum fed corn gluten feed. Limit-fed beef heifers did not have compromised feed efficiency than ad-libitum fed heifers with 0.135 versus 0.124 gain:feed, kg/kg, respectively, and the diets allowed all heifers to achieve a moderate rate of gain. Schwartzkopf-Genswein et al. (2002) stated that even though cattle consumed more DM when fed ad libitum, restricted-fed cattle consumed more feed during the first 3 h period after feed delivery. Improvement in feed efficiency has been observed in beef cattle managed under restricted feeding programs (Hicks et al., 1990; Loerch, 1990), with

an increase of over 15% observed in cattle fed a high-grain ration. That is mainly related to the higher digestibility of high grain diets than high forage diets (Klinger et al., 2007).

Feed restriction also occurs in dairy calves, which are rarely fed ad-libitum in commercial production, with most receiving only 10-15% of their body weight (Jasper and Weary, 2002). It has been shown that this amount is insufficient to satisfy hunger, and calves under restricted feeding make more visits to milk feeder, but that ensure optimal growth and development (De Paula Vieira et al., 2008). Studies have reported that managing dairy heifer under a typical limit feeding program leads to more efficient dry matter digestibility and less manure output without sacrificing growth or performance (Driedger and Loerch, 1999; Hoffman et al., 2007). Driedger and Loerch (1999) reported that limit-fed nonlactating dairy cows resulted in a 15% greater DM digestibility. In this study, DMI was restricted by 30% for cows fed the high-corn diet (6.8 kg/d) compared with the high-forage diet (9.6 kg/d). Also, they found a reduction in DM, N, and manure excretion, and they attributed the decrease in fecal output to a reduction in nitrogen output as ruminant animals fed a low-forage diet have been shown to have improved nitrogen retention and efficiency (Driedger and Loerch, 1999; Moody et al., 2007). Hoffman et al. (2007) observed an improvement of 28.9% in feed efficiency of limit-fed dairy heifer than the ad-libitum-fed diet. Zanton and Heinrichs (2007) fed Holstein heifers a high concentrate diet versus a high forage diet and found a decrease in their DMI by 0.64 kg/d. It has been reported in several studies that a limit-fed diet can successfully control ADG to ensure reaching an optimum weight and age at calving, perhaps much more effectively than ad-libitum feeding. Hoffman et al. (2007) stated no

differences in ADG and body condition score in limit-fed heifers than ad libitum-fed heifers. Zanton and Heinrichs (2007) also observed no differences in ADG between heifers fed an HC or HF diet.

Although the limit-feeding program has various benefits such as reducing feed costs and more significant economic and nutrient management benefits, there have been welfare concerns associated with this method. Greter et al. (2015) conducted a study comparing heifers' behavior on a high concentrate, low forage, limit fed diet to those on a traditional high forage diet. It was reported that heifers on the limit fed diet had increased motivation for access to a low-nutritive feedstuff and increased time spent standing without eating. In natural conditions where feed is not available ad-libitum, cattle typically participate in foraging behavior only for 4 to 9 hours per day (Hafez and Bouissou, 1975). Therefore, this change in behavior in heifers undergoing a high concentrate limit-feeding program can be attributed to either insufficient gut fill or inadequate foraging time. However, feeding a low nutritive feedstuff to limit-fed heifers may improve behavioral concerns. Kitts et al. (2011) provided a low-nutritive feedstuff to examine the heifers' behavioral and growth effects with a limit-fed high-concentrate ration. Wheat straw was mixed with TMR, offered on the side, or not offered, and TMR was fed at 2.02% of BW. Adding straw to the diets increased feeding time, rumination time, decreased inactive standing time, maintained ADG, and improved feed efficiency with 6.3 vs. 9.9 DMI/ADG in limit-fed and TMR mixed with straw fed heifers, respectively (Kitts et al., 2011). Therefore, feeding heifers a wheat straw can help their natural foraging behavior. A study published by Hoffman et al. (2007) also expressed

welfare concerns for limit fed dairy heifers, stating that the diet was associated with increased vocalizations and oral stereotypes that may suggest hunger and frustration. However, Zanton and Heinrichs (2007) state that this increase in vocalization will diminish 10 to 14 days after implementing the diet and that this behavior is due to a moderate reduction in rumen and gut size.

*Precision Feeding*

Recent research has focused on nutritional changes by increasing the diets' energy density while reducing DMI (limit-feeding). That alters and improves dairy heifers' feed efficiency without compromising rumen fermentation and milk production (Zanton and Heinrichs, 2005; Hoffman et al., 2007; Hall, 2008; Zanton and Heinrichs, 2009b). Wild ruminants can select a diet that is appropriate to their nutrient requirements. As an innate antipredator behavior, they have adapted to intermittent feeding cycles to avoid grazing at night; therefore, forages consumed result in slower passage rate and more efficient digestion (Jensen, 2017). Limit feeding an energy-dense diet that provides isocaloric and isonitrogenous nutrients required for optimal growth with a targeted ADG of about 800 g/d in dairy heifers is a feeding program known as precision feeding. Precision diets are selected on cost, availability, and nutrient composition, but the metabolizable energy (ME) and nitrogen (N) content should stay constant to meet the dairy heifer requirements, reduce the expenses of growth energy, and improve feed efficiency (Zanton and Heinrichs, 2009b). Based on Lascano and Heinrich (2009), precision-feeding programs provide the heifer with precise nutrients to reach the targeted average daily gain (ADG). Approximately 800 g/d is recommended prepubertal ADG for large breed dairy heifers to

maximize first lactation milk yields. In several studies, a precision feeding system has shown improves feed efficiency, reduces nutrient losses, and decreases manure production (Hoffman et al., 2007, Moody et al., 2007; Lascano et al., 2009; Zanton and Heinrichs, 2009b; Pino and Heinrichs, 2016). Moody et al. (2007) and Lascano et al. (2009) observed an increase in DM digestibility and feed efficiency in dairy heifers fed with HC diets compared to the LC diet. Rumen fermentation characteristics were similar, with no effects on animal's health.

Previous research has demonstrated that limit-feeding dairy heifers do not negatively impact growth characteristics. Zanton and Heinrichs (2007) conducted a study to investigate the effect of limit-fed LC or HC rations on 42 dairy heifers with approximately 4 months of age and similar prepubertal rates ADG. Diets were formulated using corn silage and grass and were limit-fed to achieve 800 g/d ADG. They observed no differences in BW gain, withers height, heart girth, body length, or hip-width between treatments. The high concentrate diet was not expected to result in more significant visceral fat. Also, it has been demonstrated that future lactation performance did not decrease when heifers were limit-fed. Hoffman et al. (2007) conducted research on 54 gravid Holstein heifers to evaluate the effects of limit-feeding on growth, feed efficiency, behavior, and subsequent lactation performance. Eating time was higher for heifers fed the control diet than those fed the 90 and 80% limit-fed diets, 19.3, 15.7, and 10.3% of the time, respectively. Additionally, limit-feeding heifers slowed the passage rate and resulted in greater ruminal retention time and increased ruminal degradation and nutrient utilization. Also, they found no differences in milk yield, fat milk yield, milk fat

percentage, milk protein percentage, or milk protein yield. They suggested that limit-fed heifers did not decrease the lactation performance, and based on the projected first 305 d lactation data, it may improve milk and fat yield.

Lascano et al. (2009) concluded that limit-feeding prepubertal dairy heifers high concentrate diets did not significantly affect most structural growth characteristics. This study also reported a difference in the bacterial numbers between HC and LC diets, but the total ruminal VFA concentrations stayed constant. Also, they reported that a high concentrate diet decreased the wet and dry rumen mass, which has been supported by evidence from other studies (Hoffman et al., 2007; Lascano and Heinrichs, 2009; Greter et al., 2015). In addition, milk yield and its component showed equal or improvement at 150 d of lactation as heifers limit-fed compared to heifers fed traditional high forage diets for equal ADG. In the precision feeding program, every kg reduction in dry matter intake equals 2.6 kg decrease in manure excretion (Zanton and Heinrichs, 2008a). Lascano and Heinrichs (2009) and Lascano et al. (2009) reported that limit-fed dairy heifers high concentrate diets significantly reduced manure output. The most important thing from this reduction in manure excretion is that the nutrient losses are reduced, also reduces the expenses related to the labor of manure management and disposal.

*Feed Efficiency*

Several factors can affect feed efficiencies, such as nutrient digestibility, forage quality, growth rate, age, body condition, physical activity, gestational stage, temperature, and genetics (Zanton and Heinrichs, 2008b). Feed efficiency in dairy cattle has a lower heritability than beef cattle because of the selection (Arthur et al., 2001; Van Arendonk et al., 1991). Dry matter intake, protein, energy, other nutrient requirements, and average body size of dairy cows had increased when genetic selection towards greater milk production started (Gabler et al., 2000). The effect of DMI on feed efficiency has been widely studied in dairy heifers. Feeding dairy heifers with NRC (2001) recommendations generating over-conditioned dairy heifers by greatly exceeding the optimum ADG, even though the energy intake is limited under the traditional low-energy, high-forage diets because of the high fiber content (NRC, 2001) as well as prevent fat deposition in the pre-calving heifers (Hoffman et al., 2007; Anderson et al., 2015; Akins, 2016). Limiting feed intake in dry cows helps improve dry matter digestibility and reduce feed costs (Driedger and Loerch, 1999). Similarly, reducing feed intake in dairy heifers were observed to control growth rates without affecting milk yield at first lactation (Lammers et al., 1999).

Maximizing energy intake does not maximize feed efficiency because the relationship is not linear between both (Ferrell and Jenkins, 1998). Feed efficiency under limited feeding is improved by nutrient utilization management (Loerch, 1990; Galyean et al., 1999). As dry matter intake by animals increase, the metabolic nutrient cost of

digestion increases as well. Nutrient digestion and absorption use a great portion of dietary energy due to the intense oxidative metabolism requirement, and the remaining energy is used for maintenance, growth, and productivity (Pino and Heinrichs, 2016). The gastrointestinal tract, liver, spleen, and pancreas use around 40 to 50% of body oxygen consumption. Metabolic activity and oxygen consumption increase as the amount of nutrients to digest increase (Huntington and Reynolds, 1983; Reynolds et al., 1991b). Reducing intake to 20 or 30% from ad-libitum in growing steers improved feed efficiency by 30% while the diets had the same NE for maintenance and growth, and animals sustained the same ADG (Loerch, 1990). The reduction in the rumen passage rate is the reason behind the improvement in feed efficiency when DMI is reduced, allowing an increase in nutrient digestibility (Tamminga et al., 1979; Loerch, 1990). The increase in nutrients digestibility help to reduce the nutrients lose and any decrease in DMI result a reduction in liver and gut sizes (Hoffman et al., 2007; Reynolds et al., 1991b), and that could help reduce the energy requirements for maintenance and increase the availability of energy for growth (Loerch, 1990; Hoffman et al., 2007). Wertz et al. (2001) observed improved feed efficiency as DMI reduced in heifers fed energy-dense diets. Feed efficiency improved about 23.7 and 28.9%, respectively, when heifers feed intake decreased around 10 or 20% compared to ad-libitum diets; the manure output decreased by 12.9 and 34.6% (Hoffman et al., 2007).

Overall, dairy heifer feed efficiency is improved without any adverse effects on growth, health, and milk production under the precision feeding program (Zanton and Heinrichs, 2009b). Therefore, precision feeding fat to dairy heifers could help decrease

the amount of DMI further, reducing the passage rate in the rumen, increasing the digestion and absorption of nutrients, and decreasing the nutrient loss and manure excretion. However, there is no information about optimal lipid dietary concentration and sources when using precision feeding in dairy heifers.

*Average Daily Gain*

The age at first calving (AFC) determines the optimum growth rate for dairy heifers, and the suggested AFC (23 to 24.5 months) has not changed over the years (Swanson, 1967; Heinrichs, 1993; Ettema and Santos, 2004). Increasing the AFC will increase the nonproductive life of heifer and their raising cost as well. Therefore, one of the strategies used to reduce the raising dairy heifers costs is reducing the period of growth by increasing the prepubertal average daily gain (ADG) to reach puberty at an earlier age and decrease the AFC before 20 months (Swanson, 1967). However, very low AFC could potentially affect future lactation potential by preventing the normal development of the mammary gland, decreasing first lactation milk production and overall performance of the dairy heifer (Swanson, 1960; Roy, 1978; Radcliff et al., 2000; Zanton and Heinrichs, 2005; Davis Rincker et al., 2008). Increasing prepubertal ADG may reduce mammary development (Sejrsen et al., 1982). Prepubertal ADG and body weight at calving and first lactation culling need to be considered in raising dairy heifers (Hoffman and Funk, 1992; Ettema and Santos, 2004; Zanton and Heinrichs, 2005). In general, an allometric growth rate occurs in the mammary gland before puberty, followed by an isometric growth rate after the onset of puberty (Sinha and Tucker, 1969). When

high energy diets are fed, the insulin-like growth factor-1 receptors become less sensitive (Sejrsen and Purup, 1997).

It has been suggested that the optimum growth can be achieved by restriction of good-quality feed (Swanson, 1967). Meyer et al. (2006) conducted a study on heifers fed an elevated or restricted level of nutrients to support 950 or 650 g/d of ADG and its effect on mammary development. They reported that elevated nutrient intake did not influence the mammary epithelial cell proliferation during the prepubertal period. Also, they observed that when heifers were between 250 and 300 kg of BW, there was a 50% reduction in mammary parenchyma, indicating that the mammary gland was transitioning from allometric to isometric growth. Van Amburgh et al. (1998) fed heifers three different energy diets with varied protein sources within each energy treatment. These diets were designed to achieve ADG of 0.6, 0.8, and 1.0 kg/d from 90 to 320 kg of BW. They reported that the protein source did not show any differences in ADG or milk yield between treatments. However, the results showed that heifers grown at an excess of 0.7 kg/d during the prepubertal period had a 5% decrease in milk yield. Additionally, heifers had greater first lactation milk yield when they reached a bodyweight (BW) 82-90% of mature size at calving. Furthermore, they concluded that the protein supplementation and adequate energy might have synchronized in a better way to meet the tissue requirements to increase gain and enable heifers to reach breeding at an earlier weight without any adverse effects on mammary development (Van Amburgh et al., 1998). Zanton and Heinrichs (2005) conducted a meta-analysis on Holstein heifers to determine the effects of prepubertal ADG on milk production, fat corrected milk yield, milk fat, and milk

protein during the first lactation. They reported that the optimum average daily gain for Holstein heifers was 799 g/d and should be restricted to this level to avoid negative effects on lactation potential. Also, that maximizes milk and protein production at the first lactation in heifers weighing between 150 and 320 kg of body weight (BW).

In addition, postpubertal ADG should also be controlled to avoid any over conditioning at calving because it can be detrimental to future lactation performance (Hoffman et al., 1996; Nor et al., 2013). It has been shown if the dairy farmers in the second year of the heifer rearing period made any dietary mistakes will lead to impaired lactation performance, such as reduced daily milk yield and compromised fertility (Meyer et al., 2006). Roche et al. (2000) found an impairment in cow fertility due to poor feeding management between 12 to 18 months of age. An inadequate feeding and synchronizing between energy and protein can negatively affect fertility and increase early embryonic death. It is essential to adjust the diet to ADG around 800 g/day and support the body frame growth and suppress fat deposition after the breeding and breed at 360-400 kg BW. Meyer et al. (2006) recommended a similar ADG of about 816 g/day. Additionally, St-Pierre (2002) suggested a target BW of adult Holstein cows to be around 630-820 kg. According to Spiekers et al. (2009), carbohydrate supply should be adjusted to prevent fat deposition in the older heifers, mainly starch, and non-digestible starch should be kept at acceptable levels. It has been suggested that heifer feeding around the 7th month of pregnancy is similar to dry cow feeding, and nutrient concentrations in the diet should be increased to levels similar to the production diet only 3 weeks before calving. Patterson et al. (1992) suggested that heifers should receive a well-balanced high-energy diet for

adequate heifer and fetus growth, without over-conditioning the heifer in order to avoid an increased risk of dystocia and metabolic disorders after parturition.

*Passage Rate and Digestibility of Nutrients*

In ad-libitum feeding, the feed intake is usually high, which increases liquid and solid passage rate through the rumen and the GI tract in ruminants (Balch and Campling, 1965; Colucci et al., 1990). The passage rate increases as dietary fiber increase in the diets due to the rumen load increase and evacuation stimulus (Clauss and Hummel, 2005). Whereas, in precision feeding, with the reduction in intake, the rumen passage rate decreases, and the diet retention time increase (Tamminga et al., 1979; Wertz et al., 2001; Lascano and Heinrichs, 2009). In this case, the diet components will further interact with rumen microorganisms, increasing rumen digestion and fermentation (Colucci et al., 1990; Dijkstra, 1992; Zanton and Heinrichs, 2008a; Zanton and Heinrichs, 2009b; Lascano et al., 2016a). The ratio between organs and gut surface to digesta volume stays constant in ad-libitum feeding, while this ratio increases due to changes in the GI tract volume in precision feeding (Clauss and Hummel, 2005). That increases the retention time of nutrients in the rumen and nutrients digestibility because of a greater contact surface with gut enzymes for digestion and absorption (Clauss and Hummel, 2005). Heifers under precision feeding consume and digest less intake than traditional ad-libitum feeding and retain more energy by reducing heat production associated with digestive metabolism, which can be used for growth by tissues (Reynolds et al., 1991b). Dry matter intake is controlled in precision-fed heifers and energy-dense diets to cover the energy and N requirements (Murphy et al., 1994). The reduction in passage rate in precision-fed

dairy heifers will reduce microbial protein flow to the small intestine, compensated by higher protein digestion and N retention (Zanton and Heinrichs, 2008a). Firkins et al. (1986) and Merchen et al. (1986) reported a decrease in rumen digestibility as DMI increase with changes in the pattern of ruminal fermentation in steers and sheep fed different levels of intake.

Colucci et al. (1990) conducted studies on sheep and beef cattle comparing different F:C with low intake diets and reported that nutrient digestibility increase as concentrates increase in the diet due to longer retention time in the rumen. Fecal and urine excretion are reduced as nutrient digestibility increases, which is accompanied by a reduction in emissions; thus, nutrient loss is reduced (Reynolds et al., 1991b). In addition, any reduction in manure production will lead to easier manure management and decrease farm expenses in general. Four different levels of DMI as a high forage diet were offered to dairy heifers to evaluate the passage rate (Zanton and Heinrichs, 2008a). They observed that the rumen passage rate increased as DMI increased up to ad-libitum levels. Additionally, they have observed a higher feed efficiency in limit-fed heifers as DM, OM, and NDF digestibility increased as intake decreased. Lascano et al. (2016a) showed that low forage diets (LF) had a lower turnover rate for solid and liquid fractions than high forage diets (HF). Also, as dietary fiber increased in the diets, the passage rate increased linearly. In addition, as dietary fiber increased in the diets, the DM, OM, and cellulose digestion decreased linearly, whereas a higher DM, OM, NDF, ADF, cellulose, and starch digestibility in LF diets due to the retention time changes. The effect of 3 different intake levels and 3 F:C were evaluated on dairy heifers by Lascano and

Heinrichs (2009). They have observed a lower DM turnover rate and a higher rumen retention time in heifers that consumed the smallest F:C. Pino and Heinrichs (2016) conducted a study to evaluate 4 different DMI with 4 different starch concentrations in dairy heifers' diets. They have reported that the DMI decreased linearly as dietary starch concentration increased. As starch concentration increased in this study, the DM, hemicellulose, and starch digestibility increased linearly. However, treatments did not show any effects on NDF and ADF digestibility. Low OM digestibility and high ruminal passage rate were observed in dairy heifers at 8 and 20 mo of age fed with low energy diets and high DMI (Zanton and Heinrichs, 2016). They have also observed a higher N digestion and retention in heifers that received high energy diets with low DMI compared to low energy diets with high DMI. Passage rate plays a major role in the nutrients digestibility under this system as giving the nutrients a more retention time in the rumen to be fermented and digested.

*Forage to Concentrate Ratio*

Dairy farmers traditionally fed dairy heifers with ad-libitum, high forage components, low energy diets. However, the high fiber-based diets may decrease diet digestibility and result in energy and protein inefficiency (Moody et al., 2007; Zanton and Heinrichs, 2007; Zanton and Heinrichs, 2008b). Precision feeding systems are more cost-effective per unit of energy and protein than forages despite containing more concentrates (Zanton and Heinrichs, 2007). High concentrate (HC), high energy diets have been shown to reduce DMI in dairy heifers, reduce the nutrient loss in ad-libitum diets, and have greater efficiency of using metabolizable energy (ME; Blaxter and Wainman, 1964).

These reductions can help reduce the cost of dairy heifers and benefit the farm economically (Zanton and Heinrichs, 2009b).

The use of high concentrate components has been broadly described in ruminant diets (Lascano and Heinrichs, 2009; Suarez-Mena et al., 2015; Lascano et al., 2016a). When steers consumed HC diets with the same level of ME intake, it has been observed that a reduction in heat production and more energy was used for growth. Additionally, nutrient digestibility increased as diet concentrate increased (Reynolds et al., 1991b; Huntington et al., 1996). Reynolds et al. (1991b) observed that the apparent digestibility of DM, OM, CP, EE, NDF, ADF, and hemicellulose increased in steers that consumed a low forage (25% DM) diet. Similar results were observed in lambs as the apparent digestibility of DM, OM, ADF, NDF, and starch increased linearly when the concentrate proportion increased to 92% of the ration (Murphy et al., 1994). Furthermore, as dietary concentrate increased in cow and sheep diets, the apparent digestibility of DM, NDF, ADF, and hemicellulose increased linearly at low DMI (Colucci et al., 1989).

In dairy heifers, Zanton and Heinrichs (2008a) found that nutrient utilization efficiency was increased as intake decreased even though dairy heifers were fed HF diets but with limit feeding intake at the level needed for maintenance. Additionally, Reynolds et al. (1991b) fed beef heifers with a constant ME from an HC (25:75) or LC (75:25). They found that the HC diet had less heat energy production and retained more tissue energy. It has been suggested that HC diets can still meet the nutrient requirements even though the DMI of the animal is reduced, which is necessary in order to avoid increased ADG (Zanton and Heinrichs, 2007). Feeding HC diets had no negative effect on rumen

fermentation, milk yields, and lower manure output (Hoffman et al., 2007; Moody et al., 2007; Zanton and Heinrichs, 2007; Lascano and Heinrichs, 2009). When HC diets (energy-dense diets) were used, DMI was reduced, and propionate increased at the expense of acetate. Also, microbial N and MPS' efficiency increased (Merchen et al., 1986; Colucci et al., 1990; Zanton and Heinrichs, 2008a). Several studies in precision-fed heifers have proven that reduction in DMI as a high concentrate (HC) included in the diets do not affect rumen pH and fiber digestion (Moody et al., 2007; Lascano and Heinrichs, 2009; Lascano et al., 2014; Ding et al., 2015; Pino and Heinrichs, 2016). Furthermore, ruminal pH was higher for precision-fed dairy heifers compared to ad-libitum feeding. Dry matter and OM apparent digestibility were observed to be higher when dairy heifers fed HC diets (Suarez-Mena et al., 2015; Lascano et al., 2016a; Zanton and Heinrichs, 2016). In addition, it has been reported that the apparent starch digestibility increased with HC diets (Lascano and Heinrichs, 2011; Lascano et al., 2016a; Pino and Heinrichs 2016). Also, Lascano and Heinrichs (2011) and Lascano et al. (2016a) observed that the HC diets increased NDF and ADF digestibility. In contrast, Zanton and Heinrichs (2009a) did not observe any effect on NDF, ADF, and hemicellulose digestibility. It has been clarified that feeding HC diets could limit fiber digestibility by shifting the rumen bacteria towards amylolytic bacteria at fibrinolytic bacteria's expenses (Mertens and Loften, 1980; Calsamiglia et al., 2008).

Furthermore, feeding different F:C diets may show differences in nitrogen (N) partitioning and utilization (Zanton and Heinrichs, 2009b). Lascano et al. (2016a) fed dairy heifers with HC diets and observed no N digestion differences, but N excretion was

reduced, and N retention was higher. In contrast, a high CP and N digestibility was observed by Zanton and Heinrichs (2009a). Therefore, the HC diets lead to an increase in N efficiency by reducing N excretion and increase in N retention (Murphy et al., 1994; Moody et al., 2007; Zanton and Heinrichs, 2009b). In beef and dairy heifers, HC diets have higher N efficiency and retention, even though the N intake is lower compared to LC diets (Reynolds et al., 1991a; Zanton and Heinrichs, 2008a; Lascano and Heinrichs, 2011). When feeding HC diets, fecal DM, urine, and urinary N excretion were mostly lower (Huntington et al., 1996; Zanton and Heinrichs, 2007). Zanton and Heinrichs (2007) concluded that N's use is more efficient when HC diets were fed to the dairy heifer and observed that 1.8 g N intake/kg BW0.75 is the maximum N efficiency in dairy heifers. In general, due to more energy availability in the rumen as in HC diets and rapid growth of microbes that can degrade nutrients faster, the DM, OM, and starch digestibility is higher than in diets with HF components (Merchen et al., 1986). Additionally, dairy heifers fed HC diets under the precision feeding program will reduce DMI and stimulate rumen retention to provide a higher digestion response (Zanton and Heinrichs, 2009b).

*Feed Costs*

Raising dairy heifer is one of the highest routine costs on a dairy farm (Gabler et al., 2000). Dairy heifers are fed, housed, and bred over two years until they calve and produce milk. Heifer market prices vary broadly with the many different systems used in the rearing process to raise these heifers. The cost of raising dairy heifer based on research journals and extension articles varied greatly over the last 20 years, raising each

animal ranging from $1,134.06 to $2,241.00 (Gabler et al., 2000; Tranel, 2019). Sometimes, the costs exceed 14.4% of what producers calculated (Mohd Nor et al., 2015). The cost of raising dairy heifers accounts for 15 to 20% of the total annual expenses in dairy farms and often represents the second greatest cost to the dairy farm (Heinrichs, 1993). The heifer raising cost has increased over the last 5 years ranging from $1,730.29 to $2,241.00 in dairy farms in the USA (Tranel, 2019). Reducing extra expenditures on raising heifers may reduce the whole-farm expenses (Zanton and Heinrichs, 2009b).

Feed costs are the most considerable expense in raising dairy heifers representing over 60% of the total cost (Gabler et al., 2000; Harsh et al., 2001; Heinrichs, 2013). The high contribution of feed cost to the total cost associated with raising dairy heifers makes it an opportunity to search for alternative, more efficient feed management practices and less expensive by-products that reduce feed expenses. To substantially reduce feeding costs, a reduction in nutrient intake by feeding animals to meet their requirements (precision feeding program) is necessary. Therefore, nutritional needs are covered while nutrient losses are minimized (Hoffman et al., 2007; Zanton and Heinrichs, 2008a). Accordingly, precision feeding improves feed efficiency by reducing DMI and providing the heifer with the minimum amount of nutrients to reach the targeted average daily gain (ADG). Thus, controlling heifer growth and minimizing nutrient discharge to the environment (Loerch, 1990; Galyean et al., 1999; Hoffman et al., 2007; Zanton and Heinrichs, 2008a). The precision feeding program is the most traditional way to reduce

feed expenses that have been reported in beef cattle (Koch et al., 1963; Loerch, 1990; Galyean et al., 1999).

On the other hand, different fat sources could be included in a heifer's diet under precision feeding conditions in order to increase the energy density. Based on USDA (March 2020), the National Agricultural Statistics Service, the US soybean oil production in 2019 was 12063.6 tons, with an average price of $645 per ton, while the coconut oil production was 428.7 tons, with an average price of $890 per ton. The palm oil production was 1161.0 tons, with an average price of $745 per ton, while the palm kernel oil production was 125.2 tons, with an average price of 1320 per ton. In addition, by-products from numerous industries, such as the poultry industry by-products, can be utilized by ruminants. Poultry fat is a by-product of chicken processing and extensively produced world-wide and a potential source of valuable nutrients, such as energy. Using PF in dairy diets can be an economical energy source. The US PF production was 1249.9 tons, with an average price of $546 per ton, while the yellow grease production was 1110.8 tons, with an average price of $434 per ton. Also, animal fat prices were $241 per ton (USDA, March 2020). However, more research in the dairy heifer nutrition area under precision-feeding programs and its outcomes could further reduce feed costs.

## DIETARY FAT

Feeding fat has gained interest in the last few decades, and adding fat to dairy diets became common practice for its potential to increase the energy density in diets, improve palatability and reduce feed dustiness, which may provide some benefits to the animal's health (Azain, 2004). The advantages of the addition of fat into dairy rations include potential increased energy intake for high milk production (Ostergaard et al., 1981; Ruesegger et al., 1985), the efficiency of energy utilization (Brumby et al., 1978). Also, improving rumen fermentation by optimizing starch to fiber ratio (Palmquist and Conrad, 1978) without the risk of feeding excessive fermentable carbohydrates (Jenkins and McGuire, 2006).

The literature has noted that feeding fat has some positive effects on beef cow's reproduction (McCullough, 2015) and may help with heat stress during warm and hot temperatures and humid environmental weather. Madison et al. (1994) observed that feeding supplemental fat during the summertime increased milk production more than it did during winter. Furthermore, Skaar et al. (1989) reported that the lactating cows were improved their lactation performance when they were fed diets supplemented with fat. They suggested that the metabolic heat during digestion and metabolism in fat is less than in proteins and carbohydrates. However, supplemental fat can negatively affect dry matter intake, milk yield, and milk components if added to a ration in excess amounts (Rico et al., 2014).

Typical heifer's diets are low in fat due to high forage content and low amounts of fat in those forages. Usually, the mixture of cereal grains and forages contain about 3%

fat. The total dietary fat does not exceed 6-7% of the DMI, with traditional dairy heifer diets typically containing between 2 to 3% fat (NRC, 2001; Moran, 2005). Fats have more energy-dense with gross energy of 9.0 kcal/g compared to carbohydrate and protein (4.0, 3.2 kcal/g; respectively), which provides energy to ruminants without increasing the energy lost heat increment (McCullough, 2015). Several studies have explored different feeding fat strategies for dairy cows (Rabiee et al., 2012). However, there is limited research regarding feeding fat on the growing dairy heifer under the precision feeding system. Therefore, DMI can be reduced further by using fat as an energy source in the precision feeding program as long as other nutrients are adjusted to provide the required amounts.

*Fat Sources*

Various lipid sources can be grouped into two major categories: natural fats (including plant and animal fats) and commercial or specialty fats, which is a unique preparation made by using animal or plant fats (Eastridge, 2002).

Oilseeds are the primary plant-sourced fats such as cottonseed, sunflower, canola, flax, and soybeans. Whole oilseeds are commonly used for a dietary fat source because it is relatively high in protein, fiber, and energy with a relatively low cost (Schossow, 2019). However, in order to increase the utilization in the rumen for protein, extruding, or roasting is very beneficial (Stern et al., 1985). Also, caution should be exercised in feeding pure vegetable oils since they may reduce fiber digestion and milk fat percentage (Mohamed et al., 1988). Further research is being conducted to see if encapsulating or hydrogenating the vegetable oil will help bypass the rumen and become more readily

available to the mammary gland and milk fat synthesis. It is important to mention that grass and corn silages contain about 1-3% fat, and the amount of fat within plant species depends on maturity at harvest, season, and environment. In contrast, the fat content of grains and by-products depending on processing (Boerman and Lock, 2014). Silages and preserved forages are commonly added along with commercial fat supplements since they make up a significant part of a dairy cow ration. Based on Jenkins and Harvatine (2014), the three predominant glycerol-based lipids in animal feed ingredients are triglycerides, galactolipids, and phospholipids. The major storage fat in oilseeds is triglycerides. Therefore the concentrate feedstuffs are high, while galactolipids make up a major portion of the glycolipids within forages (Van Soest, 1994).

The primary animal fats fed to dairy cattle are tallow, lard, poultry fat, fish meal, and grease. Tallow and lard are solid or semi-solid at room temperature, contain more saturated fatty acids than the plant-based oilseeds, and high in oleic acid as well (Jenkins and Jenny, 1989; Bisphlinghoff, 1990). Tallow can be of different qualities and grades and can be readily purchased in barrels with heating instruments to melt the fat for mixing purposes (Eastridge, 2002). Poultry fat is a by-product of chicken processing and extensively produced world-wide and a potential source of valuable nutrients, including energy and protein. Fatty acids composition of rendered animal fats is presented in table 2.1 (Rouse, 2003). Using poultry fat in dairy diets can be an economical energy source and can benefit the poultry industries by providing a market for their by-products (Hutchison et al., 2006; Swisher, 2015). The fish meal should be restricted to a maximum of 2 to 3% of dietary DM since it contains a significant amount of 20 and 22-carbon

polyunsaturated FA, which are very toxic to ruminal bacteria (Hoover et al., 1989). Yellow grease is a grease that is wasted from food service operations, and it usually contains a mixture of vegetable and animal fats and is used as a fat source for livestock and pet foods (Eastridge, 2002). It has been reported that including animal fat in high fiber diet decreased concentrations of short and medium-chain fatty acids and increased the milk fat (Lucy et al., 1993).

Several commercial fat preparations are available and commonly sold as rumen bypass fats or inert fats such as Energy Booster 100, contained 98 % of total fatty acids, which is mainly of stearic acid (C18:0), and Megalac (calcium salt), made from free fatty acids of palm oil and calcium (high in C16:0); soybean oil (high in C18:2), or blend of fat sources (Eastridge, 2002; Rico et al., 2014). Specialty fats are developed to minimize the detrimental effects on rumen fermentation and the risk of decreasing fiber digestion (Palmquist and Jenkins, 1982; Jenkins and Harvatine, 2014). As mentioned before, some other ways of protecting fatty acids have been reported in the literature, including physical and chemical modifications such as encapsulating the unsaturated FA within a saturated FA shell or combining unsaturated FA with casein and cross-linking with formaldehyde (Jenkins and Bridges, 2007). The difference between specialty fats and plant and animal fats that they contain mostly saturated fats compared to unsaturated fatty acids (Schossow, 2019). It has been reported that using these specialty fats with high saturated fats led to minimizing negative effects on milk fat production, rumen fermentation, and feed intakes (Jenkins and Jenny, 1989). They related to the fact that hydrogenated yellow grease was more palatable than yellow grease. The hydrogenated

one had lower effect on ruminal fermentation and did not affect the intake by maintaining gut fill as in the yellow grease.

Several other alternative feed sources from ethanol, biodiesel, and vegetable oil industry such as hominy, canola meal, linseed meal, and dry distillers grain with solubles with a moderate amount of fat are on the rise (Eastridge, 2002; Schossow, 2019). In a study conducted by Anderson et al. (2015), the dietary fat was up to 7% when fed high fat from traditional dried distillers grains (DDGS) to dairy heifers. Distillers grains and canola meal were observed to maintain lactating dairy cow diets (Schingoethe et al., 2009; Christen et al., 2010). Also, the distiller's wet grains were observed to improve the efficiency of converting feed to milk by decrease the intakes with similar milk production between treatments when fed in place of soybean meal and corn (Schingoethe et al., 1999). Fat sources with different saturated and unsaturated fatty acids can affect in many ways DMI, nutrient digestibility, and rumen fermentation. Each of these fat sources differs in how they metabolize, digest, and absorb in ruminants.

*Fat Metabolism in the Rumen*

After feed consumption, dietary fat undergoes an important modification once entering the rumen. In the rumen, dietary fat is exposed to two major processes; hydrolysis of ester linkages and biohydrogenation (Lock et al., 2006; Figure 2.1). As mentioned before, forages and cereal grains contain FA in triglycerides and galactolipids, representing the typical ruminant’s diets. Therefore, lipids must become free from the coating matrix through mastication and microbial digestive and followed by hydrolysis of ester linkages (Palmquist et al., 2005). Rumen bacteria such as A. Lipolytica are

responsible for hydrolyzing the lipid molecules by microbial lipases resulting in the formation and release. The cleavage of the ester bonds between FA and the glycerol backbone in triglycerides, glycolipids, and phospholipids is the hydrolysis (Lourenço et al., 2010). The low ruminal pH (less than 6.0) as a result of feeding high concentrate diets can negatively affect fat hydrolysis by reducing it even though the rate and extent of hydrolysis usually are about 85% (Gerson et al., 1985; Van Nevel and Demeyer, 1996; Doreau and Ferlay, 1994; Beam et al., 2000). Van Nevel and Demeyer (1996) suggested that the low pH can inhibit lipolytic bacteria's growth and metabolism or directly affect their lipase activity. Additionally, as melting point and dietary fat concentration are increased, the hydrolysis of fat is decreased (Palmquist et al., 2005; Beam et al., 2000); as the saturation of fatty acids increases with an increase in the fat concentration, the lipolysis activity of these saturated fatty acids decreases.

Triglycerides and glycolipids are digested in the rumen and broken down into glycerol and FFA, and two or more sugar molecules, while galactolipids are broken down into galactose and diacylglycerol, which are metabolized into VFA (McCullough, 2015). Saturated fatty acids (SFA) remain unmodified and form carboxylate salts after binding with positively charged molecules to pass to the small intestine. In contrast, unsaturated fatty acids (UFA) pass to the small intestine after going through the biohydrogenation (BH) process and forming carboxylate salts (McCullough, 2015). Following hydrolysis, UFA, such as linoleic and linolenic acids, undergo BH by ruminal microbes. The BH is a multi-step process involving several isomerization and reduction steps. The end-products

are mostly the formation of SFA, C18:0 (stearic acid), and C18:1 (oleic acid; NRC, 2001; Harfoot and Hazlewood, 1997).

Linoleic acid in HF diets is typically biohydrogenated to form conjugated linoleic acid (CLA), then to vaccenic acid, and finally to stearic acid. Whereas, in HC diets, where rumen pH is low, typically biohydrogenated linoleic acid to CLA isomers without continuing down the pathway to stearic acid, thus milk fat depression can occur (Baumgard et al., 2000; Lock and Bauman, 2004; Chilliard et al., 2007). It has been suggested that the toxic effects of UFA (disrupt cell integrity and limit bacterial growth) lead to a mechanism response by ruminal bacteria to deal with it by both BH and cis-trans isomerization of dietary UFA (Maia et al., 2007 and Heipieper et al., 2010). In addition, the toxic effects increase as unsaturation increases when linolenic is higher than linoleic (Maia et al., 2007; Maia et al., 2010). It is estimated that ruminal BH of linoleic and linolenic acids ranges from 70 to 95% and 85 to 100%, respectively, and is higher in plant oils than animal oils (NRC, 2001; Lock et al., 2006). The SFA could have some potential adverse effects on decreasing the ruminal NDF digestibility due to increasing the rumination rate. That increases the passage rate of more rapidly fermentable NDF (Harvatine and Allen, 2006). It has been reported that the SFA does not negatively influence bacterial plasma membrane function and has less detrimental to rumen fermentation than UFA (Jenkins, 1993). On the other hand, even though there are less than 1% of CLA in milk and beef, but due to its potential benefits in human such as an anticarcinogen, antioantherogen, as well as anti-obesity, the CLA isomer has been extensively researched by many researchers (McGuire et al., 2000).

*Fat Digestion in the Small Intestine*

The end-products of rumen hydrolysis and BH that reach the duodenal are mainly FFA (85-90%), where SFA (C16:0 and C18:0) represent (65%) of them. The remaining lipids (10-15%) are microbial phospholipids that are typically found as potassium, sodium, or calcium salts due to neutral conditions in the rumen plus small amounts of triglycerides and glycolipids from residual feed material (Doreau and Ferlay, 1994; NRC, 2001; Lock et al., 2006). Most lipids are digested in the small intestine with 5% in the duodenum, 20% in the upper jejunum, 25% in the mid and lower jejunum, the rest 50% is digested in the ileum (Leat and Harrision, 1975). After passing the rumen and the low pH in the abomasum and duodenum (2.0 to 2.5), the FFA and salts dissociate and attach to feed particles (Drackley, 2005). The pancreatic lipases are not active at low pH in ruminants; therefore, it cannot break down the FA. Instead of that, micelles must be formed to allow certain parts of the bile salts and FFA to interact with the aqueous environment (Davis, 1990). Micelles are facilitated by both bile and pancreatic juice activity, secreted into the upper duodenum, where FA digestion begins. Bile contains bile salts and lecithin (phosphatidylcholine), lecithin is converted to lysolecithin (lysophosphatidylcholine; an emulsifier for SFA) by the pancreatic phospholipase A2 provided by the pancreatic juice, which provides the bicarbonate as well to raise the pH (Lock et al., 2006; Figure 2.2). The FA that is attached to feed particles and bacteria adsorb by lysolecithin and bile salts and transfer lipids to a soluble micellar phase, which is required for FA absorption to happen (Moore and Christie, 1984).

The results from 20 different independent studies on lactating dairy cattle were compiled; total and individual FA digestibility was calculated (Lock et al., 2006). They have reported total FA digestibility ranging from 58% to 86%, with an average of 74%. In addition, the mean digestibility values of individual FA were as shown in figure 2.3 below. Based on results by Doppenberg and Palmquist (1991), it has been reported that as the supply of FA increases, the true digestibility of FA may decline. Furthermore, in a review paper by Bauchart (1993), it has been discussed that when the LCFA flow is elevated, the pancreatic phospholipase A2 activity and bile salts may become restrictive for their, which leads to a decrease in their digestibility as well. On the other hand, it has been reported that the digestibility of C16:0 in the small intestine was more than C18:0 (72.5 vs. 54.6%, respectively; Ferlay et al., 1993). In contrast, Enjalbert et al. (1997) reported no significant differences between the digestibility of C16:0 and C10:0 when feeding Ca-soaps of FA from palm oil or rapeseed at the same inclusion in the ration. It seems important to consider duodenal flow differences when comparing these two FA's digestibility due to the higher C18:0 flow to the duodenum compared to C16:0. As mentioned before, the amount of saturated and unsaturated fatty acids and its representative in different fat sources can determine these fats in ruminants' digestibility.

*Fat Absorption in Ruminants*

There is no significant absorption, or any modifications occur during the transit of LCFA through the omasum and abomasum (Moore and Christie, 1984). The absorption of FA happens in the small intestine, mainly in the jejunum portion. Upon the enterocyte’s entry, the acyl-CoA synthetase converts the absorbed FA with chain length

>C10 to their coenzyme A derivatives and re-esterified them by the α-glycerolphosphate pathway to triglycerides (Bach and Babayan, 1982), and along with phospholipids, cholesterol, and apoproteins, they will be formed into chylomicrons, and very-low-density lipoproteins (VLDL; Bauchart, 1993). Lipoproteins (chylomicrons and VLDL) cannot be absorbed directly by intestinal cells due to their large size. Therefore, they must leave the cell by pinocytosis and secret into the lymph system and then into the bloodstream by way of the intestinal and thoracic lymph ducts (Moore and Christie, 1984). Figure 2.4 is showing the absorption of fat in the enterocytes of ruminants (Navarrete, 2013). It is very important to note that the monoglyceride pathway is not active because there is no 2-monoglycerides absorption in functioning ruminants. The stearic acid (C18:0) is the main FA reaching the small intestine; the high ability of ruminant animals to absorb SFA is related to the dependence on lysolecithin as the major micelle stabilizer. In comparison to other amphiphiles, lysolecithin was the only one that significantly increased the distribution of C18:0 into the micellar phase and away from the particulate phase (Freeman, 1984). However, it has been previously shown that about 7 to 9% of the stearic acid (C18:0) that enter the enterocyte is desaturated to oleic acid (C18:1) in the intestinal mucosa OF sheep (Bickerstaffe and Annison, 1969). Lipoproteins travel to specific target tissues after the blood oxygenation, including muscle, adipose, and mammary tissue. These tissues contain the lipoprotein lipase, which is the enzyme responsible for breaking down the chylomicrons and VLDL. Also, lipoprotein lipase brake down the FFA as well, which are then small enough to enter cells

and be transformed back into triglycerides; thus, triglycerides can be used as an energy source for cell functions (Bauchart, 1993; McCullough, 2015).

On the other hand, the micelles' formation allows shorter chain FA = / <C10 to be absorbed into intestinal cells, with most being absorbed in the jejunum. These FA will leave the enterocyte mostly unmodified because they are not easily esterified or incorporated into lipoproteins and enter the venous portal system bound to albumin (Bach and Babayan, 1982).

*Effects of Fat Feeding*

*Dry Matter Intake, Satiety, and Palatability*

Some factors determine the effects of dietary fat on DMI, such as type and form of fat, chain length, and FA profile. In some studies, the DMI of dairy cows was depressed by added fat (Choi and Palmquist, 1996; Schauff and Clark. 1992). Allen (2000) reported that the DMI decreased when calcium salts of palm FA were fed in TMR with ranges from 7% to 9% of DM to dairy cows in 11 out of 24 comparisons, and unprocessed animal fats decreased DMI as well whereas adding hydrogenated fats did not affect DMI. Calcium salts of palm FA had the strongest effect on DMI, approximately twice that observed for the unprocessed animal fats. Several authors have reported the hypophagic effect of UFA of calcium salts and related it to a different reason: i) Ngidi et al. (1990) suggested that the calcium salts diet has lower acceptability, ii) Drackley et al. (1992) reported that the gastrointestinal motility had a depression effect by calcium salts diet, iii) Allen (2000) stated a that the fiber digestion was reduced with calcium salts diet which is responsible for stimulating the distension of reticolo-rumen, or the greater

absorption of UFA compared to SFA have a metabolic regulation of DMI. Regarding the chain length effect on DMI, the greater hypophagic effects of calcium salts of palm FA are probably not related to higher C16:0 content. Even though it has a high content of C16:0 relative to other FA sources, there is no evidence that C16 FA is more hypophagic than C18 FA (Allen, 2000). Additionally, the C16 to C18 FA ratio did not significantly affect the DMI of dairy cows in a regression analysis reported in the literature (Firkins and Eastridge, 1994). The FA profile of the dietary fat is another important factor determining DMI response to fat. Also, the hypophagic effects of some unsaturated fat sources should be reduced by the extensive BH of FA happen in the reticulo-rumen (RR; Dawson and Kemp, 1970; Viviani, 1970). However, BH is increased with the degree of unsaturation of C18 FA (Kalscheur et al., 1997; Wu et al., 1991) and is decreased as the amount of added unsaturated fat increases (Christensen et al., 1998). Drackley et al. (1992) reported that the amount of UFA reaching the duodenum affects DMI. Litherland et al. (2005) showed that the depression in feed intake was greater after the infusion of UFA soy oil in the abomasum than the infusion of unsaturated triglycerides. Palmquist and Jenkins (1980) suggested that adding SFA may be a particularly useful fat source because these FA have minimal effects on rumen microbial activity.

The mechanisms that fat reduces feed intake could involve releasing gut hormones, fat oxidation in the liver, fat effects on ruminal fermentation and gut motility, and palatability and acceptability of diets containing fat. Fat plays a role as a strong stimulator of releasing the gut peptide cholecystokinin (CCK) hormone (Liddle et al., 1985). It has been observed that DMI was decreased by feeding fat to dairy cows, and

postprandial plasma concentrations of insulin decreased while CCK increased (Choi and Palmquist, 1996). Additionally, feed intake of sheep was depressed by intravenous injection of exogenous CCK (Grovum, 1981). Choi et al. (1996) reported that DMI of heifers fed a high-fat diet increased by 92% during the first 2-h post-injection of MK-329, a nonpeptide CCKA receptor antagonist. The direct action of brain CCK on brain satiety centers or peripheral action of gut CCK is considered the CCK's hypophagic effects (Reidelberger, 1994). The peripheral action of gut CCK includes inhibition of gastric emptying and increase distention (Reidelberger, 1994). The signals generated by hepatic vagal afferent nerves to brain centers to signal satiety are affected by the liver's FA oxidation rate (Scharrer and Langhans, 1986). The DMI of rats fed a diet containing 18% fat was increased when beta-oxidation of FA was inhibited by mercaptoacetate, an inhibitor of acyl CoA dehydrogenases, but that did not affect the DMI of rats fed a 3.3% fat (Scharrer and Langhans, 1986). In contrast, injection mercaptoacetate decreased heifers DMI (Choi et al., 1997). Also, Litherland et al. (2005) indicated that the concentration of plasma glucagon-like peptide 1 increased, whereas CCK's plasma concentration did not change when DMI was decreased. Fat is linked to increased propionate concentration due to more energy-efficient and rumen fermentation (Manthey et al., 2016). There are less methane and carbon dioxide production in propionate than acetate (Fahey and Berger, 1988). When propionate was infused into the steers' mesenteric vein, the feed intake was reduced (Elliot et al., 1985). Based on Baile's (1971) experiments where propionate was injected into sheep and goats' ruminal vein, the DMI was decreased. They suggested that propionate receptors in the rumen might control the

feed intake. Also, propionate resulted in decreased DMI of dairy cows when infused in isocaloric amounts with long-term ruminal infusions (Sheperd and Combs, 1998). It has been reported that insulin is associated with a reduction in DMI of sheep (Foster et al., 1991), and propionate stimulates and increases plasma insulin secretion in sheep (Grovum, 1995).

Satiety centers in the brain integrate all the stimuli to signal the end of a meal, such as distension, which stimulates stretch receptors in the RR wall (Harding and Leek, 1972; Forbes, 1996). Dietary fat is one of the several dietary factors with possible distension effects (Choi and Palmquist, 1996). Fat can inhibit fiber digestion in the RR resulting in a reduction in the passage rate, increasing distension, and stimulating receptors in the RR, reducing DMI (Palmquist and Jenkins, 1980). However, in several experiments, there were no interactions between fat and fiber level on DMI (Canale et al., 1990; Jerred et al., 1990; Klusmeyer et al., 1991; Tackett et al., 1996). Also, the DMI was decreased in low fiber diets more than in high fiber diets when fat was added to the diet (Elliott et al., 1995; Grant and Weidner, 1992). As mentioned before, high-fat diets increased plasma CCK in dairy cows (Choi and Palmquist, 1996), and there is evidence that CCK contributes to satiety (Reidelberger, 1994) and suppresses feed intake by inhibiting gastric emptying (Moran and McHugh, 1982). Furthermore, the motility of the RR was inhibited when unsaturated LCFA was infused in sheep (Nicholson and Omer, 1983). The greater release of CCK stimulated by high UFA reaching the duodenum is the reason for greater hypophagic effects compared to SFA. Additionally, UFA might be absorbed and oxidized in the liver more quickly than SFA, generating reducing

equivalents and satiety faster. On the other hand, there is evidence that absorbed propionate affects satiety. Anil and Forbes (1980) reported that the feed intake was reduced over 80% compared with control when propionate was infused into sheep's portal vein.

The acceptability of different fat sources depends on the differences in their hypophagic effects. Fat was found to vary in acceptability while fed alone or top-dressed; Ca-PFA had the lower acceptability followed by tallow, encapsulated dry tallow, or prilled LCFA (Grummer et al., 1990). These differences between fat were decreased when they mixed with grain, except for Ca-PFA. The effects of fat sources on the acceptability of diets are probably small when fat is included in TMR unless the inclusion rates are very high. Furthermore, fat acceptability increased following an adaptation period. For example, heat-treated beans are more palatable when top-dressed than other fat sources. The top-dressed method may take longer for cows to adapt to whole cotton seeds, tallows, or specialty fats. It has been observed when 10% of tallow or animal vegetable blend was added to grain mixes with restricted feeding time, the length and size of initial meals were reduced, which limits the consumption (Heinrichs et al., 1982). A study conducted by (Grummer et al., 1990) compared to intake of different fat sources such as booster fat, calcium salts (megalac), energy booster, and tallow and fed to dairy cows in different ways, either alone, top dressed on grain, mixed with the grain at the 10% level, or alone with adaptation period. They observed that tallow had the highest intake between fats fed alone without adaptation. Intake of calcium salts (megalac) was lower than booster fat or energy booster with adaptation. Specialty fats intake was similar

when top-dressed or fed as part of the grain mix. Diluting fat sources with other feed ingredients as TMR and gradually adapting cows to the fat may reduce consumption problems and palatability differences between fat sources.

An important consideration is that the FA content of diets in most of these studies ranges from 7 to 9% of DM. Also, not all fat sources induced the same responses. Rabiee et al. (2012) reported in meta-analysis a great variation between different fat types with the same supplement across different diets and studies. The range in responses being as much as 5 standard deviations from the mean with positive or negative responses differed between fats. However, they concluded that fat inclusion in the diets decreased the DMI while increased milk and milk fat production and milk efficiency in general. Overall, including dietary fat in the diets can affect the intake amount as a dense energy source. The palatability of fat depends on the different sources of fat and the way of offering it to the animals.

*Fiber, Soluble Carbohydrate, and Protein Digestibility*

Rumen fermentation is not affected when fat levels are low in the diets because rumen microbes can saturate FA, but this capacity can be exceeded at higher levels, and UFA can accumulate in the rumen and interfere with rumen fermentation (NRC, 2001). Therefore, the digestibility of nonlipid energy sources is reduced (Jenkins, 1993). It has been reported that the ruminal digestion of structural carbohydrates can be reduced up to 50% or more by adding less than 10% of fat to the diet (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984). The reduction in fiber digestibility is accompanied by decreased methane production, hydrogen, VFA, and lower acetate to propionate ratio

(Boggs et al., 1987; Chalupa et al., 1984; Czerkawski and Clapperton, 1984; Ikwuegbu and Sutton, 1982). Limited hindgut fermentation may lower the fiber digestibility depression in the whole digestive tract when fat addition inhibits ruminal fermentation (Boggs et al., 1987; Jenkins and Fotouhi, 1990).

In contrast, several current studies showed different fat effect results on fiber digestibility using different feeding systems. Manthey and Anderson (2018) reported no effects on fiber apparent digestibility when heifers limit fed DDGS with ad libitum grass hay. They related that to feeding grass hay as ad libitum, which resulted in a slightly different limit feed program than the typical one. Ranathunga et al. (2012) observed that the ruminal digestion of NDF was improved in HF diets containing DDGS in dairy cows compared with LF diets containing DDGS. They attributed that to fat from DDGS to bound in the feed particle and slowly introduced to the rumen. A study conducted by Suarez-Mena et al. (2015) observed a quadratic NDF and ADF digestibility response to increasing levels of DDGS up to 14% inclusion in the diets. Also, Anderson et al. (2015) reported a higher digestibility of NDF and ADF when heifers limit-fed a high-fat DDGS compared to a low-fat DDGS. It was suggested that the high-fat DDGS diet contains a lower starch content than the low-fat DDGS resulted in higher efficiency of fiber utilization and improved total-tract digestion. However, these results did not agree with a study conducted by Lascano et al. (2016b) using two levels of fat in a continuous culture fermenter, where they did not observe any effects on ADF digestibility between the two levels of fat in the diets. Koch (2017) reported depression in NDF and ADF digestibility when continuous culture fermenter fed high soybean oil compared to low soybean oil.

Koch stated that dietary polyunsaturated fatty acids had been shown to depress fiber dC by limiting fiber digestion bacteria (Van Soest, 1994).

Dietary fat has a less detrimental effect on the digestibility of nonstructural carbohydrates in comparison with fiber digestibility (Jenkins, 1993). Bock et al. (1991) conducted a study on steers-fed treatments consisted of no added fat, 3.5% tallow, and soybean oil soap stock. They reported that adding fat did not affect starch digestibility in the rumen, even though DM or fiber digestibility was depressed. In addition, Zinn (1988) conducted a study by feeding 4% yellow grease to beef steers and reported normal starch digestion in the rumen of steers that were fed additional fat. This observation of normal starch digestibility under fat supplementation supports previous observations by McAllan et al. (1983).

The rumen digestibility of protein is also altered when fat addition interferes with rumen fermentation (Jenkins, 1993). It has been observed that the protein digestibility in the rumen was decreased when linseed oil was infused into the rumen of sheep. Also, ammonia concentration decreased as protein digestibility decreased, increasing N flow to the duodenum (Ikwuegbu and Sutton, 1982). Similar results were reported when sheep were fed additional lipid as com oil or lecithin (Jenkins and Fotouhi, 1990). The changes in protein digestibility are usually accompanied by increasing microbial protein synthesis efficiencies in the rumen (Jenkins, 1993). However, the increase in microbial protein efficiency has been attributed to a reduction in protozoal numbers in the rumen as well as less bacterial N recycling (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984), or a higher solids dilution rate in the rumen because of the fat addition (Boggs et al., 1987).

Anderson et al. (2015) reported a higher digestibility of protein when heifers limit-fed a high-fat DDGS compared to a low-fat DDGS and control diet (73.1% vs. 70.1 and 69.8, respectively). It was suggested that the high-fat DDGS diet contains a lower starch content than the control, and low-fat DDGS resulted in higher efficiency of protein utilization and improved total-tract digestion.

*By-product Dietary Fat*

Typical dairy diets such as forage or TMR contain low amounts of fat, but many by-products such as distiller's grains, bakery waste, poultry fat, vegetable oil, fish meal grease, and tallow may be used as a source of fat in the diet in addition to other feed product such as commercially inert fat.

There is limited research regarding the effects of feeding fat to the growing dairy heifers. In a study conducted by Anderson et al. (2015), the dietary fat was up to 7% when dairy heifer fed high fat from traditional dried distillers grains (DDGS). In another study by (Anderson et al., 2009), the diet's fat was close to 5% when a large portion of the heifer diet was supplied by wet distillers grains and soybean hulls. Diets with full or low-fat DDGS, included at approximately 20 or 30% of dry matter, have been observed to maintain ADG and overall growth performance; and similar total-tract nutrient digestion in dairy heifers compared with control diets containing corn and soybean meal fed ad-libitum (Schroer et al., 2014). Suarez-Mena et al. (2015) conducted a study using incremental DDGS proportions included in different forage levels. They reported that DMI could be reduced as more DDGS was added by indirectly increasing dietary fat content with no negative effects on nutrient utilization. Also, the inclusion of DDGS did

not show any effects on microbial CP flow. Manthey and Anderson (2018) reported no effects on apparent digestibility when heifers limit fed DDGS with ad libitum grass hay. Several studies showed a decrease in the total VFA concentrations and acetate concentrations while increasing propionate concentrations and a reduction in the A:P ratio on dairy heifer limit-fed DDGS (Suarez-Mena et al., 2015; Manthey et al., 2016; Manthey and Anderson, 2018). They suggested the higher propionate concentration is related to more energy-efficient and rumen fermentation in heifers fed DDGS diets. Leupp et al. (2009) and Suarez-Mena et al. (2015) observed a linear decrease in fecal outputs as DDGS level increased in limit-fed dairy heifers' diets.

Feeding pure vegetable oils can reduce fiber digestibility and milk fat percentage (Mohamed et al., 1988). They suggested that feeding FFA as oils are more likely to induce MFD than feeding whole oilseeds. In an in vitro study, soybean oil was fed at either 3% or 6% of DM. The diet digestibility was increased during the first 24 hours after feeding fat, but by 48 hours, the response was decreased (Whitney et al., 2000). They attributed the decline in digestibility to the amount of UFA, which plays as antimicrobial effects. Hess et al. (2001) reported that the ruminal and total tract NDF and OM digestibility were reduced while the microbial efficiency was increased when heifers fed soybean oil. Gould et al. (2000) reported the same results where the post ruminal and total tract OM and NDF digestibility decreased in lambs-fed soybean oil. In addition, The OM, NDF, and intestinal disappearance were not affected. Simultaneously, an increase in FA's duodenal flow was reported when lambs were fed varying amounts of safflower oil (Atkinson et al., 2006). They attributed the increase in C16:0 flow to the higher dietary

intake and the microbial FA as well, while the increase in C18:0 to the higher BH of USF. Also, Carter et al. (2002) reported when beef heifers were fed cracked corn with soybean oil, an increase in the total FA flow from the duodenal was observed. Brokaw et al. (2000) found no difference in OM intake of forage when beef heifers fed normal or high oil corn, whereas there was an increase in digestible OM intake. Furthermore, the OM and NDF digestibility were less for high oil corn even though the OM and NDF disappearance did not differ between treatments (Brokaw et al., 2000).

Including animal fat in a high fiber diet was observed to increase the milk fat while decreased short and medium-chain FA concentrations (Lucy et al., 1993). A study conducted by Zali et al. (2020) investigated the effects of feeding calcium salts of poultry oil on dairy cows. The DMI was greater for cows fed calcium salts of poultry oil and higher milk production than a palmitic acid-enriched fat and a mix between the two. In addition, fiber and protein digestibility were similar between treatments. They concluded that even though poultry oil's calcium salts improved dairy cows' production but decreased feed efficiency. Shike (2013) fed whole raw soybean, flax, or hydrolyzed animal fat to beef heifers starting at 7 mo of age and observed no differences in the percentage of pubertal heifers at 10, 12, or 14 months. Hutchison et al. (2006) conducted a study on steers fed either 4% tallow or 4% poultry fat. They observed that fat addition did not affect ADG, and steers consumed poultry fat gained more efficiently than tallow. Also, they stated that replacing tallow with poultry fat is a more economical energy source with no effects on performance. Only two studies across a summary of more than 20 dairy studies showed depression in feed intake when feeding tallows or greases (Allen,

2000). Onetti and Grummer (2004) suggested a relationship between tallow and forage source on the intake effects. The intake was reduced when tallow added to corn silage diets and did not increase milk production, while it was the opposite with alfalfa-based diets. The true digestibility of tallow was assigned about 68%, while vegetable oils and calcium salts were assumed to 86% (NRC, 2001). Jenkins (2006) reported in an independent literature review of tallow digestibility compared to other fat sources that only tallow and calcium salts of palm FA had numerically higher digestibility than other fat sources examined. That could be related to the level of saturation in these fat sources.

Typical rumen-protected fats are carboxylate salts (soap) forming from the bound between the free FA, Ca++, and Mg++. That form has specific properties, such as a more saturated, higher melting point, and lower solubility, which gives it the ability to escape from the rumen biohydrogenation (McCullough, 2015). Jenkins and Jenny (1989) reported that using these specialty fats led to fewer negative effects on rumen fermentation, feed intakes, and milk fat production. In addition, several studies reported that the DMI of dairy animals was not affected by bypass fat (Naik et al., 2007; Tyagi et al., 2009b; Thakur and Shelke, 2010; Sirohi et al., 2010; Mudgal et al., 2012). However, Chouinard et al. (1997) reported a decrease, while Tyagi et al. (2009a) reported an increase in DMI of dairy animals fed bypass fat. Furthermore, these studies reported that bypass fat did not affect the digestibility of DM, OM, CP, NDF, ADF, and cellulose. However, CP's digestibility was increased when Ca-LCFA was fed to dairy animals (Schauff and Clark, 1992). Also, the EE digestibility was increased when bypass fat was fed to dairy animals (Thakur and Shelke, 2010; Sirohi et al., 2010). Ngidi et al. (1990)

reported an increase in NDF digestibility when Ca soap's level increased in the diet. It has been suggested that the higher apparent total tract digestibility of NDF in cows-fed Ca-LCFA was related to an increase in post-ruminal degradation (Chouinard et al., 1998). The digestibility of ADF under bypass fat addition may be either increased (Naik et al., 2009) or not affected (Thakur and Shelke, 2010; Sirohi et al., 2010). It has been recommended that the ADF digestibility varies depending on the level of fat addition with no effect at a low-fat level (Schauff and Clark, 1989). Naik et al. (2007) reported that fat did not influence buffaloes' cellulose digestibility.

Precision-feeding has shown to be advantageous because it improves feed efficiency, decreases the amount of wasted feed, decreases nutrient excretion, and maintains growth performance. However, most research regarding limit-feeding has been conducted using corn and soy-based diets (Zanton and Heinrichs, 2009b). Very limited research has investigated precision-feeding heifers using alternative dietary by-products such as poultry fat (PF).

## DAIRY BREED (HOLSTEIN AND JERSEY)

Based on the Council of Dairy Cattle Breed (CDCB, 2015), Holstein and Jersey are the two most common dairy breeds in the USA and represent approximately 90% of the dairy cows. Holstein represents 83.9% of the dairy cow population, and it is the most popular breed used in U.S. dairy farms (CDCB, 2015). Holstein breed is well known for producing milk in high amounts, including milk fat and protein. This breed's mature cow weighs about 680 kg, and in a 305-d period of lactation, produces approximately 11500 kg of milk, 420 kg of fat, and 340 kg of protein (USH, 2009). The average age at first calving of Holstein is around 26.8 months, and about 38% of them remain alive at 5 years of age (Garcia-Peniche et al. 2006). Therefore, the average Holstein productive life is approximately 4 years (USH, 2009). Due to the intensive selection process for milk production, such as longevity, fertility, and resistance to diseases, Holstein cows may present some health issues (Lucy, 2001; Mackey et al., 2007; Xue et al., 2011). Furthermore, due to the continuing increase in an inbreeding level in various Holstein populations, some dairy farms have been started crossbreeding between Jersey and Holstein to improve milk composition and reproductive performance and longevity (Hansen, 2000; Xue et al., 2011).

Jersey is the second breed most popular in the U.S., and its population increased from 4.9% to 6.4% from 2009 to 2014 while the Holstein population decreased from 89.6% to 83.9% during the same period (CDCB, 2015). It has been reported that this increase in the Jersey population in comparison to Holstein is due to the higher capacity of Jerseys to produce greater milk components (milk fat, true protein, and other solids)

since the milk prices depend on these components (Capper and Caddy, 2012). Jersey cows produce an average of 7455 kg milk, 347 kg of fat, and 268 kg of protein in 305-d production (AJCA, 2009). Mature Jersey cows weigh between 400 to 450 kg, the average age at first calving is around 25.8 months, and the average productive life is approximately 3.5 years (AJCA, 2009). It has been observed that Jersey × Holstein had fewer calving problems, larger estrus periods, fewer services per conception, and shorter calving intervals than Holstein (Auldist et al., 2007). Additionally, under heat stress conditions, the Jersey cows showed less variability in milk components and smaller declines in milk production than Holstein (Smith et al., 2013). Espinoza et al. (2009) suggested that Jerseys may require less energy for thermoregulation than Holstein; therefore, they show more resistance to heat stress.

Due to the lack of research, the current guidelines for feeding dairy cows in the U.S. (NRC, 2001) do not make specific recommendations for Jerseys. It is not clear if adding Jersey cows to the herd would increase income to the overall production. Therefore, including Jersey heifers in the current project is to identify the breed differences and its efficiency, which may help farms improve economic benefits by decreasing feeding costs and potentially adapting/modifying the current feeding practices according to breed.

*Dry Matter Intake and Nutrient Digestibility*

The average DMI varies between cow's breed depending on the bodyweight of the animal. Jerseys typically consume more than the larger breeds of cows as a % of BW. In a study conducted by Blake et al. (1986), they found that Jersey cows consumed more

DM as a percentage of body weight than Holsteins, even though Holstein cows consumed one-third and one-fifth more DM than Jersey cows in the first and second trimesters, respectively. They attributed that to the relative capacity of the gastrointestinal tract that increases proportionally with body weight. In contrast, Heins et al. (2008) observed different results when they compared the DMI between Jersey × Holstein crossbred cows with pure Holstein cows during the first 150 days of the first lactation. They stated that the DMI of the Jersey × Holstein cows did not differ from the Holstein cows during any period postpartum. Also, they consumed similar DMI as a percentage of BW, while Anderson et al. (2007) reported lower DMI as a percentage of BW for pure Jersey cows.

Aikman et al. (2008) reported that the passage rate was faster but more digestion rate efficient in Jersey than that of the Holsteins. They also noticed that the Jerseys had a longer period to ruminate because they allowed more feed to be supplied to the rumen throughout the day. Holstein consumed more feed than the Jersey cows; therefore, they could not get enough feed with the time given (Aikman et al., 2008). As a result, the Jerseys spent most of their time ruminating rather than trying to consume more feed. That is an indication that there is a marked difference in the eating and ruminating behavior between these two breeds. However, the daily eating time did not differ between breeds, but Jerseys spent more time eating per unit of ingested feed (Aikman et al., 2008). They attributed that to the fact of smaller mouths that Jerseys have compared it to Holstein. They need a larger number of mouthfuls to process an equal volume of feed. They concluded that Jerseys seem to have a better way of breaking down the feed materials and utilizing them more appropriately. Also, Jerseys tend to be more efficient than Holsteins.

On the other hand, there are indications that Jerseys have higher digestibility than the Holstein. Olijhoek et al. (2018) observed that the Jersey cows had a higher total-tract apparent digestibility of DM and OM than the Holstein cows fed two levels of F:C diets. Similarly, higher OM and NDF digestibility for Jersey compared to Holstein-Friesian cows were observed by Beecher et al. (2014). Aikman et al. (2008) conducted a study on Holsten and Jersey cows fed TMR ad libitum during 3 periods, far-off, close-up, and lactation. They observed that DM, OM, ADF, and apparent starch digestibility did not differ between breeds. However, NDF digestibility was higher in Jersey than Holstein cows, and the DM and OM digestibility were numerically higher in Jerseys. When external markers were used, Jersey cows showed a higher digestion rate and efficiency in utilizing the diet because of a larger gastrointestinal tract weight relative to BW or a higher chewing rate per unit of meal consumed, suggesting particle breakdown and rumen outflow were faster in Jersey compared to Holstein (Aikman et al., 2008; Beecher et al., 2014). Some other studies reported that Jerseys have a higher feed utilization efficiency than large breeds such as Holstein (Oldenbroek, 1988; Grainger and Golddard, 2004). According to Van Soest (1994), a relatively large gastrointestinal tract as a proportion of the BW in Jerseys would indicate a larger area available for nutrient absorption; therefore, higher digestibility would be expected. Several studies conducted on Holstein and Jersey cows where the N digestibility did not differ between the two breeds (Kauffman and St-Pierre, 2001; Aikman et al., 2008; Knowlton et al., 2010; Olijhoek et al., 2018). Based on these studies' indications, Jersey heifers might be more

efficient in nutrient utilization than Holstein heifers, and comparing these two breeds under a precision feeding system is our topic of interest.

*Nutrient and Manure Excretion*

Many nutrients can be excreted in feces and urine due to an excessive amount of feeding dietary nutrients, which results in a greater emission of pollutants to the environment (Chandler, 1996; Castillo et al., 2013). Based on NRC (2001), any feeding system should ideally provide nutrients in amounts that maximize ruminal fermentation and growth of rumen microbes while minimizing nutrients losses to the environment.

Manure excretion is equal to the sum of fecal and urine production (NRC, 2001). It has been reported that dairy cow's manure production on a wet basis based on data set from metabolic studies was 66.3 ± 14.4 kg/d and ranged between 27.7 to 114.4 kg/d. In contrast, urine production was 23.1 ± 7.19 kg/d, representing one-third of the total manure excretion (Nennich et al. 2005). Knowlton et al. (2010) stated that the wet manure excretion was higher for Holstein than for Jersey (74.3 and 49.8 ± 2.34 kg/d, respectively). On the other hand, the fecal DM of dairy cows ranged from 6.2 to 7.4 kg/d as stated by Tomlinson et al. (1996), which is similar to those were reported by Nennich et al. (2005) and Weiss and Wyatt (2004) (7.3 ± 1.63 and 6.9 ± 1.5 kg/d, respectively). The fecal DM excretion observed by Knowlton et al. (2010) was also higher for Holstein compares to Jersey (8.11 and 5.67 ± 0.32 kg/d, respectively). Furthermore, Knowlton et al. (2010) indicated that differences in fecal DM output between the two breeds were relative to DMI and BW differences. Also, it has been observed that the manure excretion had a linear relationship with DMI (Nennich et al., 2005; Figure 2.5).

Nitrogen (N) excretion is one of the main concerns from the environmental perspective (NRC, 2001). The N that is secreted in milk accounts for 25 to 35% of the N that dairy cows consume in the diet (Chase, 1994; Chandler et al., 1996). Almost the rest of the remaining N is excreted in feces and urine (NRC, 2001). In several studies, it was reported that greater N excretion is due to a higher N intake (Tomlinson et al., 1996; James et al., 1999; Krober et al., 2000; Frank et al., 2002; Nennich et al., 2005). Kauffman and St-Pierre (2001) observed that the retention of ingested and absorbed N tended to be lower in lactating Jerseys compared to Holsteins. They attributed that to the differences in the dynamics of rumen digesta flow, the rate of passage, the breed response to type, and protein concentration in the diet. Knowlton et al. (2010) observed that fecal N excretion was higher for Holstein than for Jersey (243 and 162 ± 10 g/d, respectively) as well as the urinary N (213 and 161 ± 6 g/d, respectively). Also, they stated that approximately 50% of the total N is excreted in feces and the other 50% in urine. Therefore, and based on this observation, Jerseys are showing a more efficient performance than Holsteins and might give an interesting result under a precision feeding system.

# CONCLUSIONS

Dairy heifers usually grow over 22-24 months of age, when the first calving and lactation onset generally occurs (Ettema and Santos, 2004). After that, they enter the milk production system and begin generating income for the operation; therefore, any improvement in efficiency is valued (Heinrichs, 1993). It is necessary to manage dairy heifer appropriately to allow them to reach reproductive age in a timely manner at an optimal rate of gain to enhance mammary development and avoid any risk of metabolic disorders (Zanton and Heinrichs, 2005). That is very important since about 40% of the lactating herd is replaced by heifers each year (Kitts et al., 2011).

Precision feeding can improve feed efficiency by providing highly digestible feedstuffs and energy-dense diets while reducing DMI and maintain animal's requirements. Reduce DMI can decrease the passage rate of nutrients in the rumen, diets stay longer in the rumen, microbes have a longer time to digest nutrients, thus increase nutrient digestibility. Feed costs can be reduced through the reduction of feed intake used under precision feeding. Minimal refusals and nutrient losses have been reported with a concurrent decrease in manure output.

This literature review's objective was to cover the possibility of evaluating different dietary fat sources with different F:C ratio into dairy heifer's diets under precision feeding systems. That would further reduce DMI, which could help reduce the impact of high feed costs on raising heifers. Also, to evaluate the breed differences under precision feeding program since neither dietary fat nor breed have been evaluated in precision-fed heifer diets. Therefore, more research is needed in-vivo and in-vitro to

determine the impact of poultry fat inclusion in precision-fed Holstein and Jersey dairy heifer's diets on digestibility, rumen fermentation, and nutrient excretion.

Table 2.1. Fatty acids composition of rendered animal fats (Modified from Rouse, 2003).

| Fatty Acid | Tallow | Lard | Grease | Poultry Fat |
|---|---|---|---|---|
| Myristic acid C14:0 | 3.0 | 1.5 | 1.5 | 1.5 |
| Palmitic acid C16:0 | 25.0 | 27.0 | 23.0 | 21.0 |
| Palmitoleic acid C16:1 | 2.5 | 3.0 | 3.5 | 6.5 |
| Stearic acid C18:0 | 21.5 | 13.5 | 11.0 | 8.0 |
| Oleic acid C18:1 | 42.0 | 43.4 | 40.0 | 43.0 |
| Linoleic acid C18:2 | 3.0 | 10.5 | 18.0 | 19.0 |
| Linolenic acid C18:3 | N/A | 0.5 | 1.0 | 1.5 |
| Saturated | 49.5 | 42.0 | 35.5 | 30.0 |
| Unsaturated | 47.5 | 57.4 | 62.5 | 70.0 |

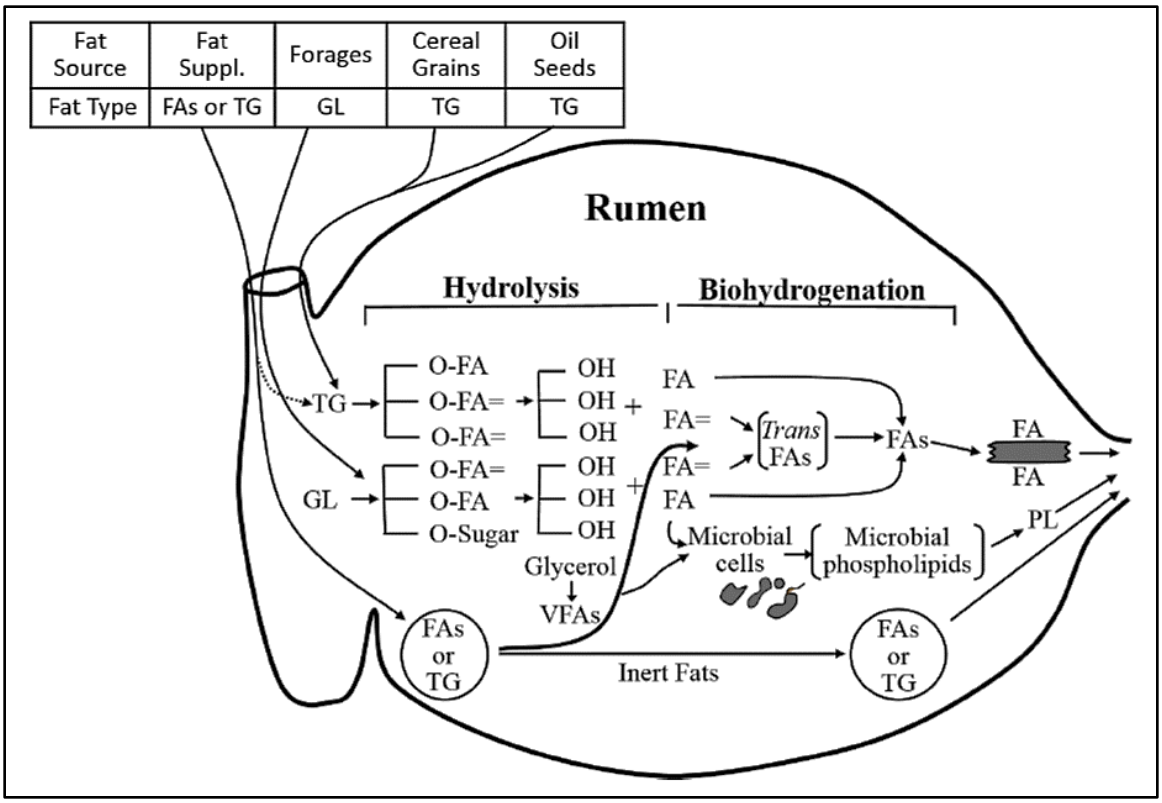


Figure 2.1. Fat metabolism in the rumen (Adapted from Lock et al., 2006).

Abbreviations: Triglycerides (TG), glycolipids (GL), phospholipids (PL), trans fatty acids (trans FA), mixture of fatty acids (FAs), and volatile fatty acids (VFA).

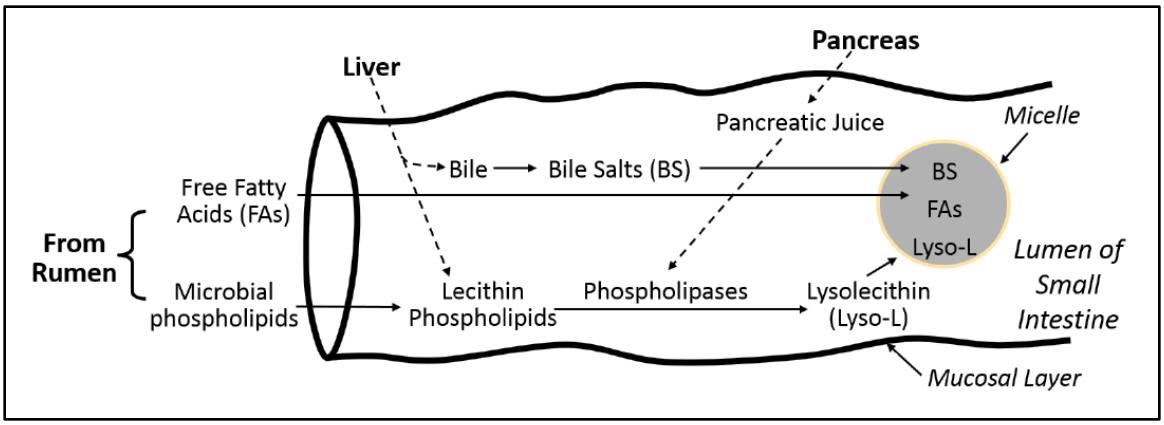


Figure 2.2. Fat digestion in the small intestine of ruminants (Adapted from Lock et al., 2006).

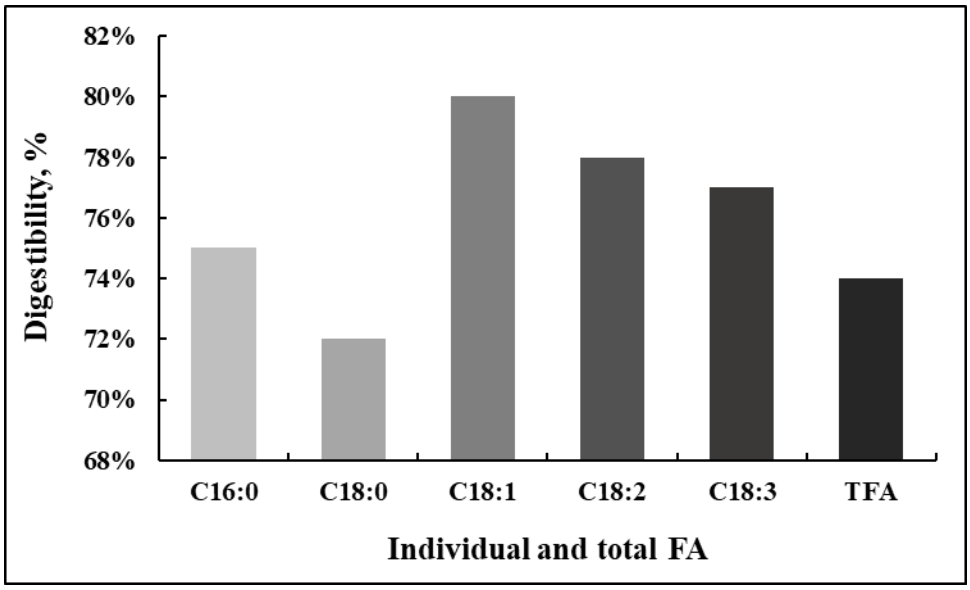


Figure 2.3. Individual and total fatty acid digestibility in dairy cows (Modified from Lock et al., 2006).

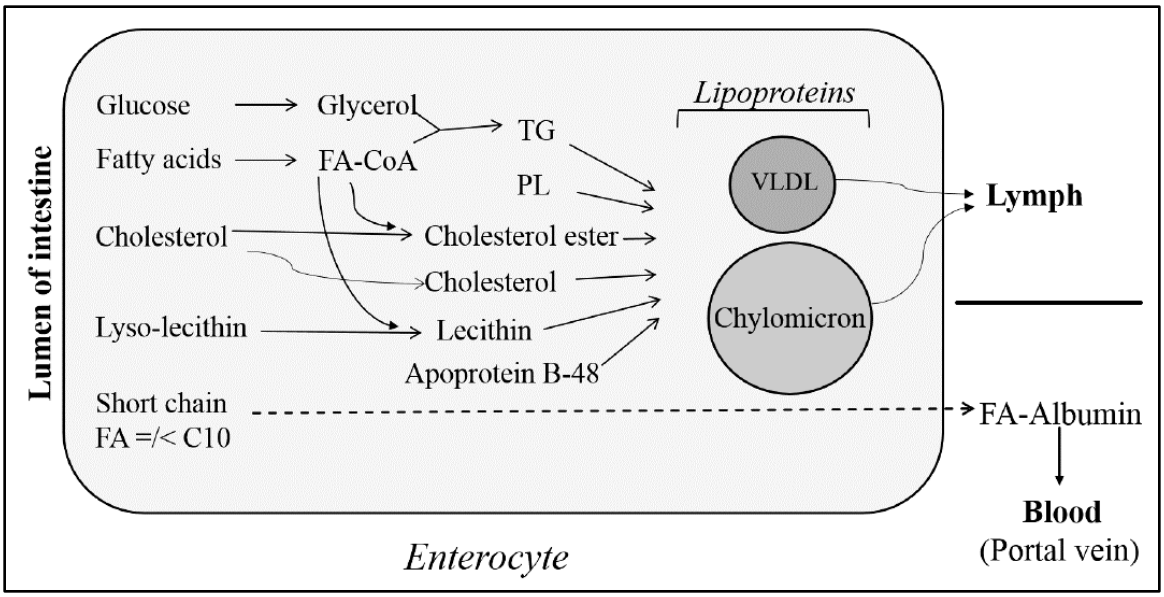


Figure 2.4. Fat absorption in the enterocytes of ruminants (Adapted from Navarrete, 2013).

Abbreviations: Fatty acids (FA); Tryglycerides (TG); Phospholipids (PL); fatty acid CoA (FA CoA), Very low-density lipoprotein (VLDL).

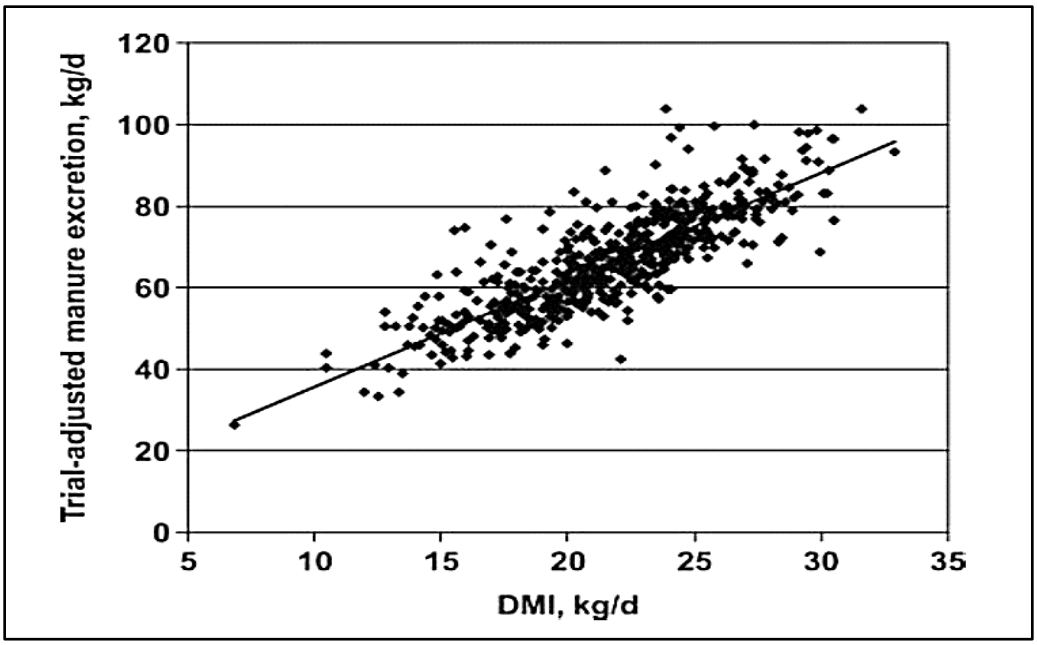


Figure 2.5. Relationship between DMI and trial-adjusted manure excretion for lactating cows (Adapted from Nennich et al., 2005). (Manure excretion (kg/d) = DMI (kg/d) × 2.63 + 9.4).

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# CHAPTER THREE

## SCREENING DIETARY FAT SOURCES AND LEVELS ADDED TO LOW AND HIGH FORAGE DIETS USING AN IN-VITRO GAS PRODUCTION SYSTEM


ABSTRACT

Including dietary fat can increase the energy density of diets fed to ruminants, reducing dry matter intake (**DMI**). Not all fat sources have detrimental effects on nutrient digestion and fermentation and can vary depending on the forage to concentrate ratio (**F:C**). Therefore, this study's objective was to screen the effects of including different fat types to high and low forage diets in vitro digestibility and fermentation. We hypothesized that incorporating fat in low forage diets can improve nutrient utilization without affecting digestibility and fermentation in-vitro gas production (**GP**). Treatments included either low forage (**LF**; 35%) or high forage (**HF**; 70%) with 2 fat levels (6 or 9% DM) screening for 6 different fat sources plus control (**CON**). The CON diet had a basal level of fat in the diet (3% fat; 0% fat inclusion); and fat sources were added to attain 6% or 9% fat and consisted of [Coconut oil, CO; Poultry fat, PF; Palm oil, PO; Palm kernel oil, PKO; Ca Salts, MEG; Soybean oil, SOY]. GP's modules were randomly assigned to treatments in a 2×2×7 factorial design and incubated for four 24 h runs. The CO-fed module had the highest DM apparent digestibility (**AD**), followed by SOY and PF. The true DM digestibility (**IVTDMD**) and OM AD were the highest in CO than the other fat types. The AD for DM, OM, NDF, and ADF was higher in LF. The 6% fat inclusion had a higher GP (109.6 vs. 103.5 mL ± 2.44). Total VFA concentration was lower in different fat types than the CON and the acetate molar proportion. The propionate was the lowest for the CON, which increased the A:P ratio. The results

suggest that an LF diet with high fat concentration can be utilized, and different fat sources may improve DM and fiber digestibility.

# INTRODUCTION

Feeding fat has gained interest in the last few decades. Adding fat to dairy diets became common practice for its potential to increase energy density in diets, improve palatability, and reduce feed dustiness (Azain, 2004). The advantages of fat addition to dairy rations include a potential increase in energy intake for high milk production (Ostergaard et al., 1981; Ruesegger et al., 1985). Also, improve rumen fermentation by optimizing starch to fiber ratio (Palmquist and Conrad, 1978) without the risk of feeding excessive fermentable carbohydrates (Jenkins and McGuire, 2006). The use of fat can enhance high forage-based diets (Reynolds et al., 1991; Zanton and Heinrichs, 2007; Naik et al., 2010). Modifying the forage to concentrate ratio (F:C) and manipulating nutrient fractions allow precision-fed dairy heifers to achieve adequate nourishment. Even though high concentrate precision fed diets showed improvement in N and OM digestibility (Zanton and Heinrichs, 2009), and resulted in similar effects on rumen fermentation (Lascano and Heinrichs, 2009; Lascano et al., 2009). However, in addition to the increase in grain costs, fiber intake can be decreased, and acidosis can occur because of feeding rapidly fermented NFC to dairy cattle (Palmquist and Jenkins 1980; Nocek, 1997).

Cost-effective by-products from numerous industries, such as poultry industry by-products, can be utilized by ruminants. Poultry fat (PF) is a by-product of chicken processing and extensively produced worldwide, can be a potential energy source. In contrast, soybean oil (SO) can decrease fiber digestion by inhibiting rumen microbes (Jenkins, 1993; Pantoja et al., 1994). Whereas coconut oil (CO) might improve rumen

fermentation (Machmuller et al., 2003; Pilajun and Wanapat, 2013). Several commercial fat preparations are available as rumen bypass fats or inert fats such as Megalac (calcium salts), made from palm oil (Eastridge, 2002; Rico et al., 2014). Specialty fats are developed to minimize the detrimental effects on rumen fermentation and the risk of decreasing fiber digestion (Palmquist and Jenkins, 1982; Jenkins and Harvatine, 2014). It has been reported that using these specialty fats with high saturated fats led to minimizing adverse effects on milk fat production, rumen fermentation, and feed intakes (Jenkins and Jenny, 1989).

In a study conducted by Elliott et al. (1997) on the effects of saturation of fat sources in steers, they reported that increasing fat sources' saturation tended to increase the NDF and ADF rumen digestibility. Other studies have reported no differences in ruminal or total tract digestibility of OM or fiber in lactating cows fed diets with increasing amounts of dietary fat or different sources (Palmquist, 1991; Drackley and Elliott, 1993). Oldick and Firkins (2000) reported that acetate responded quadratically as the fat sources' unsaturation degree increased. Several studies have explored various strategies for feeding fat to dairy cows (Rabiee et al., 2012). However, there is limited research regarding the effects of feeding fat on the growing dairy heifers, and to what extent can be strategically incorporated is unknown. Therefore, this study's objective was to evaluate the effects on digestibility and fermentation, including different types of fat with different F:C ratios using the in vitro gas production system. We hypothesized that incorporating fats in low forage diets can improve nutrient utilization without compromising fermentation and digestibility in a gas production system.

# MATERIALS AND METHODS

*Treatments and Experimental Design*

Treatments were two F:C combinations, either low forage (LF; 35%, DM) or high forage (HF; 70%, DM) with two dietary fat concentrations (6% or 9%) and six different fat source treatments plus control (CON). The CON diet had a basal level of fat in the diet [3% fat (0% fat inclusion); and fat sources were added to attain 6% or 9% fat and consisted of (Coconut oil, CO; Nature's oil, Streetsboro, OH; Poultry fat, PF; Valley proteins, Inc., Ward, SC; Palm oil, PO; Nature's oil, Streetsboro, OH; Palm kernel oil, PKO; Nature's oil, Streetsboro, OH; Ca Salts, MEG; Megalac regular; Soybean oil, SOY; Nature's oil, Streetsboro, OH)]. The experiment was conducted using an in vitro ANKOMRF gas production (GP; Ankom Technology, Macedon, NY) system. Treatments were randomly assigned to one of twenty-eight modules and allocated to a different module during each run to remove any module-specific differences. To allow the CON to be compared to the other fat treatments using a factorial modeling approach, it was assumed in the statistical analysis that the CON had the same fat levels as the other treatments, 6% and 9% (not just the 3%). That resulted in a 2×2×7 factorial treatment design and a randomized complete experiment design (run was the blocking factor) with 4 replicates per treatment as incubated for four 24 h runs. Each run was started with a clean module and inoculated with fresh ruminal contents collected from two cannulated Holstein cows. All diets were fed to the modules as total mixed rations (TMR) and predicted nutrient composition determined using NRC (2001). Dietary ingredients and chemical composition are presented in Table 3.1. Rations were grounded using a Wiley

Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 1-mm sieve, and 1 g of the premixed rations were placed in ANKOM F57 filter bags, sealed, and placed into the module glass bottle at the beginning of the 24 h incubation.

*Module Culture Conditions*

All procedures involving the surgical and animal care protocols were approved by the Clemson University Institutional Animal Care and Use Committee. Around 1800 h, the rumen contents were collected from two rumen cannulated Holstein cows fed a 50% forage:50% concentrate diet and strained through two-layers of cheesecloth into a prewarmed sealed container. The filtered rumen fluid was combined from both cows, mixed with a buffer in a 1:4 ratio. Homogenized together under magnetic stirrer and purged with $CO_2$ until inoculation into the GP modules. Also, during the time from when the rumen contents were collected to dilution and addition to modules (did not exceed 60 min.), the module glass bottles were maintained at 39$^{o}$C in a water bath to minimize the cold shock of microorganisms. Pre-prepared F57 filter bags (pre-rinsed F57 filter bags in acetone for 5 minutes and air-dried to remove surfactant that inhibits microbial digestion) containing 1 g of the premixed rations were placed into the module glass bottles. Exactly 100 mL of diluted inoculum (20 mL inoculum + 80 mL Cone (1998) buffer) was added to each gas production module glass bottle and placed in a 39$^{o}$C shaker water bath (70 rpm; Julabo SW22, Seelbach, Germany). They have purged continually with $CO_2$ directly into the bottle's top until the $CO_2$ filled the module glass bottle and then reattached the module to the glass bottle. The module cultures were connected to the computer by a radio frequency modem that allows each module to communicate remotely with the

computer. After calibrating the gas production sensors, we started recording data using the GP software and maintained for a 24 h of incubation and then stopped recording data, and data were saved in an excel spreadsheet.

*Sample Collection and Analysis*

After 24 h of incubation of each run, the F57 filter bags were removed from the module glass bottles and rinsed out twice with distilled water and gently pressed to remove excess gas and water, then placed in a forced-air drying oven for 48 h at 65$^{o}$C to determine the apparent DM digestibility. Following that, F57 filter bags were placed in the Ankom Fiber Analyzer and followed the procedure for determining NDF to determine the true DM digestibility (when determining true digestibility, it is necessary to remove any remaining soluble fractions using natural detergent solution; after rinsing the bags in cold tap water until the water is clear, place them in the Ankom Fiber Analyzer; Ankom technology method 3). Also, to determine the NDF apparent digestibility. Feed samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 1-mm sieve and analyzed for DM, OM, ash, and EE (AOAC, 2000). For NDF and ADF (Van Soest et al., 1991), an ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) was used with heat resistant α-amylase and sodium sulfite utilized in the NDF procedure and adjusted for ash content. Additionally, cultural contents were mixed thoroughly in the module glass bottles during sampling to ensure adequate sampling from the cultures. Culture pH was measured and recorded after 24 h of incubation, and a 5 mL sample of culture contents was taken at the same time points for VFA and ammonia analysis. Culture samples (5 mL) were pipetted to 15 mL

centrifuge tubes containing 1 mL of metaphosphoric acid (25%; w/v), and then, these tubes were stored at -20°C until VFA and ammonia analysis, as described by Moody et al. (2007). Samples were later thawed and centrifuged at 40,000 × g for 30 min at 4°C. After centrifugation, 1 mL of the supernatant was placed in a 2-mL Eppendorf microcentrifuge tube and used to analyze $NH_3$-N, according to Chaney's methods Marbach (1962) with modifications including reduced sample and reagent volume to accommodate the use of a 96-well plate reader. Another 0.5 mL of the supernatant was combined with 0.5 mL distilled water and 100 µL of internal standard (86 µmol of 2-ethylbutyric acid/mL) in a GC vial. GC then analyzed samples for VFA–flame-ionization detection according to the methods of Yang and Varga (1989) and injected into a Hewlett-Packard 6890 gas chromatograph (San Jose, CA) equipped with a custom packed column (2 m × 0.32 cm × 2.1 mm ss; 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW).

*Statistical Analysis*

All statistical analyses were conducted in SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC) using the MIXED procedure. Data were analyzed as a 2×2×7 factorial treatment structure in a randomized complete block design with forage, fat, source, forage × fat, fat × source, and forage × fat × source as a fixed effect, and module (forage) and run as a random effect, for the following model:

$$Yijk = \mu + Fi + Ml(Fi) + Pj + Ck + FPij + PCjk + FPCijk + Rm + eijklm,$$

Where Yijk = the dependent variable, µ = the overall mean, Fi = the fixed effect of forage, Ml(Fi) = the random effect of a module within forage, Pj = the fixed effect of

fat, Ck = the fixed effect of source, FPij = the interaction between forage and fat, PCjk = the interaction between fat and source, FPCijk = the interaction between forage, fat and source, Rm = the random effect of a run, and eijklm = the residual error. The PDIFF option-adjusted by Tukey method was included in the LSMEANS statement to account for multiple comparisons. Residuals for all models were found to be normally distributed (Shapiro-Wilk test for normality). Least square means are presented in tables, and evidence for statistical significance was declared at $P \leq 0.05$, while trends for main effects and interactions are discussed at $0.10 \geq P > 0.05$.

## RESULTS AND DISCUSSION

### *Diet Composition and Nutrient Inputs*

Diet ingredients and chemical composition values are presented in Table 3.1. Diets were planned and formulated to differ mainly in providing dietary fat by adding different fat sources. The dietary NDF and ADF were lower for LF diets compared to the HF diets, whereas the NFC was higher for LF diets than for the HF diets, as was their input because of the lower level of forage and a higher level of concentration in these diets (Table 3.1). The dietary EE concentrations increased gradually in the diets up to 9% with different fat inclusion. The fat inclusion replaced the ground corn in the control diet of both LF and HF diets, and that resulted in a decrease in NFC in the other different types of fat treatments. All other components of the rations were formulated to be similar between treatments.

### *Digestibility of Nutrients*

#### *Forage Effect*

Apparent digestibility coefficients (dC), true dry matter digestibility (IVTDMD), and cumulative gas production (GP) are outlined in Table 3.2. The dC of DM, OM, NDF, and ADF were greater for the LF-fed module than for the HF-fed module. These observations are consistent with results reported in a study conducted on Holstein dairy heifers fed LF or HF diets composed of a combination of 40 or 80% CS and corn stover (Lascano and Heinrichs, 2011) where DM and OM dC were higher for LF compared to HF diets. Two levels of F:C diets were fed to dairy heifers by Lascano et al. (2016b) and observed higher DM and OM dC for LF compared to HF diets. Similarly, higher DM and

OM digestibilities for LF compared to HF diets were observed by (Suarez-Mena et al., 2015). The greater digestibility of the LF-fed module can be attributed to the greater digestibility of these diets' ingredients (NRC, 2001). Other studies have shown an increase in DM and OM dC when LF and HF diets have been fed restrictively (Colucci et al., 1989; Reynolds et al., 1991; Murphy et al., 1994). These results did not agree with a study conducted on Holsten heifers fed low forage (45% forage) and high forage (60% forage) where DM and OM dC did not differ between LF and HF diets (Koch et al., 2017). The dC of NDF and ADF disagreed with Ranathunga et al. (2012) findings where the ruminal digestion of NDF was improved in HF diets containing DDGS in dairy cows compared with LF diets containing DDGS. They attributed that to the ability of fat from DDGS to bound in the feed particle and slowly introduced to the rumen. Furthermore, this could be attributed to the lower pH level for LF diet because cellulolytic bacteria are very sensitive to pH and their activity and growth start to decline under pH 6.0 (Russell and Wilson, 1996), but the pH in the current study was similar because of the type of buffer used in the experiment. Koch et al. (2017), Suarez-Mena et al. (2015), and Zanton and Heinrichs (2009) observed that the ADF dC did not differ between LF and HF diets, but in agreement with other studies where NDF dC was greater for LF diets (Zanton and Heinrichs, 2009; Lascano and Heinrichs, 2011; Lascano et al., 2016b). The LF-fed module showed a higher cumulative gas production compared to the HF-fed module. The current finding agrees with Kim et al. (2018), where they conducted an in-vitro study to measure the total gas production of rumen fluid collected from non-lactating cows fed three levels of concentrate diet. They reported that the high proportion of concentrate

produced the highest total gas after 24 h of incubation. They attributed that to the fact that the concentrate digestibility is faster than forage digestibility, which explains the higher total gas production observed in a high proportion of concentrate. Also, Pilajun and Wanapat (2014) reported a higher accumulated gas production as a concentrate level increased in the diet when feeding four different F:C ratios and using a gas fermentation production technique for 96 h of incubation.

*Fat Effect*

The level of fat inclusion did not show any effect on nutrients dC. These findings did not agree with a study conducted by Anderson et al. (2015), where they have observed a higher dC of NDF and ADF when heifers limit-fed a high-fat DDGS compared to a low-fat DDGS, whereas the DM and OM did not differ between the treatments. It was suggested that the high-fat DDGS diet contains a lower starch content compared to the low-fat DDGS, which is the case with NFC in our study (Table 3.1) resulted in higher efficiency of utilization of fiber and improve total-tract digestion. Also, Suarez-Mena et al. (2015) observed a quadratic DM, OM, NDF, and ADF dC response to increasing levels of DDGS up to 14% inclusion in the diets. These results agreed with a study conducted by Lascano et al. (2016a) using two levels of fat with no added fat or 3.3% added soybean oil in continuous culture fermenter. They did not observe any effects on DM and ADF dC between the two diets' two levels of fat. Koch (2017) reported a depression in DM, OM, NDF, and ADF dC when continuous culture fermenters were fed high soybean oil compared to low soybean oil. Feeding excess FA has been reported to depress fiber digestibility (Rico et al., 2014). Also, the dietary polyunsaturated fatty acid

as in soybean oil has been related to limiting the growth of fiber digesting bacteria, which reduce ruminal fiber digestibility (Van Soest, 1994). Manthey and Anderson (2018) reported no effects on dC when heifers limit fed DDGS with ad libitum grass hay. They related that to feeding grass hay as ad libitum, which resulted in a slightly different limit feed program than the typical one. The cumulative gas production was decreased as the level of fat inclusion increased in the diet. The supplementation of plant oil decreased gas fermentation production in a study conducted by Pilajun and Wanapat (2014) using two different plant oil inclusion levels. Palmquist (1994) reported that when ruminants receive diets with a fat content higher than 7% of DM, the fiber digestion could be restricted, which might explain the current finding.

*Source Effect*

The dC of DM, OM, NDF, ADF, and TDM were affected by the type of fat with greater DM dC for the CO-fed, followed by SOY, PF, CON, PKO, and PO-fed. Also, IVTDMD and OM dC were the highest with the CO-fed module, followed by all MEG, SOY, PF, PO, CON, and PKO-fed modules. Furthermore, the NDF dC was higher in MEG and CO-fed modules followed by PO, SOY, PF, CON, and PKO-fed module, whereas the ADF dC was similar fat types except for the PKO-fed module with the lowest value. These observations are consistent with results reported in a study conducted by Elliott et al. (1997) on the effects of steers' saturation of fat sources. It has been reported that increasing saturation of fat sources (tallow, partially hydrogenated tallow, hydrogenated tallow, blend of hydrogenated tallow and hydrogenated fatty acids, and hydrogenated fatty acids) tended to increase the NDF and ADF digestibility in the rumen.

Several other studies have reported no differences in ruminal or total tract digestibility of OM or fiber in lactating cows fed diets with different fat sources (Palmquist and Conrad, 1978; Ohajuruka et al., 1991; Palmquist, 1991; Drackley and Elliott, 1993). It has been reported in an in-vitro study that the CO, which contains high saturated medium-chain fatty acids, had no adverse effect on DM digestibility in the in-vitro gas fermentation production technique (Pilajun and Wanapat, 2014). That is in agreement with other studies on CO's effect on swamp buffalo by the same group (Pilajun and Wanapat, 2013). A study conducted by Lascano et al. (2016a) using 3.3% added SO in a continuous culture fermenter. They did not observe any effects on DM and ADF dC compared to the control diet. Whereas, Koch (2017) reported depression in DM, OM, NDF, and ADF dC when continuous culture fermenter fed high SO compared to low SO. Koch stated that the dietary polyunsaturated fatty acids had been shown to depress fiber dC by limiting the growth of fiber digestion bacteria (Van Soest, 1994). This finding is common in the literature (Rico et al., 2014). Bock et al. (1991) conducted a study on steers fed treatments consisted of no added fat, 3.5% tallow, and soybean oil soap stock. They reported that adding fat did depress DM and fiber digestibility. Zali et al. (2020) investigated the effects of feeding calcium salts of poultry oil on dairy cows compared to a palmitic acid-enriched fat and a mix between the two. They observed that the fiber and protein digestibility were similar between treatments. They concluded that even though the calcium salts of poultry oil improved dairy cows' production but decreased feed efficiency. Jenkins (2006) reported a literature review of tallow digestibility compared to other fat sources that only tallow and calcium salts of palm FA had numerically higher

digestibility than other fat sources examined. Ngidi et al. (1990) reported an increase in NDF digestibility when Ca soap's level increased in the diet. It has been suggested that the higher apparent total tract digestibility of NDF in cows-fed Ca-LCFA was related to an increase in post-ruminal degradation (Chouinard et al., 1998). The digestibility of ADF under bypass fat addition may be either increased (Naik et al., 2009) or not affected (Thakur and Shelke, 2010; Sirohi et al., 2010). It has been reported that the ADF digestibility varies depending on the level of fat addition with no effect at a low-fat level (Schauff and Clark, 1992). Naik et al. (2007) reported that bypass fat did not influence buffaloes' cellulose digestibility. Erickson et al. (1992) reported that hemicellulose digestibility was improved with the addition of Ca-LCFA and caused an increase in NDF and a decrease in ADF digestibility in dairy cows. Cumulative gas production was affected by the different types of fat with the highest value for the CO-fed module and SOY-fed module, followed by all of the PF-fed module, CON-fed module, and PO-fed module and then MEG-fed module and PKO-fed module. These results did not agree with Pilajun and Wanapat (2014) and Pilajun and Wanapat (2013), where they reported a reduction in gas production when CO was included in the diets. They attributed that to the negative effect of medium-chain fatty acids on the fermentation as they are small enough to be readily dissolved and disrupt the cell membranes and inhibit enzymes involved in energy production and lead to the microbial death cell (Machmuller, 2006).

*Characteristics of Fermentation*

*Forage Effects*

Culture VFA profile, $NH_3N$, and pH are shown in Table 3.3. The total VFA concentration was lower for the LF-fed module than for the HF-fed module, mainly because of the lower acetate molar proportion for the LF-fed module compared to the HF-fed module. In contrast, propionate and butyrate molar proportions were higher for the LF-fed module than the HF-fed module. As a result, acetate:propionate ratio was lower in the LF-fed module than in the HF-fed module. The lower total VFA concentration for LF-fed fermenters did not agree with in-vitro and in-vivo studies (Fuentes et al., 2009; Lascano et al., 2016b). Calsamiglia et al. (2008) concluded that the main factor influencing VFA concentration is the interaction between pH and F:C in the diets. Also, Moody et al. (2007) stated that the VFA concentrations were higher in LF than HF when pH was affected by F:C. In the current study, the pH was similar between the LF-fed module and the HF-fed module and is mainly related to the type of buffer used in the study to keep the culture in the same pH level 24 h incubation. While, the greater acetate molar proportion for HF-fed fermenter is consistent with previous studies (Martinez et al., 2010; Gudla et al., 2012; Suarez-Mena et al., 2015; Lascano et al., 2016b). Acetate results of structural carbohydrate fermentation by cellulolytic bacteria and these bacteria can be inhibited by lower NDF inputs as in the present study, which may explain the lower acetate molar proportion for LF-fed fermenter (Martin et al., 2002). Several studies showed that the F:C ratio did not affect propionate and butyrate (Rodriguez-Prado et al., 2004; Gudla et al., 2012; Suarez-Mena et al., 2015). Cultural pH

was similar between the LF-fed module compared to the HF-fed module. The pH values did not agree with a study conducted using Rusitec fermenters as they reported a higher pH in HF-fed fermenter than for LF-fed fermenter (Martinez et al., 2010). The NH3N concentration was lower for the LF-fed module than for the HF-fed module. These findings agree with several studies used different F:C ratio in continuous culture fermenter (Calsamiglia et al., 2008; Fuentes et al., 2009; Martinez et al., 2010). The lower $NH_3N$ in the LF-fed module than in the HF-fed module could be due to ammonia for AA's de novo synthesis.

*Fat Effects*

The fat inclusion level in the diets decreased the total VFA concentrations with a lower value for the 9% fat-fed module than the 6% fat-fed module. In contrast, the acetate, propionate, butyrate molar proportions, and the acetate:propionate ratio were not affected by fat inclusion in the diets. Rumen fermentation is not affected when fat levels are low in the diets because rumen microbes are able to saturate FA, but this capacity can be exceeded at higher levels, and FA can accumulate in the rumen and interfere with rumen fermentation (NRC, 2001). The different levels of fat inclusion decreased the culture pH. Suarez-Mena et al. (2013) reported a similar rumen pH between treatments as DDGS increased in the diets. In contrast, Manthey et al. (2016) observed a linear decrease in rumen pH as DDGS increased in the diets, and they attributed that to the F:C ratio. Ammonia concentration was increased as the fat inclusion increased in the diets. Suarez-Mena et al. (2015) and Manthey et al. (2016) observed similar results, and they attributed that to the lower ME intake with the addition of DDGS. Therefore, the

microbial capacity to assimilate amino acids and ammonia was negatively affected and NH3 accumulated in the rumen (NRC, 2001). Yang et al. (2009) attributed the higher NH3N concentration as fat included in the diets is due to the greater proteolytic bacteria.

*Source Effects*

The different types of fat in the diets decreased the total VFA concentrations in the modules compared to the CON-fed module. Elliott et al. (1997) reported that the total VFA concentration decreased when different fat sources were fed compared to the control diet. Pilajun and Wanapat (2014) reported a lower total VFA concentration in the in-vitro gas production technique after 48 h incubation with CO diet. They attributed that to the negative effect of medium-chain fatty acids on the fermentation. Also, the higher total VFA concentration for CON-fed fermenters could be related to the pH as it is the main factor influencing VFA concentrations (Calsamiglia et al., 2008). In the current study, the pH was lower for the CON-fed module compared to the CO-fed module and PF-fed module. Oldick and Firkins (2000) reported that the acetate responded quadratically as the fat sources' unsaturation degree increased (tallow, partially hydrogenated tallow, and animal-vegetable fat). In addition, Elliott et al. (1997) reported a decrease in acetate's molar proportion when different saturation fat (tallow, partially hydrogenated tallow, hydrogenated tallow, blend of hydrogenated tallow, and hydrogenated fatty acids, and hydrogenated fatty acids) was fed and increased linearly as saturation increased.

In contrast, the propionate molar proportion was higher for the CO-fed module and PF-fed module compared to CON-fed module and other fat type treatments. The

propionate finding did not agree with a study conducted by Pilajun and Wanapat (2014) as they reported a lower propionate in the CO diet after 48 h incubation. Also, with a study by Oldick and Firkins (2000), Holstein heifers fed a different degree of fat saturation (tallow, partially hydrogenated tallow, and animal-vegetable fat). Elliott et al. (1997) reported a linear decrease in propionate as saturation increased. As a result of the increase in propionate molar proportion, the acetate:propionate ratio was the lowest in the CO-fed module and PF-fed module compared to the CON-fed module and the other fat types treatments. Some studies have reported that feeding fat can decrease the acetate:propionate ration (Oldick and Firkins, 2000; Elliott et al., 1997) or unchanged (Tjardes et al., 1998). They related the decrease in acetate:propionate ratio to the reduction in ruminal NDF dC, which is not the case in the current study. Ruminal fermentation has been frequently shifted to greater propionate in cows fed fats such as tallow, yellow grease, or animal-vegetable blends and resulted in lower acetate:propionate ratio (Jenkins and Jenny, 1989; Ohajuruka et al., 1991; Weisbjerg et al., 1991; Schauff et al., 1992; Elliott et al., 1993). Butyrate molar proportion was lower in the CON-fed module compared to the different types of fat treatments. Manthey and Anderson (2018) suggested that the differences in starch contents and intake are the reason behind the shift in VFA concentrations and the decrease in acetate and increase in propionate. They also suggested that higher propionate is related to more energy-efficient and rumen fermentation in heifers fed DDGS diets (Manthey et al., 2016). There are less methane and carbon dioxide production in propionate as compared with acetate (Fahey and Berger, 1988). Cultural pH was lower for the CON-fed module compared to the

different types of fat-fed module. Chibisa et al. (2015) stated that the drop in pH with high starch diets is common in the literature. The inclusion of different fat sources in the diets increased the cultural pH with the highest pH values were observed at the CO-fed module, followed by the PF-fed module compared to the CON-fed module. That agrees with a study conducted by Elliott et al. (1997), where they reported an increase in ruminal pH as different saturated fat were fed, and they attributed that to the lower fermentable carbohydrate content in these diets. The $NH_3N$ concentration was similar between the different types of fat compared to the CON-fed module except for the MEG-fed module, which had the highest $NH_3N$ concentration. Elliott et al. (1997) reported a linear increase in $NH_3N$ concentrations as the degree of saturation increased (tallow, partially hydrogenated tallow, hydrogenated tallow, blend of hydrogenated tallow and hydrogenated fatty acids, and hydrogenated fatty acids), and they suggested that the dietary triglycerides became more unsaturated, and the ruminal protein digestion inhibited. These results could be related to better synchrony between N and energy availability for microorganism's activity. These results agreed with previous studies where the ruminal $NH_3N$ concentrations were not affected by supplemental fat or fat source (Doreau and Ferlay, 1995; Pantoja et al., 1995; Oldick and Firkins, 2000).

# CONCLUSIONS

Screening different types of unsaturated fat with different inclusion levels in both low and high forage diets using a gas production system showed differential effects on culture fermentation. Acetate molar proportion decreased, and the A:P ratio while maintaining higher pH in more saturated than the unsaturated fat and control treatments. The type of fat had some minor effect on the cumulative gas production, whereas the high forage and high-fat inclusion decreased the gas production. Results from this study demonstrate that poultry fat inclusion along with coconut oil, calcium salt, and palm oil inclusion improved true dry matter digestibility significantly in comparison to unsaturated soybean oil and the control diet, which has lower dietary fat, while the level of fat inclusion had no detrimental impact on nutrients digestibility. These results showed that the LF-fed modules consistently resulted in higher nutrient utilization and the apparent digestibility of most nutrients. Therefore, we can conclude that the high concentrate diet up to 65% with high-fat inclusion up to 6% from by-products dietary poultry fat or coconut oil can be successfully included in rations for precision-fed dairy heifers without negative effect on nutrient digestibility and fermentation characteristics.

Table 3.1. Ingredient and chemical composition of low (LF) and high (HF) forage diets containing unsaturated fat sources with different fat concentration (CON 3%, CO 6%, PF 6%, PO 6%, PKO 6%, MEG 6%, SOY 6%, CO 9%, PF 9%, PO 9%, PKO 9%, MEG 9%, SOY 9% DM) fed to in vitro gas production system.

| | | Fat type, % in the diet | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient, % | Forage | CON 3% | CO 6% | PF 6% | PO 6% | PKO 6% | MEG 6% | SOY 6% | CO 9% | PF 9% | PO 9% | PKO 9% | MEG 9% | SOY 9% |
| Coastal hay | LF | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| | HF | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Corn silage | LF | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| | HF | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Ground corn | LF | 51.8 | 46.4 | 46.4 | 46.4 | 46.4 | 46.4 | 46.4 | 40.8 | 40.8 | 40.8 | 40.8 | 40.8 | 40.8 |
| | HF | 24.4 | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 | 18.6 | 12.6 | 12.6 | 12.6 | 12.6 | 12.6 | 12.6 |
| Soybean meal (SBM) | LF | 11.2 | 13.7 | 13.7 | 13.7 | 13.7 | 13.7 | 13.7 | 16.4 | 16.4 | 16.4 | 16.4 | 16.4 | 16.4 |
| | HF | 3.60 | 6.33 | 6.33 | 6.33 | 6.33 | 6.33 | 6.33 | 9.20 | 9.20 | 9.20 | 9.20 | 9.20 | 9.20 |
| Mineral mix | LF | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| | HF | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Fat inclusion | LF | 0.00 | 2.80 | 2.80 | 2.80 | 2.80 | 2.80 | 2.80 | 5.79 | 5.79 | 5.79 | 5.79 | 5.79 | 5.79 |
| | HF | 0.00 | 3.04 | 3.04 | 3.04 | 3.04 | 3.04 | 3.04 | 6.19 | 6.19 | 6.19 | 6.19 | 6.19 | 6.19 |
| Chemical composition | | | | | | | | | | | | | | |
| DM % | LF | 91.1 | 90.9 | 91.6 | 91.9 | 92.3 | 92.1 | 91.8 | 92.0 | 91.8 | 92.3 | 92.5 | 92.3 | 92.1 |
| | HF | 91.9 | 91.7 | 92.2 | 91.9 | 92.2 | 92.0 | 92.0 | 92.0 | 92.5 | 92.3 | 92.6 | 92.4 | 92.2 |
| OM, % | LF | 95.3 | 95.5 | 95.0 | 95.1 | 95.3 | 94.8 | 94.7 | 95.1 | 95.2 | 95.2 | 95.0 | 94.7 | 94.7 |
| | HF | 93.9 | 94.1 | 94.0 | 94.0 | 94.1 | 93.6 | 94.1 | 94.0 | 93.9 | 93.8 | 94.0 | 93.5 | 94.0 |
| CP, % | LF | 12.0 | 13.8 | 13.2 | 13.2 | 13.4 | 13.2 | 13.5 | 14.1 | 13.8 | 14.5 | 14.3 | 14.0 | 13.8 |
| | HF | 9.40 | 11.2 | 11.4 | 11.0 | 11.8 | 11.3 | 10.7 | 12.0 | 12.0 | 11.9 | 11.3 | 12.1 | 12.1 |
| NDF, % | LF | 22.7 | 20.2 | 22.3 | 22.9 | 22.7 | 21.1 | 22.7 | 20.5 | 23.2 | 22.4 | 21.2 | 21.7 | 23.1 |
| | HF | 37.2 | 34.3 | 36.8 | 37.9 | 36.9 | 36.1 | 36.6 | 35.2 | 36.9 | 39.4 | 37.1 | 34.3 | 36.5 |
| ADF, % | LF | 11.4 | 11.4 | 11.5 | 11.6 | 11.2 | 10.5 | 11.1 | 12.0 | 11.8 | 11.3 | 10.7 | 10.7 | 11.7 |
| | HF | 20.4 | 19.8 | 20.2 | 20.7 | 20.1 | 19.7 | 19.8 | 21.9 | 20.3 | 21.3 | 20.5 | 18.9 | 20.0 |
| EE, % | LF | 3.32 | 5.58 | 5.78 | 5.76 | 5.84 | 4.97 | 5.76 | 8.59 | 8.64 | 8.84 | 8.40 | 7.56 | 8.50 |
| | HF | 3.08 | 5.48 | 5.33 | 5.35 | 5.51 | 4.67 | 5.25 | 8.21 | 8.19 | 8.53 | 8.38 | 7.77 | 8.46 |
| NFC, % | LF | 57.2 | 55.9 | 53.7 | 53.2 | 53.3 | 55.5 | 52.7 | 52.0 | 49.5 | 49.4 | 51.1 | 51.4 | 49.4 |
| | HF | 44.2 | 43.1 | 40.4 | 39.6 | 39.8 | 41.5 | 41.6 | 38.6 | 36.7 | 34.0 | 37.2 | 39.4 | 36.9 |
| Ash, % | LF | 4.63 | 4.50 | 4.96 | 4.85 | 4.68 | 5.18 | 5.22 | 4.86 | 4.76 | 4.77 | 4.96 | 5.26 | 5.25 |
| | HF | 6.04 | 5.90 | 5.99 | 5.98 | 5.86 | 6.38 | 5.82 | 5.95 | 6.05 | 6.14 | 5.92 | 6.45 | 5.96 |
| TDN | LF | 77.1 | 80.7 | 81.6 | 82.4 | 81.9 | 80.3 | 81.9 | 83.8 | 85.2 | 84.7 | 84.6 | 78.4 | 84.3 |
| | HF | 69.4 | 72.7 | 73.0 | 73.3 | 69.1 | 70.8 | 73.1 | 74.8 | 77.3 | 77.0 | 77.6 | 71.3 | 77.5 |
| ME, Mcal/Kg | LF | 2.81 | 2.94 | 2.98 | 3.01 | 2.99 | 2.93 | 2.99 | 3.06 | 3.11 | 3.09 | 3.09 | 2.86 | 3.07 |
| | HF | 2.53 | 2.65 | 2.66 | 2.67 | 2.52 | 2.58 | 2.67 | 2.73 | 2.82 | 2.81 | 2.83 | 2.60 | 2.83 |

[1]All diets were ground to 1 mm
[2]NFC: non-fiber carbohydrates = 100 - (CP + ether extract + NDF + Ash)
[3]ME calculated using modified equations from NRC (2001), using TDN values as reported by Cumberland Valley Analytical Services, Inc., Waynesboro, PA. ME = (TDN × 4.409 × 1.01 – 0.45) × 0.82. To represent better the increase in energy as fat increased in the diets, another modified equation from NRC (2001) was used. ME = (TDN × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82

Table 3.2. Nutrient apparent digestibility of in vitro gas production system fed low (LF) and high (HF) forage diets containing different fat sources with different fat concentration (CON 3%, CO 6%, PF 6%, PO 6%, PKO 6%, MEG 6%, SOY 6%, CO 9%, PF 9%, PO 9%, PKO 9%, MEG 9%, SOY 9% DM).

| | Fat type | | | | | | | Forage | | Fat % | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Digestibility, % | CON | CO | PF | PO | PKO | MG | SOY | LF | HF | 6% | 9% | SE | Type | F:C | Fat |
| DM | 50.6 $^{c}$ | 54.5 $^{a}$ | 50.6 $^{c}$ | 49.5 $^{d}$ | 50.1 $^{cd}$ | 49.7 $^{d}$ | 51.8 $^{b}$ | 54.6 | 47.3 | 51.2 | 50.8 | 0.48 | <0.01 | <0.01 | 0.10 |
| IVTDMD | 72.7 $^{d}$ | 76.8 $^{a}$ | 73.0 $^{cd}$ | 72.9 $^{cd}$ | 71.2 $^{e}$ | 74.5 $^{b}$ | 73.6 $^{c}$ | 80.3 | 66.7 | 73.4 | 73.6 | 0.56 | <0.01 | <0.01 | 0.31 |
| OM | 71.0 $^{d}$ | 75.4 $^{a}$ | 71.2 $^{cd}$ | 71.2 $^{cd}$ | 69.4 $^{e}$ | 72.7 $^{b}$ | 71.8 $^{c}$ | 78.9 | 64.6 | 71.7 | 71.9 | 0.35 | <0.01 | <0.01 | 0.21 |
| NDF | 58.1 $^{b}$ | 59.2 $^{ab}$ | 58.0 $^{b}$ | 59.7 $^{ab}$ | 52.2 $^{c}$ | 60.8 $^{a}$ | 58.9 $^{ab}$ | 66.2 | 49.9 | 58.1 | 58.2 | 0.86 | <0.01 | <0.01 | 0.78 |
| ADF | 53.1 $^{a}$ | 53.6 $^{a}$ | 53.1 $^{a}$ | 54.4 $^{a}$ | 45.7 $^{b}$ | 55.0 $^{a}$ | 53.5 $^{a}$ | 62.6 | 42.5 | 53.1 | 52.2 | 1.08 | <0.01 | <0.01 | 0.17 |
| GP[1] mL | 110 $^{ab}$ | 114 $^{a}$ | 109 $^{ab}$ | 101 $^{ab}$ | 99.1 $^{b}$ | 100 $^{b}$ | 113 $^{a}$ | 111 | 101 | 109 | 103 | 5.07 | 0.03 | 0.01 | 0.03 |

[1]Cumulative gas production in mL; gas pressure was converted to mole using the ideal gas law, n = p (V/RT), and then converted to milliliter using the Avogadro's law, gas produced in mL = n × 22.4 × 1000

Table 3.3. Volatile fatty acids, $NH_3N$, pH, and GP of in vitro gas production system fed low (LF) and high (HF) forage diets containing different fat sources with different fat concentration (CON 3%, CO 6%, PF 6%, PO 6%, PKO 6%, MEG 6%, SOY 6%, CO 9%, PF 9%, PO 9%, PKO 9%, MEG 9%, SOY 9% DM).

| | Fat type | | | | | | | Forage | | Fat % | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Culture fermentation | CON | CO | PF | PO | PKO | MG | SOY | LF | HF | 6% | 9% | SE | Type | F:C | Fat |
| Total VFA, mM | 89.6 $^{a}$ | 82.8 $^{b}$ | 68.9 $^{c}$ | 69.4 $^{c}$ | 69.1 $^{c}$ | 70.3 $^{c}$ | 71.2 $^{c}$ | 73.1 | 77.7 | 80.9 | 69.9 | 1.20 | <0.01 | <0.01 | <0.01 |
| VFA, mol/100 mol | | | | | | | | | | | | | | | |
| Acetate | 67.7 $^{a}$ | 56.5 $^{e}$ | 58.2 $^{d}$ | 60.3 $^{c}$ | 61.5 $^{bc}$ | 62.2 $^{b}$ | 62.0 $^{b}$ | 56.3 | 66.1 | 60.9 | 61.4 | 0.58 | <0.01 | <0.01 | 0.19 |
| Propionate | 20.8 $^{d}$ | 29.0 $^{a}$ | 26.8 $^{b}$ | 24.4 $^{c}$ | 24.8 $^{c}$ | 23.9 $^{c}$ | 24.5 $^{c}$ | 27.9 | 21.9 | 24.9 | 24.9 | 0.45 | <0.01 | <0.01 | 0.94 |
| Butyrate | 11.5 $^{c}$ | 14.5$^{ab}$ | 15.0 $^{a}$ | 15.2 $^{a}$ | 13.7 $^{b}$ | 14.0 $^{ab}$ | 13.5 $^{b}$ | 15.9 | 11.9 | 14.2 | 13.7 | 0.52 | <0.01 | <0.01 | 0.17 |
| Acetate:propionate | 3.29 $^{a}$ | 2.00 $^{d}$ | 2.25 $^{c}$ | 2.56 $^{b}$ | 2.63 $^{b}$ | 2.69 $^{b}$ | 2.60 $^{b}$ | 2.09 | 3.06 | 2.59 | 2.56 | 0.06 | <0.01 | <0.01 | 0.37 |
| pH | 6.56 $^{b}$ | 6.62 $^{a}$ | 6.63 $^{a}$ | 6.61 $^{ab}$ | 6.62 $^{a}$ | 6.59 $^{ab}$ | 6.61 $^{ab}$ | 6.60 | 6.61 | 6.62 | 6.59 | 0.02 | 0.29 | 0.84 | 0.01 |
| $NH_3N$, mg/dL | 10.6 $^{b}$ | 10.8 $^{b}$ | 10.1 $^{b}$ | 10.2 $^{b}$ | 10.3 $^{b}$ | 11.6 $^{a}$ | 10.2 $^{b}$ | 8.70 | 12.3 | 10.1 | 10.9 | 0.44 | 0.01 | <0.01 | 0.04 |

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# CHAPTER FOUR

## SIMULATING PRECISION FEEDING OF HIGH CONCENTRATE DIETS WITH HIGH FAT INCLUSION AND DIFFERENT PLANT-BASED SATURATED, UNSATURATED, AND ANIMAL FAT SOURCES IN CONTINUOUS CULTURE FERMENTORS


ABSTRACT

Controlling dry matter intake (**DMI**) is one strategy to reduce feed costs and increase efficiency. Including fat at a high concentrate level can increase the energy density of diets fed to ruminants, thus reducing DMI further. Therefore, the objective of this study was to evaluate the effects on fermentation and nutrient digestion of including different fat sources when high concentrate diets with high-fat inclusion are used under simulating precision feeding in continuous culture. We hypothesized that incorporating different fat sources to the aforementioned program can improve nutrient utilization without affecting rumen fermentation. Four treatments were randomly assigned to 8 continuous culture in a randomized complete block design and ran for 2 periods of 10 d. Diets included high concentrate (**HC**; 65%) with high-fat inclusion starting with a basal level of fat as control [3% fat (0% fat; **CON**); and 9% fat (6% poultry fat; **PF**, coconut oil; **CO**, and soybean oil; **SO**)]. Data were analyzed using the MIXED procedure of SAS with repeated measures. The DM, OM, NDF, ADF, and hemicellulose digestibility coefficients (**dC**) were higher for PF and CO, followed by SO and then the CON. Starch and FA dC were higher for different fat sources than for the CON. The total VFA concentration was higher for CON. There was a reduction in acetate and propionate with different fat sources. Mean culture pH and $NH_3N$ were the highest for CO, followed by PF, then SO, and CON. Protozoa population was higher for CON than for the other fat

treatments, followed by CO, PF, and SO. These results suggest that simulated precision feeding with high concentrate diets up to 65% and high fat up to 6% can improve nutrient digestibility.

# INTRODUCTION

High fiber-based diets are inefficient in terms of energy and protein utilization and lower digestibility compared to concentrates (Reynolds et al., 1991; Moody et al., 2007; Zanton and Heinrichs, 2007). However, that can be potentially enhanced by incorporating concentrate, fat, or both to make the diets more energy-dense (Naik et al., 2010). The use of high concentrate precision-fed diets showed improvement in OM digestibility (Zanton and Heinrichs, 2009) and resulted in similar effects on rumen fermentation (Lascano and Heinrichs, 2009; Lascano et al., 2009). However, in addition to the increase in grain costs, negative effects of high-level feeding of concentrates on dairy cattle's fiber intake and acidosis incidence can occur (Palmquist and Jenkins 1980; Nocek, 1997). Feeding fat has gained interest in the last few decades. Adding fat to dairy diets became common practice for its potential to increase the energy density in diets, improve palatability, and reduce feed dustiness (Azain, 2004). Unsaturated fatty acids, as in soybean oil (SO), can decrease fiber digestion (Jenkins, 1993; Pantoja et al., 1994). Whereas saturated medium-chain fatty acids, as in coconut oil (CO), may improve rumen fermentation (Machmuller et al., 2003; Pilajun and Wanapat, 2013). Additionally, cost-effective by-products from numerous industries, such as the poultry industry, can be utilized by ruminants. Poultry fat (PF) is a by-product of chicken processing and extensively produced world-wide with a potential source of energy (Hutchison et al., 2006; Swisher, 2015).

On the other hand, the total dietary fat should not exceed 6-7% of the dry matter intake (DMI), and the traditional dairy heifer diets typically contain between 2 to 3% fat (NRC, 2001). In a study conducted by Anderson et al. (2015), the dietary fat reached up

to 7% when fed high fat from traditional dried distillers grains (DDGS) to dairy heifers. Also, in another study by Anderson et al. (2009), the diet's fat was close to 5% when a large portion of the heifer diet was supplied by wet distillers grains and soybean hulls.

The level of fat saturation showed an effect on digestibility and fermentation. In a study conducted by Elliott et al. (1997) on the effects of saturation of fat sources in steers, they reported that increasing fat sources' saturation tended to increase the NDF and ADF digestibility in the rumen. Other studies have reported no differences in ruminal or total tract digestibility of OM or fiber in lactating cows fed diets with increasing amounts of dietary fat or different sources of fat (Palmquist and Conrad, 1978; Ohajuruka et al., 1991; Palmquist, 1991; Drackley and Elliott, 1993). Oldick and Firkins (2000) reported that acetate responded quadratically as the fat sources' unsaturation degree increased. In addition, Elliott et al. (1997) reported a decrease in acetate's molar proportion when different saturation fat was fed and increased linearly as saturation increased. The objective of this study was to evaluate the effects on fermentation and nutrient digestion of including different unsaturated fat sources when high concentrate diets with high-fat inclusion are used when simulating precision feeding in continuous culture. We hypothesized that incorporating different fat sources at a high concentrate diet to the aforementioned program can improve nutrient utilization without compromising fermentation and fermenters' digestibility.

## MATERIALS AND METHODS

### *Treatments and Experimental Design*

Diets included high concentrate (HC; 65%) and high-fat inclusion with four different fat sources starting with a basal level of fat in the diet as control [3% fat (0% fat; CON); and 9% fat (6% poultry fat; PF; Stabilized poultry fat; Valley proteins, Inc., Ward, SC); 9% fat (6% soybean oil; SO; Pure soybean oil; Nature's oil, Streetsboro, OH); and 9% fat (6% coconut oil; CO; Fractionated coconut oil; Nature's oil, Streetsboro, OH)]. The experiment was designed as a randomized complete block design consisting of 4 experimental diets split into two blocks of 4 dual-flow continuous culture fermenters during 2 periods of 10 d with a total of 4 replicates per treatment. Each period was started with a clean fermenter and inoculated with ruminal contents collected from 2 cannulated Holstein cows. Adaptation to treatment rations was made over the first 7 d of each period and 3 d for sampling collection. Treatments were randomly assigned to one of 4 continuous culture fermenters in each block and allocated to a different fermenter during each period to remove any fermenter-specific differences. All diets were fed to the fermenters as total mixed rations (TMR) and predicted nutrient composition determined using NRC (2001). Diets were formulated to simulate a precision feeding program in continuous culture fermenters to restrict intake. Also, to provide equal amounts of ME and N to supply 1.70 g N/kg $BW^{0.75}$ in Holstein heifers, which has been observed to maximize N utilization and allow for 800 g/d of ADG (Zanton and Heinrichs, 2009; Lascano and Heinrichs, 2011). Dietary ingredients and chemical composition are presented in Table 4.1. Fermenter receiving the CON treatment were fed greater amount

of TMR [(CON; 3% fat; 53.4 g/d as-fed)] than the other treatments [(PF; 9% fat; 47.7 g/d; (SO; 9% fat; 47.7 g/d; (CO; 9% fat; 47.7 g/d as-fed]. That was because of different energy concentrations of the diets and different levels of fat inclusion between the control and the other treatments required to maintain isocaloric intake. Rations were prepared and mixed in advance, split into two equal amounts, and fed to the continuous culture fermenters daily at 0900 and 2100 h.

*Continuous Culture Conditions*

All procedures involving the surgical and animal care protocols were approved by the Clemson University Institutional Animal Care and Use Committee. Around 1800 h, the entire rumen contents were collected from two rumen cannulated Holstein cows fed a 50% forage:50% concentrate diet and strained through two-layers of cheesecloth into a prewarmed sealed container. The filtered rumen fluid was combined from both cows, mixed with a buffer in a 1:1 ratio according to the methods of Slyter et al. (1966), and purged with $CO_2$ until inoculation into the continuous culture fermenters. Moreover, the time from inoculum collection to fermenter inoculation did not exceed 60 min. Approximately 750 mL of diluted inoculum was added to each dual-flow fermenter. The fermenters' design and operation were based on a previous design outlined by Teather and Sauer (1988), with some modifications include the use of an overflow sidearm that angled downward at approximately 45$^{o}$ to facilitate emptying. In addition, a faster stirring rate (45 rpm) that still allowed the stratification of particles into three layers; an upper mat layer, a middle liquid layer of small feed particles, and a lower layer of dense particles (Koch, 2017). A higher feeding rate for the control treatment (53.4 g/d as fed;

26.7 g/feeding) to a lower feeding rate in the other treatments to simulate the restricted intake was utilized. The buffer solution was also delivered continuously to the cultures using a peristaltic pump. Also, it was manipulated to achieve different liquid and solid passage rates [($kp_l$; 8.6%/h for CON, and 7.7%/h for other treatments); ($kp_s$; 3.8%/h for CON, and 3.2%/h for other treatments; Appendix A)] to simulate a precision feeding program in dairy heifers based on an in-vivo study by Lascano et al. (2016b). The buffer solution was used to dilute the inoculum (Slyter, 1966) in a 1:1 ratio and was selected based on previous works in our lab and included a greater level of $NaHCO_3$ to maintain culture pH. The cultures were maintained for 10 d, 7 d for adaptation duration to obtain a steady-state fermentation in the cultures, and 3 d for culture sampling (Lascano et al., 2016a). Fuentes et al. (2009) reported that the cultures' microbial population requires a 5 d adaptation period. These durations are commonly used in continuous culture experiments (Jenkins et al., 2014; Brandao et al., 2018; Dai et al., 2019). The fermenters' temperature was maintained at 39°C by a recirculating water bath. Each fermenter was continuously purged with $CO_2$ at a rate of 20 mL/min to maintain anaerobic conditions, and gas flow rates were checked before the morning and evening feeding to ensure consistency. Culture's pH was monitored using handheld pH probes and calibrated at the start of each period. Oxidation-reduction potential (E*h*) was measured using the redox probe (Traceable 4277 pH/ORP Meter, Control Company, Webster, TX) during the sampling day at the same time points of pH measuring. The relative hydrogen score (rH) was calculated using the Clark equation for deriving rH from pH and E*h*.

*Sample Collection and Analysis*

On d 8, 9, and 10 of each period, liquid and solid digesta overflow from each fermenter were collected in a 2 L Erlenmeyer flask immersed and covered in an ice bath to stop the microbial activity. The overflow flasks were weighed, and the total volume was recorded once daily at 2030 h. A 20% aliquot of the overflow was collected in a pre-labeled container and immediately frozen at - 20$^{o}$C. The 3 d composited overflow samples were later thawed, homogenized, and subsampled for later analysis of DM, OM, NDF, ADF, and LCFA. On the last day (d 10) of each period, cultural contents were mixed thoroughly (120 rpm) during sampling to ensure an adequate sample from the cultures. Culture pH and E*h* were measured and recorded at 0 (before feeding), 2, 4, 6, 8, 10, and 12 h, and a 5 mL sample of culture contents were taken at the same time points for protozoa (kept in the fridge at 4$^{o}$C), VFA, and ammonia analysis (frozen at - 20$^{o}$C).

Feed and dried overflow samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 2-mm sieve and analyzed for DM, OM, ash, and EE (AOAC, 2000). And through a 1-mm sieve for NDF and ADF (Van Soest et al., 1991) using an ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with heat resistant α-amylase and sodium sulfite utilized in the NDF procedure. Starch was analyzed on reground samples (< 0.5-mm screen) using an enzymatic procedure (Bach Knudson, 1997). Culture samples (5 mL) were pipetted to 15 mL centrifuge tubes containing 1 mL of metaphosphoric acid (25%; w/v), and then, these tubes were stored at -20°C until VFA and ammonia analysis, as described by Moody et al. (2007). Samples were later thawed and centrifuged at 40,000 × g for 30 min at 4°C.

After centrifugation, 1 mL of the supernatant was placed in a 2-mL Eppendorf microcentrifuge tube and used for the analysis of $NH_3N$ according to the methods of Chaney and Marbach (1962) with modifications including reduced sample and reagent volume to accommodate the use of a 96-well plate reader. Another 0.5 mL of the supernatant was combined with 0.5 mL distilled water and 100 μL of internal standard (86 μmol of 2-ethylbutyric acid/mL) in a GC vial.

Samples for VFA were then analyzed by GC–flame-ionization detection according to the methods of Yang and Varga (1989) and injected into a Hewlett-Packard 6890 gas chromatograph (San Jose, CA) equipped with a custom packed column (2 m × 0.32 cm × 2.1 mm ss; 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW). Additionally, a 4 mL culture sample was pipetted and preserved in 4 mL of methyl green formalin-saline solution (1:2 dilution) and stored in darkness at 4°C for protozoa counting (Ogimoto and Imai, 1981). Dried ground feed and overflow samples were sent to the Multi-User Analytical Laboratory and Metabolomics Core, Clemson University, SC, for the LCFA analysis. Quantities of individual fatty acids present in the cultures were determined on a Shimadzu GC-2010 gas chromatograph with a flame ionization detector. It was equipped with an SLB-IL111 (Sigma, St. Louis, MO) fused silica capillary column (L x I. D. 100 m x 0.25 mm) with 0.2 um film thickness. The initial temperature was held at 140°C for 3 min then increased by 3.7°C per min up to 220°C for 60 min. The carrier gas was helium purged at 20 cm/s. Fatty acid peaks were identified and separated by comparison of the retention times to known standards.

*Calculations and Statistical Analysis*

Fractional passage rates were calculated according to Lascano et al. (2016b) as follows:

The liquid passage rate in the in-vivo study by Lascano et al. (2016b) was 8.93%/h for LF (45% forage). Therefore, we assumed 8.60%/h would be the control diet's liquid passage rate (35% forage) in our study.

Liquid passage rate was decreased based on the decreased dry matter intake as we increased the fat inclusion in the diets.

Liquid passage rate (%/h) = drymatter intake (g/d) × liquid passage rate for the control (mL/h) × dry matter intake for the control (g/d),

Buffer input (mL/h) was calculated as follows:

Buffer input (mL/h) = liquid passage rate (%/h) × fermenter volume (mL),

In the same way, the solid passage rate was calculated and based on the results of our study.

Metabolizable energy intake (Mcal/d) was calculated as follows:

ME (Mcal/d) = (digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) × 0.82, assuming that digestible OM intake and total digestible nutrient intake were equal.

That equation was used for the control diet, which was modified from NRC (2001). To represent better the increase in energy as fat increased in the diets, another modified equation from NRC (2001) was used as follows:

ME (Mcal/d) = (digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82].

All statistical analyses were conducted in SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC) using the MIXED procedure. Data were analyzed as a randomized complete block design with period and fats as a fixed effect, fermenters as a random effect, and repeated measures as needed for the following model:

$$Yijk = \mu + Fi + Pj + Ck + eijk,$$

Where Yijk = the dependent variable, $\mu$ = the overall mean, Fi = the fixed effect of fat, Pj = the fixed effect of the period, Ck = the fermenter's random effect and, eijk = the residual error. The PDIFF option adjusted by the Tukey method was included in the LSMEANS statement to account for multiple comparisons. For observations where multiple repeated measures occurred in a period, the fixed effects of time and its interaction with other fixed effects were included in the model based on a repeated measures analysis (Littell et al., 1998). Covariance structures of simple, autoregressive, or compound symmetry were chosen for use in the repeated measures analysis based on the lowest values of Akaike's Information Criterion and Schwartz's Bayesian Criterion. Residuals for all models were found to be normally distributed (Shapiro-Wilk test for normality). Least square means are presented in tables, and evidence for statistical significance was declared at $P \leq 0.05$, while trends for main effects and interactions are discussed at $0.10 \geq P > 0.05$.

## RESULTS AND DISCUSSION

### *Diet Composition and Nutrient Inputs*

Diet ingredients and chemical composition values are presented in Table 4.1. The dietary EE concentrations increased in the diets up to 9% with the inclusion of different lipid sources and, consequently, ME concentration; therefore, daily feeding amount decreased as different lipid sources increased. The addition of different lipid sources to the diets resulted in 2 different proportions of FA concentrations in the diets, and its input increased as well. As planned, the fat inclusion replaced the ground corn in the control diet, resulting in a decrease in starch and NFC in the other three different fat treatments. All other components of the rations were formulated to be similar between treatments.

Daily starch and NFC inputs were decreased as fat included in the diets and were the opposite with EE input as increased to achieve the planned diets. Consequently, there was an input fat effect on OM, NDF, ADF, starch, and NFC to maintain the isoenergetic and isonitrogenous treatment design. The liquid and solid passage rates were lower for different fat-fed fermenters compared to the control-fed fermenter (Appendix A). Passage rates of diets can be slower when intake is limited (Eng et al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990), and we expected to be even slower when fat is added to the diets.

### *Digestibility of Nutrients*

Apparent digestibility coefficients (dC) are outlined in Table 4.3. The dC of DM, OM, NDF (Figure 4.1), ADF, and hemicellulose were greater for CO-fed fermenter and PF-fed fermenter followed by SO-fed fermenter and then CON-fed fermenter. These

observations are consistent with results reported in a study conducted by Elliott et al. (1997) on the effects of saturation of fat sources in steers; it has been reported that increasing saturation of fat sources tended to increase the NDF and ADF digestibility in the rumen. Several other studies have reported no differences in ruminal or total tract digestibility of OM or fiber in lactating cows fed diets with increasing amounts of dietary fat (up to 5.7% total FA ~ 7% EE) or different sources of fat (Palmquist and Conrad, 1978; Ohajuruka et al., 1991; Palmquist, 1991; Drackley and Elliott, 1993). In contrast, it has been reported in a meta-analysis by Weld and Armentano (2017) that adding 3% of saturated fats or calcium salts to the diets increased total-tract NDF digestibility. Whereas medium-chain fats and unsaturated vegetable oil decreased total-tract NDF digestibility of lactating dairy cows. It has been reported in an in-vitro study that the CO up to 5%, which contains a high saturated medium-chain fatty acid, had no adverse effects on DM digestibility in the in-vitro gas fermentation production technique (Pilajun and Wanapat, 2014). In a study conducted by Anderson et al. (2015), the authors reported a higher dC of NDF and ADF when heifers were limit-fed a high-fat DDGS (7.00% EE) compared to a low-fat DDGS (3.08% EE), whereas the DM and OM did not differ between the treatments. It was suggested that the high-fat DDGS diet contains a lower starch content compared to the low-fat DDGS, which is the case in our study (Table 4.1), which resulted in a higher pH and an efficiency of utilization of fiber and improved the total-tract digestion. Also, Suarez-Mena et al. (2015) observed a quadratic DM, OM, NDF, and ADF dC response to increasing levels of DDGS up to 14% inclusion in the diets (4.99% total FA ~ 6% EE). These results did not agree with a study conducted by Lascano et al.

(2016a) using two levels of fat with no added fat, or 3.3% added SO in continuous culture fermenter where they did not observe any effects on DM and ADF dC between the two levels of fat in the diets. In the present experiment, fat represented 3% and 9% of the diets, respectively, which is larger than what has been reported in the above study. Koch (2017) reported depression in DM, OM, NDF, and ADF dC when continuous culture fermenters were fed high SO compared to low SO. Koch (2017) stated that the dietary polyunsaturated fatty acids had been shown to depress fiber dC by limiting the growth of fiber digestion bacteria (Van Soest, 1994), and this finding is common in the literature (Rico et al., 2014). Manthey and Anderson (2018) reported no effects on dC when heifers were limit fed DDGS with ad libitum grass hay. They related that to feeding grass hay as ad libitum, which resulted in a slightly different limit feed program than the typical one. Also, the passage rates of diets can be slower when intake is limited (Eng et al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990), and we expected to be even slower as fat added to the diets and as we have it in the current study (Appendix A).

The greater digestibility of fat-fed fermenter can be attributed to the greater digestibility of the ingredients in these diets. Also, to a higher retention time of these diets in the culture fermenter (Leaver et al., 1969; Colucci et al., 1990) as we decreased the passage rate with lower intake, as we planned in our study. On the other hand, the lower NDF, ADF, and hemicellulose dC in the CON-fed fermenter could be related to the availability of rapidly fermented ingredients such as starch and NFC (Table 4.1). That could also be related to more numerous amylolytic bacteria populations associated with CON diets (Brown et al., 2006). Furthermore, this could be attributed to the lower pH

level for the CON-fed fermenter (Table 4.6) because cellulolytic bacteria are very sensitive to pH and their activity and growth start to decline under pH 6.0 (Russell and Wilson, 1996). The FA (Figure 4.2) and starch dC were higher in the fat inclusion-fed fermenters than the CON-fed fermenter. That is mainly because of the lower starch and NFC content (Table 4.1) and their inputs (Appendix A) as corn was replaced with fat in the diets. Also, the lower passage rate and higher retention time resulted in more efficient fat and starch utilization in the continuous culture fermenter system.

*Fatty Acid Flows and Biohydrogenation*

The overflows of major fatty acids are detailed in Table 4.4. The inclusion of CO showed an increase in the overflow of individual saturated FA C12 and C14. That is mainly because the CO is relatively high in saturated medium-chain fatty acids such as C12 and C14, as we can see from Table 4.2. That agrees with a study conducted by Potu et al. (2011) using continuous culture fermenters and different fats supplement. They observed that the C14 flow was the highest when fish oil was fed, which is relatively higher in C14 compared to animal fat (Rumo-fat) and SO. Whereas the overflow of saturated FA C16, C18, and C22 was the highest with PF inclusion. Similarly, that can be attributed to PF high saturated long-chain fatty acids such as C16 and C18, as shown in Table 4.2 and their inputs in Table 4.3. These observations also agree with an in-vivo study where animal fat (Rumo-fat) showed the highest C18 flow (Potu et al., 2011). Lascano et al. (2016a) and Koch (2017) observed a reduction in saturated FA C12, C14, C20, C22, and C24 when fermenters were fed, increasing starch degradability. Also, Lascano et al. (2016a) reported increased daily outflows of individual saturated and total

fatty acids when fermenters fed high-fat diets compared to low-fat diets. Similarly, in a study conducted on feeding two levels of fat (no added fat or 3.64% of DM) to continuous culture fermenters, the high fat-fed fermenters showed a higher outflow of C:16, C20, C22, and C24 compared to low fat-fed fermenters (Jenkins et al., 2014).

The SO-fed fermenter showed the highest flow of individual unsaturated FA C18:1, C18:2, and C18:3. That can be attributed to the fact that the SO is relatively high in unsaturated long-chain fatty acids (Table 4.2). Potu et al. (2011) reported in an in-vitro study that the SO inclusion resulted in the highest flow of C18:1, and they attributed that to the highest proportion of C18 unsaturated FA, among other treatment diets. Other studies also reported similar increases in C18:1 flows in the rumen (Loor et al., 2002; Varadyova et al., 2007) and duodenal (Kucuk et al., 2008) with SO inclusion in ruminant animal's diet. The decrease in C18:1 and C18:2 overflow in CO-fed fermenter and PF-fed fermenter is partially related to replacing ground corn with fat and the high biohydrogenation efficiency of high-fat diets (Schmidely et al., 2008). That is in agreement with a study on dry dairy cows fed two levels of crude fat (2.9% and 7.6%) and showed a decrease in C18:2 FA (Zened et al., 2013).

Additionally, the CON-fed fermenter showed a lower total fatty acid flow compared to the other treatments. That is due to the higher content of starch and NFC, as well as the lower fat content. Lascano et al. (2016a) and Koch (2017) reported an increase in the outflow of C18:2 and C18:3 from the fermenters fed high starch, which resulted in a lower extent of biohydrogenation. Cultures under low pH conditions (5.65) showed less disappearance of C18 unsaturated FA (AbuGhazaleh and Jacobson, 2007). Martin et al.

(2002) and Jenkins et al. (2008) stated that most rumen microbial growth and enzyme activities could be impacted under low rumen pH conditions. In the current study, the lower pH in CON-fed fermenters (Table 4.5) may have affected culture bacteria and reduced the biohydrogenation rates.

Part of the differences in the unsaturated fatty acids flow is related to the differences in the dietary contribution of C18:1, C18:2, and C18:3, while the other part is related to the rate of biohydrogenation. The Biohydrogenation rate of C18:2 was decreased with a CON-fed fermenter. That aligns with our observations with a lower amount of C18:0 flows for CON-fed fermenter and indicating a reduction in the biohydrogenation pathway to completion at C18:0. Based on PF's effect on unsaturated FA C18, the PF-fed fermenter showed the highest percentage in the biohydrogenation of C18:2 and C18:3, followed by both of SO-fed fermenter and CO-fed fermenter and then CON-fed fermenter. These results agree with several in-vivo and in-vitro studies as they observed that the biohydrogenation rated of unsaturated fatty acids increased as the inclusion of fat increased in the diets (Zened et al., 2013; Jenkins et al., 2014; Lascano et al., 2016a).

*Characteristics of Fermentation*

Culture VFA profile, $NH_3N$, pH, reduction potential (E*h*), relative hydrogen score (rH), and total protozoa counts are shown in Table 4.5. The inclusion of different lipid sources in the diets decreased the total VFA concentrations with the lowest at CO-fed fermenter compared to the CON diet. Elliott et al. (1997) reported that the total VFA concentration decreased when different fat sources were fed compared to the control diet.

They attributed that to the lower fermentable carbohydrate content in fat-fed diets as corn was replaced with fat and as in the current study to maintain the isocaloric intake. Pilajun and Wanapat (2014) reported a lower total VFA concentration in the in-vitro gas production technique and after 48 h incubation with 5% CO in the diet. They attributed that to the negative effect of medium-chain fatty acids on the fermentation. Machmuller (2006) stated that the medium-chain fatty acids in the CO are small enough to penetrate and disrupt the cell membranes by readily dissolve in the lipid phase. Also, inhibit the enzymes involved in energy production and nutrient transfer, leading to reversible and irreversible changes that could lead to the microbial cell's death. Also, the higher total VFA concentration for CON-fed fermenters could be related to the pH (Calsamiglia et al., 2008). In the current study, the pH was the lowest with the CON-fed fermenter than for other treatments. In addition, as DM inputs decrease with fat inclusion, the passage rate decrease and the retention time increase in the continuous culture fermenter as planned in the current study, and that could be the reason behind the lower total VFA as fat increased in the diets (Appendix A). Furthermore, this reduction could be mainly because of the reduction in acetate concentrations as fat included in the diets, specifically with SO-fed fermenter and CO-fed fermenter. Even though the dC of NDF and ADF were the highest by CO inclusion, the reduction in fiber intake and the starch intake is the reason behind the reduction in acetate concentration (Manthey and Anderson, 2018). Acetate production within the rumen results from the fermentation of structural carbohydrates by cellulolytic bacteria (Enjalbert et al., 1999). Oldick and Firkins (2000) reported that the acetate responded quadratically as the fat sources' unsaturation degree increased. In

addition, Elliott et al. (1997) reported a decrease in acetate's molar proportion when different saturation fat was fed and increased linearly as saturation increased. Furthermore, acetate results of structural carbohydrate fermentation by cellulolytic bacteria and these bacteria can be inhibited by lower NDF inputs as in the present study, which may explain the lower acetate concentration for fat-fed fermenter (Martin et al., 2002). Rumen fermentation is not affected when fat levels are low in the diets because rumen microbes are able to saturate FA, but this capacity can be exceeded at higher levels, and FA can accumulate in the rumen and interfere with rumen fermentation (NRC, 2001).

However, the propionate concentrations were not affected with fat inclusion except the CO-fed fermenter, which was lower than the CON-fed fermenter. The propionate observation agrees with a study conducted by Pilajun and Wanapat (2014) as they reported a lower propionate concentration in the CO diet after 48 h incubation. Also, agrees with Oldick and Firkins (2000) when Holstein heifers fed different degree of fat saturation (tallow, partially hydrogenated tallow, and animal-vegetable fat). Elliott et al. (1997) reported a linear decrease in propionate as saturation increased. Even though the acetate:propionate ratio was not affected and was similar between the treatments. Some studies have reported that feeding fat can decrease the acetate:propionate ration (Oldick and Firkins, 2000; Elliott et al., 1997) or unchanged (Tjardes et al., 1998). Butyrate, valerate, and isobutyrate concentrations were lower in the CON-fed fermenter than the CO-fed fermenter but were not different from the PF and SO-fed fermenter. The reduction in valerate concentration in CON-fed fermenter could be related to the higher

liquid fraction kp accompanied with lower retention time for CON-fed fermenter (Eun et al., 2004; Fuentes et al., 2009). In the present study, the CON-fed fermenter showed a lower $NH_3N$ concentration. If energy is available, the AA can be incorporated into bacteria without deamination (Russell et al., 1991), which would explain the lower isobutyrate concentration as lower deamination in the CON-fed fermenter compare to CO-fed fermenter. These results are comparable to those reported by several studies conducted on dairy heifer limit-fed DDGS (Suarez-Mena et al., 2015; Manthey et al., 2016; Manthey and Anderson, 2018). In addition, these reports could be due to the decline in the culture bacteria population with fat inclusion, as suggested by Suarez-Mena et al. (2015), and this is supported by the decline in total protozoa counts as fat included in the diets in the current study. Manthey and Anderson (2018) suggested that the differences in starch contents and intake are the reason behind the shift in VFA concentrations and the decrease in acetate and increase in propionate concentrations. Also, they suggested the higher propionate concentration is related to more energy-efficient, and rumen fermentation in heifers fed DDGS diets (Manthey et al., 2016) because there are less methane and carbon dioxide production in propionate as compared with acetate (Fahey and Berger, 1988).

The ammonia concentration increased as the different unsaturated fat included in the diets compared to the CON-fed fermenter with the highest concentration in CO-fed fermenter followed by PF and then SO-fed fermenter. The lower $NH_3N$ in CON-fed fermenter than in fat-fed fermenters could be due to the use of ammonia for the de novo synthesis of AA. Suarez-Mena et al. (2015) and Manthey et al. (2016) observed similar

results, and they attributed that to the lower ME intake with the addition of DDGS; therefore, the microbial capacity to assimilate amino acids and ammonia was negatively affected and NH3 accumulated in the rumen (NRC, 2001). Additionally, Elliott et al. (1997) reported a linear increase in $NH_3N$ concentrations as the degree of saturation increased (tallow, partially hydrogenated tallow, hydrogenated tallow, blend of hydrogenated tallow, and hydrogenated fatty acids, and hydrogenated fatty acids). They suggested that the dietary triglycerides became more unsaturated, and the ruminal protein digestion inhibited. These results could be related to better synchrony between N and energy availability for microorganism's activity. These results did not agree with previous studies where the ruminal $NH_3N$ concentrations were not affected by supplemental fat or fat source (Doreau and Ferlay, 1995; Pantoja et al., 1995; Oldick and Firkins, 2000).

Cultural pH was lower for the CON-fed fermenter compared to the fat-fed fermenters. Chibisa et al. (2015) stated that the drop in pH with high starch diets is common in the literature. The inclusion of fat in the diets increased the cultural pH with the highest pH values were observed at CO-fed fermenter, followed by PF-fed fermenter and SO-fed fermenter compared to the CON-fed fermenter. That agrees with a study conducted by Elliott et al. (1997), where they reported an increase in ruminal pH as different saturated fat were fed, and they attributed that to the lower fermentable carbohydrate content in these diets. Suarez-Mena et al. (2013) reported a similar rumen pH between treatments as DDGS increased in the diets. In contrast, Manthey et al. (2016) observed a linear decrease in rumen pH as DDGS increased in the diets, and they

attributed that to the F:C ratio. The E*h* was the lowest for CO-fed fermenter than the other two fat treatments and CON-fed fermenter, and the opposite was with the rH. The relative H score range from 0 to 42, and 28 is the mid-point because lower than 28 is reducing, and higher than 28 is oxidizing. Julien et al. (2010) stated that there is a relationship between pH and E*h*, and it seemed that the ruminal E*h* moved toward higher E*h* when pH dropped, which is exactly the case in the present study. We observed that the lowest reducing E*h* (-360.64) was observed when pH was the highest (6.13) with a CO-fed fermenter. These findings could be related to the rapidly fermentable carbohydrates for the CON-fed fermenter (Huang et al., 2018).

The total protozoa count was decreased with fat inclusion in the diets, and it was the lowest with SO-fed fermenter followed by PF-fed fermenter, CO-fed fermenter, and then the highest was the CON-fed fermenter. Oldick and Firkins (2000) reported a decrease in total rumen protozoa when fat was supplemented (tallow, partially hydrogenated tallow, and animal-vegetable fat) and as the supplemental fat source became more unsaturated as in the case of SO-fed fermenter in the current study. Whereas, Karnati et al. (2009) observed that total protozoa count was higher with high-fat diets (5% animal-vegetable fat). They attributed that the direct incorporation of preformed FA might have spared more energy for cell growth, or the BH would have decreased FA's toxic concentrations below the threshold. Also, Mathew et al. (2011) reported an increase in total protozoa counts in diets containing 4% fat from DDGS and monensin. Koch (2017) reported that the polyunsaturated fatty acids did not affect total protozoa counts; however, they found an increase in genera Epidinium spp. With high

polyunsaturated fatty acids treatment, and they stated that the reason behind that is unclear. Yang et al. (2009) reported a decrease in protozoa when Holstein dairy cows fed 4% supplemental soybean as a source of linoleic acid and linseed oil as a source of linolenic acid in diets. Also, they observed a lower number of cellulolytic bacteria and a higher proteolytic bacteria number. Ferlay et al. (1993) and Oldick and Firkins (2000) reported that the polyunsaturated fatty acids showed a more negative effect than the saturated fatty acids on the metabolism of cellulolytic bacteria and a direct effect on ruminal protozoa. Several in-vitro and in-vivo studies showed a toxic effect of linoleic acid on ruminal protozoa with a consistent decrease in protozoa counts (Sutton et al., 1983; Hristov et al., 2004; Newbold and Chamberlain, 1988). In addition, Maia et al. (2007) observed that the linolenic acid was more toxic on ruminal bacteria than the linoleic acid. The effect of fatty acid on bacteria can directly disrupt the microbial cell membrane, lipid coating of bacteria and feed particles, and antimicrobial effects on the bacterial population (Jenkins, 1993).

# CONCLUSIONS

Simulating precision feeding high concentrate and high-fat inclusion with different unsaturated fat sources diet in continuous culture fermenter had some effects on ruminal fermentation. The total VFA concentration and protozoa population were decreased while maintaining higher pH and ammonia concentration in more saturated than the unsaturated fat sources and control treatments. This study demonstrates that dietary poultry fat inclusion and coconut oil inclusion improved apparent digestibility significantly compared to soybean oil and the control diet. Therefore, we can conclude that the saturated fatty acids as in the by-products dietary poultry fat, or the saturated medium-chain fatty acids as in coconut oil can be successfully included in rations for precision-fed dairy heifers up to 6% and reduce the DMI further while improving nutrient digestibility.

Table 4.1. Ingredient and chemical composition of high concentrate diets, high-fat inclusion, and different lipid sources (CON 3%, PF 9%, SO 9%, CO 9% DM) fed to continuous culture fermenters.

| | Fat type, % in the diet | | | |
|---|---|---|---|---|
| Ingredient,[1] % | CON 3% | PF 9% | SO 9% | CO 9% |
| Coastal hay | 5.00 | 5.00 | 5.00 | 5.00 |
| Corn silage | 30.0 | 30.0 | 30.0 | 30.0 |
| Ground corn | 51.8 | 40.8 | 40.8 | 40.8 |
| Soybean meal (SBM) | 11.2 | 16.4 | 16.4 | 16.4 |
| Mineral mix | 2.00 | 2.00 | 2.00 | 2.00 |
| Fat inclusion | 0.00 | 5.79 | 5.79 | 5.79 |
| Chemical composition | | | | |
| DM % | 90.5 | 90.6 | 90.7 | 90.0 |
| OM, % | 95.6 | 95.2 | 94.8 | 95.5 |
| CP, % | 12.8 | 14.0 | 14.2 | 14.2 |
| Soluble P, % CP | 23.4 | 24.3 | 24.3 | 23.7 |
| NDF, % | 20.8 | 19.8 | 20.2 | 20.4 |
| ADF, % | 9.84 | 9.20 | 9.58 | 9.71 |
| Hemicellulose,[2] % | 10.9 | 10.6 | 10.6 | 10.7 |
| Starch, % | 39.3 | 31.9 | 31.9 | 31.7 |
| Ether extract, % | 3.52 | 8.56 | 8.69 | 8.31 |
| NFC,[3] % | 58.5 | 52.8 | 51.7 | 52.6 |
| TDN | 78.9 | 86.0 | 85.4 | 82.4 |
| ME,[4] Mcal/Kg | 2.88 | 3.14 | 3.11 | 3.01 |
| Ash, % | 4.41 | 4.83 | 5.18 | 4.55 |

[1]All diets were ground to 2 mm

[2]Hemicellulose = NDF - ADF

[3]NFC: non-fiber carbohydrates = 100 - (CP + ether extract + NDF + Ash)

[4]ME calculated using TDN values as reported by Cumberland Valley Analytical Services, Inc., Waynesboro, PA. ME = (TDN × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (TDN × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)

Table 4.2. Fatty acid profile of high concentrate diets, high-fat inclusion, and different lipid sources (CON 3%, PF 9%, SO 9%, CO 9% DM) fed to continuous culture fermenters.

| | Fat type, % in the diet | | | |
|---|---|---|---|---|
| Fatty Acid, % | CON 3% | PF 9% | SO 9% | CO 9% |
| C8:0 | 0.05 | 0.07 | 0.03 | 0.06 |
| C10:0 | 0.01 | 0.02 | 0.01 | 0.04 |
| C12:0 | 0.05 | 0.06 | 0.02 | 34.5 |
| C14:0 | 0.11 | 0.44 | 0.07 | 28.3 |
| C14:1T | 0.01 | 0.01 | 0.00 | 0.12 |
| C14:1 | 0.08 | 0.08 | 0.04 | 0.01 |
| C16:0 | 14.7 | 23.9 | 11.7 | 6.00 |
| C18:0 | 0.04 | 4.59 | 0.03 | 0.99 |
| C18:1 | 25.7 | 32.2 | 17.3 | 7.59 |
| C18:1-11C | 1.68 | 2.65 | 19.9 | 0.90 |
| C18:2 | 51.9 | 31.3 | 44.9 | 18.9 |
| C18:3 | 4.40 | 2.26 | 4.17 | 1.80 |
| C22:0 | 0.24 | 0.30 | 0.19 | 0.07 |
| C24:0 | 0.49 | 1.25 | 0.96 | 0.37 |
| C22:2 | 0.01 | 0.34 | 0.41 | 0.26 |
| C22:6 | 0.51 | 0.51 | 0.26 | 0.06 |
| Total, mg/g | 28.9 | 80.2 | 82.3 | 78.2 |

Table 4.3. Nutrient apparent digestibility of continuous culture fermenters fed high concentrate diets, high-fat inclusion, and different lipid sources (CON 3%, PF 9%, SO 9%, CO 9% DM).

| | Fat type, % in the diet | | | | |
|---|---|---|---|---|---|
| Digestibility, % | CON 3% | PF 9% | SO 9% | CO 9% | SE |
| DM | 69.0 [c] | 80.1 [a] | 76.3 [b] | 80.9 [a] | 0.35 |
| OM | 74.5 [c] | 84.6 [a] | 81.4 [b] | 85.4 [a] | 0.28 |
| ADF | 33.9 [c] | 50.6 [a] | 46.6 [b] | 51.1 [a] | 0.81 |
| Hemicellulose | 48.7 [c] | 68.0 [a] | 60.6 [b] | 69.3 [a] | 0.68 |
| Starch | 99.7 [c] | 99.9 [ab] | 99.9 [b] | 99.9 [a] | 0.01 |

Table 4.4. Daily fatty acids flow and biohydrogenation of continuous culture fermenters fed high concentrate diets, high-fat inclusion, and different lipid sources (CON 3%, PF 9%, SO 9%, CO 9% DM).

| | Fat type, % in the diet | | | | |
|---|---|---|---|---|---|
| FA outflow, mg/d | CON 3% | PF 9% | SO 9% | CO 9% | SE |
| Saturated | | | | | |
| C8:0 | 16.5 [a] | 12.8 [a] | 10.4 [ab] | 2.15 [b] | 2.90 |
| C10:0 | 3.52 [a] | 1.17 [b] | 1.96 [ab] | 2.32 [ab] | 0.64 |
| C12:0 | 1.78 [b] | 2.09 [b] | 1.99 [b] | 641 [a] | 18.9 |
| C14:0 | 2.41 [b] | 8.70 [b] | 3.00 [b] | 476 [a] | 25.8 |
| C16:0 | 145 [c] | 419 [a] | 248 [b] | 72.9 [c] | 27.0 |
| C18:0 | 29.3 [c] | 501 [a] | 233 [b] | 22.3 [c] | 37.9 |
| C22:0 | 2.20 [c] | 6.66 [a] | 4.04 [b] | 1.62 [c] | 0.54 |
| C24:0 | 2.70 [b] | 4.91 [ab] | 6.18 [a] | 2.36 [b] | 0.97 |
| Unsaturated | | | | | |
| C18:1 | 237 [ab] | 230 [b] | 316 [a] | 117 [c] | 29.1 |
| C18:2 | 434 [b] | 227 [c] | 587 [a] | 241 [c] | 44.9 |
| C18:3 | 24.5 [b] | 13.5 [b] | 43.8 [a] | 16.1 [b] | 3.81 |
| Total | 1106 [b] | 2628 [a] | 2609 [a] | 2422 [a] | 102 |
| Biohydrogenation,[1] % | | | | | |
| C18:2 | 42.1 [c] | 78.9 [a] | 63.3 [b] | 62.1 [b] | 3.39 |
| C18:3 | 60.3 [b] | 82.8 [a] | 70.6 [b] | 69.4 [b] | 3.63 |

[1]Expressed as milligrams of input - milligrams of outflow/milligrams of input for 18:2 and 18:3

Table 4.5. Volatile fatty acids, $NH_3N$, pH, E*h*, and protozoa population of continuous culture fermenters fed high concentrate diets, high-fat inclusion, and different unsaturated fat sources (CON 3%, PF 9%, SO 9%, CO 9% DM).

| | Fat type, % in the diet | | | | |
|---|---|---|---|---|---|
| Culture fermentation | CON 3% | PF 9% | SO 9% | CO 9% | SE |
| Total VFA, m*M* | 111.9 [a] | 83.4 [b] | 88.0 [b] | 66.3 [c] | 4.39 |
| Individual VFA, mol/100 mol | | | | | |
| Acetate | 49.9 [a] | 47.4 [ab] | 45.2 [bc] | 44.3 [c] | 1.26 |
| Propionate | 31.4 [a] | 30.2 [ab] | 31.5 [a] | 27.1 [b] | 1.19 |
| Butyrate | 11.8 [b] | 15.7 [ab] | 14.3 [b] | 18.9 [a] | 1.24 |
| Valerate | 6.05 [bc] | 5.65 [c] | 8.22 [ab] | 8.64 [a] | 0.81 |
| Isobutyrate | 0.68 [b] | 0.80 [ab] | 0.71 [ab] | 0.98 [a] | 0.12 |
| Acetate:propionate | 1.62 | 1.58 | 1.46 | 1.66 | 0.08 |
| $NH_3N$, mg/dL | 4.84 [d] | 5.64 [b] | 5.09 [c] | 5.91 [a] | 0.02 |
| pH | 5.78 [d] | 6.05 [b] | 5.94 [c] | 6.13 [a] | 0.01 |
| E*h*,[1] mV | -296 [a] | -265 [a] | -279 [a] | -360 [b] | 17.9 |
| rH[2] | 8.35 [a] | 9.92 [a] | 9.22 [a] | 6.90 [b] | 0.60 |
| Protozoa, $10^3$/mL | 26.0 [a] | 19.4 [c] | 16.9 [d] | 22.1 [b] | 0.55 |

[1]E*h* = Redox potential

[2]rH, Clark's exponent = ((E*h* + 200) / 30) + (2 × pH)

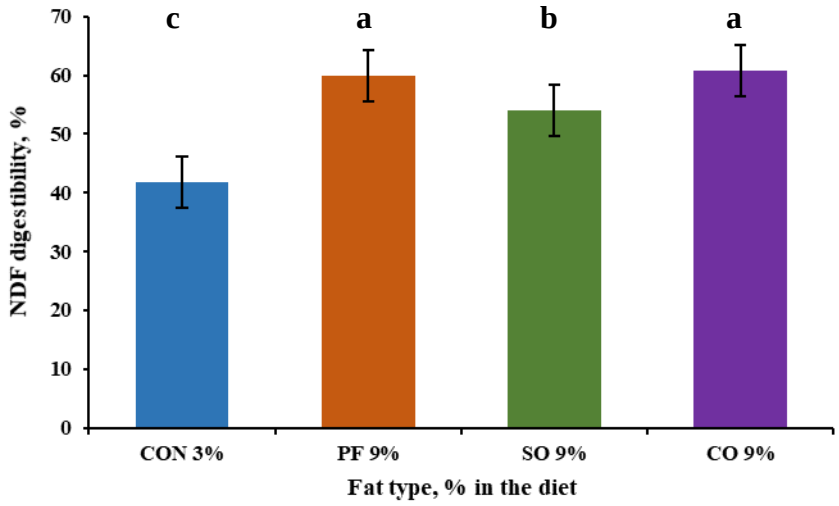


Figure 4.1. Neutral detergent fiber apparent digestibility of continuous culture fermenters fed high concentrate diets, high-fat inclusion, and different unsaturated fat sources (CON 3%, PF 9%, SO 9%, CO 9% DM).

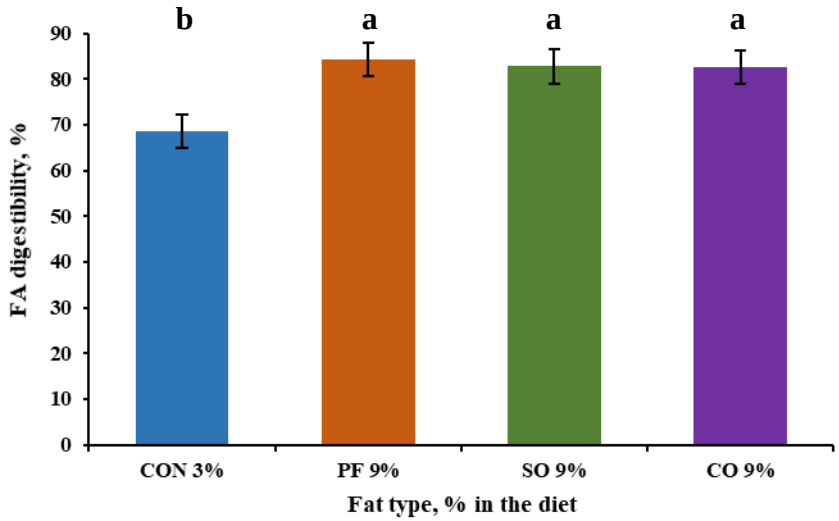


Figure 4.2. Fatty acid's apparent digestibility of continuous culture fermenters fed high concentrate diets, high-fat inclusion, and different unsaturated fat sources (CON 3%, PF 9%, SO 9%, CO 9% DM).

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# CHAPTER FIVE

## SIMULATING PRECISION FEEDING OF HIGH AND LOW FORAGE DIETS WITH INCREASING POULTRY FAT INCLUSION IN CONTINUOUS CULTURE FERMENTORS


### ABSTRACT

Diets used for precision-feeding are more nutrient-dense, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss. Modifying the forage to concentrate ratio (**F:C**) and manipulating nutrient fractions allow precision-fed dairy heifers to achieve adequate nourishment. Including dietary fat can increase the energy density of diets, reducing intake further. Therefore, the objective of this study was to evaluate the effects of simulated precision feeding different levels of poultry fat (**PF**) at different F:C ratio on digestibility and fermentation in continuous cultures. We hypothesized that including PF at low forage diets would further reduce intake without compromising fermentation and digestibility in fermenters. Treatments included 2 forage combinations, low (**LF**; 35% forage), and high (**HF**; 70% forage) and 4 levels of PF starting with a basal level of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments were randomly assigned to 8 fermenters in a 2×4 factorial design and ran for 4, 10 d periods. Data were analyzed using the MIXED procedure of SAS. The LF-fed fermenter had higher DM, OM, N, starch, and NFC digestibility coefficients (**dC**) than HF. Nutrients digestibility increased linearly with PF inclusion. Bacterial efficiency was higher in HF than LF, and the PF inclusion decreased the efficiency. The total VFA concentration was higher in LF, and there was a reduction in acetate with LF. The PF inclusion decreased acetate and increased propionate linearly.

Protozoa population was higher in HF than LF, and the PF inclusion decreased the protozoa population linearly. These results suggest increasing PF inclusion in precision fed LF or HF can alter rumen fermentation and improve digestibility.

# INTRODUCTION

Nutrition determines the dairy heifer growth rate and efficiency and affects the time necessary for the animal to attain an optimal size. Therefore, it is important to find strategies to raise dairy heifers economically and efficiently to increase dairy industry profitability (Lascano et al., 2009). Typical dairy heifers are fed high forage-based diets with a large inefficiency inherent with this feeding method; however, this can be enhanced by incorporating either energy-dense sources such as concentrates or fat (Naik et al., 2010). Modifying the forage to concentrate ratio (F:C) and manipulating nutrient fractions allowed precision-fed dairy heifers to achieve adequate nourishment, improved N and OM digestibility (Zanton and Heinrichs, 2009), and resulted in similar effects on rumen fermentation (Lascano and Heinrichs, 2009; Lascano et al., 2009). Adverse effects of feeding rapidly fermented NFC to dairy cattle on fiber digestion and acidosis incidence are commonly reported in the literature (Palmquist and Jenkins 1980; Nocek, 1997).

Cost-effective by-products from other agricultural industries can be utilized as sources of energy. The use of fat and other nutrients in dairy diets is increasing due to higher energy demands of dairy cows and higher availability of fat supplements (NRC, 2001). Poultry fat (PF) is a by-product of chicken processing and extensively produced world-wide and a potential economical source of energy for dairy diets (Hutchison et al., 2006; Swisher, 2015). The PF's fatty acid composition is high in unsaturated fatty acids, such as Oleic acid 43% and Linoleic acid 19%, also high in saturated fatty acids such as Palmitic acid 21% (Rouse, 2003). Several studies conducted on dairy heifers were fed dietary fat up to 5% and 7% from traditional wet, dried, high or low-fat distillers' grains

(DDGS; Anderson et al., 2009, 2015; Schroer et al., 2014; Suarez-Mena et al., 2015). They observed similar total-tract nutrient digestion in dairy heifers compared with control diets; DMI can be reduced as more fat from DDGS were added. Also, found to maintain ADG and overall growth performance. However, there is limited research regarding the effects of feeding fat on the growing dairy heifers, and to what level can be strategically incorporated into precision feeding is unknown.

Continuous culture systems have been extensively used and improved over the years to address major limitations (Koch, 2017). Continuous culture systems are relatively inexpensive to operate, provide an advantage for the quick and safe assessment of experimental treatments, and provide a cheaper alternative to test preliminary hypotheses compared to running an in vivo trial (Hristov et al., 2012). Even though omasal sampling allows researchers to evaluate FA concentration flowing out of the rumen and available to the animal (Shingfield et al., 2012), this technique/method can be difficult and labor-intensive. In contrast, continuous culture systems contain a reaction vessel that simulates the rumen, while the omasum in a cow is simulated by the overflow port where the effluent is removed. Furthermore, continuous culture systems can be easily adjusted to simulate the precision feeding system in dairy heifers by manipulating the passage rate based on the DMI and buffer infusion. Therefore, the objective of this study was to evaluate the effects of simulated precision feeding of different levels of PF at different F:C ratio on digestibility and fermentation in continuous culture fermenters. We hypothesized that including PF in low forage diets would further reduce intake without compromising digestibility and fermentation in fermenters.

## MATERIALS AND METHODS

*Treatments and Experimental Design*

Treatments included two F:C combinations, low (LF; 35% forage) and high (HF; 70% forage), and four different levels of dietary PF inclusion (Stabilized poultry fat; Valley proteins, Inc., Ward, SC) starting with a basal level of fat in the diet [3% fat (0% PF inclusion); 5% fat (2% PF inclusion); 7% fat (4% PF inclusion); and 9% fat (6% PF inclusion)]. The experiment was designed as a 2×4 factorial design consisting of eight experimental diets fed to eight dual-flow continuous culture fermenters. Continuous culture fermenters were run in 4 replicated periods of 10 d. Each period was started with a clean fermenter and inoculated with fresh ruminal contents collected from 2 cannulated Holstein cows. Adaptation to treatment rations was made over the first 7 d of each period and 3 d for sampling collection. Treatments were randomly assigned to one of eight continuous culture fermenters and allocated to a different fermenter during each period to remove any fermenter-specific differences. All diets were fed to the fermenters as total mixed rations (TMR) and predicted nutrient composition determined using NRC (2001). Diets were formulated to simulate the precision feeding program in continuous culture fermenters to restrict intake. Also, to provide equal amounts of ME and N to supply 1.70 g N/kg BW 0.75 in Holstein heifers, which has been observed to maximize N utilization in dairy heifers and allow for 800 g/d of ADG (Zanton and Heinrichs, 2009; Lascano and Heinrichs, 2011). Dietary ingredients and chemical composition are presented in Table 5.1. Fermenter fed greater amount for the basal diet treatment [(47.46; LF 3% fat); (54.19; HF 3% fat) g/d DM basis] than the other treatments [(45.69; LF 5% fat); (44.25;

LF 7% fat); (42.67; LF 9% fat); (51.92; HF 5% fat); (49.83; HF 7% fat); (47.89; HF 9% fat) g/d DM basis] because of different energy concentration of the diets between F:C combinations and different levels of PF inclusion (Table 5.1 and Appendix B). Rations were prepared and mixed in advance and split into two equal amounts, and were fed to the continuous culture fermenters daily at 0730 and 1930 h.

*Continuous Culture Conditions*

All procedures involving the surgical and animal care protocols were approved by the Clemson University Institutional Animal Care and Use Committee. Prior to the morning feeding, the whole rumen contents were collected from two rumen cannulated Holstein cows fed a 50% forage:50% concentrate diet and strained through two-layers of cheesecloth into a prewarmed sealed container. The filtered rumen fluid was combined from both cows, mixed with a buffer in a 1:1 ratio according to the methods of Slyter et al. (1966), and purged with $CO_2$ until inoculation into the continuous culture fermenters. Approximately 750 mL of diluted inoculum was added to each dual-flow fermenter. The fermenter's design and operation were based on a previous design outlined by Teather and Sauer (1988), with some modifications include the use of an overflow sidearm that angled downward at approximately 45$^o$ to facilitate emptying. In addition, a faster stirring rate (45 rpm) that still allowed stratification of particles into three layers; an upper mat layer, a middle liquid layer of small feed particles, and a lower layer of dense particles (Koch, 2017). A higher feeding rate for the control treatment (60 g/d as fed; 30 g/feeding) to a lower feeding rate in the other treatments to simulate the restricted intake was utilized (Appendix B). The buffer solution was also delivered continuously to the cultures

using a peristaltic pump and manipulated to achieve different liquid and solid dilution rates for each treatment (Appendix B) to simulate a precision feeding program in dairy heifers as reported by Lascano et al. (2016b). The buffer solution was used to dilute the inoculum (Slyter, 1966) in a 1:1 ratio and was selected based on previous works in our lab and included a greater level of $NaHCO_3$ to maintain culture pH. The cultures were maintained for 10 d, 7 d for duration of adaptation to obtain a steady-state fermentation in the cultures, and 3 d for culture sampling (Lascano et al., 2016a). Fuentes et al. (2009) reported that the cultures require a 5 d of adaptation period to adapt thoroughly. These durations are commonly used in continuous culture experiments (Jenkins et al., 2014; Brandao et al., 2018; Dai et al., 2019). The fermenters' temperature was maintained at 39°C by a recirculating water bath, and each fermenter was continuously purged with $CO_2$ at a rate of 20 mL/min to maintain anaerobic conditions and gas flow rates were checked before the morning and evening feeding to ensure consistency. Culture's pH was monitored using handheld pH probes and calibrated at the start of each period. Oxidation-reduction potential (E*h*) was measured using the redox probe (Traceable 4277 pH/ORP Meter, Control Company, Webster, TX) during the sampling day at the same time points of pH measuring. The relative hydrogen score (rH) was calculated using the Clark equation for deriving rH from pH and E*h*. A custom $CH_4$ sensor system monitored gas production for $CH_4$ analysis, and daily $CH_4$ output (mmol/d) was estimated.

*Sample Collection and Analysis*

On d 5 prior to evening feeding, samples were collected from the overflow flasks after mixing and homogenizing the liquid and solid digesta to determine the background

15N abundance. Pulse dose of 0.047 g of $(15NH_4)_2SO_4$ with 10.2% atom excess of 15N (Sigma-Aldrich Co., St. Louis, MO) was mixed with the diet of each treatment and infused into each fermenter to label the $NH_3N$ pool instantaneously. Also, the buffer solution was replaced with a pre-prepared and reformulated buffer that contains 0.075 g/L of the enriched $(15NH_4)_2SO_4$ to replace an isonitrogenous amount of urea to obtain a steady-state 15N enrichment of the $NH_3$ pool in the fermenters (Calsamiglia et al., 1996).

On d 8, 9, and 10 of each period, liquid and solid digesta overflow from each fermenter were collected in a 2 L Erlenmeyer flask and immersed and covered in an ice bath to stop the microbial activity. The overflow flasks were weighed, and the total volume was recorded once daily at 1900 h, and a 20% aliquot of the overflow was collected in a pre-labeled container and immediately frozen at - 20°C. The 3 d composited overflow samples were later thawed, homogenized, and subsampled for later analysis of DM, OM, NDF, ADF, and LCFA. Twenty-four hours before d 10 of each period, 20 mL of 50% $H_2SO_4$ was added to each overflow flasks to prevent further microbial and enzymatic activities. On d 10 of each period, cultural contents were mixed thoroughly (160 rpm) during sampling to ensure an adequate sample from the cultures. Culture pH, E*h*, and $CH_4$ were measured and recorded at 0 (before feeding), 2, 4, 6, 8, 10, and 12 h, and a 5 mL sample of culture contents were taken at the same time points for VFA, protozoa, and ammonia analysis. On the last day at the end of culture sampling of each period, the entire culture contents were strained through 2 layers of cheesecloth into a pre-labeled container. Then, centrifuged at 1,000 × g for 10 min at 5°C, and the supernatant was collected into a new centrifuge tube and centrifuged at 20,000 × g for 20

min at 5°C. Bacteria pellets were collected after discarding the supernatant and freeze-dried and stored for 15N, N, and OM analysis (Bach et al., 2008). In addition, 5 mL samples from the overflow were taken for ammonia analysis and to calculate the N flows.

Feed and dried overflow samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 2-mm sieve and analyzed for DM, OM, ash, and EE (AOAC, 2000). And through a 1-mm sieve for NDF and ADF (Van Soest et al., 1991) using an ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with heat resistant α-amylase and sodium sulfite utilized in the NDF procedure. Starch was analyzed on reground samples (< 0.5-mm screen) using an enzymatic procedure (Bach Knudson, 1997). Feed, enriched 15N overflow, background overflow, and freeze-dried bacteria pellets were sent to the Ohio State University for N and 15N analysis (Thermo EA/IRSM). Culture samples (5 mL) were pipetted to 15 mL centrifuge tubes containing 1 mL of metaphosphoric acid (25%; w/v), and then, these tubes were stored at -20°C until VFA and ammonia analysis, as described by Moody et al. (2007). Samples were later thawed and centrifuged at 40,000 × g for 30 min at 4°C. After centrifugation, 1 mL of the supernatant was placed in a 2-mL Eppendorf microcentrifuge tube and used for the analysis of $NH_3N$ according to the methods of Chaney and Marbach (1962) with modifications including reduced sample and reagent volume to accommodate the use of a 96-well plate reader. Another 0.5 mL of the supernatant was combined with 0.5 mL distilled water and 100 μL of internal standard (86 μmol of 2-ethylbutyric acid/mL) in a GC vial. Samples for VFA were then analyzed by GC–flame-ionization detection according to the methods of Yang and Varga (1989)

and injected into a Hewlett-Packard 6890 gas chromatograph (San Jose, CA) equipped with a custom packed column (2 m × 0.32 cm × 2.1 mm ss; 10% SP-1200/1% $H_3PO_4$ on 80/100 Chromosorb WAW). Additionally, 4 mL culture sample was pipetted and preserved in 4 mL of methyl green formalin-saline solution (1:2 dilution) and stored in darkness at 4°C for protozoa counting (Ogimoto and Imai, 1981). The LCFA in dried ground feed and overflow samples were sent to (Multi-User Analytical Laboratory and Metabolomics Core, Clemson University, SC). Quantities of individual fatty acids present in the cultures were determined on a Shimadzu GC-2010 gas chromatograph with a flame ionization detector and equipped with an SLB-IL111 (Sigma, St. Louis, MO) fused silica capillary column (L x I. D. 100 m x 0.25 mm) with 0.2 um film thickness. The initial temperature was held at 140°C for 3 min then increased by 3.7°C per min up to 220°C for 60 min. The carrier gas was helium purged at 20 cm/s. Fatty acid peaks were identified and separated by comparison of the retention times to known standards.

*Calculations and Statistical Analysis*

The relative hydrogen score (rH) was calculated from pH and E*h* by using the Clark's exponent as follows:

$$rH = ((Eh + 200) / 30) + (2 \times pH)$$

$CH_4$ output (mmol/d) was estimated by using the following equation:

$CH_4$ percentage measured in fermenter headspace (%) × $CO_2$ gas flow through fermenter headspace (20 mL/min) × 60 min × 24 h / 22.4 gas constant (mol/L) / 1000

Bacterial N flow and bacterial efficiency were calculated according to Calsamiglia et al. (1996) as follows:

Sample 15N enrichment (atom percentage excess) = sample 15N atom % - background 15N atom %,

Bacterial N flow (g/d) = (NAN flow × atom percentage excess of 15N of overflow) / (atom percentage excess of 15N of bacteria), and

Bacterial efficiency = bacterial N flow (g) / OM truly digested (kg)

Nitrogen flows were calculated as follows:

NH3-N flow (g/d) = mg/dL of overflow $NH_3$-N × (g of total overflow flow / 100),

NAN flow (g/d) = g of overflow N - g of overflow $NH_3$-N,

Dietary N flow (g/d) = g of overflow NAN - g of overflow bacterial N,

RUP N flow (g/d) = total N flow - overflow bacterial N flow, and

RDP N supply (g/d) = total N intake – RUP N flow

Fractional passage rates were calculated according to Lascano et al. (2016b) as follows:

The liquid passage rate in the in-vivo study by Lascano et al. (2016b) was 8.93%/h and 10.40%/h for LF (45% forage) and HF (90% forage), respectively. Therefore, we assumed 9.75%/h would be the control diet's liquid passage rate (HF; 70% forage) in our study.

Liquid passage rate was decreased based on the decreased dry matter intake as we increased the fat inclusion in the diets.

Liquid passage rate (%/h) = drymatter intake (g/d) × liquid passage rate for the control (mL/h) × dry matter intake for the control (g/d),

Buffer input (mL/h) was calculated as follows:

Buffer input (mL/h) = liquid passage rate (%/h) × fermenter volume (mL),

In the same way, the solid passage rate was calculated and based on the results of our study.

Metabolizable energy intake (Mcal/d) was calculated as follows:

ME (Mcal/d) = (digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) × 0.82, assuming that digestible OM intake and total digestible nutrient intake were equal.

That equation was used for the control diet, which was modified from NRC (2001). To represent better the increase in energy as fat increased in the diets, another modified equation from NRC (2001) was used as follows:

ME (Mcal/d) = (digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82].

All statistical analyses were conducted in SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC) using the MIXED procedure. Data were analyzed as a 2×4 factorial design with fixed effects of period, forage, PF inclusion, and forage × PF interaction, and a random effect of fermenters (forage) and repeated measures as needed for the following model:

$Yijklm = \mu + Fi + Tj + Pk + FTij + Cl(Fi) + eijklm,$

where Yijklm = the dependent variable, μ = the overall mean, Fi = the fixed effect of forage F:C, Tj = the fixed effect of PF sequence, Pk = the fixed effect of period, FTij = the interaction between F:C and PF, Cl(Fi) = the random effect of fermenter with forage F:C and, eijklm = the residual error. Linear and quadratic polynomial contrasts were utilized to analyze the PF main effects and interactions further. For observations where

multiple repeated measures occurred in a period, the fixed effects of time and its interaction with other fixed effects were included in the model based on a repeated measures analysis (Littell et al., 1998). Covariance structures of simple, autoregressive, or compound symmetry were chosen for use in the repeated measures analysis based on the lowest values of Akaike's Information Criterion and Schwartz's Bayesian Criterion. Residuals for all models were found to be normally distributed (Shapiro-Wilk test for normality). Least square means are presented in tables, and evidence for statistical significance was declared at $P \leq 0.05$, while trends for main effects and interactions are discussed at $0.10 \geq P > 0.05$.

## RESULTS AND DISCUSSION

### *Diet Composition and Nutrient Inputs*

Diet ingredients and chemical composition values are presented in Table 5.1. The dietary NDF and ADF were lower for LF diets compared to the HF diets, whereas the starch and NFC were higher for LF diets than for the HF diets, as was their input because of the lower level of forage and a higher level of concentrate in these diets (Table 5.1 and Appendix B). The EE concentrations increased gradually in the diets with PF addition and, consequently, ME concentration; therefore, daily feeding amount decreased as PF inclusion increased. The addition of PF to LF and HF diets resulted in 8 different proportions of FA concentrations in the diets, and its input increased (Tables 5.1 and Appendix B). The PF replaced the ground corn in both LF and HF diets, resulting in a gradual decrease in starch and NFC in treatments. All other components of the rations were formulated to be similar between treatments.

By design, daily as-fed and DM input were higher for the HF-fed fermenter compares to the LF-fed fermenter based on the nutrient density differences between the two diets (Hoffman et al., 2007; Appendix B) to maintain the isoenergetic nature of the diets. Similar results were reported by Lascano and Heinrichs (2009), Lascano et al. (2009), and Zanton and Heinrichs (2009), where DMI was higher for HF in controlled nutrient intake at different F:C ratio. Inputs of ME and N were similar between treatments as planned to maintain isoenergetic and isonitrogenous diets, and as recommended by Zanton and Heinrichs (2009) for optimum N utilization in precision-fed dairy heifer diets. Daily DM, starch, and NFC inputs were decreased as PF included in the diets and were

the opposite with EE input as increased to achieve the planned diets. Consequently, there was an input effect of F:C and PF inclusion on OM, Ash, NDF, ADF, starch, and NFC to maintain the isoenergetic and isonitrogenous design of the treatments. To achieve a better simulation of the precision feeding program using the continuous culture fermenters, we manipulated and decreased the liquid and solid passage rate (kp) by reducing the buffer rate as the DM input decreased based on in-vivo experiment results by (Lascano et al., 2016b). The liquid and solid turnover rates were lower for the LF-fed fermenter compared to the HF-fed fermenter (Appendix B). Passage rates in LF diets can be slower compared to HF diets when intake is limited (Eng et al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990), and we expected to be even slower as more fat was added to the diets.

*Apparent Digestibility of Nutrients*

*Forage to Concentrate Effect*

Apparent digestibility coefficients (dC) are shown in Table 5.3. The dC of DM, OM (Figure 1), N starch, and NFC were greater for the LF-fed fermenter than for the HF-fed fermenter ($P < 0.01$). These observations are consistent with results reported in a study conducted on Holstein dairy heifers fed LF or HF diets composed of a combination of 40 or 80% CS and corn stover (Lascano and Heinrichs, 2011). They reported a higher DM, OM, and starch dC for LF compared to HF diets. High rumen degradable protein diet with 2 levels of F:C diets were fed to dairy heifers by Lascano et al. (2016b) and observed higher DM and OM dC for LF compared to HF diets. Similarly, higher DM and OM dC for LF compared to HF diets were observed by (Suarez-Mena et al., 2015).

Greater dC of LF-fed fermenter can be attributed to the greater utilization of the ingredients in theses diets (NRC, 2001) and to an increased retention time in the rumen of LF diets (Leaver et al., 1969; Colucci et al., 1990) as often occurs under controlled intake conditions. Other studies have shown increased DM, OM, and starch dC when LF and HF diets have been fed restrictively (Colucci et al., 1989; Reynolds et al., 1991; Murphy et al., 1994). These results did not agree with a study conducted on Holsten heifers fed low forage (45% forage) and high forage (60% forage) where DM and OM dC did not differ between LF and HF diets (Koch et al., 2017). Forage represented 35% and 70% of the present experiment's diets, respectively, which is a larger difference in F:C than what has been reported in the above study. The dC of NDF, hemicellulose, and ash were higher for HF-fed fermenter than for LF-fed fermenter whereas, ADF and EE dC did not differ between the two groups. The higher NDF dC in HF-fed fermenter agreed with Ranathunga et al.'s (2012) findings where the ruminal digestion of NDF was improved in HF diets containing DDGS in dairy cows compared with LF diets containing DDGS. They attributed that to HF diets' ability to decrease the detrimental effect of fat from DDGS on rumen microbes by attaching the fat into the feed particle and slowly introduced to the rumen. Furthermore, this could be attributed to the lower pH level for LF-fed fermenter (Table 5.6) because cellulolytic bacteria are very sensitive to pH and their activity and growth start to decline under pH 6.0 (Russell and Wilson, 1996). These results agree with Koch et al. (2017), Suarez-Mena et al. (2015), and Zanton and Heinrichs (2009) where ADF dC did not differ between LF and HF diets, but conflicts with other studies where NDF and hemicellulose dC was greater for LF diets (Zanton and

Heinrichs, 2009; Lascano and Heinrichs, 2011; Lascano et al., 2016b). The lower NDF and hemicellulose dC in the LF-fed fermenter could be related to the availability of rapidly fermented ingredients. Also, more numerous amylolytic bacteria populations were associated with LF diets (Brown et al., 2006). We observed a higher starch and NFC dC in the LF-fed fermenter compared to the HF-fed fermenter in the current study.

*Poultry Fat Effect*

Dry matter, OM, N, NDF, ADF, NFC, hemicellulose, ash showed a linear increase in dC ($P < 0.01$) as PF inclusion increased in both F:C diets. Suarez-Mena et al. (2015) observed a quadratic DM, OM, NDF, and ADF dC response to increasing levels of DDGS up to 14% inclusion in the diets (4.99% total FA ~ 6% EE). Also, Anderson et al. (2015) reported a higher dC of NDF, ADF, and N when heifers limit-fed a high-fat DDGS (7.00% EE) compared to a low-fat DDGS (3.08% EE), whereas the DM and OM did not differ between the treatments. It was suggested that the high-fat DDGS diet contains lower starch content compared to the low-fat DDGS resulted in higher efficiency of utilization of fiber and CP and improve the total-tract digestion. These results did not agree with a study conducted by Lascano et al. (2016a) using two levels of fat (1.01% and 2.73% FA ~ 2% and 4% EE) in continuous culture fermenter where they did not observe any effects on DM and ADF dC. Koch (2017) reported a depression in DM, OM, NDF, and ADF dC when continuous culture fermenter fed high soybean oil compared to low soybean oil. Koch stated that the dietary polyunsaturated fatty acids had been shown to depress fiber dC by limiting the growth of fiber digestion bacteria (Van Soest, 1994), and this finding is common in the literature (Rico et al., 2014). Manthey and Anderson

(2018) reported no effects on dC when heifers limit fed DDGS with ad libitum grass hay. They related that to feeding grass hay as ad libitum, which resulted in a slightly different limit feed program than the typical one. The EE and starch dC increased linearly with an increase of PF inclusion. This is mainly because the linear decrease in starch and NFC content and their inputs with PF increase in the diets resulted in more efficient fat and starch utilization in the continuous culture fermenter system. The passage rates of diets can be slower when intake is limited (Eng et al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990), and we expected to be even slower as fat added to the diets and as we have it in the current study. A linear interaction was observed in the present study for CP, EE, NDF, ADF, and hemicellulose dC. The HF-fed fermenter had a higher EE, NDF, and ADF dC at 3% and 5% inclusion of fat compared to the LF-fed fermenter at the same fat inclusion, but the LF-fed fermenter showed a higher dC at 7% and 9% inclusion of fat compared to the HF-fed fermenter at the same fat inclusion. That is mainly due to a higher fat input in the fermenter as a higher PF inclusion as required to maintain isoenergetic diets in HF diets compared to LF diets, which may have a negative effect on microbial growth (Jenkins, 1993; Maia et al., 2007). Therefore, the rumen fermentation is not affected when fat levels are low in the diets, as in the case of 3% and 5% fat inclusion because rumen microbes are able to saturate FA. However, this capacity can be exceeded at higher levels, and FA can accumulate in the rumen and interfere with rumen fermentation as in the case of 7% and 9% of fat inclusion, especially with HF-fed fermenter (NRC, 2001); therefore, the digestibility of nonlipid energy sources is reduced (Jenkins, 1993).

*Fatty Acid Flows and Biohydrogenation*

*Forage to Concentrate Effect*

The overflows of major fatty acids are detailed in Table 5.4. Outflows of individual saturated FA C14, C16, C18, C22, and C24 were all reduced with LF-fed fermenter compared to HF-fed fermenter. Gudla et al. (2012) reported a reduction in FA C16 and C18 flow when a continuous culture fermenter fed low forage diets compared to high forage diets. Similarly, in a study conducted on ewes fed five ratios of F:C, the duodenal flow of C18 increased linearly with high forage diet, but C16 was not affected (Kucuk et al., 2001). Lascano et al. (2016a) and Koch (2017) observed a reduction in saturated FA C12, C14, C20, C22, and C24 when fermenters were fed, increasing starch degradability. That could be related to the prevalence of microbial species that thrive in the rumen when high forage diets are fed (Tajima et al., 2001). The high saturated FA flow in HF-fed fermenter in the current study can also result in higher PF inclusion in HF diets compared to LF diets since the PF is relatively high in saturated FA (Table 5.2). The overflow of the unsaturated FA C18:1 and C18:2 were increased with LF-fed fermenter compared to HF-fed fermenter. Part of that is related to the differences in the dietary contribution of C18:1and C18:2, while the other part is related to the rate of biohydrogenation. Biohydrogenation rates of C18:2 and C18:3 were decreased with LF-fed fermenter ($P < 0.01$). That is aligned with our observations with decreased the amount of C18:0 flows for LF-fed fermenter and indicating a reduction in the biohydrogenation pathway to completion at C18:0. These results are in agreement with Gudla et al. (2012) as they reported greater concentrations of C18:1 and C18:2 in

fermenters fed with low forage diets with no effect on C18:3, also that reflected a reduction in the biohydrogenation of theses FA. Kucuk et al. (2001) observed a decrease in C18:1 and C18:2, but an increase in C18:3 of duodenal flow of ewes fed increased dietary forage biohydrogenation of these FA increased as dietary forage increased as well. Similarly, Lascano et al. (2016a) and Koch (2017) reported an increase in the outflows of C18:2 and C18:3 from the fermenters fed high starch and resulting in lower extents of biohydrogenation. In addition, biohydrogenation of unsaturated FA in the ruminal fluid of lactating dairy cows was reduced when they switched their diets from high to a low forage diet, and they related that to a low rumen pH (Latham et al., 1972). Cultures under low pH conditions showed less disappearance of C18 unsaturated FA (AbuGhazaleh and Jacobson, 2007). Martin et al. (2002) and Jenkins et al. (2008) stated that most rumen microbial growth and enzyme activities could be impacted under low rumen pH conditions. In the current study, the lower pH in LF-fed fermenters (Table 5.6) may have impacted culture bacteria and reduced the biohydrogenation rates.

*Poultry Fat Effect*

The inclusion of PF showed a linear increase in the overflows of saturated FA C14, C16, C18, and C22. That is mainly due to the gradual increase of PF inclusion in both LF and HF diets resulted in a higher overflow of these saturated FA, also due to the lower passage rate as PF inclusion increased in the diets (Appendix B). Lascano et al. (2016a) reported increased daily outflows of individual saturated and total fatty acids when fermenters fed high-fat diets compared to low-fat diets. Similarly, in a study conducted on feeding two levels of fat to continuous culture fermenters, the high fat-fed

fermenters showed a higher outflow of C:16, C20, C22, and C24 compared to low fat-fed fermenters (Jenkins et al., 2014). Additionally, the PF inclusion showed a linear and quadratic effect on C18:1 overflow. The inclusion of PF showed an opposite effect on individual unsaturated FA C18:2 overflow with a linear decrease for LF-fed fermenters. The linear decrease in C18:2 overflow as PF inclusion increase is partially related to the replacement of ground corn by PF as well as to the high biohydrogenation efficiency of high-fat diets (Schmidely et al., 2008). That is in agreement with a study on dry dairy cows fed two levels of crude fat (2.9% and 7.6%) and showed a decrease in C18:2 FA (Zened et al., 2013). Based on PF's effect on unsaturated FA C18, the PF showed a linear increase in the biohydrogenation of C18:2 and C18:3. These results agree with several in-vivo and in-vitro studies as fat increases in the diets (Zened et al., 2014; Jenkins et al., 2014; Lascano et al., 2016a). In the current study, the biohydrogenation of C18:2 and C18:3 showed a quadratic interaction with a lower biohydrogenation rate increase for HF-fed fermenters as PF inclusion increase compared to the LF-fed fermenters. These results could explain a better combination between forage and PF than for concentrate diet, taking into account a higher PF inclusion in HF diets even though the biohydrogenation rate for HF diets was higher than the LF diets as well as the stearic acid overflow.

*Nitrogen Flows and Metabolism*

*Forage to Concentrate Effect*

Nitrogen flows, N dC, and bacteria efficiency are shown in Table 5.5. Total N flows did not differ by F:C ratio ($P = 0.73$), and because of the little differences in N

inputs between the F:C ratio, the N dC was higher for LF-fed fermenter compared to HF-fed fermenter ($P = 0.01$). The N dC in our study agrees with previous studies (Hill et al., 2007; Zanton and Heinrichs, 2009), where the N dC was higher at lower F:C. They attributed the improvement in N dC of LF diets to a lower N excreted level in feces. In contrast, Suarez-Mena et al. (2015), Lascano et al. (2016b), and Koch et al. (2017) did not find an effect of F:C on N dC in heifers precision fed low and high forage diets. The $NH_3$-N concentration, ammonia flows, bacteria N flows, and bacteria efficiency were all lower for LF-fed fermenter than for HF-fed fermenter, whereas the non-ammonia N flows, dietary N flows, RUP N and RDP N were higher for LF-fed fermenter compared to HF-fed fermenter. These findings agree with several studies used different F:C ratio in continuous culture fermenter (Calsamiglia et al., 2008; Fuentes et al., 2009; Martinez et al., 2010). The lower $NH_3$-N and ammonia flow in LF-fed fermenter compared to HF-fed fermenter could be due to the use of ammonia for the de novo synthesis of AA, which agrees with the higher non-ammonia N flows observed in the LF-fed fermenter, or to the lower protein degradability in forage relative to concentrate. However, the bacteria efficiency was lower in LF-fed fermenter, and that is because of the higher OM dC compared to the HF-fed fermenter (Table 5.3). In addition, pH has an impact on bacteria N flow; bacteria tend to spend part of the available energy on maintaining the proton motive force across the cell membrane more than on their growth at lower pH (Wallace and Cotta, 1989), which is the case with LF-fed fermenter (Table 5.6). Calsamiglia et al. (2008) reported a lower bacteria efficiency in concentrate diet than in forage diet, and they attributed that to the decreased synthesis of microbial protein, which is confirmed by

the lower bacteria N flow in LF-fed fermenter in the current study. Also, the lower passage rate with higher retention time in LF-fed fermenter could be the reason behind the lower bacteria N flow.

*Poultry Fat Effect*

The inclusion of PF in the diets decreased the total N flows linearly ($P < 0.01$), which reflected in increasing the N dC linearly *(*$P < 0.01$). Several studies had reported increased total tract N dC when fat was supplemented (Palmquist and Conrad, 1978; Klusmeyer et al., 1991; Ohajuruka et al., 1991; Pantoja et al., 1994). Anderson et al. (2015) reported a higher N dC when heifers limit-fed a high-fat DDGS compared to a low-fat DDGS and control diet (73.1% vs. 70.1 and 69.8, respectively). In contrast, Suarez-Mena et al. (2015) did not observe any effect on N dC as DDGS increased in the diets. It was suggested that the high-fat DDGS diet contains a lower starch content than the control and low-fat DDGS resulted in higher efficiency of N and improved total-tract digestion. Ammonia concentration was linearly increased as the PF inclusion increased in the diets ($P < 0.01$). Suarez-Mena et al. (2015) and Manthey et al. (2016) observed similar results, and they attributed that to the lower ME intake with the addition of DDGS; therefore, the microbial capacity to assimilate amino acids and ammonia was negatively affected and $NH_3$ accumulated in the rumen (NRC, 2001). Additionally, Elliott et al. (1997) reported a linear increase in ammonia concentrations as the degree of saturation increased. They suggested that the dietary triglycerides became more unsaturated, and the ruminal protein digestion inhibited. Ammonia N, non-ammonia N, dietary N flows, bacteria efficiency, and RUP N were all decreased linearly as PF

inclusion increased in the diets and tended to decrease bacteria N flow (P = 0.06) while increased RDP N. When steers were fed fat supplemented diets the microbial N flow tended to decrease, which corresponds to the decreased OM dC, whereas the total N and non-ammonia N flows did not differ (Elliott et al., 1997). Several studies reported that feeding fat did not affect bacteria efficiency (Elliott et al., 1997; Oldich and Firkins, 2000; Vargas et al., 2020). The linear decrease in bacteria efficiency in the current study is mainly due to the increase of OM dC as PF inclusion increased. In the present study, the bacteria N flow and bacteria efficiency showed a quadratic effect as PF included in the diets (P = 0.01) with a higher bacteria N flow and bacteria efficiency at 5% added fat. These results could be related to better synchrony between N and energy availability for microorganism's activity. Also, as DM inputs decrease with the increase of PF inclusion, the passage rate decreases, and the retention time increases in the continuous culture fermenter as planned in the current study. That could be the reason behind the lower bacteria N flow and bacteria efficiency as PF increased in the diets.

*Culture Fermentation and Protozoa Population*

*Forage to Concentrate Effect*

Culture VFA profile, methane, pH, reduction potential (E*h*), relative hydrogen score (rH), and total protozoa counts are shown in Table 5.6. The total VFA concentration was higher for LF-fed fermenter than for HF-fed fermenter (*P* = 0.01). Acetate molar proportion was lower for LF-fed fermenter than for HF-fed fermenter (*P* = 0.04). Whereas propionate, butyrate molar proportions, and A:P ratio were not affected by F:C (Figure 5.1). Valerate molar proportion was higher, but isobutyrate molar

proportion was lower for LF-fed fermenter. Even though A:P ratio was not affected significantly by F:C, LF-fed fermenters were numerally lower than those of the HF-fed fermenter (1.41 vs. 1.47). The higher total VFA concentration for LF-fed fermenters agrees with in-vitro and in-vivo studies (Fuentes et al., 2009; Lascano et al., 2016b). Calsamiglia et al. (2008) concluded that the main factor influencing VFA concentration is the interaction between pH and F:C in the diets. In addition, Moody et al. (2007) stated that the VFA concentrations were higher in LF than HF when pH was affected by F:C. In the current study, the pH was decreased as the concentrate increased in the diet ($P < 0.01$), which is consistent with the higher OM dC in LF-fed fermenter than in HF-fed fermenter. The greater acetate molar proportion for HF-fed fermenter is consistent with previous studies (Rodriguez-Prado et al., 2004; Fuentes et al., 2009; Martinez et al., 2010; Gudla et al., 2012; Suarez-Mena et al., 2015; Lascano et al., 2016b). Acetate results of structural carbohydrate fermentation by cellulolytic bacteria and these bacteria can be inhibited by lower pH as in the present study along with lower NDF inputs, which may explain the lower acetate proportion for LF-fed fermenter (Martin et al., 2002). The no-effect on propionate and butyrate by F:C is similar to other studies by (Rodriguez-Prado et al., 2004; Gudla et al., 2012; Suarez-Mena et al., 2015). In the present study, the LF-fed fermenter showed a lower $NH_3N$ concentration and flow (Table 5.5). If energy is available, the AA can be incorporated into bacteria without deamination (Russell et al., 1991). That would explain the lower isobutyrate concentration as lower deamination in the LF-fed fermenter than the HF-fed fermenter. The reduction in valerate concentration

with HF-fed fermenter could be related to the increase in liquid fraction kp accompanied with lower retention time for HF-fed fermenter (Eun et al., 2004; Fuentes et al., 2009).

*Poultry Fat Effect*

The inclusion of PF in the diets decreased the total VFA concentrations linearly (P = 0.01), and this is mainly because of the linear reduction in acetate molar proportion as the PF level increased in the diets (Figure 5.1; $P < 0.01$). However, the propionate, butyrate, and isobutyrate molar proportions increased linearly with PF inclusion ($P < 0.01$). That resulted in a linear reduction in A:P ratio (Figure 5.2 and 5.3; $P = 0.04$). These results are comparable to those reported by several studies conducted on dairy heifer limit-fed DDGS (Suarez-Mena et al., 2015; Manthey et al., 2016; Manthey and Anderson, 2018). These findings could be due to the decline in the culture bacteria population as suggested by (Suarez-Mena et al., 2015), and this is supported by the bacteria efficiency (Table 5.5) and total protozoa counts in the current study. Manthey and Anderson (2018) suggested that the differences in starch contents and intake are the reason behind the shift in VFA concentrations and the decrease in acetate and increase in propionate. Also, they suggested the higher propionate is related to more energy-efficient and rumen fermentation in heifers fed DDGS diets (Manthey et al., 2016).

Methane production was lower for HF-fed fermenter than LF-fed fermenter, which could be related to the higher dilution rate with HF-fed fermenter than for LF-fed fermenter. Isaacson et al. (1975) observed a lower methane formation as the dilution rate increased. Additionally, since methanogens' growth rate is relatively slow, the higher dilution rate could result in a reduction in methanogenic archaea numbers (Eun et al.,

2004). Martinez et al. (2009) did not report any effect on methane production when different dilution rates were used in Rusitec fermenters. The inclusion of PF in the diets did not affect methane production ($P = 0.52$). Cultural pH was lower for LF-fed fermenter compared to the HF-fed fermenter ($P < 0.01$). Chibisa et al. (2015) stated that the drop in pH with high starch diets is common in the literature. Also, the higher retention time in LF-fed fermenter could explain the reduction in pH as the bacteria had more time for fermentation, reflecting higher total VFA concentration. The pH values agree with a study conducted using Rusitec fermenters; they reported a higher pH was observed in HF-fed fermenter than LF-fed fermenter (Martinez et al., 2010). The inclusion of PF in the diets increased the cultural pH quadratically (P = 0.02), with the highest pH values were observed at 7% fat inclusion in LF-fed fermenter and 5% fat inclusion in HF-fed fermenters. Suarez-Mena et al. (2013) reported a similar rumen pH between treatments as DDGS increased in the diets. In contrast, Manthey et al. (2016) observed a linear decrease in rumen pH as DDGS increased in the diets, and they attributed that to the F:C ratio. The E*h* was higher for LF-fed fermenter than for HF-fed fermenter (P = 0.02). These are in agreement with the observation of several studies in sheep (Marounek et al., 1982), goats (Giger-Reverdin et al., 2006), dairy cows (Julien et al., 2010; Michelland et al., 2011; Friedman et al., 2017) and dairy heifers (Monteils et al., 2009). They all showed that the ruminal environment tended to be less reducing in animals fed a concentrate diet than those fed a forage diet. Julien et al. (2010) stated that there is a relationship between pH and E*h*, and it seemed that the ruminal E*h* moved toward higher E*h* when pH dropped, which is exactly the case in the present study. We

found that the lowest reducing E*h* (-262.49) was observed when pH was the highest (6.47) with 5% fat inclusion for HF-fed fermenter. In contrast, the higher E*h* (-222.27) was recorded when pH was the lowest (5.80) with 3% fat for LF-fed fermenter. These findings could be related to the higher NDF inputs for HF-fed fermenter or rapidly fermentable carbohydrates for LF-fed fermenter (Huang et al., 2018). Total protozoa counts were lower for LF-fed fermenter compared to the HF-fed fermenter ($P < 0.01$), and this is mainly attributed to the higher concentrate diets up to 65% incorporated in LF-fed fermenter (Table 5.1). High concentrate diets provide a source of rapidly fermentable carbohydrates for microorganisms, produce more VFA, and reduce the pH (Abe et al., 1973; Mackie et al., 1978; Wedekind et al., 1986; Franzolin and Dehority, 1996). Mackie et al. (1978) stated that protozoa concentrations decrease when the concentrate diets exceed 60%. Also, the low pH in some cases can lead to a complete disappearance of the protozoa (Latham et al., 1971; Vance et al., 1972; Abe et al., 1973; Mackie et al., 1978; Lyle et., 1981). The total protozoa count decreased linearly with PF inclusion in the diets ($P < 0.01$). Oldick and Firkins (2000) reported a decrease in total rumen protozoa when fat was supplemented, whereas Karnati et al. (2009) observed that total protozoa count was higher with high-fat diets. Jouany et al. (1988) stated that protozoa defaunation is usually related to the increase in propionate and the decrease in butyrate with fat-feeding. Even though that was the case with propionate in the current study, it wasn't with butyrate. Also, as Jenkins (1993) suggested, the effect of fatty acids on bacteria can be as direct disruption of the microbial cell membrane, lipid coating of bacteria and feed particle, and as antimicrobial effects on bacterial population. Yang et al. (2009) reported

a decrease in protozoa when Holstein dairy cows fed 4% supplemental soybean and linseed oil in diets and observed a lower number of cellulolytic bacteria and a higher proteolytic bacteria number. They attributed the higher protein degradation, and a higher $NH_3N$ concentration as fat included in the diets is due to the greater proteolytic bacteria. Also, the decrease in microbial protein synthesis could be associated with high ruminal $NH_3N$ concentration.

# CONCLUSIONS

Simulating precision feeding dietary poultry fat in continuous culture fermenter in both low and high forage diets affected ruminal fermentation by decreasing acetate molar proportion and reducing the A:P ratio. Results from this study demonstrate that the dietary poultry fat inclusion improved apparent digestibility while increased the biohydrogenation rate and decreased bacterial efficiency. In the present results, the LF-fed fermenter consistently resulted in higher nutrient utilization and apparent digestibility of most nutrients, but the HF-fed fermenter showed a higher fiber apparent digestibility and protozoa population. Therefore, we can conclude that by-products of dietary poultry fat can be successfully included in rations for precision-fed dairy heifers and further reduce dry matter intake.

Table 5.1. Ingredient and chemical composition of low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets fed to continuous culture fermenters.

| | LF | | | | HF | | | |
|---|---|---|---|---|---|---|---|---|
| Ingredient,[1] % | 3% | 5% | 7% | 9% | 3% | 5% | 7% | 9% |
| Coastal hay | 5.00 | 5.00 | 5.00 | 5.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Corn silage | 30.0 | 30.0 | 30.0 | 30.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Ground corn | 51.8 | 48.5 | 44.4 | 40.8 | 24.4 | 20.6 | 16.6 | 12.6 |
| Soybean meal (SBM) | 11.2 | 12.7 | 14.7 | 16.4 | 3.60 | 5.40 | 7.25 | 9.20 |
| Mineral mix | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Poultry fat (PF) | 0.00 | 1.74 | 3.85 | 5.79 | 0.00 | 1.98 | 4.09 | 6.19 |
| Chemical composition | | | | | | | | |
| DM % | 88.8 | 89.0 | 89.2 | 89.4 | 90.3 | 90.5 | 90.7 | 90.9 |
| OM, % | 94.6 | 95.1 | 95.4 | 95.5 | 94.8 | 94.1 | 93.6 | 93.4 |
| CP, % | 12.2 | 12.7 | 13.3 | 13.8 | 9.90 | 10.3 | 10.9 | 11.6 |
| Soluble P, % CP | 23.6 | 22.2 | 21.8 | 20.4 | 37.5 | 36.4 | 35.5 | 34.5 |
| NDF, % | 20.1 | 20.7 | 21.3 | 21.8 | 33.2 | 33.8 | 35.1 | 35.7 |
| ADF, % | 9.18 | 10.3 | 10.6 | 11.1 | 18.4 | 18.7 | 19.6 | 19.9 |
| Hemicellulose,[2] % | 10.9 | 10.4 | 10.7 | 10.7 | 14.8 | 15.1 | 15.4 | 15.8 |
| Starch, % | 40.1 | 37.9 | 35.1 | 32.9 | 27.3 | 24.9 | 22.3 | 20.1 |
| Ether extract, % | 3.24 | 4.78 | 6.77 | 8.34 | 3.08 | 5.14 | 6.82 | 8.77 |
| NFC,[3] % | 59.1 | 56.9 | 53.9 | 51.6 | 48.7 | 44.9 | 40.9 | 37.2 |
| TDN | 76.3 | 78.8 | 81.0 | 83.2 | 69.7 | 72.1 | 73.9 | 75.6 |
| ME,[4] Mcal/Kg | 2.78 | 2.87 | 2.95 | 3.03 | 2.54 | 2.63 | 2.69 | 2.76 |
| Ash, % | 5.38 | 4.87 | 4.63 | 4.52 | 5.19 | 5.93 | 6.36 | 6.59 |

[1]All diets were ground to 2 mm

[2]Hemicellulose = NDF - ADF

[3]NFC: non-fiber carbohydrates = 100 - (CP + ether extract + NDF + Ash)

[4]ME calculated using TDN values as reported by Cumberland Valley Analytical Services, Inc., Waynesboro, PA. ME = (TDN × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (TDN × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)

Table 5.2. Fatty acid profile of low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets fed to continuous culture fermenters.

| | LF | | | | HF | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty Acid, % | 3% | 5% | 7% | 9% | 3% | 5% | 7% | 9% |
| C12:0 | 0.12 | 0.08 | 0.09 | 0.08 | 0.28 | 0.20 | 0.14 | 0.11 |
| C14:0 | 0.29 | 0.39 | 0.59 | 0.62 | 0.44 | 0.57 | 0.64 | 0.78 |
| C14:1T | 0.19 | 0.15 | 0.09 | 0.08 | 0.04 | 0.03 | 0.02 | 0.13 |
| C14:1 | 0.05 | 0.05 | 0.09 | 0.07 | 0.25 | 0.13 | 0.17 | 0.14 |
| C16:0 | 15.8 | 20.2 | 24.5 | 25.6 | 18.6 | 21.8 | 23.7 | 25.6 |
| C16:1T | 0.12 | 0.17 | 0.25 | 0.23 | 0.20 | 0.25 | 0.35 | 0.31 |
| C18:0 | 2.42 | 4.20 | 5.94 | 6.06 | 3.49 | 5.30 | 6.08 | 7.75 |
| C18:1 | 22.4 | 26.5 | 28.6 | 29.3 | 19.9 | 26.4 | 28.1 | 29.8 |
| C18:1-11C | 2.62 | 2.54 | 2.54 | 2.87 | 2.12 | 2.65 | 2.45 | 2.39 |
| C18:2 | 50.2 | 39.8 | 31.2 | 28.2 | 46.5 | 35.8 | 31.91 | 27.3 |
| C20:2 | 0.18 | 0.26 | 0.28 | 0.34 | 0.24 | 0.31 | 0.30 | 0.30 |
| C18:3 | 2.61 | 2.10 | 1.84 | 1.80 | 5.32 | 3.97 | 3.09 | 1.93 |
| C22:0 | 0.28 | 0.35 | 0.37 | 0.43 | 0.31 | 0.36 | 0.41 | 0.44 |
| C22:1 | 0.29 | 0.33 | 0.32 | 0.33 | 0.06 | 0.09 | 0.12 | 0.17 |
| C20:4 | 0.88 | 0.43 | 0.18 | 0.20 | 1.47 | 1.52 | 1.74 | 1.83 |
| C24:0 | 0.22 | 0.27 | 0.27 | 0.31 | 0.24 | 0.28 | 0.32 | 0.47 |
| C22:2 | 1.16 | 1.92 | 2.57 | 2.76 | 0.13 | 0.11 | 0.25 | 0.40 |
| C22:6 | 0.24 | 0.30 | 0.33 | 0.69 | 0.27 | 0.24 | 0.19 | 0.17 |
| Total, mg/g | 25.8 | 40.7 | 60.6 | 76.3 | 24.9 | 45.1 | 61.3 | 80.7 |

Table 5.3. Nutrient apparent digestibility of continuous culture fermenters fed low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets.

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| Digestibility, % | Forage | 3% | 5% | 7% | 9% | SE | F:C | Linear | Quadratic | Linear | Quadratic |
| DM | LF | 66.2 | 68.7 | 76.9 | 82.7 | 1.16 | <0.01 | <0.01 | 0.19 | 0.21 | 0.85 |
| | HF | 59.8 | 63.0 | 66.8 | 73.5 | | | | | | |
| OM | LF | 70.2 | 73.1 | 80.2 | 85.9 | 0.88 | <0.01 | <0.01 | 0.12 | 0.76 | 0.89 |
| | HF | 61.6 | 65.8 | 70.3 | 77.7 | | | | | | |
| N | LF | 54.1 | 55.0 | 61.1 | 68.4 | 1.15 | 0.01 | <0.01 | 0.15 | 0.04 | 0.77 |
| | HF | 53.3 | 53.4 | 55.5 | 61.0 | | | | | | |
| EE | LF | 59.0 | 61.2 | 70.1 | 78.7 | 2.00 | 0.62 | <0.01 | 0.17 | 0.02 | 0.71 |
| | HF | 65.2 | 66.5 | 67.3 | 72.9 | | | | | | |
| NDF | LF | 35.9 | 39.8 | 54.3 | 66.0 | 1.71 | 0.01 | <0.01 | 0.13 | 0.02 | 0.60 |
| | HF | 46.2 | 50.2 | 54.5 | 63.8 | | | | | | |
| ADF | LF | 26.7 | 33.1 | 47.8 | 59.6 | 1.93 | 0.51 | <0.01 | 0.15 | 0.04 | 0.92 |
| | HF | 34.8 | 38.4 | 44.0 | 53.6 | | | | | | |
| Hemicellulose | LF | 43.7 | 46.4 | 60.8 | 72.7 | 1.67 | <0.01 | <0.01 | 0.11 | 0.01 | 0.32 |
| | HF | 60.5 | 64.8 | 67.9 | 76.6 | | | | | | |
| Starch | LF | 98.7 | 98.7 | 98.8 | 98.9 | 0.01 | <0.01 | <0.01 | 0.67 | 0.34 | 0.89 |
| | HF | 97.7 | 97.7 | 97.8 | 97.8 | | | | | | |
| NFC | LF | 78.2 | 82.0 | 89.9 | 93.7 | 1.17 | <0.01 | <0.01 | 0.66 | 0.51 | 0.67 |
| | HF | 66.6 | 72.1 | 77.7 | 84.6 | | | | | | |

Table 5.4. Daily fatty acids flow and biohydrogenation of continuous culture fermenters fed low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets.

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| FA flow, mg/d | Forage | 3% | 5% | 7% | 9% | SE | F:C | Linear | Quadratic | Linear | Quadratic |
| Saturated | | | | | | | | | | | |
| C12:0 | LF | 4.84 | 4.78 | 4.47 | 4.28 | 0.58 | 0.53 | 0.67 | 0.24 | 0.51 | 0.29 |
| | HF | 4.21 | 5.64 | 5.00 | 4.56 | | | | | | |
| C14:0 | LF | 10.8 | 13.2 | 15.8 | 18.9 | 1.32 | 0.01 | <0.01 | 0.45 | 0.72 | 0.23 |
| | HF | 15.6 | 22.2 | 21.0 | 24.2 | | | | | | |
| C16:0 | LF | 187 | 306 | 372 | 464 | 25.5 | 0.01 | <0.01 | 0.07 | 0.01 | 0.22 |
| | HF | 192 | 393 | 506 | 599 | | | | | | |
| C18:0 | LF | 169 | 239 | 286 | 335 | 32.4 | 0.04 | <0.01 | 0.78 | 0.02 | 0.41 |
| | HF | 336 | 415 | 513 | 636 | | | | | | |
| C22:0 | LF | 4.43 | 4.59 | 5.59 | 4.18 | 1.30 | 0.06 | 0.05 | 0.62 | 0.06 | 0.18 |
| | HF | 6.76 | 8.81 | 7.34 | 12.7 | | | | | | |
| C24:0 | LF | 4.40 | 5.19 | 4.70 | 3.93 | 0.61 | 0.02 | 0.35 | 0.19 | 0.97 | 0.64 |
| | HF | 6.26 | 6.08 | 6.46 | 5.54 | | | | | | |
| Unsaturated | | | | | | | | | | | |
| C18:1 | LF | 198 | 368 | 399 | 419 | 25.8 | 0.01 | <0.01 | 0.04 | 0.94 | 0.14 |
| | HF | 127 | 212 | 258 | 339 | | | | | | |
| C18:2 | LF | 1337 | 342 | 323 | 237 | 20.9 | <0.01 | 0.03 | 0.12 | 0.02 | 0.16 |
| | HF | 160 | 157 | 169 | 161 | | | | | | |
| C18:3 | LF | 12.4 | 14.3 | 14.2 | 12.7 | 1.25 | 0.89 | 0.99 | 0.29 | 0.83 | 0.41 |
| | HF | 13.4 | 13.1 | 13.6 | 12.9 | | | | | | |
| Total | LF | 1014 | 1601 | 2194 | 2202 | 119 | 0.01 | <0.01 | 0.19 | 0.07 | 0.16 |
| | HF | 1026 | 1690 | 2207 | 2987 | | | | | | |
| Biohydrogenation,[1] % | | | | | | | | | | | |
| C18:2 | LF | 43.1 | 46.7 | 48.9 | 63.9 | 3.32 | <0.01 | <0.01 | 0.73 | 0.54 | 0.01 |
| | HF | 59.7 | 75.6 | 82.1 | 83.3 | | | | | | |
| C18:3 | LF | 59.9 | 65.6 | 64.0 | 69.6 | 2.30 | <0.01 | 0.01 | 0.07 | 0.37 | 0.05 |
| | HF | 70.6 | 82.2 | 84.9 | 83.2 | | | | | | |

[1]Expressed as milligrams of input - milligrams of outflow/milligrams of input for 18:2 and 18:3

Table 5.5. Daily nitrogen flow and bacteria efficiency of continuous culture fermenters fed low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets.

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| N flow, g/d | Forage | 3% | 5% | 7% | 9% | SE | F:C | Linear | Quadratic | Linear | Quadratic |
| Total N | LF | 0.43 | 0.42 | 0.37 | 0.30 | 0.01 | 0.73 | <0.01 | 0.16 | 0.07 | 0.50 |
| | HF | 0.40 | 0.39 | 0.38 | 0.35 | | | | | | |
| $NH_3N$ | LF | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | <0.01 | 0.01 | 0.57 | 0.07 | 0.40 |
| | HF | 0.05 | 0.05 | 0.05 | 0.04 | | | | | | |
| NAN | LF | 0.41 | 0.40 | 0.35 | 0.30 | 0.01 | 0.01 | <0.01 | 0.13 | 0.06 | 0.28 |
| | HF | 0.35 | 0.34 | 0.34 | 0.24 | | | | | | |
| Bacteria N | LF | 0.20 | 0.23 | 0.18 | 0.19 | 0.01 | 0.03 | 0.06 | 0.01 | 0.93 | 0.31 |
| | HF | 0.23 | 0.27 | 0.24 | 0.21 | | | | | | |
| Dietary N | LF | 0.21 | 0.17 | 0.17 | 0.10 | 0.01 | <0.01 | 0.04 | 0.82 | 0.07 | 0.21 |
| | HF | 0.12 | 0.09 | 0.10 | 0.09 | | | | | | |
| RUP N | LF | 0.23 | 0.19 | 0.18 | 0.11 | 0.02 | 0.05 | 0.03 | 0.82 | 0.06 | 0.19 |
| | HF | 0.17 | 0.13 | 0.14 | 0.13 | | | | | | |
| RDP N | LF | 0.70 | 0.75 | 0.76 | 0.83 | 0.02 | 0.02 | <0.01 | 0.60 | 0.07 | 0.39 |
| | HF | 0.69 | 0.72 | 0.72 | 0.75 | | | | | | |
| $NH_3$-N, mg/dL | LF | 4.78 | 4.98 | 5.36 | 5.82 | 0.09 | <0.01 | <0.01 | 0.17 | <0.01 | 0.32 |
| | HF | 5.02 | 5.57 | 6.80 | 7.82 | | | | | | |
| Digestibility, % | LF | 54.1 | 55.0 | 61.1 | 68.5 | 1.15 | 0.01 | <0.01 | 0.15 | 0.04 | 0.77 |
| | HF | 53.4 | 53.5 | 55.5 | 61.1 | | | | | | |
| Bacteria efficiency[1] | LF | 18.7 | 20.0 | 13.9 | 12.0 | 1.36 | <0.01 | <0.01 | 0.01 | 0.99 | 0.34 |
| | HF | 23.8 | 25.6 | 21.6 | 16.5 | | | | | | |

[1]Calculated as g of bacteria N / kg of OM truly digested

Table 5.6. Volatile fatty acids, methane, pH, E*h*, and protozoa population of continuous culture fermenters fed low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets.

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| Culture fermentation | Forage | 3% | 5% | 7% | 9% | SE | F:C | Linear | Quadratic | Linear | Quadratic |
| Total VFA, m*M* | LF | 100 | 96.2 | 92.9 | 95.8 | 3.35 | 0.01 | 0.01 | 0.08 | 0.86 | 0.09 |
| | HF | 76.9 | 73.2 | 72.4 | 68.8 | | | | | | |
| Individual VFA, mol/100 mol | | | | | | | | | | | |
| Acetate | LF | 47.4 | 47.8 | 46.5 | 45.9 | 0.99 | 0.04 | 0.04 | 0.09 | 0.82 | 0.42 |
| | HF | 52.0 | 50.9 | 51.0 | 49.0 | | | | | | |
| Propionate | LF | 33.5 | 34.7 | 34.4 | 36.5 | 1.06 | 0.54 | 0.01 | 0.09 | 0.32 | 0.82 |
| | HF | 33.8 | 35.0 | 34.0 | 35.7 | | | | | | |
| Butyrate | LF | 10.4 | 10.6 | 12.4 | 11.2 | 0.88 | 0.29 | 0.02 | 0.96 | 0.38 | 0.63 |
| | HF | 9.24 | 9.89 | 9.68 | 10.2 | | | | | | |
| Valerate | LF | 8.20 | 7.35 | 6.28 | 5.83 | 0.39 | 0.01 | <0.01 | 0.75 | 0.08 | 0.07 |
| | HF | 4.52 | 4.16 | 4.46 | 4.46 | | | | | | |
| Isobutyrate | LF | 0.34 | 0.34 | 0.41 | 0.48 | 0.02 | 0.01 | <0.01 | 0.13 | 0.42 | 0.15 |
| | HF | 0.43 | 0.44 | 0.44 | 0.54 | | | | | | |
| Methane, mmol/d | LF | 30.5 | 30.6 | 30.4 | 30.5 | 0.11 | <0.01 | 0.52 | 0.51 | 0.29 | 0.10 |
| | HF | 29.9 | 29.7 | 30.2 | 29.8 | | | | | | |
| pH | LF | 5.80 | 5.93 | 6.11 | 5.94 | 0.03 | <0.01 | 0.18 | <0.01 | <0.01 | 0.19 |
| | HF | 6.20 | 6.47 | 6.26 | 6.13 | | | | | | |
| E*h*,[1] mV | LF | -222 | -238 | -229 | -224 | 6.93 | 0.02 | 0.34 | 0.01 | 0.73 | 0.69 |
| | HF | -242 | -262 | -244 | -243 | | | | | | |
| rH[2] | LF | 10.8 | 10.5 | 11.2 | 11.1 | 0.21 | 0.79 | 0.20 | 0.91 | 0.17 | 0.46 |
| | HF | 10.9 | 10.8 | 11.0 | 10.8 | | | | | | |
| Protozoa, $10^3$/mL | LF | 22.2 | 20.0 | 17.9 | 16.2 | 0.66 | <0.01 | <0.01 | 0.15 | 0.11 | 0.37 |
| | HF | 27.8 | 24.2 | 20.9 | 17.5 | | | | | | |

[1]E*h* = Redox potential

[2]rH, Clark's exponent = ((E*h* + 200) / 30) + (2 × pH)

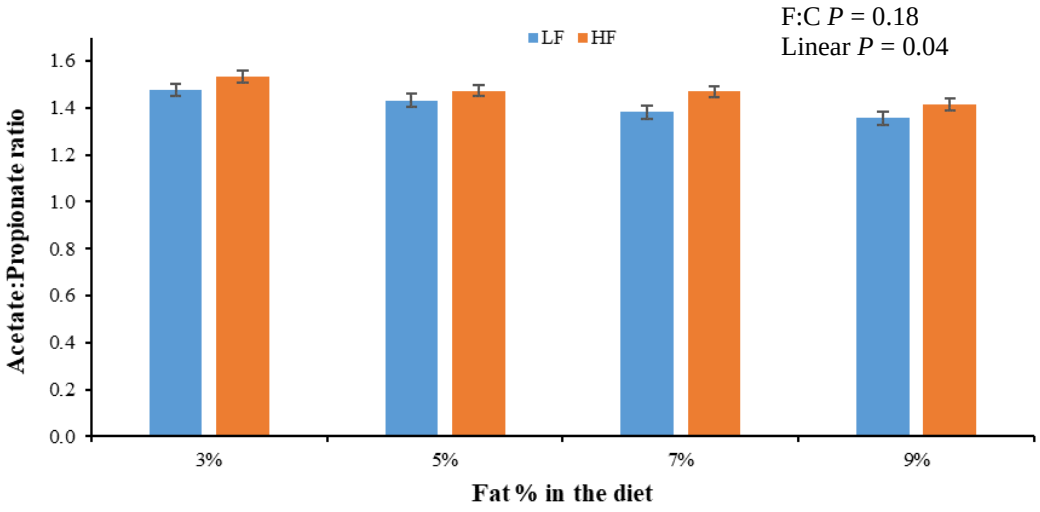


Figure 5.1. Acetate:propionate ratio of continuous culture fermenters fed low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as a gradual increase of dietary poultry fat (PF) in the diets.

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# CHAPTER SIX

## EFFECTS OF PRECISION FEEDING HOLSTEIN AND JERSEY DAIRY HEIFERS A GRADUAL INCREASE OF DIETARY POULTRY FAT ON TOTAL-TRACT NUTRIENT DIGESTIBILITY AND RUMEN FERMENTATION PARAMETERS


### ABSTRACT

Fat inclusion can increase the energy density of diets fed to ruminants, reducing dry matter intake (**DMI**) required to meet caloric demands. Diets used for precision-feeding are more nutrient-dense, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss. Also, there are indications that Jerseys have higher digestibility than the Holstein. Therefore, the objective of this study was to evaluate the effects on nutrient digestion and rumen fermentation of including different levels of poultry fat (**PF**) in precision fed Holstein and Jersey dairy heifers. We hypothesized that including PF would reduce intake without compromising digestibility and rumen fermentation in dairy heifers. Four Holstein and four Jersey cannulated heifers were randomly assigned to 4 treatments, included a 55% forage diet with 4 increasing PF inclusion starting with a basal concentration of fat in the diet [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments were administered according to a split-plot, 4×4 Latin square design for 4 periods of 21 d. Data were analyzed using the MIXED procedure of SAS. Holsteins had a lower apparent digestibility (**AD**) than Jerseys. The inclusion of PF did not affect most of AD. The PF inclusion showed a linear decrease in manure output. Estimated microbial CP flow was higher for Holstein, whereas the PF inclusion did not affect microbial CP. Total VFA, acetate decreased linearly as PF increased; concurrently, there was a linear increase in propionate resulting

in a linear reduction on A:P ratio. These results suggest that Jerseys utilized nutrients more efficiently than Holsteins. Also, increasing PF inclusion up to 6% in the rations can reduce DMI further in precision-fed heifer without negatively affecting digestibility.

# INTRODUCTION

Wild ruminants have the capacity to select a diet that is appropriate to their nutrient requirements. As an innate antipredator behavior, they have adapted to intermittent feeding cycles to avoid grazing at night; therefore, forages consumed result in slower passage rate and more efficient digestion (Jensen, 2017). This eating habit results in restricted feeding. On the other hand, cost-effective by-products from other agricultural industries can be utilized as sources of energy. Ruminants can utilize by-products from numerous industries, such as the poultry industry. Poultry fat (PF) is a by-product of poultry processing and extensively produced world-wide and a potential source of energy (Swisher, 2015). Using PF in dairy diets can be an economical energy source and can benefit the poultry industries by providing a market for their by-products (Hutchison et al., 2006). The use of fat in dairy diets increases due to higher energy demands of dairy cows and higher availability of fat supplements (NRC, 2001). Several studies have explored different strategies for feeding fat to dairy cows (Rabiee et al., 2012); however, there is limited research regarding the effects of feeding fat to the growing dairy heifer.

In a study conducted by Anderson et al. (2015), the dietary fat reached 7% DM by using dried distillers grains (DDGS) at the highest dietary incorporation fed to dairy heifers. They reported an increase in CP and NDF digestibility with the high-fat diets with no effect on DM and OM digestibility. Limit-fed animals are given energy and nutrients adjusted to allow the animal to reach a targeted ADG (Zanton and Heinrichs, 2005). The inclusion of more energy-dense ingredients results in lower DMI while higher

nutrient (starch, protein, fiber) digestibility (Lascano and Heinrichs, 2011). However, fat added to rations can reduce feed intake, inhibit microbial activity in the rumen, and reduced nutrient digestibility (Palmquist and Jenkins, 1980), but to what extent dietary fat can be strategically incorporated into precision feeding is not known.

On the other hand, due to the lack of research, the current guidelines for feeding dairy cows in the U.S. (NRC, 2001) do not make a specific recommendation for feeding growing Jersey heifer. Based on the Council of Dairy Cattle Breeding (CDCB; 2015), Jersey is the second most popular breed in the U.S., and its percentage of the cow population increased from 4.9 to 6.4 % from 2009 to 2014, while Holstein population decreased from 89.6 to 83.9% because of milk fat level. Also, there are indications that Jerseys have higher digestibility than Holsteins. Olijhoek et al. (2018) reported that Jersey had a higher DM and OM digestibility than Holstein cows fed two F:C ratio diets. Based on the previous in-vitro studies results, DMI can potentially be reduced further by using fat as an energy source in the precision feeding program as long as other nutrients are adjusted to provide the required amounts. To our knowledge, to what extent this happens in either Jersey or Holstein heifer is not known. Therefore, the objective of this study was to evaluate the effects of including a gradual increase of PF inclusion in precision feeding dairy heifers on breed, nutrient digestibility, and rumen fermentation. We hypothesized that including PF would further reduce intake and improve nutrients digestibility in dairy heifers.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

All procedures involving animals' use were approved by the Clemson University Institutional Animal Care and Use Committee (AUP Protocol #: 2019-007). Four Holstein heifers (16.7 ± 0.5 mo of age and 453.3 ± 9.4 kg of BW at the start of the experiment) and four Jersey heifers (18.08 ± 0.4 mo of age and 343.2 ± 7.5 kg of BW at the start of the experiment) were surgically fitted with 7.62 cm ruminal cannulas (Bar Diamond, Parma, ID) under local anesthesia 5 mo before the start of the study and replaced later with 10.16 cm cannulas (Bar Diamond, Parma, ID). Heifers were randomly assigned to four treatments, that included 55% forage diet with four increasing levels of dietary PF inclusion [3% fat (0% PF); 5% fat (2% PF); 7% fat (4% PF); and 9% fat (6% PF)]. Treatments were administered according to a split-plot, 4×4 Latin square design for four periods of 21 d. The whole plot factor in this study was the breed (Holstein and Jersey; H:and J), whereas the subplot factors were the different levels of dietary PF inclusion. All diets were offered as total mixed rations (TMR) and predicted nutrient composition determined using NRC (2001). Diets were formulated to restrict intake and provide equal amounts of ME and adjusted weekly to allow for 800 g/d of ADG (Zanton and Heinrichs, 2009; Lascano and Heinrichs, 2011). A similar N intake was provided to supply 1.70 g N/kg BW 0.75 for Holstein heifers, which has been observed to maximize N utilization in dairy heifers (Zanton and Heinrichs, 2009). Also, 1.30 g N/kg BW 0.75 for Jersey heifers, which is relatively based on the ADG recommendation for Jersey heifers (600 g/d; Heinrichs and Jones, 2017). Adaptation to treatment rations (PF) was

made over the first 15 d of each period and 5 d for sampling collection starting from d 16. Heifers were weighed weekly 2 h before feeding time, and the amount of feed offered for the next 7-d interval was adjusted based on the weighted averages. No refusals were observed in the present experiment. Rations were mixed daily at 0900 h by preparing each diet individually with dietary PF (Stabilized poultry fat; Valley proteins, Inc., Ward, SC) mixed with a portion of the TMR and were offered to heifers daily at 1000 h. Heifers were housed in individual stalls (150 × 300 cm) in a naturally ventilated tie-stall barn with rubber mattress bedding. They were allowed access to an exercise lot for 2 h before the 1000 h feeding on non-sampling days. Total time (min.) required to complete the daily amount of feed offered was recorded, and water was available at all times.

*Sample Collection and Analyses*

Feces and urine were totally collected from d 16 to 20 immediately after feeding for 4 d. Urine was collected using a modified non-invasive urine device (Lascano et al., 2010), connected to a container containing acidified distilled water (To avoid the formation of precipitates). The pH of collected urine was checked hourly and 12 N HCl was added to acidify the urine pH to less than 2 if needed, to minimize $NH_3N$ volatilization (Zanton and Heinrichs, 2009). Urine was weighed and sub-sampled daily before feeding. Approximately 250-mL urine subsample was frozen immediately at -20ºC for later analyses. Feces were collected whenever the heifers were dropping dung and stored in airtight containers. Every 24 h, the total collection of feces was mixed, weighed, and sub-sampled. Feedstuffs, TMR, fecal, and urine, were composited by each period as a proportion of the daily amount excreted during the sampling days.

Feces, urine N, TMR, and diet ingredients were sent to a commercial lab for analyses (Cumberland Valley Analytical Services, Inc., Waynesboro, PA) and analyzed for dry matter (DM, method 930.15, AOAC, 2000), and N (method 984.13, ACOC, 2000). Also, for crude fat (EE, method 2003.05, 18$^{th}$ edition, AOAC, 2006) using Tecator Soxtec System HT 14043 Extraction unit and modified to use anhydrous ether. Samples shipped back from Cumberland Valley Analytical Services were analyzed for OM (OM, DM-ash) and ash (method 942.05, AOAC, 2000). Also, neutral detergent fiber (NDF) and acid detergent fiber (ADF; Van Soest et al., 1991) using an ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with heat resistant α-amylase and sodium sulfite utilized in the NDF procedure and corrected for ash content. Starch was analyzed on reground samples (< 0.5-mm screen) using an enzymatic procedure (Bach Knudson, 1997). The thawed urine samples were diluted with distilled water (1:10) and analyzed for uric acid (Cat No. 1045-225, Stanbio Laboratory, Boerne, TX) and allantoin (Chen and Gomes, 1992). Urinary purine derivative (PD; allantoin and uric acid) excretion was used to estimate duodenal microbial N flow (Chen and Gomes, 1992). Metabolizable energy intake (Mcal/d) was calculated for each heifer within each period using [(digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) × 0.82], assuming that digestible OM intake and total digestible nutrient intake were equal. That equation was used for the control diet, which was modified from NRC (2001). In addition to that equation, another equation was modified from NRC (2001) to represent better the increase in energy as fat increased in the diets [(digested OM intake × 4.409 (Mcal/Kg) × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82].

Rumen contents were obtained from 5 places in the rumen (dorsal, ventral, anterior, caudal, and central) at -2, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h after the 1000-h feeding on d 20 to 21. Rumen contents were mixed and strained through 2 layers of cheesecloth, pH of rumen fluid was immediately recorded using a pH-specific electrode meter (Hanna Instruments, Woonsocket, RI). A 4 mL was pipetted from the strained fluid and added to 15 mL centrifuge tubes containing 1 mL of metaphosphoric acid (25%; w/v). These tubes were stored at -20°C until VFA and $NH_3N$ analysis. Rumen samples were thawed and centrifuged at 40,000 × g for 30 min at 4°C. After centrifugation, 1 mL of the supernatant was placed in a 2-mL Eppendorf microcentrifuge tube and used for the analysis of $NH_3N$ according to the methods of Chaney and Marbach (1962). Another 0.5 mL of the supernatant was combined with 0.5 mL distilled water and 100 μL of internal standard (86 μmol of 2-ethylbutyric acid/mL) in a GC vial. Samples for VFA were then analyzed by GC–flame-ionization detection according to the methods of Yang and Varga (1989) and injected into a Hewlett-Packard 6890 gas chromatograph (San Jose, CA) equipped with a custom packed column (2 m × 0.32 cm × 2.1 mm ss; 10% SP-1200/1% $H_3PO_4$ on 80/100 Chromosorb WAW). At the end of each 21-d period, rumen contents were evacuated 3 h after the 1000-h feeding, and mass and volume of the total contents were recorded.

*Statistical Analyses*

All statistical analyses were conducted in SAS version 9.2 for Windows (SAS Institute Inc., Cary, NC) using the MIXED procedure. Data were analyzed as a split-plot, 4×4 Latin square design, breed as the whole plot and PF level as the subplot with fixed

effects of period, breed, PF inclusion, and breed × PF interaction, and a random effect of heifer (breed) and repeated measures as nedded for the following model:

$$Yijklm = \mu + Bi + Hm(i) + Fj + BFij + Pk + eijklm,$$

where Yijklm = the dependent variable, μ = the overall mean, Bi = the fixed effect of breed H:J, Hm(i) = the random effect of heifer within breed H:J, Fj = the fixed effect of PF sequence, BFij = the interaction between H:J and PF, Pk = the fixed effect of period, and, eijklm = the residual error. Linear and quadratic polynomial contrasts were utilized to analyze the PF main effects and interactions further. Because animals were observed for four periods, the PF inclusion was randomized across periods consistent with a Latin Square design to allow for the period to be included in the model and evaluate the carryover effect with respect to previous PF inclusion. The previous treatment's fixed effect was initially included in the model but was found to be non-significant and removed from the final model.

For observations where repeated measures occurred in a period, the fixed effects of time and its interaction with breed and PF were included in the model based on a repeated measures analysis (Littell et al., 1998) or a split-split plot design with time as the sub-sub-plot factor. The covariance structures of simple, autoregressive, or compound symmetry were chosen for use in the repeated measures analysis based on the lowest values of Akaike's Information Criterion and Schwartz's Bayesian Criterion. Residuals for all models were found to be normally distributed (Shapiro-Wilk test for normality). Least square means are presented in tables, and evidence for statistical significance was

declared at $P \leq 0.05$, while trends for main effects and interactions are discussed at $0.10 \geq P > 0.05$.

## RESULTS AND DISCUSSION

### *Diet Composition and Nutrient Intakes*

Diet ingredients and chemical composition values are shown in Table 6.1. Diets were formulated and resulted in isoenergetic and isonitrogenous intakes. Diets were planned to differ mainly in dietary fat by adding different levels of PF. Intake was greater for the basal diet group than the other groups because we restricted intake as the energy from PF's inclusion increased. The PF addition resulted in 4 different proportions of EE concentrations in the diets, and fat intake increased linearly (Tables 6.1 and 6.2). Per design, PF replaced the ground corn in the diets, resulting in a gradual decrease in starch and NFC in treatments. All other components of the rations were formulated to be similar between treatments.

### *Breed Effect*

Heifers were fed diets that provided 16.84 and 10.74 ± 0.44 Mcal of ME/d for Holstein and Jersey, respectively. Mean heifer BW in the treatment groups differed between breed ($P < 0.01$) but did not differ by the addition of PF throughout the experiment ($P = 0.64$; Table 6.2). The time required to consume the daily amount of diet offered to the heifers differed between the H:J ($P = 0.05$) with longer time needed to finish the meal for H-group than the J-group (65.3 vs. 59.9 ± 1.64 min) and that is related to the higher DMI based on the breed BW size (492.63 vs. 373.27 ± 3.88 kg). These results did not agree with a study conducted on Holstein and Jersey cows, where the daily eating time did not differ between breeds, but Jerseys spent more time eating per unit of ingested feed (Aikman et al., 2008). They attributed that to Jersey’s small mouth

compared to Holstein, so they require several mouthfuls to process an equal volume of feed. The present study scenario is different because of the precision feeding program that provides the heifers a limited intake, whereas the study mentioned above used ad libitum feeding. Dry matter intake was higher for H-group than J-group because of the BW differences between the two breeds and intake requirements. Consequently, there was a significant intake effect of H:J and PF inclusion on OM, Ash, NDF, ADF, starch, and NFC to maintain the isoenergetic and isonitrogenous design of the treatments. The ADG for H-group (753 ± 43.6 g/d; data not shown) were close to the recommendation by (Zanton and Heinrichs, 2009; Lascano and Heinrichs, 2011). Also, J-group was close to achieving a targeted ADG 600 g/d (580 ± 47.9 g/d; data not shown) as a prediction to reach the optimum BW at first calve (Heinrichs and Jones, 2017). In the present study, a linear interaction was observed for EE and NFC intake. As planned, EE intakes were increased linearly in both Holstein and Jersey heifers while the NFC intakes were decreased linearly in both breeds.

*Poultry Fat Effect*

Daily DM, starch, and NFC intakes were decreased linearly with the planned PF inclusion to maintain isoenergetic intakes and was the opposite with EE intake, which increased linearly to achieve the planned diets. That is mainly related to the higher PF inclusion, and the replacement with ground corn as PF increased in the diet. The time required to finish the meal decreased linearly with increased PF inclusion in the diets ($P$ = 0.01).

*Apparent Digestibility of Nutrients*

*Breed Effect*

Apparent total-tract nutrient digestibility (AD) are shown in Table 6.3. The total-tract AD of DM, OM, NDF, ADF, and NFC was greater for J-group than H-group, but not for starch ($P$ = 0.01). These observations are consistent with results reported in a study conducted on Holstein and Jersey dairy cows fed 2 levels of F:C diets (Olijhoek et al., 2018). Similarly, higher OM and NDF dC for Jersey compared to Holstein-Friesian cows were observed by (Beecher et al., 2014). Greater digestibility of J-group can be attributed to a higher digestion rate through the rumen and total tract, as Ingvartsen and Weisbjerg (1993) stated. These results did not agree with a study conducted on Holstein and Jersey cows fed ad libitum TMR during 3 periods, far-off, close up, and lactation. They reported that DM, OM, ADF, and starch AD did not differ between breeds; however, NDF AD was higher in Jersey compared to Holstein cows, and the DM and OM AD were numerically higher in Jerseys as well (Aikman et al., 2008). Nitrogen AD did not differ between H:J ($P$ = 0.44), and this is in agreement with several studies conducted on Holstein and Jersey cows where the N AD did not differ between the two breeds (Kauffman and St-Pierre, 2001; Aikman et al., 2008; Knowlton et al., 2010; Olijhoek et al., 2018). When external markers were used, Jersey cows showed a higher digestion rate and efficient in utilizing the diet because of a larger gastrointestinal tract weight relative to BW or a higher chewing rate per unit of meal consumed, suggesting particle breakdown and rumen outflow were faster in Jersey compared to Holstein (Aikman et al., 2008; Beecher et al., 2014). Some other studies reported that Jerseys have a higher feed

utilization efficiency than large breeds such as Holstein (Oldenbroek, 1988; Grainger and Golddard, 2004). According to Van Soest (1994), a relatively large gastrointestinal tract as a proportion of the BW in Jerseys would indicate a larger area available for nutrient absorption; therefore, higher AD would be expected.

*Poultry Fat Effect*

The PF inclusion did not show any linear or quadratic effects on DM, OM, NDF, and ADF AD, but we noticed a numerical increase in N AD as the PF level increased in the diets. The EE (not presented) and starch AD increased linearly with increased levels of PF inclusion, and the effect was the opposite with NFC AD. However, EE AD is not a good indication of total-tract fat digestibility because fecal fat is mostly FFA and calcium salts. The increase in starch AD could be mainly related to the linear decrease in starch intake as corn was replaced with PF in the diets, resulting in more efficient starch utilization in the rumen and total-tract digestive system. The passage rates of diets can be slower when intake is limited (Eng et al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990), and we expected to be even slower as fat was added to the diets and the intake reduced further. The AD for N and hemicellulose did not differ with PF inclusion, but N AD tended to increase linearly with PF inclusion ($P = 0.06$). Anderson et al. (2015) reported a higher AD of N when heifers limit-fed a high-fat DDGS compared to a low-fat DDGS and control diet (73.1 vs. 70.1 and 69.8 ± 0.88%, respectively). It was speculated that is because of the higher efficiency of N utilization when the starch content decreased in the high-fat DDGS diets compared to the control; therefore, that improved the total-tract digestion. However, further research would be necessary to test this hypothesis. The

AD of EE showed a linear interaction with an increase in the EE AD as PF inclusion increased in the diets and in both Holstein and Jersey heifers. That could be related to the lower DMI as PF increased in the diets, which reduced the passage rate and resulted in more efficient fat utilization in the total-tract digestive system.

*Nitrogen Intake and Dynamics*

*Breed Effect*

Nitrogen intake, AD, and dynamics are shown in Table 6.4. Daily N intakes differed by breed, and that is mainly because of the BW sizes and to maintain the planned isonitrogenous diets. Fecal, urine and total excreted N were higher for H-group than the J-group ($P$ <0.01). That is related to the higher amount of N intakes based on BW size and requirements; however, the N retention (% of intake and % of digested) was higher in H-group compared to the J-group. In the study of Knowlton et al. (2010), Jersey cows were observed to have lower N in feces and urine with 33, and 24% reduced compared to Holstein cows. Also, Blake et al. (1986) observed a 30% reduction while Kauffman and St-Pierre (2001) reported a 27% reduction in fecal N for Jerseys compared to Holsteins. They attributed that to the differences in digestion rate and the breed response to type and concentration of protein in the diet.

*Poultry Fat Effect*

As the PF inclusion increased in the diets, the fecal and total excreted N decreased linearly ($P$ = 0.01), and the retention of N increased numerically, specifically in H-group. The fecal N excretion agreed with Suarez-Mena et al. (2015), where N excretion in fecal was linearly decreased as DDGS levels increased, but the N retention was decreased.

They attributed that to the linear increase in urine N excretion, which was not the case in the current study. The reduction in total N excretion and the numerical improvement in N retention could be related to better synchrony of N and energy available for the microorganism in the rumen. That is consistent with the numerical improvement in microbial protein synthesis (Table 6.6). There was a linear interaction for urine N excretion with a linear decrease in Holstein heifers while a linear increase in Jersey heifers. Despite that, the total N excretion showed a linear decrease in both breeds, which is mainly related to the lower fecal N excretion as PF increased in the diets and the lower DMI.

*Nutrient Excretion and Estimated Microbial Protein*

*Breed Effect*

Excretion parameters data are shown in Table 6.5, and estimated microbial CP flow to the duodenum is given in Table 6.6. Wet, dry, water fecal, and manure outputs were higher for H-group compares to J-group ($P$ <0.01), and that is related to the higher amount of intake to meet the requirements based on BW differences between breeds. That could also explain the reduction in nutrient AD for H-group in the current study (Table 6.3). However, the urine excretion was higher for the J-group than for the H-group ($P$ <0.01), which is consistent with the reduction in urine N for J-group, and because of that, the total water excretion did not differ between H:J ($P$ = 0.92). Even though the water consumption was not measured in the current study, but from a researcher note, we speculated the differences in the urine outputs between the two breeds are related to water consumption and behavior, which was higher for J-group than for H-group. These results

agree with a study conducted on Holstein and Jersey cows by Knowlton et al. (2010). They observed a lower manure excretion for J-group than H-group with 35% less wet feces.

There were higher allantoin, uric acid, and total PD concentrations for H-group compared to J-group heifers ($P < 0.01$), which resulted in greater amounts of microbial CP flow. That is mainly related to the BW and DMI differences between the two breeds. However, the allantoin as a percentage of PD did not differ between H:J.

*Poultry Fat Effect*

All the excretion parameter outputs were decreased linearly as the PF inclusion level increased. That is mainly related to the lower amount of DMI as the PF inclusion increased in the diets and the lower NDF intake since the NDF helps increase water excretion in feces. That was also an inverse relationship to the ADs responses in the current results (Table 6.3). These results agreed with several studies (Leupp et al., 2009; Suarez-Mena et al., 2015), where they observed a linear decrease in fecal outputs as DDGS level increased (fat level increased). They related that to a similar trend in OM intake, which is the case in the current study (Table 6.2).

The inclusion of PF did not show any effects on microbial CP flow, and this agrees with a study conducted by Suarez-Mena et al. (2015) on dairy heifers fed different levels of DDGS. However, we noticed a numerical but not significant quadratic effect on total PD and microbial CP flow. The greatest microbial CP flow was observed at the 9% fat in the diets compared to the other fat concentrations. These observations could be related to the passage rates of diets, which can be slower when intake is limited (Eng et

al., 1964; Owens and Isaacson, 1977; Colucci et al., 1990). Also, we expected to be even slower as fat was added to the diets, which give more time for microbial CP synthesis. Also, the reduction in NDF intakes in the 9% fat in the diets, as high NDF intakes has been linked to show a reduction in microbial CP flow (Valadares et al., 1999; Pina et al., 2009; Lascano et al., 2016).

*Rumen Fermentation, Contents, and Volume*

*Breed Effect*

Rumen VFA profile, $NH_3N$, pH, and rumen pool sizes are shown in Table 6.7. There were no differences between H:J for total ruminal VFA, acetate, propionate, butyrate, valerate, and isobutyrate molar proportions. The lack of differences in acetate and propionate were reflected in acetate to propionate ratio, which also did not differ between H:J. Rodriguez et al. (1997) observed no differences in A:P ratio when Holstein and Jersey cows fed diets differing in fat and rumen undegradable protein content, which agrees with the current study. Even though the current results were not significant, but H-group numerically tended to be lower in acetate (65.65 vs. 66.90 ± 0.68), higher in propionate (22.54 vs. 21.77 ± 0.41), and lower in A:P ratio (3.04 vs. 3.16 ± 0.12) than J-group. Similar results with significant effects were observed by (Olijhoek et al., 2018) on VFA parameters. They attributed the differences between the two breeds in rumen fermentation to the rumen's microbial community structure.

Mean ruminal $NH_3N$ concentration did not differ between H:J ($P = 0.38$). Ruminal pH did not differ between the two breeds ($P = 0.12$). These results agree with Rodriguez et al. (1997), where they observed no differences in ruminal $NH_3N$ and pH

between Holstein and Jersey cows fed diets differing in fat and rumen undegradable protein content. We observed differences in rumen mass, volume and tended to have a higher density for the H-group than the J-group. That is due to the differences in BW sizes and the amount of DMI.

*Poultry Fat Effect*

The inclusion of PF in the diets decreased the total VFA concentrations linearly ($P = 0.03$), and this is mainly because of the linear reduction in acetate molar proportion as the PF level increased in the diets ($P < 0.01$). Even though the AD of NDF and ADF were not affected by PF inclusion, the linear reduction in fiber intake and the starch intake could be the reason behind the reduction in acetate concentration (Manthey and Anderson, 2018). Acetate production within the rumen results from the fermentation of structural carbohydrates by cellulolytic bacteria (Enjalbert et al., 1999). Also, the higher total VFA concentration for the CON diet could be related to the pH (Calsamiglia et al., 2008). In the current study, the pH was the lowest with the CON-fed group than for other treatments. In addition, as DM intakes decrease with PF inclusion, the passage rate decrease and the retention time increase in the rumen, and that could be the reason behind the lower total VFA as PF increased in the diets. However, the propionate, valerate, and isobutyrate molar proportions increased linearly with PF inclusion ($P < 0.01$). Therefore, the propionate increasing as PF inclusion increased in the diets resulted in a linear reduction in A:P ratio (Figure 6.1 and 6.2; $P < 0.01$). These results are comparable to those reported by several studies conducted on dairy heifer limit-fed DDGS (Suarez-Mena et al., 2015; Manthey et al., 2016; Manthey and Anderson, 2018). These

observations could be due to the decline in the rumen bacteria population, as suggested by (Suarez-Mena et al., 2015); however, this is not supported by the estimated microbial CP flow in the current study (Table 6.6). Manthey and Anderson (2018) suggested that the differences in starch contents and intake are the reason behind the shift in VFA and the decrease in acetate and increase in propionate. Also, they suggested the higher propionate is related to more energy-efficient, and rumen fermentation in heifers fed DDGS diets (Manthey et al., 2016) because there are less methane and carbon dioxide production in propionate as compared with acetate (Fahey and Berger, 1988). Jenkins (1993) stated that there is a lower A:P ratio at a greater fat intake to the reduction in nonlipid energy sources AD, as we observed in NFC AD in the current study (Table 6.3).

Ammonia concentration linearly increased as the PF inclusion increased in the diets ($P = 0.05$). Suarez-Mena et al. (2015) and Manthey et al. (2016) observed similar results, and they attributed that to the lower ME intake with the addition of DDGS, which is numerically decreased in the current study as PF inclusion increased in the diets (Table 6.2). Also, Suarez-Mena et al. (2015) stated that the effect of lower ME intake could have been aggravated as DDGS increased by greater energy coming from fat and providing lower carbohydrates to the bacteria. Therefore, the microbial capacity to assimilate amino acids and ammonia was negatively affected and $NH_3$ accumulated in the rumen (NRC, 2001). Additionally, the bacterial growth is generally affected negatively by fat by disrupting the integrity of the membrane of the bacteria (Doreau and Ferlay, 1995; Maia et al., 2010); however, this is not supported by the estimated microbial CP flow in the current study (Table 6.6).

The inclusion of PF in the diets increased the ruminal pH linearly (Figure 6.3 and 6.4; $P = 0.02$). That is mainly because of the reduction in DMI as planned in the current study and the reduction in starch intake as corn was replaced with PF in the diets. Suarez-Mena et al. (2013) reported a similar rumen pH between treatments as DDGS increased in the diets, whereas Manthey et al. (2016) observed a linear decrease in rumen pH as DDGS increased in the diets, and they attributed that to the F:C ratio. Chibisa et al. (2015) stated that the drop in pH with high starch diets is common in the literature as in the control diet in the current study. Also, Elliott et al. (1997) reported an increase in ruminal pH as different saturated fat were fed, and they attributed that to the lower fermentable carbohydrate content in these diets.

The inclusion of PF in the diets showed a linear decrease in the rumen mass, volume, and density, which is related to the reduction in DMI as PF inclusion increased. These results did not agree with (Suarez-Mena et al., 2015) as they did not find any effects on rumen contents and volume as DDGS increased in the diets. Overall, increasing PF inclusion in the diets did not appear to negatively affect rumen fermentation to change growth performance and may have shifted fermentation towards more efficient energy utilization in the current study. In the current study, there was a tendency for a linear interaction in propionate concentration. Propionate concentration tended to increase linearly in both Holstein and Jersey heifers, and this could be related to the more energy-efficient in heifers fed a decreased amount of DMI.

# CONCLUSIONS

Increasing dietary poultry fat in precision-fed Holstein and Jersey dairy heifer diets had some effects on ruminal fermentation, as evidenced by the decrease in acetate and increase in propionate. That might shift the rumen fermentation towards more efficient energy utilization by reducing the A:P ratio. This study demonstrates that the dietary poultry fat inclusion up to 6% does not impact apparent total tract digestibility, N dynamics, and microbial protein synthesis. In addition, the poultry fat inclusion in precision feeding dairy heifers decreased intake and manure excretion. The present results followed the same pattern in both Holstein and Jersey dairy heifers. However, Jersey heifers consistently resulted in higher nutrient utilization and apparent total tract digestibility of most nutrients, but Holstein heifers showed higher nitrogen retention. Therefore, we can conclude that dietary poultry fat can be successfully included in rations up to 6% DM in Holstein and Jersey heifers when precision-feeding is utilized and might help dairy farmers economically.

Table 6.1. Ingredient and chemical composition of 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF) in Holstein and Jersey dairy heifers' diets.

| | Fat % in the diet | | | |
|---|---|---|---|---|
| Item | 3% | 5% | 7% | 9% |
| Ingredient, % | | | | |
| Coastal bermudagrass hay | 5.00 | 5.00 | 5.00 | 5.00 |
| Corn silage | 50.0 | 50.0 | 50.0 | 50.0 |
| Ground corn | 33.7 | 30.4 | 26.5 | 22.6 |
| Soybean meal (SBM) | 10.1 | 11.6 | 13.4 | 15.2 |
| Mineral mix[1] | 1.10 | 1.10 | 1.10 | 1.10 |
| Poultry fat | 0.00 | 1.78 | 3.91 | 6.04 |
| Chemical composition | | | | |
| DM % | 49.1 | 48.1 | 48.8 | 48.9 |
| OM, % of DM | 94.7 | 94.7 | 94.5 | 94.4 |
| CP, % of DM | 13.1 | 13.2 | 13.3 | 13.8 |
| Soluble P, % of CP | 34.1 | 28.9 | 31.5 | 32.9 |
| NDF, % of DM | 27.7 | 28.6 | 29.0 | 28.4 |
| ADF, % of DM | 16.6 | 17.5 | 17.8 | 17.4 |
| Hemicellulose,[1] % of DM | 11.1 | 11.0 | 11.2 | 11.0 |
| Lignin, % of DM | 2.35 | 2.71 | 2.89 | 2.37 |
| Starch, % of DM | 32.6 | 30.6 | 29.4 | 28.0 |
| Ether extract, % of DM | 3.38 | 5.05 | 7.09 | 8.92 |
| NFC,[2] % of DM | 50.4 | 47.8 | 45.0 | 43.2 |
| TDN | 75.6 | 77.7 | 79.3 | 83.5 |
| ME,[3] Mcal/Kg | 2.76 | 2.83 | 2.89 | 3.05 |
| Ash, % of DM | 5.30 | 5.29 | 5.45 | 5.57 |

[1]Hemicellulose = NDF - ADF

[2]NFC: non-fiber carbohydrates = 100 - (CP + ether extract + NDF + Ash)

[3]ME calculated using TDN values as reported by Cumberland Valley Analytical Services, Inc., Waynesboro, PA. ME = (TDN × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (TDN × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)

Table 6.2. Feed intake of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic | Linear | Quadratic |
| BW, Kg | H | 491 | 492 | 492 | 493 | 4.12 | <0.01 | 0.64 | 0.82 | 0.69 | 0.75 |
| | J | 373 | 372 | 374 | 372 | | | | | | |
| Time to finish a meal,[2] min | H | 70.9 | 63.6 | 63.4 | 63.3 | 2.33 | 0.05 | 0.01 | 0.04 | 0.92 | 0.91 |
| | J | 66.3 | 57.0 | 58.8 | 57.5 | | | | | | |
| Intake, % of BW | H | 1.63 | 1.58 | 1.50 | 1.46 | 0.02 | <0.01 | <0.01 | 0.20 | 0.38 | 0.27 |
| | J | 1.34 | 1.25 | 1.20 | 1.19 | | | | | | |
| Intake, Kg/d | | | | | | | | | | | |
| As fed | H | 17.0 | 16.9 | 15.8 | 15.4 | 0.23 | <0.01 | <0.01 | 0.60 | 0.15 | 0.15 |
| | J | 10.6 | 10.01 | 9.57 | 9.52 | | | | | | |
| DM | H | 8.02 | 7.78 | 7.40 | 7.20 | 0.13 | <0.01 | <0.01 | 0.24 | 0.18 | 0.33 |
| | J | 5.01 | 4.63 | 4.46 | 4.45 | | | | | | |
| OM | H | 7.59 | 7.37 | 6.99 | 6.81 | 0.12 | <0.01 | <0.01 | 0.26 | 0.17 | 0.35 |
| | J | 4.74 | 4.39 | 4.22 | 4.20 | | | | | | |
| CP | H | 1.05 | 1.03 | 0.99 | 1.00 | 0.02 | <0.01 | 0.07 | 0.19 | 0.49 | 0.82 |
| | J | 0.65 | 0.62 | 0.61 | 0.62 | | | | | | |
| EE | H | 0.27 | 0.39 | 0.53 | 0.65 | 0.01 | <0.01 | <0.01 | 0.35 | <0.01 | 0.25 |
| | J | 0.18 | 0.22 | 0.33 | 0.41 | | | | | | |
| NDF | H | 2.22 | 2.22 | 2.15 | 2.04 | 0.04 | <0.01 | 0.01 | 0.59 | 0.54 | 0.16 |
| | J | 1.40 | 1.32 | 1.29 | 1.25 | | | | | | |
| ADF | H | 1.33 | 1.38 | 1.32 | 1.26 | 0.02 | <0.01 | 0.02 | 0.05 | 0.41 | 0.27 |
| | J | 0.83 | 0.82 | 0.82 | 0.78 | | | | | | |
| Hemicellulose | H | 0.89 | 0.85 | 0.82 | 0.78 | 0.02 | <0.01 | 0.03 | 0.34 | 0.93 | 0.29 |
| | J | 0.58 | 0.51 | 0.47 | 0.47 | | | | | | |
| Starch | H | 2.62 | 2.40 | 2.19 | 2.02 | 0.05 | <0.01 | <0.01 | 0.21 | 0.05 | 0.52 |
| | J | 1.65 | 1.41 | 1.33 | 1.24 | | | | | | |
| NFC | H | 4.04 | 3.73 | 3.33 | 3.12 | 0.06 | <0.01 | <0.01 | 0.05 | 0.02 | 0.47 |
| | J | 2.52 | 2.23 | 2.00 | 1.92 | | | | | | |
| Ash | H | 0.43 | 0.41 | 0.40 | 0.40 | 0.01 | <0.01 | 0.05 | 0.05 | 0.47 | 0.30 |
| | J | 0.27 | 0.24 | 0.24 | 0.25 | | | | | | |
| TDN | H | 5.09 | 5.25 | 5.10 | 5.09 | 0.13 | <0.01 | 0.51 | 0.56 | 0.67 | 0.17 |
| | J | 3.47 | 3.27 | 3.11 | 3.34 | | | | | | |
| ME,[3] Mcal/d | H | 17.6 | 17.6 | 16.5 | 16.2 | 0.44 | <0.01 | 0.07 | 0.59 | 0.75 | 0.19 |
| | J | 11.6 | 11.0 | 10.1 | 10.5 | | | | | | |

[1] Breed effect Holstein:Jersey (H:J).

[2] Eating time calculated from feeding to completion of the meal.

[3]ME (Mcal/d) calculated as ME = (digested OM × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (digested OM × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)

Table 6.3. Nutrient apparent digestibility of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | Interaction | |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic | Linear | Quadratic |
| Digestibility, % | | | | | | | | | | | |
| DM | H | 62.0 | 64.2 | 63.0 | 62.8 | 1.54 | 0.01 | 0.99 | 0.73 | 0.78 | 0.56 |
| | J | 67.3 | 67.7 | 66.3 | 67.3 | | | | | | |
| OM | H | 63.6 | 65.8 | 64.6 | 64.5 | 1.60 | 0.02 | 0.97 | 0.73 | 0.84 | 0.59 |
| | J | 68.9 | 69.3 | 68.2 | 69.0 | | | | | | |
| N | H | 61.1 | 65.7 | 64.0 | 67.9 | 2.22 | 0.44 | 0.06 | 0.96 | 0.86 | 0.81 |
| | J | 63.5 | 65.7 | 65.6 | 68.8 | | | | | | |
| NDF | H | 43.1 | 51.0 | 49.9 | 49.0 | 2.44 | 0.01 | 0.21 | 0.24 | 0.63 | 0.34 |
| | J | 55.7 | 55.2 | 58.6 | 57.3 | | | | | | |
| ADF | H | 37.6 | 41.8 | 41.2 | 39.8 | 2.82 | 0.01 | 0.34 | 0.28 | 0.62 | 0.93 |
| | J | 44.8 | 47.3 | 51.0 | 48.8 | | | | | | |
| Hemicellulose | H | 66.6 | 67.5 | 71.7 | 69.7 | 2.77 | 0.46 | 0.65 | 0.38 | 0.36 | 0.80 |
| | J | 69.8 | 71.2 | 72.2 | 68.2 | | | | | | |
| Starch | H | 94.6 | 95.3 | 95.8 | 95.8 | 0.45 | <0.01 | 0.01 | 0.98 | 0.39 | 0.46 |
| | J | 92.3 | 92.9 | 93.2 | 94.4 | | | | | | |
| NFC | H | 74.7 | 72.8 | 70.5 | 68.2 | 1.85 | 0.04 | 0.01 | 0.91 | 0.81 | 0.99 |
| | J | 76.9 | 77.3 | 72.3 | 72.3 | | | | | | |

[1] Breed effect Holstein:Jersey (H:J).

Table 6.4. Nitrogen intake, apparent digestibility, and dynamics of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic | Linear | Quadratic |
| Intake, g/d | H | 168 | 164 | 158 | 160 | 3.75 | <0.01 | 0.07 | 0.15 | 0.41 | 0.85 |
| | J | 103 | 98.4 | 96.5 | 99.5 | | | | | | |
| Digestibility, % | H | 61.1 | 65.7 | 64.0 | 67.9 | 2.22 | 0.44 | 0.06 | 0.96 | 0.86 | 0.81 |
| | J | 63.5 | 65.7 | 65.6 | 68.8 | | | | | | |
| Fecal N, g/d | H | 65.2 | 56.1 | 56.7 | 50.4 | 2.72 | <0.01 | 0.01 | 0.60 | 0.33 | 0.92 |
| | J | 38.8 | 33.1 | 33.9 | 30.3 | | | | | | |
| Urine N, g/d | H | 61.8 | 51.7 | 57.1 | 49.8 | 2.19 | <0.01 | 0.70 | 0.13 | 0.02 | 0.45 |
| | J | 41.8 | 35.6 | 44.6 | 46.8 | | | | | | |
| Total excreted N, g/d | H | 127 | 107 | 113 | 100 | 3.55 | <0.01 | 0.01 | 0.18 | 0.01 | 0.69 |
| | J | 80.6 | 68.8 | 78.4 | 77.1 | | | | | | |
| Retained N, g/d | H | 41.5 | 56.1 | 44.0 | 58.7 | 5.23 | <0.01 | 0.68 | 0.91 | 0.10 | 0.91 |
| | J | 24.8 | 28.6 | 15.9 | 21.6 | | | | | | |
| Retained N, % of intake | H | 24.3 | 34.2 | 27.3 | 36.1 | 3.61 | 0.01 | 0.73 | 0.67 | 0.07 | 0.81 |
| | J | 23.7 | 29.2 | 19.3 | 20.8 | | | | | | |
| Retained N, % of digested | H | 39.4 | 52.0 | 42.3 | 52.8 | 4.43 | 0.02 | 0.84 | 0.53 | 0.04 | 0.72 |
| | J | 36.9 | 44.4 | 29.3 | 29.4 | | | | | | |

[1] Breed effect Holstein:Jersey (H:J).

Table 6.5. Excretion parameters of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic |
| Wet feces, Kg/d | H | 13.1 | 12.1 | 12.0 | 11.6 | 0.31 | <0.01 | 0.05 | 0.31 |
| | J | 8.50 | 7.55 | 7.85 | 7.39 | | | | |
| Dry feces, Kg/d | H | 3.16 | 2.89 | 2.82 | 2.78 | 0.11 | <0.01 | 0.03 | 0.43 |
| | J | 1.69 | 1.55 | 1.63 | 1.49 | | | | |
| Fecal water,[2] Kg/d | H | 9.97 | 9.24 | 9.25 | 8.82 | 0.26 | <0.01 | 0.01 | 0.37 |
| | J | 6.79 | 6.01 | 6.20 | 5.90 | | | | |
| Urine, Kg/d | H | 7.28 | 6.59 | 5.77 | 5.52 | 0.28 | <0.01 | <0.01 | 0.30 |
| | J | 10.5 | 9.13 | 9.42 | 8.59 | | | | |
| Manure, Kg/d | H | 20.4 | 18.7 | 17.8 | 17.1 | 0.38 | 0.01 | <0.01 | 0.11 |
| | J | 19.0 | 16.6 | 17.2 | 15.9 | | | | |
| Total water excreted, Kg/d | H | 17.2 | 15.8 | 15.0 | 14.3 | 0.33 | 0.92 | <0.01 | 0.12 |
| | J | 17.3 | 15.1 | 15.6 | 14.4 | | | | |

[1] Breed effect Holstein:Jersey (H:J).
[2]Weight lost on drying at 60ºC.

Table 6.6. Urinary excretion of purine derivatives and estimated microbial CP of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic |
| Allantoin, mmol | H | 102 | 97.0 | 100 | 100 | 4.22 | <0.01 | 0.66 | 0.26 |
| | J | 66.5 | 56.7 | 60.1 | 60.9 | | | | |
| Uric acid, mmol | H | 13.3 | 9.98 | 10.2 | 12.5 | 1.39 | <0.01 | 0.21 | 0.12 |
| | J | 8.39 | 8.07 | 4.19 | 5.67 | | | | |
| Total PD, mmol | H | 115 | 107 | 110 | 113 | 3.94 | <0.01 | 0.38 | 0.09 |
| | J | 75.0 | 64.6 | 64.3 | 66.6 | | | | |
| Allantoin, % of PD | H | 88.3 | 90.6 | 91.0 | 88.8 | 1.53 | 0.74 | 0.24 | 0.45 |
| | J | 88.9 | 87.3 | 92.4 | 91.4 | | | | |
| Microbial CP,[2] g/d | H | 402 | 357 | 374 | 391 | 20.9 | <0.01 | 0.38 | 0.09 |
| | J | 225 | 171 | 170 | 181 | | | | |

[1] Breed effect Holstein:Jersey (H:J).
[2] Estimated according to the methods and equations of Chen and Gomes (1992).

Table 6.7. Rumen fermentation parameters and rumen pools sizes of Holsten and Jersey dairy heifers fed 4 different levels of fat (3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

| | | | | | | | Contrast, *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fat % in the diet | | | | | | Fat | | Interaction | |
| Item | Breed | 3% | 5% | 7% | 9% | SE | H:J[1] | Linear | Quadratic | Linear | Quadratic |
| Total VFA, m*M* | H | 109 | 109 | 103 | 101 | 3.34 | 0.90 | 0.03 | 0.27 | 0.58 | 0.61 |
| | J | 109 | 111 | 103 | 97.9 | | | | | | |
| Individual VFA, mol/100 mol | | | | | | | | | | | |
| Acetate (A) | H | 69.0 | 66.0 | 64.2 | 63.3 | 1.24 | 0.17 | <0.01 | 0.90 | 0.31 | 0.36 |
| | J | 70.5 | 69.1 | 65.4 | 62.4 | | | | | | |
| Propionate (P) | H | 20.2 | 21.4 | 23.8 | 24.6 | 0.62 | 0.21 | <0.01 | 0.97 | 0.07 | 0.80 |
| | J | 18.3 | 20.7 | 22.7 | 25.3 | | | | | | |
| Butyrate | H | 9.44 | 11.1 | 10.4 | 9.94 | 0.69 | 0.24 | 0.80 | 0.82 | 0.97 | 0.09 |
| | J | 10.1 | 8.89 | 9.59 | 10.0 | | | | | | |
| Valerate | H | 0.54 | 0.50 | 0.54 | 0.71 | 0.08 | 0.89 | <0.01 | 0.43 | 0.03 | 0.36 |
| | J | 0.28 | 0.35 | 0.82 | 0.87 | | | | | | |
| Isobutyrate | H | 0.72 | 0.91 | 0.96 | 1.37 | 0.09 | 0.13 | <0.01 | 0.13 | 0.69 | 0.04 |
| | J | 0.68 | 1.08 | 1.43 | 1.15 | | | | | | |
| A:P | H | 3.47 | 3.09 | 2.99 | 2.61 | 0.17 | 0.48 | <0.01 | 0.79 | 0.11 | 0.79 |
| | J | 3.82 | 3.41 | 2.84 | 2.57 | | | | | | |
| $NH_3$-N, mg/dL | H | 4.37 | 4.89 | 5.26 | 5.13 | 0.32 | 0.38 | 0.05 | 0.57 | 0.32 | 0.22 |
| | J | 4.04 | 4.52 | 4.87 | 4.41 | | | | | | |
| pH | H | 6.81 | 6.83 | 6.93 | 6.93 | 0.05 | 0.12 | 0.02 | 0.41 | 0.80 | 0.60 |
| | J | 6.68 | 6.82 | 6.84 | 6.86 | | | | | | |
| Rumen pool sizes[2] | | | | | | | | | | | |
| Mass, Kg | H | 80.7 | 78.2 | 73.3 | 69.6 | 1.00 | <0.01 | <0.01 | 0.33 | 0.67 | 0.09 |
| | J | 55.8 | 50.3 | 46.7 | 45.2 | | | | | | |
| Volume,[3] L | H | 91.3 | 86.6 | 85.1 | 81.5 | 1.67 | <0.01 | <0.01 | 0.09 | 0.81 | 0.19 |
| | J | 64.9 | 56.5 | 55.2 | 54.3 | | | | | | |
| Density, Kg/L | H | 0.89 | 0.90 | 0.86 | 0.85 | 0.01 | 0.06 | 0.03 | 0.23 | 0.69 | 0.94 |
| | J | 0.86 | 0.88 | 0.85 | 0.84 | | | | | | |

[1] Breed effect Holstein:Jersey (H:J).
[2] Determined by whole rumen contents evacuation.
[3] Rumen volume was measured by marking the level of rumen contents on a plastic container.

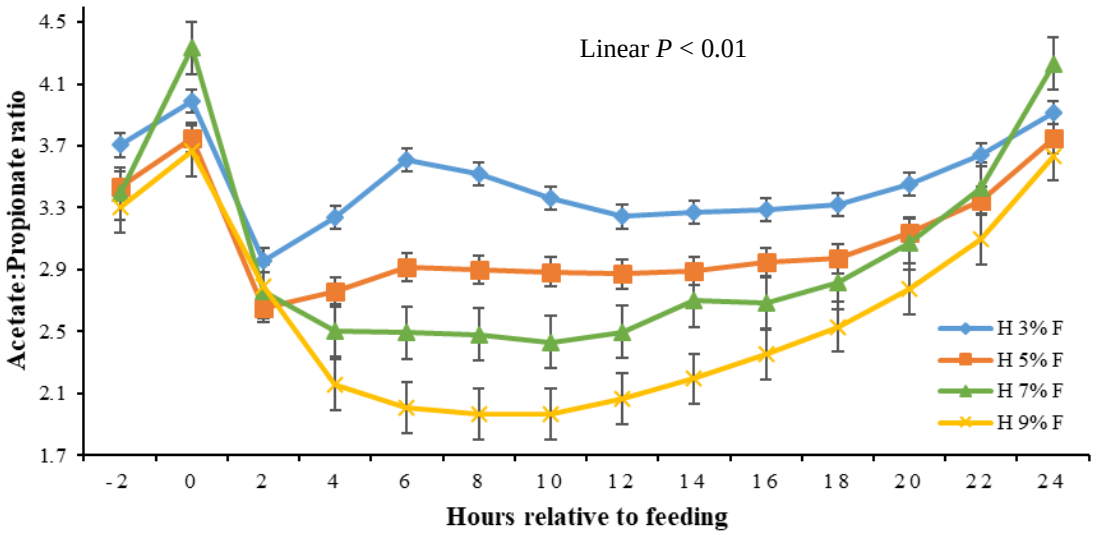


Figure 6.1. Diurnal acetate:propionate ratio of Holstein (H) heifers fed 4 different levels of fat (F; 3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

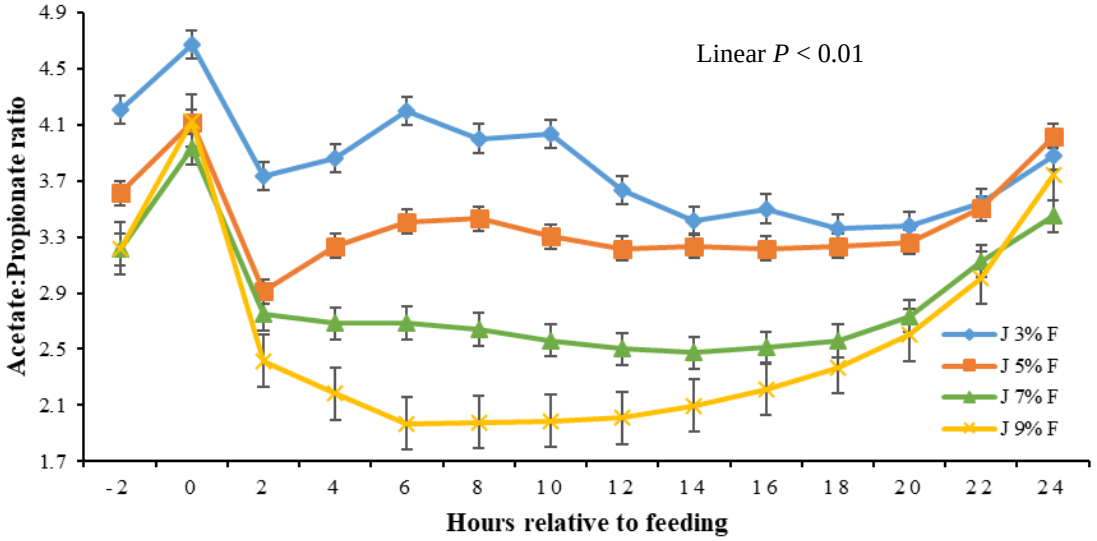


Figure 6.2. Diurnal acetate:propionate ratio of Jersey (J) heifers fed 4 different levels of fat (F; 3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

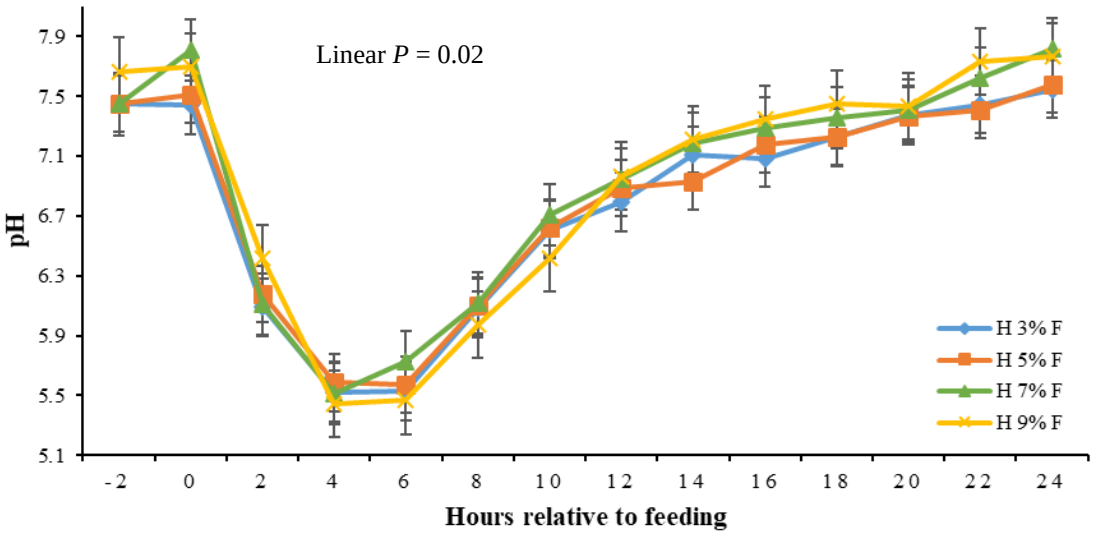


Figure 6.3. Diurnal pH of Holstein (H) heifers fed 4 different levels of fat (F; 3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

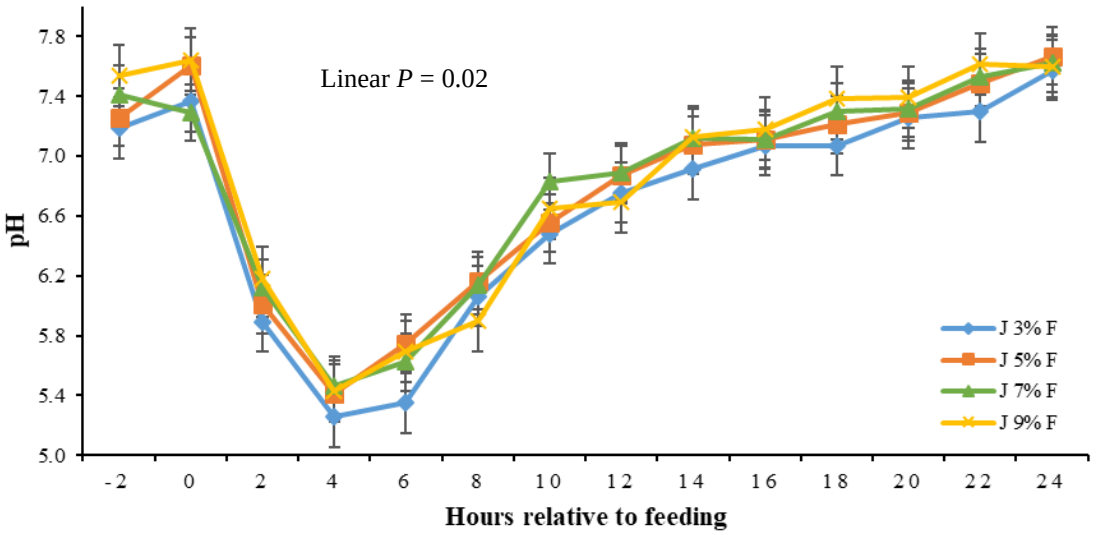


Figure 6.4. Diurnal pH of Jersey (J) heifers fed 4 different levels of fat (F; 3, 5, 7, 9% DM) containing a gradual increase of dietary poultry fat (PF).

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# CHAPTER SEVEN

# CONCLUSIONS AND IMPLICATIONS

These experiments and the literature review presented in this dissertation increased our knowledge regarding our overall objective, to determine the optimal F:C ratio and the extent of fat inclusion in the diets of precision-fed dairy heifers. Precision feeding fat to dairy heifers is a potential tool for reducing feed intake and improving nutrient efficiency with optimal performance on total-tract nutrient digestibility or rumen fermentation.

The study outlined in Chapter 3 exhibited that screening different types of fat with different extent of inclusion in both low and high forage diets using a gas production system showed some effects on culture fermentation. As evidenced by the decrease in acetate and might shift the rumen fermentation towards more efficient energy utilization by reducing A:P ratio. Results from this study demonstrate that the PF inclusion, along with CO inclusion, improved IVTDMD significantly in comparison to SOY and CON diet, while the level of fat inclusion had no detrimental impact on nutrients digestibility. The present results showed that the LF diet consistently resulted in higher nutrient utilization and most nutrients' digestibility. Also, not all fat sources are the same in how they ferment, depending on the extent of saturated and unsaturated fatty acids in these fat sources. We can conclude that the high concentrate diet with high-fat inclusion can be successfully included in rations for precision-fed dairy heifers.

In Chapter 4, it was demonstrated that increasing the fat inclusion up to 6% in stimulated precision feeding high concentrate diet in continuous culture fermenter had

some effects on ruminal fermentation, as evidenced by the decrease in total VFA and protozoa population. At the same time, maintain higher pH and ammonia concentration in PF and CO than in SO and CON treatments. Results from this study showed that the dietary PF inclusion along with CO inclusion improved digestibility significantly in comparison to SO and CON diet while increased the BH rate. In conclusion, the high concentrate diet with dietary PF can be a potential source of fat to be included in rations for precision-fed dairy heifers and reduce the dry matter intake further.

The study presented in Chapter 5 was aimed to find the optimal modification between F:C ratio in simulated precision feeding different extent of dietary PF in continuous culture fermenter. Both low and high forage diets had some effects on ruminal fermentation, as evidenced by the decrease in acetate and shift the rumen fermentation towards more efficient energy utilization by reducing A:P ratio. Results from this study demonstrate that PF inclusion improved digestibility while increased BH rate and decreased bacterial efficiency. The present results followed the same pattern in both LF and HF. However, LF consistently resulted in higher nutrient utilization and digestibility of most nutrients, but HF showed a higher fiber digestibility and protozoa population. Therefore, by-products PF is a fat source worth being included in rations for precision-fed dairy heifers in a moderate balance between F:C ratio.

Finally, Chapter 6 aimed to apply dietary PF inclusion to a different extent in precision-fed Holstein and Jersey dairy heifer diets. As expected, PF had some effects on ruminal fermentation, as evidenced by the decrease in acetate and increase in propionate and shift the rumen fermentation towards more efficient energy utilization by reducing

A:P ratio. Results from this study showed that PF inclusion does not impact apparent total-tract digestibility, N dynamics, and MCP synthesis. In addition, PF inclusion in precision feeding dairy heifers could help reduce the negative impact on the environment by decrease the manure excretion outputs. The present results followed the same pattern in both Holstein and Jersey dairy heifers. However, Jersey heifers consistently resulted in higher nutrient utilization and apparent total tract digestibility of most nutrients.

Overall, the results from these studies indicate that poultry fat can be used as a replacement for corn in precision-fed Holstein and Jersey dairy heifer diets up to 6% DM with varying forage to concentrate rations to improve efficiency. Other fat sources with different characteristics can be utilized with relative success, but further research is needed. Incorporation of supplemental fat to controlled intake strategies such as precision-feeding can lead to a reduction in feed intake for optimal growth with promising impacts on costs. Furthermore, nutrient digestibility, rumen fermentation, and animal performance can be enhanced with positive effects on environmental impact.

Future directions related to this research area should continue and focus on the effects of fat inclusion under a precision feeding system on dairy heifers after calving and specifically during the transition period since dairy cows require high energy diets during this period. Also, conducting a study on the mammary of dairy heifers, such as taking biopsy samples in order to study the effects of high-fat inclusion under precision feeding system on the mammary development. Furthermore, applying high-fat inclusion with high forage diets to dairy heifers under precision feeding program since high forage diets were showing less detrimental effects in our in-vitro studies. Finally, economic research

could be conducted studying the impact of using different fat sources with high inclusion under precision feeding programs and compared to the typical dairy heifers feeding program in the U.S. on dairy farmers.

# APPENDICES

## Appendix A

Nutrient input of high concentrate diets with high fat inclusion and different lipid sources (CON 3%, PF 9%, SO 9%, CO 9% DM) fed to continuous culture fermenters.

| | Fat type, % in the diet | | | |
|---|---|---|---|---|
| Nutrient input, g/d | CON 3% | PF 9% | SO 9% | CO 9% |
| As fed | 53.4 | 47.7 | 47.7 | 47.7 |
| DM | 48.3 | 43.2 | 43.2 | 42.9 |
| OM | 46.1 | 41.1 | 41.0 | 41.0 |
| N | 0.99 | 0.97 | 0.98 | 0.98 |
| EE | 1.70 | 3.70 | 3.76 | 3.57 |
| NDF | 10.0 | 8.57 | 8.74 | 8.74 |
| ADF | 4.76 | 3.98 | 4.15 | 4.15 |
| Hemicellulose | 5.29 | 4.59 | 4.59 | 4.60 |
| Starch | 18.9 | 13.8 | 13.7 | 13.6 |
| NFC | 28.2 | 22.8 | 22.3 | 22.5 |
| Ash | 2.13 | 2.09 | 2.24 | 1.96 |
| ME,[1] Mcal/d | 0.12 | 0.12 | 0.12 | 0.12 |
| FA input, mg/d | | | | |
| Total | 1401 | 3465 | 3563 | 3360 |
| C8:0 | 0.72 | 2.45 | 0.95 | 1.95 |
| C10:0 | 0.14 | 0.86 | 0.13 | 1.45 |
| C12:0 | 0.72 | 2.18 | 0.71 | 1157 |
| C14:0 | 1.57 | 15.34 | 2.53 | 951 |
| C16:0 | 196 | 811.2 | 408 | 191 |
| C18:0 | 0.55 | 158.8 | 0.96 | 33.2 |
| C22:0 | 3.30 | 10.52 | 6.84 | 2.24 |
| C24:0 | 6.92 | 43.25 | 34.3 | 12.3 |
| C18:1 | 359 | 1046 | 617 | 255 |
| C18:2 | 728 | 1077 | 1600 | 637 |
| C18:3 | 61.6 | 78.36 | 148 | 52.6 |
| Fractional passage rate | | | | |
| Liquid fraction, %/h | 8.60 | 7.76 | 7.76 | 7.76 |
| Solid fraction, %/h | 3.84 | 3.22 | 3.22 | 3.22 |

[1]ME (Mcal/d) calculated as ME = (digested OM × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (digested OM × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)

## Appendix B

## Nutrient input of low (LF) and high (HF) forage diets containing 4 different levels of fat (3, 5, 7, 9% DM) as gradual increase of dietary poultry fat (PF) in the diets fed to continuous culture fermenters.

| | LF | | | | HF | | | |
|---|---|---|---|---|---|---|---|---|
| Nutrient input, g/d | 3% | 5% | 7% | 9% | 3% | 5% | 7% | 9% |
| As fed | 53.4 | 51.6 | 49.5 | 47.7 | 60.0 | 57.4 | 54.9 | 52.6 |
| DM | 47.4 | 45.9 | 44.2 | 42.6 | 54.1 | 51.9 | 49.8 | 47.8 |
| OM | 44.9 | 43.7 | 42.2 | 40.7 | 51.3 | 48.8 | 46.6 | 44.7 |
| N | 0.93 | 0.93 | 0.94 | 0.94 | 0.87 | 0.87 | 0.87 | 0.89 |
| EE | 1.54 | 2.20 | 3.00 | 3.56 | 1.67 | 2.67 | 3.40 | 4.20 |
| NDF | 9.54 | 9.50 | 9.43 | 9.28 | 17.9 | 17.5 | 17.4 | 17.1 |
| ADF | 4.36 | 4.72 | 4.70 | 4.72 | 9.99 | 9.72 | 9.78 | 9.54 |
| Hemicellulose | 5.18 | 4.78 | 4.73 | 4.56 | 8.00 | 7.85 | 7.69 | 7.58 |
| Starch | 19.0 | 17.4 | 15.5 | 14.0 | 14.7 | 12.9 | 11.1 | 9.64 |
| NFC | 28.0 | 26.1 | 23.8 | 22.0 | 26.4 | 23.3 | 20.3 | 17.8 |
| Ash | 2.55 | 2.24 | 2.05 | 1.93 | 2.81 | 3.08 | 3.17 | 3.16 |
| ME,[1] Mcal/d | 0.12 | 0.12 | 0.12 | 0.13 | 0.11 | 0.12 | 0.12 | 0.13 |
| FA input, mg/d | | | | | | | | |
| Total | 1224 | 1870 | 2680 | 3257 | 1351 | 2342 | 3056 | 3863 |
| C12:0 | 1.39 | 1.64 | 1.93 | 1.94 | 2.37 | 3.73 | 4.11 | 4.47 |
| C14:0 | 2.22 | 7.70 | 12.3 | 14.3 | 2.94 | 10.9 | 18.7 | 31.0 |
| C16:0 | 186 | 378 | 470 | 525 | 159 | 406 | 691 | 1022 |
| C18:0 | 28.6 | 83.2 | 124 | 140 | 26.4 | 101 | 151 | 309 |
| C22:0 | 2.16 | 5.16 | 5.80 | 7.79 | 2.06 | 5.88 | 8.96 | 12.0 |
| C24:0 | 10.4 | 12.5 | 13.7 | 14.5 | 5.74 | 28.9 | 50.6 | 72.9 |
| C18:1 | 264 | 524 | 598 | 676 | 170 | 499 | 909 | 1187 |
| C18:2 | 593 | 642 | 632 | 656 | 398 | 644 | 943 | 962 |
| C18:3 | 30.9 | 41.5 | 41.5 | 41.8 | 45.5 | 73.8 | 90.1 | 77.1 |
| Fractional passage rate | | | | | | | | |
| Liquid fraction, %/h | 8.60 | 8.34 | 8.04 | 7.76 | 9.75 | 9.36 | 8.98 | 8.64 |
| Solid fraction, %/h | 3.84 | 3.63 | 3.38 | 3.22 | 4.68 | 4.37 | 4.06 | 3.70 |

[1]ME (Mcal/d) calculated as ME = (digested OM × 4.409 × 1.01 – 0.45) × 0.82. To represent the increase in energy as fat increased in the diets, ME = (digested OM × 4.409 × 1.01 – 0.45) + (0.0046 × (EE - 3) × 0.82 (Modified from NRC, 2001)