EVALUATION OF BREED AND DIET ON PARTIAL CARBON FOOTPRINT OF MILK PRODUCED IN CONVENTIONAL SYSTEM OF WISCONSIN

By

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Dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Dairy Science)

at the

University of Wisconsin-Madison

2019

Date of final oral examination: November 6, 2019

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Dedication

This dissertation is dedicated to my wife, Sheuly Yesmin; our daughter, Bushra; and my parents who inspired me, continuously supported me and sacrificed for me to accomplish this task.

Acknowledgements

All praise and thanks go to Almighty Allah who gave me the strength and passion to accomplish this challenging task of completing my PhD research and dissertation. It is also my immense pleasure to acknowledge the support of several people who were instrumental in helping me conduct my PhD research and complete this dissertation.

First, I would like to thank my advisor, Dr. Michel A. Wattiaux, who continuously supported and encouraged me. I truly enjoyed working with him, and I love his mentoring and teaching styles.

I would also like to acknowledge the valuable input of Dr. Rebecca Larson, who did not only help me with manure greenhouse gas emissions and lifecycle assessment study but also contributed to several discussions that helped to better shape this project and my dissertation.

I also would like to thank my dissertation committee members, Dr. Kent Weigel, Dr. Randy Shaver and Dr. Randy Jackson for serving on my committee and for their valuable input and suggestions throughout my graduate study. I also appreciate the input and valuable time from Dr. Horacio Aguirre-Villegas, who helped me out with lifecycle assessment study.

I am thankful to my teacher and mentor Dr. Harun-ur Rashid for giving me the inspiration and support during my PhD study period.

I must thank my beloved wife, Sheuly Yesmin, and our daughter, Bushra for their continuous love, support, help and sacrifice throughout my graduate study; otherwise, it would have been impossible to finish this task. I would like to give special thanks to my parents and family members for bringing me up to this stage with their love, encouragement and support. Finally, I would like to express my appreciation to all my friends and family members, my teachers and colleagues and well-wishers for their physical and mental support during my graduate study.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADF	Acid detergent fiber	**LCI**	Life cycle analysis
AS	Alfalfa silage	**LCIA**	Life cycle impact assessment
BW	Body weight	**LF**	Low FNDF
C	Carbon	**MBW**	Metabolic body weight
CH_4	Methane	**MF**	Milk fat
CO_2	Carbon di-oxide	**MFY**	Milk fat yield
CO_2-e	Carbon di-oxide equivalent	**MPY**	Milk protein yield
CP	Crude protein	**MUN**	Milk urea nitrogen
CS	Corn silage	**MY**	Milk yield
DM	Dry matter	**N**	Nitrogen
DMI	Dry matter intake	**NDF**	Neutral detergent fiber
EF	Emissions factor	**NFC**	Non-fiber carbohydrate
FE	Feed efficiency	**NH_3**	Ammonia
FN	Fecal nitrogen	**N_2O**	Nitrous oxide
FNDF	Forage neutral detergent fiber	**OM**	Organic matter
FPCM	Fat and-protein corrected milk	**SCC**	Somatic cell count
GF	GreenFeed Unit	**SM**	Soybean-meal
GHG	Greenhouse gas	**SNF**	Solids-not-fat
GTP	Global temperature change potential	**TMR**	Total mixed ration
GWP	Global warming potential	**TP**	True protein
HF	High FNDF	**UN**	Urinary nitrogen
IPCC	Intergovernmental Panel on Climate Change	**VFA**	Volatile fatty acids
LCA	Life cycle analysis		

Abstract

This dissertation is comprised of six chapters which include a literature review (chapter 1), four experimental chapters (chapters 2, 3, 4 and 5) and a future research chapter (chapter 6).

In chapter 2, measures of efficiency of Holstein and Jersey cows were assessed when fed alfalfa silage or corn silage in low or high forage fiber diets. Breed did not affect digestive and metabolic efficiencies. In contrast, methane and urinary energy (% gross energy intake) were lower for corn silage than alfalfa silage-fed cows; and compared to high, low forage fiber diets reduced loss of urinary N (g/d and % N intake). Neither breed nor dietary treatments affected methane intensity (g/kg fat-protein corrected milk).

In chapter 3, the carry-over effects of same three treatment factors (cow breed, dietary forage source and forage level) on manure greenhouse gas (GHG) emissions (methane and nitrous oxide) during 50-d storage and followed by a 50-d field application were evaluated. Compared to high, low forage-fed cows tended to emit 51 to 72% (depending on mode of expressions) greater combined (storage plus field) GHG emissions which were not affected by cow breed and forage source.

In chapter 4, we evaluated the carry-over effects of the same treatment factors on manure ammonia emissions. Compared to high, low forage fed cows emitted less ammonia expressed as per cow, per kg manure or percentage of manure N. Although, forage source did not affect ammonia emissions, cow breed did impact ammonia emissions expressed per cow being 17% greater for Holstein than Jersey.

In chapter 5, we performed a cradle-to-gate life cycle assessment to determine the carbon footprint (CF) of milk for the same treatment factors using emission factors measured in our

studies. Low forage-fed cows had 11% greater CF than high forage-fed cows whereas both forage sources and cow breeds (Holstein and Jersey) had similar CF. We concluded that GHG mitigation strategies (choice of cow breed or diet) need to be evaluated holistically using measurements specific to the production system under consideration since evaluations at the whole-farm scale led to different results than when completed at the animal scale.

CHAPTER 1. Literature Review

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Dairy production system and milk carbon footprint

Dairy products contribute to a healthy and nutritious human diet through providing essential nutrients (e.g., protein, micronutrients including Calcium, Magnesium, Vitamins B5, and B12). However, one of the major challenges that today's dairy sector is facing globally is how to reduce the negative impact of dairy production on environment. Because, livestock sector including dairy is contributing to a significant portion of anthropogenic (human-induced) greenhouse gas (**GHG**) emissions. Globally, livestock is producing 14.5% of total anthropogenic GHG (per annum 49 gigatons carbon di-oxide equivalent, **CO_2-e**) where the contribution of dairy sector itself is approximately 20% of the total livestock GHG emissions (Gerber et al., 2013). These estimates are aligned with the estimates reported in 'Livestock's long shadow' (Steinfeld et al., 2006). Dairy sector in the United States (**US**) is producing only 1.9% of the total US GHG (Thoma et al., 2013). The contribution of GHG by the US dairy sector is substantially lower than any other big sectors such as transportation sector that produces 29% of total US GHG (EPA, 2018). Yet, emphasis has been given to reduce GHG emissions from US dairy sector to make the dairy sector environmentally friendly and competitive to the world market. For instance, US dairy industry set a goal in 2009 to reduce the GHG of fluid milk by 25% within 2020 and the progress report to achieve this goal is underway. Additionally, the total GHG emissions from global dairy sector has increased by 18% over 10 years between 2005 to 2015 due to mainly increased total milk production induced by increased demand (Table 1; FAO and GDP, 2018). Further expansion of global population as predicted to reach 9.3 billion by 2050 will require to increase milk

production by 58% (FAO, 2011). This increment in milk production will further increase the total GHG emissions from dairy sector. On contrary, the carbon footprint (**CF**) of milk i.e., GHG intensity expressed as CO_2-e per kg of fat- and protein corrected milk (**FPCM**) decreased by 11% between 2005 and 2015 due to increased efficiency through improved animal genetics, better feeding and nutritional management and herd management practices (Table 1; FAO and GDP, 2018). This decrease in emission intensity (or CF) has been happening globally indicating the potential to reduce total emissions if this trend is continued (Figure 1). Most importantly, the value of milk CF varies widely across regions ranging from 1.29 (North America) to 6.66 (Sub-Saharan Africa) kg CO_2-e/kg FPCM (Figure 1). The CF value may vary even within region. For instance, milk CF value ranged from 1.02 to 1.66 kg CO_2-e/kg FPCM across four milk production regions in Europe (Battini et al., 2016). Wattiaux et al. (2019) reported a wide range of CF across 10 studies (0.84 to 1.5 kg CO_2-e/kg FPCM) from three continents namely North America, Europe and Australia. One of the first cradle-to-grave life cycle assessment (**LCA**) study conducted by Thoma et al. (2013) reported that the CF of US milk production is 2.05 (ranging from 1.77 to 2.44) kg CO_2-e/kg FPCM. The same study also reported a farm-gate CF of 1.47 kg CO_2-e/kg FPCM. These variability of milk CF within or across regions or production systems indicates the potential for further reduction of milk CF through mitigation strategies making the future dairy sector more environment friendly.

Sources of greenhouse gases from dairy production system

Three major GHG that come from dairy sector are CO_2, methane (**CH_4**) and nitrous oxide (**N_2O**) (Gerber et al., 2013) where the global warming potential (**GWP**) of CH_4 and N_2O are respectively 28 and 265 times greater than CO_2 (Myhre et al., 2013). Globally, enteric emissions (CH_4), feed production related emissions (CO_2 and N_2O) and manure management related

emissions (CH_4 and N_2O) share respectively 58.5, 29.4 and 9.5% of total milk production related emissions (FAO and GDP, 2018). According to a US-based LCA study, the contribution of enteric emissions, feed production and manure management related emissions to farm-gate milk CF were 35, 33, and 27%, respectively (Thoma et al., 2013). Regardless of system boundary (regional or global), these three emission sources mentioned in this section are the major on-farm emission sources and therefore, the following sections will be focused on these three emission hotspots.

Another important emission from dairy production system is ammonia (NH_3) which is not a GHG. But, NH_3 is an important human health hazard and it can cause soil acidification and eutrophication and can also be an indirect source of N_2O through deposition of NH_3 (van Breemen et al., 1983; Bobbink et al., 1998; Wattiaux et al., 2019). Globally, most anthropogenic NH_3 (80 to 90%) originates from agricultural activities where the share of livestock is more than 90% of agricultural NH_3 emissions (Zhang et al., 2010). A very recent US study showed that NH_3 is the greatest environmental concern for the state of Pennsylvania where dairy production accounts for approximately 50% NH_3 emissions of the state (Rotz et al., 2019; unpublished data). Since most NH_3 in dairy production comes from volatilization of manure N, therefore manure NH_3 emissions will be briefly discussed in manure GHG emission section.

Enteric methane emissions

Ruminant has the unique capability to utilize and convert human inedible fiber into milk. During fermentation process of feed, ruminants produce enteric CH_4 as a fermentation by-product. Enteric CH_4 comprises approximately 17% of global CH_4 emissions (Knapp et al., 2014). Whereas enteric CH_4 accounts for 25% of US anthropogenic CH_4 emission (EPA, 2018). Additionally, enteric CH_4 emissions also account for 2 to 12 % loss of total gross energy intake in lactating dairy

cows (Johnson and Johnson, 1995; Arndt et al., 2015). Therefore, mitigation of enteric CH_4 will not only reduce the milk CF but it may also improve energy utilization efficiency of lactating dairy cows.

Various mitigation strategies (e.g., dietary manipulation, inclusion of additive, inhibition of methanogens, manipulation of rumen microbes, and improvement of animal productivity through genetic selection) have been proposed and investigated by many researchers. Several review studies have critically evaluated and summarized enteric CH_4 mitigation strategies (Boadi et al., 2004; Beauchemin et al., 2008; Martin et al., 2010; Grainger and Beauchemin, 2011; Hristov et al., 2013; Knapp et al., 2014; Wattiaux et al., 2019). We will be focusing on feeding and nutritional management strategy particularly the effects of dietary manipulation/change on enteric CH_4 emissions.

Enteric CH_4 is produced through anaerobic methanogenesis process (as shown below) mostly in the rumen and partly in the hind gut.

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

During the methanogenesis process, CO_2 is reduced to CH_4 and this reduction reaction depends on availability of hydrogen (H_2). Therefore, dry matter intake (**DMI**) is one of the most important drivers of enteric CH_4 production since H_2 is produced through fermentation of consumed feed nutrients. Dry matter intake itself can explain about 64 % of the CH_4 production **(g/d per cow)** variability when cows are fed *ad libitum* basis (Boadi and Wittenberg, 2002). Increasing DMI increases enteric CH_4 production due to increased amount of feed to be fermented whereas CH_4 intensity **(g/kg of FPCM)** decreases due to increased FPCM (Knapp et al., 2014). Furthermore,

increasing DMI decreases organic matter (**OM**) digestibility in the rumen due to increased rate of passage (Johnson and Johnson, 1995; NRC, 2001). This reduction in OM digestibility might affect both manure composition and subsequent manure GHG emissions which will be discussed in manure management related GHG emissions section. A recent study used global enteric CH_4 data to predict enteric CH_4 showed that DMI is the most important explanatory variable for the prediction of enteric CH_4 production (Niu et al., 2018). However, inclusion of dietary neutral detergent fiber (**NDF**), starch and ether extract concentrations in the model improved prediction accuracy where NDF was positively correlated with enteric CH_4 production, starch and ether extract were negatively correlated with CH_4 production. For example, increasing 1 percentage unit of dietary NDF concentrations in dairy cows' diet under US production system increased 2.3 to 2.59 g of enteric CH_4 production. The same study also concluded that milk yield and milk composition is important for better prediction of CH_4 yield (g/kg of DMI) and intensity. Importantly, Niu et al. (2018) also reported that Intergovernmental Panel on Climate Change (**IPCC**) tier 2 model overpredicted enteric CH_4 production for the US cows by 22% when IPCC predicted CH_4 was compared to their model prediction developed using US-based data. This message is important for the practitioners of LCA study since most LCA studies use IPCC tier 2 model to predict enteric CH_4. Therefore, LCA study conducted in the US should either use US specific model to better predict enteric CH_4 production or should use measured emission factor (**EF**) for enteric CH_4 specific for the production system concerned if available.

Ruminal fermentation stoichiometry study showed that compared to starch, ruminal fermentation of NDF produces greater amount of H_2 (Wolin, 1960). Because, fermentation of NDF increases molar proportion of acetate and butyrate production which are H_2 producing pathways whereas digestion and fermentation of starch and simple sugars increases molar

proportion of propionate which is a H_2 consuming pathway (Figure 2). Since H_2 is the input for methanogenesis process, thus increased NDF (or decreased starch) in the diet increases enteric CH_4 production. For instance, increasing level of forage in the dairy cows' diet from 47 to 68% (DM basis) showed a linear increase in CH_4 production from 538 to 648 g/d per cow (Aguerre et al., 2011). Increasing level of concentrate from 52 to 72% (or increasing starch from 21 to 30%, DM basis) in the dairy cows' diet decreased acetate to propionate ratio by 44% (Agle et al., 2010). Therefore, increasing level of concentrate in the dairy cows' diet is an effective strategy for reducing enteric emissions but excessively high concentrate in the diet might also lead to a decreased ruminal pH resulting in sub-acute ruminal acidosis and milk fat depression (Agle et al., 2010). Additionally, inclusion of concentrate in the diet may increase feed cost which might be discouraging for the farmers adopting this technique unless farmers are given any incentive for reducing environmental impact. Therefore, economic and environmental trade-off of concentrate supplementation needs to be considered as well.

Quality of forage particularly forage type (e.g. grass vs. legume) and digestibility influence enteric emissions. High quality forage (e.g. high digestibility) has been shown to increase DMI and FPCM resulting decreased CH_4 yield and CH_4 intensity (Johnson and Johnson, 1995; Hammond et al., 2009). A meta-analysis based on 22 in vivo studies concluded that ruminants fed C_4 grass (e.g. containing 65% NDF) yielded 17% greater CH_4 (g/kg OM intake) than C_3 grass (e.g. containing 56% NDF). Several recent studies showed that lactating dairy cows fed corn silage (**CS**) at the expense of alfalfa silage (**AS**) or barley silage (**BS**) resulted in 10 to 14 % lower enteric CH_4 energy loss (Arndt et al. 2015; Hassanat et al., 2013; Benchaar et al., 2014). Because CS based diet had greater concentration of starch which created unfavorable rumen environment for methanogens and protozoa through reducing pH and acetate to propionate ratio. Increasing CS in

the diet also increased DMI resulting increased milk production. However, effect of increased CS in the diet on NDF digestibility was inconsistent in those studies e.g. no change or decreased NDF digestibility (Arndt et al. 2015; Hassanat et al., 2013). These findings might have been confounded by the NDF source and starch content of the diet since both varied across diets in all studies compared CS with AS. Therefore, a future research focusing on how NDF source might affect enteric emissions would be worthy. Because NDF is one of the major dietary components that has profound effect on enteric CH_4 emissions. The differential physico-chemical structure of NDF between C4 grass (e.g., CS) and C3 legume (e.g., AS) might affect enteric CH_4 production differently.

Greenhouse gas and ammonia emissions from manure management

Livestock production has been specialized and intensified worldwide to meet the increasing demand of meat, milk and eggs consequently leading to huge volume of manure excretion by different animal species (Steinfeld et al., 2006). Depending on total solids (**TS**) content dairy manure can be classified as i) solid (> 20% TS), ii) semi-solid (13 to 19% TS), iii) slurry (8 to 12% TS) and iv) liquid (1 to 7% TS) (Aguirre-Villegas and Larson, 2017). Since manure contains both inorganic N and microbially available organic C, it is a widely used organic fertilizer for cropland particularly important for today's organic farming where chemical fertilizer is restricted to apply. Manure is also very much important to maintain soil organic carbon (**SOC**) stock which is an input for national GHG inventories and has agronomic importance (Maillard and Angers, 2014). On the other hand, manure is a significant source of GHG emitted either during storage or after subsequent land application of manure.

Major GHG emitted during storage of manure is CH_4 (with little N_2O). Manure CH_4 is produced through anaerobic decomposition of organic matter by microbes (Hill et al., 2001; Ni et

al. 2008; Owen and Silver, 2017). Manure CH_4 emissions depend on manure storage facility (e.g., liquid/slurry in lagoon or pond vs. solid), manure treatment, ambient climate (e.g., oxygen concentrations, temperature and moisture), manure storage duration and manure composition (Owen and Silver, 2017; Montes et al., 2013; Rotz, 20018). Manure CH_4 emission rate during storage is much greater for large farm compared to small farm. Because, large farm store manure for longer period (> 6 months) as liquid/slurry form (anerobic) whereas small farms store manure for very short period of time as solid form (Aguirre-Villegas and Larson, 2017). Handling solid manure produces mainly CO_2 (with no or little CH_4) through aerobic decomposition. Treatment of manure (e.g. anaerobic digestion, solid-liquid separation) has significant effects on manure emissions. For instance, anaerobic digestion reduces the manure GHG emissions by 25 to 50% (Amon et al., 2006; Holly et al., 2017). However, anaerobic digestion increases manure ammonia (NH_3) emisisons substantially (> 80%; Holly et al., 2017). Because, during the anaerobic digestion, N-containing compounds such as protein, amino acids, urea are reduced to NH_3 (Bernet et al., 2000). Additionally, anaerobic process also leads to higher pH due to mineralization of organic N and VFA resulting in greater NH_3 loss (Peterson and Sommer, 2011). This trade-off between CH_4 and NH_3 emissions needs to be accounted for while evaluating the effects of anaerobic digestion on manure emissions. Solid-liquid separation can reduce CH_4 emissions during storage by 46% (Holly et al., 2017). When solid-liquid separation is combined with anaerobic digestion this reduction was even greater (68%). Furthermore, combining these two techniques may also reduce NH_3 emisions although total GHG emisisons might be increased due to increased N_2O emisions (Holly et al., 2017).

Manure OM is the main substrate for CH_4 formation which may vary between animal types or animal diets. Replacing AS with CS in lactating cows' diet reduced NDF digestibility which

might increase subsequent manure CH_4 emision during storage (Hassanat et al., 2013). Compared to conventional CS, cows-fed highly digestible brown mid-rib CS had lower enteric CH_4 emission but greater manure volatile solids yield and manure CH_4 emision during storage (Benchaar and Hassanat, 2019). This trade-off between enteric CH_4 and manure storage CH_4 emisions suggests that dietary mitigation strategies need ot be evaluated at whole-farm scale. Animal type (e.g., breed) might also affect manure emissions due to differtial manure composition. Jerseys had greater NDF digestibility than Holsteins when they were fed the similar diet (Aikman et al., 2008). Similarly, NDF digestibility was greater for efficent Holstein than ineffcient Holstein cows when similar diet was fed to both groups (Olijhoek et al., 2018). These differences in NDF digestibility within or between cow breeds might affect both manure composition (e.g., organic matter and N content) and manure GHG emisisons subsequently.

Nitrous oxide is produced either directly through nitrification and denitrification process or indirectly from redeposition of volatilized NH_3 and leached/run-off N (Chadwick et al., 2000). Manure N_2O is mostly emitted after soil application of manure to crop-field with little or no N_2O emissions during manure storage depending on crust formation (Aguerre et al., 2012). Manure GHG emissions from soils are produced via microbial processes which depend on both manure characteristics and manure application practices (e.g., methods, rate and timing of application). Soil characteristics (e.g., soil texture) and micro-climatic condition of soil (soil nutrient content, soil temperature and moisture) may also have significant impact on soil GHG emissions.

Like manure CH_4 emissions, manure N_2O emissions might also be affected by type and diet of dairy cows (Aguerre et al., 2012; Anon 2010). There are not many studies explicitly determining the carry-over effects of cows' diet on manure N_2O emissions. Yet, decreasing dietary CP in lactating cows' diet reduced manure N_2O emissions during storage but increased manure

CH_4 emissions (Kulling et al., 2001). When AS was replaced with CS in dairy cows' diet, the manure N concentration decreased linearly (Arndt et al., 2015; Hassanat et al., 2013). This reduction in manure N concentrations might lead to lower N_2O from CS fed cows' manure than AS-fed cows' manure.

There are few days of lag time required to start N_2O emissions after soil application of manure because this time is required for mineralization or nitrification to accumulate NO_3^- and conversion of organic C to available C to be utilized by microbes (Rochette et al., 2008). During this lag time, a large portion of N is lost as NH_3 within 2 to 3 days of manure application which could potentially reduce available N for N_2O formation (Webb and Misselbrook, 2004). Therefore, N_2O emission is not the function of total manure N but the function of readily available manure N. Increasing the rate of manure N application or fertilizer N application increases N_2O emissions from soils (van Groenigen et al., 2004; Cardenas et al., 2010). Because, higher N application rate increases depletion of O_2 leading to higher N_2O emissions via anaerobic denitrification process. Therefore, consideration for manure N availability in fertilizer plan is a prerequisite for mitigation of N_2O emissions (Petersen, 2018). Manure application timing may also influence N_2O emissions due to differential temperature and humidity between seasons which in turn affect microbial activity. Soil humidity is the single most important factor that affects microbial activity. Soil with 60% water filled pore space (**WFPS**) had optimal N_2O emissions while the lowest emission was reported at 30% WFPS (Gao et al., 2014). With increasing WFPS CH_4 emission also increases because methanogenesis requires strictly anaerobic condition (Gao et al., 2014). The CH_4 and N_2O emissions increase with increasing soil temperature due to decreased O_2 and thus spring applied manure reported to emit more GHG than fall application (Butterbach-Bahl et al. 2013). Therefore, crops should be supplied essential manure nutrients in a timely manner to maximize utilization

and minimize losses (Anon, 2010). Thus, optimizing manure application rate, timing and application technique could help to improve effective manure nutrients utilization resulting decreased GHG emissions (Van der Meer, 2008). Another important factor that might affect manure GHG is the method of manure application. Surface application (broadcasting) and injection are the two common manure application methods practiced by the dairy producers. Surface application is the most common method across farm sizes followed by injection (Aguirre-Villegas and Larson, 2017). Compared to surface application, injection method can reduce NH_3 loss substantially after field application (Aguirre-Villegas et al., 2015) although it increases the N_2O emissions due to creating favorable anaerobic condition for denitrifiers. Manure CH_4 emission from field is considered insignificant relative to large CH_4 emission during manure storage (Collins et al., 2011). Yet, CH_4 emission may be increased unexpectedly due to incorporation of manure which creates anaerobic condition (Flessa and Beese, 2000).

Greenhous gas emissions from feed production and land use change

Feed production and transportation contribute a significant portion of GHG (mainly CO_2 and N_2O) for milk CF. The CO_2 and N_2O accounts for 33 and 67% of feed production related GHG emissions, respectively (FAO and GDP, 2018). Feed N_2O comes from direct and indirect source of applied N whereas feed CO_2 comes from fossil fuel consumption for feed and fertilizer production, transportation, and land use change (**LUC**). Globally, LUC contributes to only 3% of dairy feed production related GHG, however this estimate may vary a lot depending on attribution of emissions to different drivers of LUC (FAO and GDP, 2018). In European Union, LUC change accounts for 7 to 28% of total milk production related GHG emissions (Yan et al., 2011). Emissions related to LUC (i.e., transformation of forest to arable land for feed production) are estimated using IPCC tier 1 model (Gerber et al., 2013). Emissions related to LUC should be

accounted for in LCA study if the concerned production system use the feed ingredients which are associated with deforestation. In spite of it's importance, most LCA studies do not account for LUC due to unavailability of clear data related to LUC (Weiss and Leip, 2012). Soybean production in Brazil and Argentina is associated with deforestation and thus, a dairy production region/system importing soybean from those countries should account for LUC. Most dairy in European Union import soybean from South America and emissions estimated for soybean related LUC for milk CF was 0.09 kg CO_2/kg FPCM (FAO, 2010). Whereas, soybean production in the USA is not associated with deforestation and therefore, soybean related LUC is not accounted for LCA of milk production in the USA (FAO, 2010).

Importance of evaluating greenhouse gas mitigation strategies at whole-farm scale

Based on above discussion, enteric CH_4, manure management related GHG and feed production related GHG emissions are three major sources of GHG from dairy production system. Almost all the nutritional and dietary management strategies focused on mitigation of animal-scale enteric CH_4 emissions. However, researchers have clearly showed and pointed the existence of potential trade-off or interactions between enteric emissions and manure management related emissions when dietary mitigation strategies were evaluated. Thus, drawing conclusions based on animal-scale evaluation of dietary strategies on enteric CH_4 emissions might be misleading. Therefore, effects of GHG mitigation strategies on whole-farm GHG emissions need to be determined to capture all the potential interactions and account for trade-off existed between sub-systems of the dairy production system. Several recent studies also recommended the evaluations of dietary mitigation strategies on whole-farm GHG emissions (Table 2).

Life cycle assessment-a holistic tool

Life cycle assessment could be used as a tool for whole-farm evaluation of GHG mitigation strategies. Because, LCA is a holistic tool and it is widely used in recent decades to determine the environmental impact and identify potential mitigation hotspots of dairy production system. Hence, the following sections will be focusing on type of LCA, phases of LCA study, important assumptions and issues of conducting LCA for milk CF. International standard organization (ISO, 2006) has defined LCA as a "compilation and evaluation of the inputs, outputs and potential environmental impacts of a product system throughout its life cycle". LCA that covers the entire life of a product starting from attainment of raw materials to disposal of that product is called cradle-to-grave LCA. For instance, Thoma et al. (2013) conducted a cradle-to-grave LCA for milk production in the USA. However, most LCA studies conducted for livestock products are cradle-to-gate LCA also called partial LCA because most of the emissions for livestock products come from on-farm activities (Yan et al., 2011; Lorenz et al., 2019). Depending on the boundary of the LCA study, the results might vary significantly. Zehetmeier et al. (2014) reported a strong influence of system boundary on both level and variation of GHG emissions. For example, most cradle to-gate LCA studies reported CF of milk ranging from 1 to 1.5 kg CO_2-e of GHG/kg FPCM whereas a cradle-to-grave LCA of US milk consumption reported a greater value of CF (2.05 kg CO_2-e of GHG/kg FPCM consumed; Thoma et al., 2013). In manufacturing industry, LCA is often used to compare the environmental impact of two products. On contrary, direct comparison may be misleading in the case of livestock product due to complexity and wide variability of production system, co-products allocation and functional unit unless results are standardized (Lorenz et al., 2019). However, LCA can be used as a potential tool in animal production system to quantify and identify potential mitigation options of a product system (FAO, 2010). For example, Thoma et al.

(2013) identified three on-farm activities (enteric emission, manure management and feeding management) as major contributor for US milk CF.

Phases of life cycle assessment

LCA is a complex procedure and therefore, ISO has established a methodological framework to simplify the complexity of LCA. LCA framework has four phases as shown in Figure 3 (ISO, 2006). In first phase, LCA practitioners need to explicitly define the goal of the study and target audiences whereas LCA scope defines the temporal (e.g., time period of data coverage), geographical (e.g., local/national or global) and technology coverage. In the second phase, input and output data are collected. This step is the most time consuming and laborious step of LCA study but is most important. Because, use of EF that has less uncertainty and represents local production system is important to determine the environmental impact accurately. For instance, use of IPPC-based EF instead of measured EF for manure management related GHG underestimated GWP of milk production by 21% (Baldini et al, 2018). Third phase of LCA is impact assessment which could be either mid-point (e.g., GWP) or end-point impact assessment (e.g., sea level rise). In most cases, LCA studies conducted for livestock products use mid-point impact assessment using GWP as a metric at 100-year time horizon whereas global temperature change potential (**GTP**) is an alternative metric (Persson et al., 2015). These metrices (GWP or GTP) are used to convert CH_4 and N_2O into CO_2-e. Over 100-year time horizon, GWP and GTP for CH_4 are 28 and 4, respectively (Myhre et al., 2013). Thus, using GTP instead of GWP would lower the CF value of milk production making the dairy sector look much better although choice of the metric should ideally be based on climate policy goal (Persson et al., 2015). Livestock including dairy production has multiple impacts on environment and the commonly used impact

categories for livestock products are GWP, acidification potential, eutrophication potential, and land use (Table 3). If multiple impact category is used in LCA, the challenge is to determine appropriate weighing factor for each impact category. Fourth and final phase of LCA is the interpretation phase where conclusions are drawn, and recommendations are made. Results of sensitivity analysis should be reflected in interpretation phase.

Types of life cycle assessment

Based on methodological principle, there are two types of LCA namely attributional LCA (**ALCA**) and consequential LCA (**CLCA**). A brief comparison of ALCA and CLCA is shown in Table 4. Most LCA conducted for livestock products are ALCA because ALCA is conceptually simpler compared to CLCA. Cederberg and Mattsson (2000) showed that both ALCA and CLCA could be performed for milk production depending on practitioner's choice. The final LCA result might vary depending on LCA methodology. For instance, replacing soybean meal with rapeseed meal in pig diet decreased land use by 14% when determined using ALCA whereas land use increased by 10% when determined using CLCA (Zanten et al., 2018).

Functional unit and co-products allocation

Functional unit (**FU**) is the tool to measure the performance of a system output (ISO, 2006). FU is a measurable reference point which is relevant for both input and output. Commonly used FU for a dairy production system is FPCM. Ton of milk produced per ha of land could be used as an alternative FU when the goal is to identify land use efficiency. A system might have more than one potential function, but the selection of a function will depend on the goal and scope of the study. Like industrial process or system, livestock production system is also multifunctional. For

example, the main product of dairy production system is milk whereas meat is an important co-product from culled animals and calves. ISO (2006) suggested to assess environmental impact of each co-product separately. But, in livestock system separate impact assessment of co-product is not possible due to mutual dependence between co-products. Therefore, emissions must be allocated among co-products using one of the four allocation methods described in the following section. Cederberg and Stadig (2003) defined these four allocation methods which are i) no allocation, ii) biological allocation or mass allocation (e.g. based on underlying physical relationship between co-products), iii) economic allocation, and iv) system expansion. No allocation means that 100% emissions are allocated to main product e.g. 100% emission burden is assigned to milk for dairy production system. Although, system expansion is recommended as the best option but it seeks for alternative way of producing co-products (Meier et al., 2015). In livestock agriculture, it is difficult to implement system expansion because LCA for an alternative system or product may not exist. Therefore, system expansion is used for CLCA only whereas either economic or mass or biological allocation is used for ALCA. Economic allocation is done based on income received from each co-product over a specified time period. Mass allocation between milk and meat can be done as per IDF (2015) recommended equation as shown in equation below.

$$\text{Allocation Factor} = 1 - 5.7717 \times R$$

Where, R = sum of total meat sold/sum of total FPCM sold. Default values for meat and milk allocation in dairy production system are 14.4 and 85.6 %, respectively. Allocation might have substantial effects on final LCA results as shown in Table 5. As discussed in earlier sections, milk CF for the dairy production system in developing countries (extensive system) are greater than

developed countries (intensive system) due to lower milk production of cows in extensive system than intensive system. However, cows in extensive system not only produces milk, meat and manure but they also provide other monetary and non-monetary benefits to farmers e.g., cows reared in extensive system provide nutrients to family members of farmer and cows are considered as bank or insurance of farmers. Therefore, all functionality should be accounted for and emissions need to be allocated to all functions to make a fair comparison between extensive system and intensive system. For example, milk CF value of Kenya was comparable with milk CF value of Europe or North America when emissions were allocated to all functionality of cows (Weiler et al., 2014).

Uncertainty and sensitivity analysis

Input parameters used to determine environmental impact assessment in LCA study could have uncertainty due to temporal or spatial variability. For example, EF for N_2O from agricultural soil is highly variable and it is also expensive to measure N_2O (IPCC, 2006). Estimation of emissions associated with carbon sequestration and LUC has very high uncertainty (Weiss and Leip, 2012). Uncertainty in inputs parameters may create uncertainty in LCA outcome. Sensitivity analysis can be performed to understand the effect of parameters' uncertainty on LCA outcome. Sensitivity analysis could be either local or global sensitivity analysis (Groen et al., 2017). In the case of local sensitivity, one input parameter is changed at a time to determine it's impact on LCA outcome. Local sensitivity analysis provides an idea about most sensitive parameters. Effect of co-products allocation methods on milk CF as shown in Table 5 could be considered as local sensitivity analysis. Assumption of LCA could be evaluated using local sensitivity analysis. Whereas in the case of global sensitivity analysis, more than one parameter is changed at a time to determine the relative effect of each parameter on LCA outcome variance. Global sensitivity could

be conducted using several different methods which perform equally; however, choice of method depends on data availability, degree of uncertainty and goal of the LCA study (Groen et al., 2017).

Issue of biogenic carbon dioxide and carbon sequestration

Biogenic CO_2 produced from respiration of animals, plants and microbes is not included in LCA study since biogenic CO_2 is not considered as anthropogenic GHG. Because biogenic CO_2 is assumed to be recycled back to the soil through photosynthesis of plant that is subsequently consumed by animal (Beauchemin and McGeough, 2013).

Soil organic carbon stock change is not accounted for in LCA study which is an important limitation of LCA study. The SOC stock balance may depend on vegetation type, soil management practices and soil characteristics (Wiesmeier et al., 2019). For instance, converting forest or grassland into cropland decreases SOC (Wei et al., 2014). Whereas, rotational cropping increases SOC when compared with monoculture cropping (Jarecki and Lal, 2003). Perennial crops and cover crops have been shown to increase SOC distinctively (Jarecki and Lal, 2003). However, most LCA studies assume a steady state condition of SOC which is not true in most cases. Therefore, accounting for SOC stock change would help to estimate LCA outcome more accurately but quantification of SOC stock change is difficult. Because, the change of SOC happens slowly and myriads of factors affect SOC stock (Beauchemin and McGeough, 2013; Wiesmeier et al, 2019).

Summary and conclusions

Enteric CH_4, manure management and feed production related GHG are the three major GHG sources from dairy production system. Among mitigation strategies, manipulation of dairy cows' diet has potential to reduce enteric CH_4 emissions. However, dietary mitigation strategies need to be evaluated on whole-farm GHG emissions because several studies clearly showed the

existence of potential trade-off (or interactions) between enteric emissions and manure management related GHG emissions. Life cycle assessment could be a great tool for whole-farm evaluation of dietary strategies. Site specific EF for the major GHG sources should preferably be used in LCA study since IPCC tier 2 models have been shown to overpredict enteric CH_4 and underpredict manure management related GHG emissions. Additionally, while conducting LCA, LUC related emissions should be accounted for whenever applicable and SOC stock change need to be accounted for depending on appropriate and accurate data availability.

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Table 1. Total greenhouse gas (GHG) emissions and carbon footprint of global milk production over a period of 10-years (Adapted from FAO and GDP, 2018).

	Year		
	2005	2010	2015
Total GHG emissions (MMT[1])	1456	1572	1712
Carbon Footprint (CO_2/kg FPCM[1])	2.8	2.7	2.5

[1]MMT: Million metric tons, [2]FPCM: Fat-and protein corrected milk

Table 2. Recent studies recommended the evaluation of dietary mitigation strategies on whole-farm greenhouse gas (GHG) emissions.

Study	Year	Study	Year
Kebreab et al.	2019	Hassanat et al.	2013
Wattiaux et al.	2019	Hristov et al.	2013
Olijhek et al.	2018	Montes et al.	2013
Little et al.	2017	Benchaar and Hassanat	2019

Table 3. Commonly used environmental impact categories in LCA of livestock products (Thomassen et al., 2008; Cederberg and Stadig, 2003).

Impact category	Description	Characterization	Indicator unit
GWP	GHG production	Estimation of CO_2, CH_4 and N_2O	kg CO_2-e
Water use	Water depletion	Water used for livestock production	Liter
Acidification Potential (AP)	SO_2, NO_x and NH_x emission to air	AP of each emission	kg SO_2-e
Eutrophication Potential (EP)	N and P emission to air, water and soil	EP of each emission	kg PO_4-e or kg NO_3-e

Table 4. Characteristics, strengths and weaknesses of attributional LCA and consequential LCA (Cederberg and Mattsson, 2000).

Items	Attributional LCA	Consequential LCA
Characteristics	• Uses average historical data • Uses either biological or economic co-product allocation • Optional system expansion	• Uses marginal future data • Never uses co-product allocation • Obligatory system expansion
Strengths/Advantages	• Conceptually simple • Requires less assumptions	• Dynamic • Determines future scenario • Outcome is more sensitive to uncertainties due to inclusion of market prospects
Weakness/Disadvantages	• Can’t determine future scenario	• Complex procedure • Rely on assumptions that affect outcome

Table 5. Effects of allocation methods on carbon footprint of milk production determined with attributional LCA (Adapted from Little et al., 2017; Battini et al., 2016).

Study	Allocation method	Emission attributed to milk	Carbon footprint (kg CO_2-e/kg FPCM)
Little et al., 2017	No allocation	100	1.24
	Economic	90	1.11
	Mass	88	1.09
Battinit et al., 2016	No allocation	100	1.40
	Economic	94	1.32
	Mass	97	1.36
	Biological	86	1.20

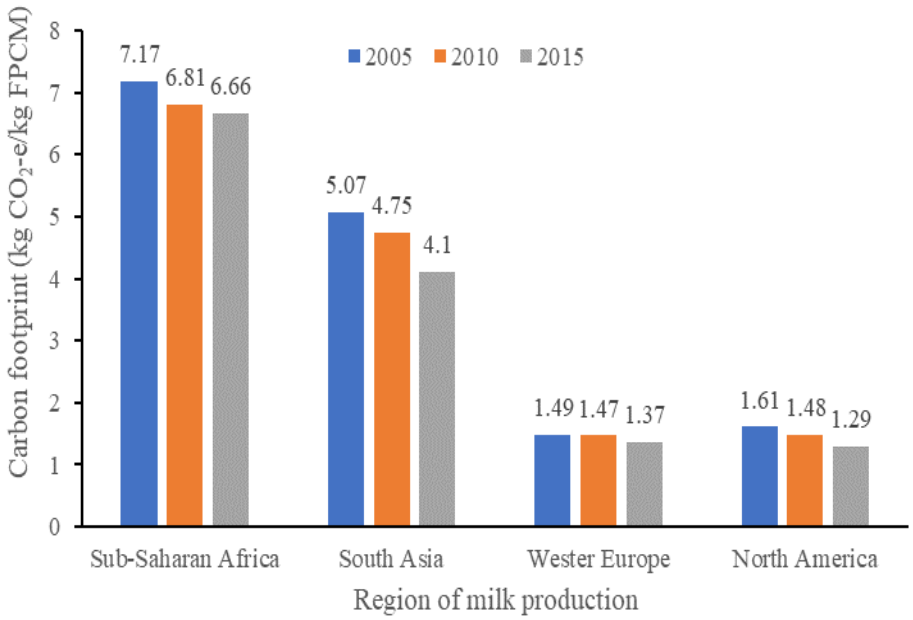

Figure 1. Carbon footprint of milk production across continents (Adapted from FAO and GDP, 2018).

Ruminal fermentation of fibrous feed: Hydrogen-producing pathways

1 Glucose $\longrightarrow$ 2 acetate $+ 4H_2$

1 Glucose $\longrightarrow$ butyrate $+ 2H_2$

Ruminal fermentation of concentrate feed: Hydrogen-consuming pathways

1 Glucose $\longrightarrow$ 2 propionate $- 2H_2$

1 Glucose $\longrightarrow$ Minor VFA $- 1H_2$

Figure 2. Showing the hydrogen producing and hydrogen consuming pathways when different type of feed is fermented in the rumen (Adapted from Wattiaux et al., 2019).

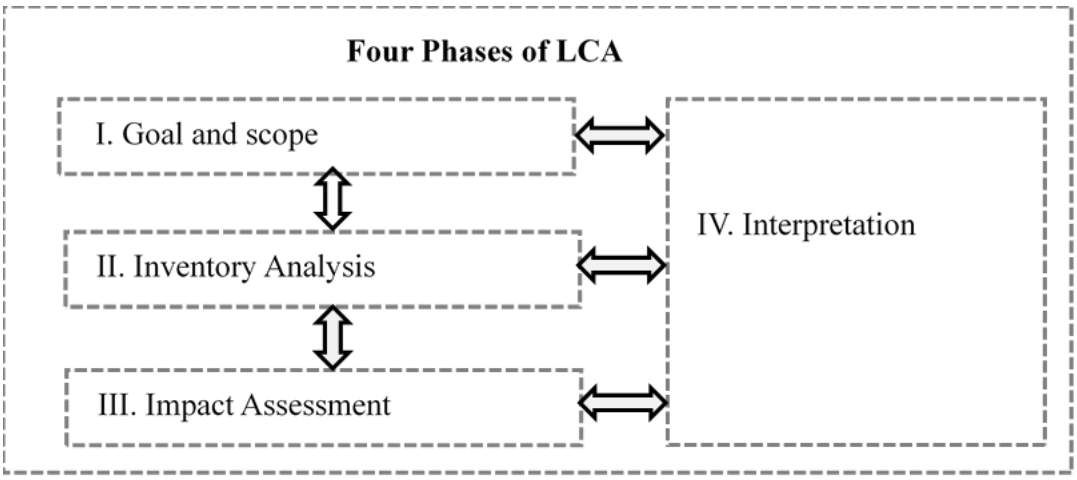

Figure 3. Phases of life cycle assessment (ISO, 2006).

CHAPTER 2. Enteric methane, lactation performances, digestibility, and metabolism of nitrogen and energy of Holsteins and Jerseys fed 2 levels of forage fiber from alfalfa silage or corn silage

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INTERPRETIVE SUMMARY

Enteric methane, lactation performances, digestibility, and metabolism of nitrogen and energy of Holsteins and Jerseys fed 2 levels of forage fiber from alfalfa silage or corn silage

By *Uddin et al., 2019.* Measures of efficiency of Holstein and Jersey cows were assessed when fed alfalfa silage or corn silage in low or high forage fiber diets. The only interaction detected was between breed and forage source for dry matter intake. Breed did not affect digestive and metabolic efficiencies. In contrast, methane and urinary energy (% gross energy intake) were lower for corn silage than alfalfa silage-fed cows; and compared to high, low forage fiber diets reduced loss of urinary N (g/d and % N intake). Neither breed nor dietary treatments affected methane intensity (g/kg fat-protein corrected milk).

ABSTRACT

Our objective was to determine the effects of replacing alfalfa silage (**AS**) neutral detergent fiber (**NDF**) with corn silage (**CS**) NDF at 2 levels of forage NDF (**FNDF**) on enteric methane (**CH_4**), performances, rumen characteristics, digestibility, and metabolism of N and energy in Holstein and Jersey cows. Twelve Holstein and 12 Jersey cows (all primiparous and mid-lactation) were used in a split-plot, triplicated 4×4 Latin square experiment where breed and diet formed the main and subplots, respectively. The 4 iso-nitrogenous and iso-starch dietary treatments were arranged as 2×2 factorial with 2 levels of FNDF [19 (low FNDF, **LF**) and 24% (high FNDF, **HF**) of dry matter] and 2 sources of FNDF (70:30 and 30:70 ratio of AS NDF:CS NDF). Soyhull (non-forge NDF) was used to keep dietary NDF similar between HF and LF diets and corn grain was used to keep dietary starch similar between the CS and AS diets. Each experimental period lasted 4 wk with wk 3 and 4 for sampling. Total collection of feces and urine over 3 days was performed on 8 cows (one Latin square from each breed). The only interaction we detected was between breed and forage source for dry matter intake (**DMI**). Compared to Jersey, Holstein cows had greater body weight (48%), DMI (34%), fat-and-protein corrected milk (**FPCM**; 31%) and CH_4 production (22%; 471 vs. 385 g/d). However, breed did not affect other CH_4 emission measures, nutrient digestibility, and the partition of intake N into milk N, fecal N and urinary N. Compared to HF, LF-fed cows had greater DMI (10%), N intake (8%) and FPCM (5%) but they were 5% less efficient (both FPCM/DMI and milk N/intake N), and they excreted 11 and 17% less urinary N (g/d and % of N intake, respectively). In spite of lower (2.5%) acetate and higher (10%) propionate (mol/100 mol ruminal volatile fatty acids) LF-fed cows had greater (6%) CH_4 production than HF-fed, due most likely to the effect of soyhulls on the aforementioned difference in DMI. Compared to AS, CS-fed cows had greater DMI (7%) and FPCM (4%) but they were less efficient (5%) and

CH_4 yield (g/kg DMI) was reduced by 8%. In addition, per unit of gross energy intake CS-fed cows lost less urinary energy (15%) and CH_4 energy (11%) than AS-fed cows. We concluded that in contrast to level and source of FNDF; breed did not affect digestive and metabolic efficiencies and furthermore neither breed nor dietary treatments affected CH_4 intensity (g/kg FPCM).

Key Words: forage source, feed efficiency, greenhouse gas, nitrogen excretion

INTRODUCTION

In the United States, milk from the dairy sector is contributing approximately 1.9% of greenhouse gas (**GHG**) emissions (Thoma et al., 2013). Although, this contribution to the national inventory is minimal, emphasis has been placed on reducing milk C footprint. Cradle-to-grave life cycle assessment suggested an average emission of 2.05 kg CO_2-eq per kg of fat-and-protein corrected milk (**FPCM**; Thoma et al., 2013). The same study revealed that the 3 major on-farm GHG sources, namely feed production, enteric methane (**CH_4**), and manure management, comprise about 70% of the recorded emissions, indicating that on-farm mitigation strategies could contribute substantially to reducing the C footprint of milk consumed in the United States (Wattiaux et al. 2019).

In the Midwest, increasing herd size without concomitant increase in the land-base has progressively led dairy farmers to rely increasingly on corn silage (**CS**) at the expense of alfalfa silage (**AS**), which historically has been a major component of their feeding (and cropping) systems. For instance, the proportion of cropland used for CS production in dairy operations has increased 75% during the 30 years between 1982 to 2012 (Martin et al., 2017). Therefore, it is important to quantify the impact of changing forages in dairy diet on animal performances, efficiency, and environmental impacts (Uddin, 2019). Methane production (g/d), yield (g/kg DMI)

and intensity (g/kg FPCM) increased when increasing forage level in the diet by increasing both AS and CS in the same ratio (Aguerre et al., 2011). However, alerting the AS:CS ratio at constant forage level resulted invariably in quadratic CH_4 emission responses (Hassanat et al. 2013; Arndt et al., 2015a). However, the varying level of dietary starch in the 3 aforementioned studies may have had confounded effects, leaving unclear the nature of the interaction between level and source of forage NDF (**FNDF**). Furthermore, greater AS:CS in the diet linearly increased manure N excretions (Hassanat et al. 2013; Arndt et al. 2015a), which likely increase nitrous oxide emission during manure storage (Külling et al., 2001) and after field application (Allen et al., 1996).

Breed may also influence undesirable C and N losses from dairy cows. Approximately 3 percentage unit greater NDF digestibility has been reported for Jersey compared to Holstein cows by Aikman et al. (2008) and Olijhoek et al. (2018). Furthermore, the latter authors reported a breed by diet interaction for CH_4 yield ($P < 0.001$) and for CH_4 intensity ($P = 0.10$) suggesting greater mitigation potential for Holstein than for Jersey cows with increasing level of concentrates in the diet. Although urinary N (% of N intake) did not differ between breed in Aikman et al. (2008) and Kauffman and St-Pierre (2001), the latter authors detected 3.5 and 4.8 percentage units lower N digestibility and productive N [(milk N + retained N) / N intake], respectively for Jersey compared to Holstein.

Therefore, we hypothesized the existence of interactions influencing C and N losses when Holstein and Jersey cows consume low (**LF**) and high (**HF**) forage fiber diets relying primarily on AS or CS. Thus, the objective of this study was to determine main effects and interactions of diets formulated with 2 levels of FNDF with either AS or CS on enteric CH_4, lactation performances, nutrient digestibility, rumen characteristics, and metabolism of N and energy of Holstein and Jersey cows.

MATERIALS AND METHODS

Institutional Animal Care and Use Committee approved protocol was followed for animal use and care during entire period of the experiment, which was conducted at the Dairy Cattle Center, University of Wisconsin-Madison.

Animals, design of experiment, and dietary treatments

Twelve primiparous Holstein (mean ± SD; 606 ± 40 kg BW and 106 ± 17 DIM) and 12 primiparous Jersey (407 ± 43 kg BW and 112 ± 15 DIM) cows housed in a tie-stall barn, fed once (0730h) and milked twice daily (0430 and 1630h), were included in this experiment. The design was a split-plot, triplicated 4×4 Latin square in which breed and diet formed the main and subplots, respectively. Cows were fed 4 diets in a 2×2 factorial arrangement with 2 levels of FNDF (19.0 and 24.0 % of DM) and 2 sources of FNDF (70:30 and 30:70 ratio of AS NDF:CS NDF). Diets were offered as TMR and included (Table 1): LF (19.0% FNDF) with a 70:30 ratio of AS NDF:CS NDF (**LFAS**), LF with a 30:70 ratio of AS NDF:CS NDF (**LFCS**), HF (24.0% FNDF) with a 70:30 ratio of AS NDF:CS NDF (**HFAS**), and HF with a 30:70 ratio of AS NDF:CS NDF (**HFCS**). To achieve similar dietary levels of starch, CP, NDF, gross energy (**GE**) as well as NRC (2001) predicted RDP, RUP and NE_L, diets with greater proportion of AS than CS had greater inclusion of corn grain, less solvent soybean meal and more expeller soybean whereas diets with higher proportion of FNDF had lower inclusion of soyhulls (Table 1). Each experimental period lasted 4 wk with sampling conducted in wk 3 and 4.

Collection and analyses of feed and milk sample

Samples of AS and CS were collected weekly for moisture determination at 60°C for 48h and TMR were adjusted accordingly at the beginning of each week throughout the experiment.

Samples of concentrate mixtures (one for each TMR), AS, CS and bait feed mixture (used for enteric CH_4 measurement) were collected daily during each sampling period. Samples were frozen at -20°C until further analysis. All feed samples were dried at 60°C for 48h in a forced-air oven and ground in Wiley mill (Arthur H. Thomas, Philadelphia, PA) to pass 1-mm screen. Each feed sample was then composited by period and analyzed in duplicates for absolute DM by drying at 100°C for 24 h, starch (Hall, 2009), total N (combustion method; Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI), amylase-treated NDF using Ankom (method 2002.04; AOAC International, 2016), ADF and lignin using Ankom (method 973.18; AOAC International, 2016), ash and OM at 600°C for 2 h (method 942.05; AOAC International, 2016), crude fat using acid hydrolysis (method 922.06; AOAC International, 2016) and GE using bomb calorimeter analyses (Parr 1241 adiabatic oxygen bomb calorimeter). Reported values for NDF, ADF and lignin were corrected for ash. Calculation of NFC was performed according to NRC (2001).

The amount of TMR offered was adjusted daily to yield 5 to 10% refusal. Daily TMR offered and refused were used to calculate DMI for each cow in each period. Body weight were measured after morning milking and before feeding starting approximately at 0630h on d 14 and d 15; and on d 21 and d 22 of each period and averaged by week for each period. Afternoon (1630 h) and morning (0430 h) milk yield (**MY**) were recorded daily. Twelve milk samples were collected for each cow and preserved using bronopol. Samples were from 6 consecutive milking in wk 3 (d 17 to d 20) and 6 consecutive milking in wk 4 (d 24 to 27). Samples were analyzed for milk fat, true protein, and lactose, SNF and MUN with infrared analyzer with a Foss FT6000 (Foss Electric, Hillerød, Denmark) and SCC using flow cytometry (Agsource Milk Analysis Laboratory, Menomonie, WI). Analytical results were weighted for corresponding yields of morning and

afternoon milk to calculate milk composition. The FPCM was calculated as described in IDF (2010). Feed efficiency was computed by dividing either MY or FPCM by DMI.

Enteric methane measurement

At the beginning of the experiment, 14 Holstein and 14 Jersey cows were trained for 10 d to adapt to the GreenFeed (C-Lock Inc., Rapid City, SD) system which is a mobile, open circuit gas measurement unit considered as non-invasive technique with minimal animal disturbance (Dorich et al., 2015). Details of the equipment, measurement protocol, and calculation of flux have been descried elsewhere (Hristov et al., 2015; Huhtanen et al., 2015). In our study, about 300 g bait feed mixture (Table 1) were dispensed in 6 aliquots at 45 s interval to entice cows to place and maintain their head inside the head chamber of the unit. After training, we randomly selected 12 Holstein and 12 Jersey cows to participate in the study. In each period, eight 3-hr interval time-point measurements (each time point lasted at least for 5 min) were obtained for each cow during a 24-h clock where feeding time (0730 h) was considered as 0 h. These 8 measurements were spread over 4 d during wk 3 between d 17 and 20 in each period. Bait feed mixture was included either in the TMR or through the grain dispenser of the GreenFeed unit during the days of enteric CH_4 measurement such that bait feed was included in DMI calculation. Calibration and CO_2 recovery test were performed at the beginning and end of measurements in each period as described by Hristov et al. (2015). The measured gas concentrations were adjusted based on the CO_2 recovery value which was 100 ± 1.3%. Enteric CH_4 production was calculated by averaging 8 time-point measurements for each cow in each period. Body weight, DMI and FPCM data of wk 3 only were used to compute the denominator when calculating CH_4 per unit of metabolic BW (**MBW**), CH_4 yield, and CH_4 intensity, respectively.

Apparent nutrient digestibility

Total collection of feces and urine was performed for 72 h using 8 cows (one randomly selected Latin square from each breed) starting at 1800 h on d 24 and ending at 1800 h on d 27 in each period. Stalls were individualized with wooden partition that included open space (window) allowing cows to socialize with their neighbors. Feces were collected in a stainless-steel pan fitted in the gutter beneath the grate. No bedding was added to stalls to avoid fecal contamination, but manure was scrapped in the pan as needed around the clock to ensure cow cleanliness and measurement precision. Cows were followed to and from the parlor to collect any feces excreted during milking. Indwelling Foley catheters (24 French, 75 ml-balloons, C.R. Bard Inc., Covington, GA) were inserted into the bladder for urine collection in closed containers with 300 ml 50% sulfuric acid solution (weight basis). Excretion of feces and urine were determined gravimetrically every 8 h, leading to 9 samples for each cow in each period. At each sampling time, samples of feces (500 g) and urine (100 mL) were collected and stored at -20°C until further analysis.

Urine samples were thawed at room temperature and composited by cow (equal volume from each sample) and period. Urine samples were then analyzed in duplicate for total N using the combustion method as described above for feed samples. Fecal samples were dried at 60°C in a forced-air oven for 96 h, ground to pass 1-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), and then composited by cow and period. Aiming to determine accurate nutrient intake during the 3 d of total collection, refusals from each cow were collected daily and stored at -20°C until further analysis. After drying and grinding individual samples as described above, a composite sample was generated for each cow in each period. Composited fecal and refusal samples were then analyzed in duplicate for absolute DM, starch, NDF, ADF, Lignin, ash, total N, crude fat, and GE as described above for feed samples.

Nutrient intake (DM, OM, NDF, ADF, hemicellulose, and cellulose) was calculated by multiplying DMI with respective nutrient concentration in the diets. Nutrient excreted via feces was calculated by multiplying fecal DM output with respective nutrient concentration in feces. Total-tract apparent digestibility was expressed on a percent basis after the amount of nutrient apparently digested was calculated by difference between intake and fecal excretion.

Energy and N digestibility and metabolism

In this experiment, we measured daily dietary energy input and output in feces, urine, methane, and milk. Thus, we used the following equation to describe energy partitioning:

$$E_B = GE_I – (E_F + E_U + E_{CH4} + NE_M + NE_L)$$

Where all terms are in Mcal/d, and E_B is energy balance which represented an aggregate estimate of energy in pool not measured and compounded errors of measurements, GE_I is GE intake, E_F is fecal energy, E_U is urinary energy, E_{CH4} is CH_4 energy, NE_M is net energy of maintenance (0.80 Mcal / kg $BW^{0.75}$; NRC 2001), NE_L is net energy of lactation per kg of milk based on Eq. 2-16 of NRC (2001).

Similarly, we measured daily N intake and output in feces, urine and milk. Thus, we used the following equation to describe N partitioning:

$$N_B = N_I – (N_F + N_U + N_L)$$

Where all terms are in g/d, N_B is N balance which represented an aggregate estimate of N in pools not measured and the compounded error of measurements, N_I is N intake, N_F is fecal N, N_U is urinary N , and N_L is milk N. Total N excretion (or manure N) was calculated as $N_F + N_U$.

Ruminal measurements

About 10 mL ruminal fluid sample was collected by rumenocentisis (Nordlund and Garret, 1994) from each cow in each period approximately 4 h after feeding on d 27 (12 cows) and d 28 (12 cows). The pH was measured immediately (Laqua Twin pH-meter model B-713; Spectrum Technologies Inc., Plainfield, IL). Then, 1-mL aliquot was transferred to a microfuge tube with containing 20 µl 50% sulfuric acid solution for ruminal VFA analysis. Another 1-mL ruminal fluid sample was transferred to another microfuge tube containing 20 µl 50% trichloroacetic acid solution for ruminal NH_3-N analysis. Both samples were stored at -20°C and thawed at room temperature the day of analysis. Ruminal NH_3-N analysis was performed as per Chaney and Marbach (1962). For VFA, thawed samples were vortexed, and centrifuged at 25,000 × *g* at 4°C for 10 min. The supernatant was transferred to vials for analysis in GC (Phenomenex ZB-FFAP 30m L x 0.32mm ID x 0.25um FT; Shimadzu GC-2010 plus, Shimadzu Corporation, Kyoto, Japan).

Statistical Analysis

All response variables were reduced to one mean value for each cow in each period and data were analyzed in SAS (version 9.4; SAS Institute Inc., Cary, NC) using Proc Mixed procedure using following model:

$$Y_{ijklmn} = \mu + B_i + Sq_{j:i} + C_{k:ji} + P_l + F_m + S_n + FS_{mn} + BF_{im} + BS_{in} + BFS_{imn} + E_{ijklmn}$$

where, Y_{ijklmn} is the response variable; µ is the overall mean; B_i is the fixed effect of i^{th} breed, i = 1, 2; $Sq_{j:i}$ is the random effect of j^{th} square within i^{th} breed, j = 1, 2, 3, ~ n (0, σ^2_{sq}); $C_{k:ji}$ is the random effect of k^{th} cow within i^{th} breed and j^{th} square, k = 1, 2,…24, ~ n (0, σ^2_c); P_l is the fixed effect of l^{th} period, l = 1, 2, 3, 4; F_m is the fixed effect of m^{th} FNDF level, m = 1, 2; S_n is the fixed

effect of n^{th} FNDF source, n= 1, 2; FS_{mn} is the interaction term of m^{th} FNDF level with n^{th} FNDF source; BF_{im} is the interaction term of i^{th} breed with m^{th} FNDF level; BS_{in} is the interaction term of i^{th} breed with n^{th} FNDF source; BFS_{imn} is three-way interaction term of i^{th} breed, m^{th} FNDF level and n^{th} FNDF source; and E_{ijklmn} is the error term ~ N (0, σ^2_e).

In the case of variables related to nutrient digestibility and metabolism the square effect was removed from the model. Significance was declared at $P \leq 0.05$. Interactions were reported and discussed only when significance was declared.

RESULTS AND DISCUSSION

Chemical composition of the diets

All 4 diets had similar content of OM, ADF, cellulose, starch, fat, NFC, GE and NE_L averaging 92.0, 22.4, 19.4, 22.5, 2.3, 41.5% of DM, 4.38 and 1.52 Mcal/kg DM, respectively (Table 1). We formulated diets to be iso-nitrogenous, but the CP content of final diets (DM basis) was slightly greater (averaged 17.0%) than we intended (16.5%) because AS was harvested at an earlier than anticipated vegetative stage. The NRC (2001) predicted RDP and RUP were similar across diets and averaged 10.8 and 6.2% (DM basis), respectively. On average, HF diets contained about 13.6 units greater forage (54.5 vs. 68% of DM), 4.8 units greater FNDF (19.2 vs. 24% of DM), and 5.6 units lower non-forage NDF (12.5 vs. 6.9% of DM) than the LF diets. As anticipated, the ratio of AS NDF:CS NDF for high AS based diets (LFAS and HFAS) and high CS-based diets (LFCS and HFCS) were maintained at 70:30 and 30:70, respectively. However, compared to AS, CS-based diets had 3 units greater NDF (30 vs. 33%) which was due to greater content of non-forage NDF (8.2 vs. 11.3%) associated primarily with greater content of soyhulls (Table 1).

Intake, production performances and feed efficiency

Main effects of diets and breed on measurements of intake, lactation performances and feed efficiency are in Table 2. The only interactions detected in this study, which indicated that the difference in DMI between breed depended on forage source (and vice-versa) are shown in Figure 1. Although Holstein had on average 34% greater DMI (kg/d) than Jersey cows (Table 2), this difference was wider when the main forage source was AS compared to CS (41 vs. 28%, respectively, Figure 1A). Similarly, CS-fed cows had on average 7% greater DMI than AS-fed cows but this difference was wider for Jersey (13.5%) than for Holstein (3%; Figure 1A). Lower rumen fill associated with greater NDF digestibility of CS vs. AS-based diet (57.8 vs. 54.7%, respectively; Table 3) may have contributed to the greater intake of DM for cows fed the former compared to the later diets. In agreement with our results, but in contrast to Arndt et al. (2015a), Hassanat et al. (2013) reported that increasing CS at the expense of AS in the diet increased DMI. Expressed as a percentage of BW, DMI was 10% lower for Holstein than Jersey cows (Table 2). Jersey cows may have spent greater rumination time per kg of DMI leading to greater rate of passage and thus greater DMI per unit of BW (Aikman et al. 2008); however, the superiority of Jersey cow was narrower when the main forage source was AS compared to CS (5 vs. 13%, Figure 1B). Compared to HF, LF-fed cows consumed 9% greater DM (Table 2). Contrary to our findings, Aguerre et al., (2011) reported no change in DMI when FNDF level gradually increased from 19 to 28% of dietary DM. This discrepancy may be explained in part by the chemical fraction that decreased as FNDF increased: starch in Aguerre et al., (2011) but non-forage NDF in this study.

Compared to Jersey, Holstein cows produced 56% more milk (33 vs. 21 kg/d), however the magnitude of difference decreased to 33% when production was expressed as FPCM (33 vs. 25 kg/d). Similar or even greater production differences between Holstein and Jersey have been

reported elsewhere (Kauffman and St-Pierre, 2001; Knowlton et al. 2010; Olijhoek et al., 2018). Compared to Jersey, Holstein cows had 16.5 % greater efficiency (MY/DMI) but both breeds had the same efficiency when expressed as FPCM/DMI. Compared to HF, LF-fed cows produced greater amount of milk fat, protein, and lactose due to in part greater MY, and in part to greater concentration of milk protein and lactose (Table 2). In addition, LF-fed cows were 5.5% (MY/DMI) and 4.8% (FPCM/DMI) less efficient than HF-fed cows. Compared to AS, CS-fed cows had greater MY, FPCM, milk component yield and milk protein concentration (Table 2) but were less efficient (4.9% for MY/DMI and 4.2% for FPCM/DMI). In contrast to our study, others found no effects of forage level or source on feed efficiency (Aguerre et al., 2011; Arndt et al., 2015a).

Total tract apparent digestibility of nutrients

Compared to Jersey, Holstein cows consumed greater amount of OM, N, NDF, ADF, hemicellulose and cellulose mainly because of 34% greater DMI ($P < 0.01$, Table 3). However, breed affected none of the apparent digestibility coefficients (Table 3). In contrast to our findings, Aikman et al. (2008) reported about 3 percentage unit greater ADF and NDF digestibility for Jersey than Holstein cows whereas several other studies did not find breed differecnes for DM and NDF digestibility (Kauffman and St-Pierre, 2001; Knowlton et al. 2010). Compared to HF, LF-fed cows also had greater N (8%), NDF (10%), ADF (8%), hemicellulose (21%) and cellulose (11%) intake again mainly due to greater DMI. Except for N, LF-fed cows had greater digestibility of all measured nutrients, most importantly for hemicellulose (10%) and cellulose (4%) than HF-fed cows (Table 3). In comparison, Olijhoek et al. (2018) reported a decrease in NDF digestibility when forage level in the diet decreased from 68 to 39% (DM basis), an effect likely due to a substantial increase in dietary starch content. In our study, however, starch content was kept

constant across diets but level of non-forage NDF vaaried when dietary forage level was manipulated. Compared to AS, CS-fed cows consumed greater amount of all nutrients (Table 3) and had greater digestibility of NDF (6%), hemicellulose (41%) and cellulose (5%). In contrast to our findings, Arndt et al. (2015a) reported a greater digestibility coefficients for AS-fed cows than CS-fed cows. The overall digestibility values of NDF and ADF across diets were also relatively high in our study compared to other studies (Aikman et al., 2008; Hassanat et al., 2013;Arndt et al. 2015a).

Enteric methane (CH_4) emissions

Enteric CH_4 emissions data (Table 4) were comparable with other studies (Arndt et al., 2015a; Sun et al., 2019). Holstein cows produced 22% more (471 vs. 385 g/d) CH_4 than Jersey cows but emission per unit of BW was 17% lower (0.77 vs. 0.93 g/kg of BW) for Holstein than Jersey cows. Breed did not affect enteric CH_4 yield and intensity, except when expressed as g/kg of MY (Table 4). Olijhoek et al. (2018) reported no difference in CH_4 intensity between breeds, however, they found greater yield of CH_4 (g/kg of DMI) for Jersey than Holstein. Thus, if the goal was to produce a fixed amount of FPCM, then GHG emission at animal level (enteric CH_4) would be similar for both breeds. Contrary to expectation, LF-fed cows had greater CH_4 production than HF-fed cows (5 to 6% depending on mode of expression), which was likely due to an overriding effect of greater DMI for the former compared to the latter diets. Thus, in this study dietary composition had little effect as FNDF level did not affect CH_4 yield (g/kg of DMI). However, the LF-fed cows had 7% lower CH_4 per kg of NDF intake (61 vs. 66 g/kg), but 21% greater CH_4 per kg of FNDF intake (101 vs. 84 g/kg) than HF-fed cows suggesting differential effect of FNDF and non-forage NDF on methanogenesis. In our study, we did not detect any breed × diet interactions. However, Olijhoek et al. (2018) reported a breed × diet interaction for CH_4 yield as their data indicated a

greater effect of concentrate supplementation on reduction of CH_4 yield in Holsteins than Jerseys. Back to our study, FNDF source did not affect CH_4 production and intensity but increasing CS NDF at the expense of AS NDF decreased CH_4 yield expressed as g/kg of DMI (8%), or g/kg of NDF intake (17%), or g/kg of FNDF intake (8%). In agreement with our results, total (Hassanat et al., 2013) and partial (Arndt et al., 2015a) replacement of AS with CS in the diet decreased CH_4 per kg of DMI. Lower CH_4 yield for CS-based diet compared to diets based on grass silage has been reported by Hammond et al. (2016). Taken together these results disagree with the suggestion of greater emission of CH_4 per kg of DMI for C4 grass (e.g., corn) than for cold season legumes such as alfalfa (Archimède et al., 2011). In contrast to the work of Hassanat et al. (2013), Arndt et al. (2015a), and Hammond et al. (2016), dietary starch was constant in our study. However, soy hulls, which was used as a source of non-forage NDF to compensate for the decrease in NDF between the HF and LF diets (and to a lesser extent to help maintain starch level constant between the CS and AS-based diets; Table 1) may have contributed to some of the emissions results reported here. Smaller particle size and lower lignification of non-forage NDF of soyhulls compared to FNDF may have enhanced rate of passage and ruminal digestibility of NDF (Table 3), respectively; both of which may have alleviated rumen fill and enhanced DMI of cows fed LF diets compared to those fed the HF diets (Table 2).

Ruminal pH, VFA and NH_3-N

Breed did not affect ruminal pH, NH_3-N, total VFA concentration and any molar proportion of VFA, except for that of iso-valerate, which was greater in Holstein than Jersey (Table 5). Compared to LF, HF-fed cows had greater ruminal pH (5.93 vs. 6.03). Aguerre et al. (2011) also reported an increase in ruminal pH from 6.38 to 6.59 when forage level increased from 47 to 68% of dietary DM. In spite of almost identical CP; compared to LF, HF-fed cows had 1.75 mg/dl more

ruminal NH_3-N concentration, which is in disagreement with the findings of Aguerre et al. (2011). In our study, altering FNDF level did not influence total VFA (mean ± SE; 123 ± 3.74 mM), a result similar to Aguerre et al. (2011). However, LF-fed cows had lower acetate, greater propionate (molar proportion) and lower acetate:propionate ratio (3.78 vs. 4.17), which may have contributed to the observed reduction in CH_4 per unit of NDF intake compared to HF-fed cows. Compared to AS, CS-fed cows had lower total VFA (119 vs. 127 mM) but greater molar proportion of butyrate (11.3 vs. 12 mol/100 mol). In other studies, increasing proportion of CS in the diet did not affect butyrate (Arndt et al. 2015a) or decreased butyrate (Hassanat et al. 2013). This inconsistency may be due in part to the strategy used when constructing experimental diets to compare AS to CS. In our study starch level was kept constant but non-forage NDF varied whereas in the 2 studies cited above starch level varied and non-forage NDF was kept constant.

Feces and urine

Holstein cows produced greater amount of feces as-is (36%), urine as-is (14%), manure as-is (29%), feces DM (37%), and urine DM (20%) than Jersey cows. These results are associated to differences in BW and DMI between the 2 breeds and they were almost of identical magnitude as reported by Knowlton et al. (2010). Level of FNDF did not affect any of the variables reported in Table 6, which agreed with the findings of Aguerre et al. (2011). However, compared to AS, CS-fed cows produced more feces as-is (14%) and less urine as-is (16%). Arndt et al. (2015a) also reported a decrease in urine volume when dietary AS was replaced with CS. This effect might be due to increased K content with inclusion of greater proportion of AS at the expense of CS in the diet. The substantially lower feces:urine ratio for AS-fed cows compared to CS-fed cows (1.65 vs. 2.21; Table 6) might lead to greater volatilization of manure NH_3 or might subsequently influence

manure nitrous oxide emissions because urinary N (mostly in the form of urea) is more labile than fecal N (Külling et al., 2001).

Digestive and metabolic partitioning of nitrogen

Intake of N and excretion via feces, urine, and manure and secretion via milk (expressed as g/d) were greater (from 34 to 38% depending on the variable) for Holstein than Jersey cows (Table 7). These results were consistent with greater DMI, feces output, urine output, manure production and FPCM reported herein for Holstein compared to Jersey cows. However, in agreement with Aikman et al. (2008) and Knowlton et al. (2010), breed did not affect N excretion in feces or urine nor milk N secretion when expressed as a percentage of N intake in this study. The absence of difference in these rates of conversion suggested that both breeds had similar digestive and metabolic efficiencies for N. Compared to HF, LF-fed cows had 8% greater N intake, lower urinary N excretion (-17 g/d), and higher milk N use efficiency (22.8 vs 21.5%, Table 7). Compared to AS, CS-fed cows had 6% greater N intake, they secreted greater milk N (+ 8.0 g/d) and exhibited lower fecal N as a percentage of N intake (26.7 vs. 28.4%). Hassanat et al. (2013) also reported an increase in milk N secretion with increasing CS in the diet. In our study, N balance (N in pools not measured and compounded error of measurements) was surprisingly large, ranging from 22 to 32% of total N intake depending on dietary treatments. Literature values for N balance (% of total N intake) in multiparous lactating cows varies from 2 to 13% (Flis and Wattiaux, 2005; Arndt et al. 2015a; Nichols et al., 2019). Because all animals used in this study were primiparous cows in mid-to-late lactation, they were gaining weight (mean ± SD; Holstein ADG: 300 ± 92 g/d and Jersey ADG: 240 ± 192 g/d) and thus were probably using some of the N intake for replenishing body tissue lost in early lactation and for further growth toward mature BW (NRC, 2001). These effects, however, were unlikely to explain entirely the high N balance. Several studies

also found positive N balance with seemingly no change in BW (Flis and Wattiaux, 2005; Kauffman and St-Pierre, 2001) and N balance ranged from -57 to 205 g/d as per the meta-analysis compiling 35 N balance studies (Spanghero and Kowalski, 1997).

Digestive and metabolic partitioning of energy

Except for energy balance, all energy related variables expressed as Mcal/d were affected by breed and were greater (22 to 43% depending on the variable) in Holstein than Jersey cows (Table 8). The most likely reasons for greater daily energy values in Holstein were related to greater body size (48%) and DMI (34%) for Holstein than Jersey. However, breed did not affect any of the energy related variables expressed as a percentage of GE_I (Table 8) with percentages comparable with other studies conducted separately with Holstein (Arndt et al., 2015b) and Jersey (Judy et al., 2018). The absence of difference in these rates of conversion suggested that both breeds had similar digestive and metabolic efficiencies for energy. Compared to HF, LF-fed cows had 6% greater GE_I (98.6 vs 93.1 Mcal/d) primarily due to greater DMI. The greater GE_I resulted in an increase of 8 and 9% of DE and ME (Mcal/d), respectively for LF-fed cows than HF-fed cows. Energy balance (energy for growth, heat production and compounded error of measurements) expressed as Mcal/d was 13% greater for LF-fed cows than HF-fed cows. When expressed as Mcal/d, all the variables except urinary energy and CH_4 energy were affected by FNDF source being greater in CS-fed cows than AS-fed cows due to mainly greater DMI. However, CS-fed cows excreted 15 and 10% lower urinary energy and CH_4 energy, respectively than AS-fed cows. These reduced energy losses resulted in greater ME in CS-fed cows than AS fed cows. Arndt et al. (2015b) reported that highly efficient cows have lower energy losses as urinary, CH_4 and heat energy than low efficient cows. On the contrary, in this study, AS-fed cows (cows with greater FPCM/DMI) excreted greater energy via urine and CH_4 than CS-fed cows

(cows with lower FPCM/DMI). Furthermore, energy balance expressed as % of GE_I did not differ between AS and CS-fed cows. However, the magnitude of the numerical difference (40.5 vs. 43.2 % of GE_I for AS-fed and CS-fed cows, respectively) may have contributed to the lower efficiency of CS-fed cows compared to AS-fed cows when expressed as FPCM/DMI (Table 2).

CONCLUSIONS

In conclusions, Holstein cows had greater DMI, FPCM and CH_4 production than Jersey cow but breed did not affect digestive and metabolic efficiencies, FPCM/DMI, CH_4 yield and intensity. In contrast to our hypothesis, there were no interactions except for a difficult-to-explain FNDF source × breed interaction for DMI. Compared to HF, LF-fed cows had lower FPCM/DMI and urinary N loss (g/d and % of N intake) with similar CH_4 intensity and yield, but had greater CH_4 production (most likely as a consequence of greater content of soy hulls as a source of non-forage NDF in LF than HF diet). Compared to AS, CS-fed cows had greater DMI and FPCM, but lower FPCM/DMI, urinary and CH_4 energy loss, and CH_4 yield without affecting CH_4 production and intensity. The trade-off between CH_4 and N losses reported here may have implications in future studies assessing environmental impact of milk production when approached from a whole-farm perspective.

ACKNOWLEDGEMENTS

This project was financially supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture Hatch Multi-state research formula fund (# WIS01941, and USDA-NIFA # 2013-68002-20525). We are thankful to fellow Lab mates Dominique D'Huvetter, Taylor Wickert, Fei Sun, Paulina Letelier, Sanjeewa Ranathunga, Fernanda De Kassia Gomes, and Benito Albarran Portillo for their suggestions and hard work during sampling. Authors are happy

to express their acknowledgement to Lab Manager, Sandy Bertics and veterinarian, Dr. Michael Maroney for their great help particularly during rumenocentesis and catheterization, respectively. Finally, authors also express their gratitude to statistical consultant Peter Crump (CALS, UW-Madison) for his help with statistical analysis.

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Table 1. Ingredients and chemical composition of dietary treatments.

	Dietary Treatments[1]			
Item	LFAS	LFCS	HFAS	HFCS
Ingredients, % of diet DM				
Forage	53.6	55.3	66.9	69.1
Alfalfa silage (AS)	36.6	15.7	45.7	19.6
Corn silage (CS)	17.0	39.6	21.2	49.5
Corn grain	22.0	9.5	20.0	4.5
Soybean meal expeller	4.0	1.5	5.6	2.2
Soybean meal solvent extract	3.2	10.5	0	9.5
Soyhulls	12.7	18.7	3.0	10.2
Blood meal	0.7	0.7	0.7	0.7
GreenFeed bait mixture[2]	2.0	2.0	2.0	2.0
Vitamins and minerals[3]	1.75	1.75	1.75	1.75
Chemical composition, % of DM unless otherwise specified				
OM	92.1	93.0	91.7	92.9
CP	17.3	16.9	17.2	16.7
RDP[4]	11.1	10.6	11.1	10.5
RUP[4]	6.2	6.3	6.1	6.2
aNDF[5]	30.5	32.9	29.0	32.8
Non-forage NDF	11.3	13.7	5.0	8.8
Forage NDF	19.2	19.2	24.0	24.0
AS NDF	13.4	5.8	16.8	7.2
CS NDF	5.8	13.4	7.2	16.8
AS NDF:CS NDF	70:30	30:70	70:30	30:70
ADF	22.5	22.5	22.0	22.8
ADL	3.2	2.4	3.7	2.8
Hemicellulose[6]	8.0	10.4	7.0	10.0
Cellulose[7]	19.3	20.1	18.2	20.0
Starch	22.1	22.9	23.0	22.7
Fat	2.4	2.2	2.5	2.3
NFC[8]	41.4	40.8	42.7	41.1
Gross Energy, Mcal/kg DM	4.37	4.36	4.39	4.40
NE_L[9], Mcal/kg DM	1.52	1.50	1.50	1.48

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]GreenFeed bait mixture, composed of 60% corn grain, 10% soybean meal and 30% molasses (DM basis) was added to the TMR except for the days of CH_4 measurement when it was used as bait via GreenFeed unit.

[3]Vitamins and minerals (DM basis) was composed of 0.5 % monocalcium phosphate (21% phosphorus), 0.25% magnesium oxide (56% magnesium), 0.25% sodium bi-carbonate (27% sodium), 0.25% salt (iodized granulated sodium chloride) and 0.5% lactating trace minerals and vitamins (87 ppm selenium, 2014540 IU/kg vitamin A, 402930 IU/kg vitamin D3 and 8543 IU/kg vitamin E).

[4]RDP and RUP calculated using NRC (2001) equation based on formulated diet.

[5]aNDF = Amylase treated NDF corrected for ash.

[6]Hemicellulose = NDF-ADF.

[7]Cellulose = ADF-ADL.

[8]NFC = 100- (CP + NDF + Fat + Ash).

[9]NE_L calculated using NRC (2001) equation based on actual diet and cow performance data.

Table 2. Effects of dietary treatments and breed on intake, milk and milk component yield, milk composition and feed efficiency (n = 24; data from wk 3 and 4).

Item	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
BW, kg	512	518	512	514	8.84	613	415	12.4	0.12	0.01	<0.01
Intake, kg/d unless otherwise specified											
DM[3]	22.5	23.6	19.9	21.9	0.66	25.2	18.8	0.81	<0.01	<0.01	<0.01
NDF	6.84	7.85	5.85	7.33	0.21	7.95	5.98	0.26	<0.01	<0.01	<0.01
DM[4], % BW	4.39	4.61	3.94	4.33	0.12	4.11	4.53	0.14	<0.01	<0.01	0.10
NDF, % BW	1.34	1.53	1.16	1.45	0.04	1.30	1.44	0.05	<0.01	<0.01	0.09
Milk and milk component yield, kg/d											
Milk	27.5	28.0	26.0	27.0	0.76	33.2	21.3	1.04	<0.01	<0.01	<0.01
FPCM[5]	29.2	29.9	27.4	29.0	0.80	32.7	24.9	1.06	<0.01	<0.01	<0.01
Fat	1.26	1.28	1.18	1.25	0.04	1.37	1.12	0.05	<0.01	0.02	0.02
Protein	0.87	0.91	0.81	0.85	0.02	0.97	0.76	0.03	<0.01	<0.01	<0.01
Lactose	1.33	1.36	1.25	1.30	0.04	1.61	1.01	0.05	<0.01	0.02	<0.01
Milk composition, % unless otherwise specified											
Fat	4.74	4.69	4.63	4.78	0.10	4.13	5.29	0.12	0.89	0.38	<0.01
Protein	3.26	3.33	3.18	3.23	0.04	2.93	3.56	0.06	<0.01	0.02	<0.01
Lactose	4.82	4.83	4.76	4.78	0.04	4.86	4.74	0.05	0.02	0.49	0.14
SCC[6]	121	145	145	159	67.7	63.0	222	93.4	0.28	0.27	0.29
MUN, mg/dl	13.9	13.5	13.9	13.7	0.22	14.1	13.4	0.26	0.43	0.06	0.11
Feed Efficiency[7]											
Milk/DMI	1.24	1.18	1.31	1.25	0.04	1.34	1.15	0.04	<0.01	<0.01	0.05
FPCM/DMI	1.33	1.26	1.38	1.34	0.04	1.31	1.35	0.04	<0.01	0.01	0.56

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]Significant interactions are shown in footnotes.

[3]Forage NDF source×breed interaction ($P = 0.04$) is shown in Figure 1A.

[4]Forage NDF source×breed interaction ($P < 0.01$) is shown in Figure 1B.

[5]FPCM: Fat-and-protein corrected milk calculated as per IDF (2010).

[6]SCC: $\times 10^3$ cells/ml

[7]Feed efficiency calculated as [Milk yield or FPCM (kg/d)]/[DMI(kg/d)].

Table 3. Effects of dietary treatments and breed on nutrient intake and total-tract apparent digestibility (n = 8; wk 4 data).

Items	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF[3] Level	FNDF Source	Breed
Intake, kg/d unless otherwise specified											
DM	23.0	24.0	20.3	22.1	0.69	25.6	19.1	0.84	<0.01	<0.01	<0.01
OM	19.9	22.1	18.6	20.6	0.90	23.4	17.2	1.12	0.04	<0.01	<0.01
Nitrogen, g/d	608	650	566	595	0.03	698	512	0.03	<0.01	0.04	<0.01
NDF	6.65	7.91	5.93	7.35	0.33	7.99	5.93	0.38	<0.01	<0.01	<0.01
ADF	4.90	5.32	4.43	5.03	0.22	5.66	4.19	0.27	<0.01	<0.01	<0.01
Hemicellulose	1.80	2.62	1.40	2.26	0.09	2.32	1.72	0.11	0.04	<0.01	<0.01
Cellulose	4.22	4.84	3.73	4.45	0.19	4.95	3.67	0.23	<0.01	<0.01	<0.01
Apparent digestibility, %											
DM	73.9	74.5	72.2	73.2	0.93	73.4	73.5	1.02	0.03	0.25	0.98
OM	75.1	75.5	73.4	74.4	0.88	74.5	74.7	0.97	0.04	0.26	0.88
Nitrogen	72.6	73.7	70.6	72.8	1.01	72.4	72.5	1.03	0.09	0.05	0.95
NDF	56.9	59.3	52.4	56.3	1.72	55.7	56.8	1.99	<0.01	0.02	0.70
ADF	57.9	58.1	55.0	57.1	1.45	56.5	57.6	1.71	0.04	0.23	0.65
Hemicellulose	48.5	61.7	38.9	61.1	2.68	52.8	51.8	2.30	0.03	<0.01	0.75
Cellulose	62.9	66.1	60.4	63.8	1.56	62.5	64.2	1.68	0.05	<0.05	0.48

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]P-values for all main effects (significant plus non-significant) are presented, but non-significant interaction effects are not shown in this Table.

[3]FNDF: Forage NDF.

Table 4. Effects of dietary treatments and breed on enteric methane (CH_4) emission (n = 24; wk 3 data).

Item	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
DMI, kg/d	22.5	23.8	20.2	22.3	0.66	25.4	19.0	0.80	<0.01	<0.01	<0.01
NDFI, kg/d	6.84	7.85	5.85	7.33	0.21	7.95	5.98	0.26	<0.01	<0.01	<0.01
FNDFI[3], kg/d	4.30	4.57	4.83	5.36	0.15	5.44	4.09	0.17	<0.01	<0.01	<0.01
Enteric CH_4 production, g/kg unless otherwise specified											
Daily, g/d	440	439	413	418	16.2	471	385	21.0	<0.01	0.74	0.04
CH_4/BW	0.88	0.86	0.82	0.83	0.03	0.77	0.93	0.03	<0.01	0.84	0.03
CH_4/MBW[4]	4.14	4.08	3.87	3.92	0.13	4.82	4.18	0.17	<0.01	0.96	0.21
Enteric CH_4 yield, g/kg unless otherwise specified											
CH_4/DMI[5]	20.2	18.6	20.8	19.2	0.69	18.8	20.6	0.80	0.21	<0.01	0.17
CH_4/NDFI[6]	66.0	56.0	72.0	59.0	2.18	60.0	66.0	2.56	<0.01	<0.01	0.17
CH_4/FNDFI	105	97	87	80	3.10	88.0	97.0	3.57	<0.01	<0.01	0.15
CH_4/Cellulose	105	93	114	96	3.58	97.0	107.0	4.13	<0.01	<0.01	0.17
CH_4/Hemicellulose	252	179	297	192	8.25	219	241	9.50	<0.01	<0.01	0.17
Enteric CH_4 intensity, g/kg unless otherwise specified											
CH_4/milk yield	16.7	16.2	16.4	16.0	0.59	14.3	18.3	0.73	0.42	0.17	0.02
CH_4/FPCM[7]	15.3	15.0	15.4	14.7	0.50	14.6	15.6	0.62	0.84	0.09	0.34
CH_4/MFY[8]	355	352	359	343	13.0	354	351	16.0	0.73	0.19	0.91
CH_4/MPY[9]	509	492	518	504	18.6	497	515	21.2	0.40	0.23	0.60

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]None of the interaction effects were significant (data not shown).

[3]FNDFI: Forage NDF intake.

[4]MBW: Metabolic body weight which was calculated as $BW^{0.75}$.

[5]Based on DMI measured on week 3 (same week as enteric CH_4 measurement).

[6]NDFI: NDF Intake.

[7]FPCM: Fat-protein corrected milk which was calculated as per IDF (2010) formula.

[8]MFY: Milk fat yield.

[9]MPY: Milk protein yield.

Table 5. Effects of dietary treatments and breed on characteristics of ruminal fluid (n = 24; wk 4 data).

Item	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
pH	5.96	5.91	6.03	6.03	0.05	5.94	6.02	0.05	0.04	0.62	0.27
VFA, mol/100 mol unless otherwise specified											
Total, mM	124	116	129	122	3.74	128	117	3.15	0.12	0.04	0.07
Acetate (A)	67.0	65.7	68.4	67.7	0.62	66.7	67.7	0.51	<0.01	0.08	0.27
Propionate (P)	17.6	18.8	16.6	16.5	0.50	17.9	16.9	0.39	<0.01	0.23	0.15
Butyrate[3]	11.6	11.9	11.1	12.0	0.26	11.5	11.8	0.22	0.38	0.02	0.44
Iso-butyrate	0.74	0.64	0.73	0.66	0.03	0.66	0.73	0.02	0.86	<0.01	0.08
Valerate[4]	1.38	1.37	1.45	1.24	0.05	1.34	1.38	0.05	0.48	0.01	0.58
Iso-valerate	1.52	1.50	1.64	1.78	0.08	1.76	1.45	0.07	<0.01	0.38	0.04
A:P ratio	3.89	3.66	4.19	4.14	0.11	3.84	4.10	0.08	<0.01	0.17	0.11
NH_3-N, mg/dl	11.4	11.0	13.4	12.5	0.89	11.4	12.8	0.97	0.01	0.36	0.36

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]Significant interactions are shown in footnotes.

[3]Significant FNDF level×breed (P=0.02).

[4]Significant FNDF level×FNDF source (P=0.03).

Table 6. Effects of dietary treatments and breed on manure production and characteristics (n = 8; wk 4 data).

Item	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Production, kg/d unless otherwise specified											
Feces as-is	38.1	43.9	39.8	45.1	1.65	48.0	35.4	1.82	0.25	<0.01	<0.01
Urine as-is	24.1	19.3	23.9	21.2	0.93	23.6	20.7	0.77	0.35	<0.01	0.03
Manure[3] as-is	62.0	63.0	64.0	66.0	1.90	72.0	56.0	2.00	0.15	0.25	<0.01
Feces DM	5.59	6.02	5.62	6.80	0.20	6.65	4.86	0.24	0.44	0.02	<0.01
Urine DM	1.39	1.27	1.39	1.38	0.05	1.48	1.23	0.04	0.29	0.28	<0.01
Ratios[4], kg/kg											
Feces/Urine	1.62	2.26	1.67	2.15	0.09	2.08	1.77	0.09	0.72	<0.01	0.07
Manure/FPCM[5]	2.30	2.20	2.40	2.31	0.08	2.26	2.34	0.08	0.12	0.16	0.52

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]None of the interaction effects were significant (data not shown).

[3]Manure: Sum of feces plus urine only but no bedding materials.

[4]Ratios: Feces, urine and manure are expressed as-is basis.

[5]Manure/FPCM: Fat-and protein corrected milk used in this ratio was calculated as per IDF (2010) formula.

Table 7. Effects of dietary treatments and breed on N intake and partitioning (n = 8; wk 4 data).

Item	Dietary Treatments[1]					Breed			P value[2]		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Daily N (intake or excretion), g/d unless otherwise specified											
Intake N[3]	607	650	565	595	28.5	697	512	35.1	<0.01	0.04	<0.01
Fecal N (N_F)	165	170	165	156	6.68	190	139	8.06	0.12	0.66	<0.01
Urine N (N_U)	135	129	144	154	7.10	164	118	7.05	0.01	0.73	<0.01
Man[4] N (N_M)	300	300	310	311	10.8	354	256	13.4	0.09	0.96	<0.01
Milk N (N_L)	130	139	127	134	5.92	152	113	7.88	0.11	<0.01	0.01
UR[5] N (N_B)	178	211	129	150	20.7	191	143	21.5	<0.01	0.11	0.16
Daily N excretion as % of N intake unless otherwise specified											
Fecal N	27.4	26.3	29.4	27.1	1.01	27.6	27.5	1.04	0.09	0.05	0.95
Urine N	23.0	20.2	25.9	26.0	1.33	24.0	23.5	1.08	<0.01	0.32	0.80
Man N	50.0	46.5	55.3	53.1	1.89	52.0	51.0	1.87	<0.01	0.07	0.85
Milk N	21.5	21.6	22.5	23.1	0.80	24.0	23.5	1.08	<0.01	0.41	0.88
UR N	28.0	32.0	22.0	24.0	2.36	26.0	27.0	2.55	<0.01	0.15	0.94

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]None of the interaction effects were significant (data not shown).

[3]Intake of N reported in this Table (calculated using data from 8 cows collected during total collection in wk 4 data) was numerically different but statistically same than N intake shown in Table 2 (calculated using data from all 24 cows based on both wk 3 and wk 4 data).

[4]Man N: Manure N which was calculated as the sum of fecal plus urinary N without including bedding N.

[5]UR N: Unaccounted and retained N = N_B = Intake N - (Fecal N + Urinary N + Milk N).

Table 8. Effects of dietary treatments and breed on energy (E) intake and partitioning (n = 8; wk 4 data).

	Dietary Treatments[1]					Breed			P value[2]		
Item	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Mcal/d unless otherwise specified											
GE_I[3]	94.2	103	89.0	97.2	4.28	110	81.2	5.34	0.02	<0.01	<0.01
Fecal E	25.0	27.2	25.4	25.8	0.93	30.0	22.0	1.16	0.31	0.02	<0.01
DE[4]	69.2	76.0	63.3	71.2	3.76	80.4	59.4	4.51	0.03	<0.01	0.01
Urinary E	2.75	2.52	2.76	2.74	0.09	2.96	2.42	0.07	0.27	0.25	<0.01
CH_4 E	5.41	5.45	5.35	5.11	0.26	6.29	4.40	0.29	0.31	0.61	<0.01
ME[5]	61.0	68.0	55.2	63.4	3.80	71.2	52.3	4.60	0.03	<0.01	0.03
Milk NE_L[6]	20.6	21.6	20.3	21.6	0.86	24.0	18.0	4.16	0.07	0.03	<0.01
Maint. E[7]	8.48	8.58	8.49	8.52	0.17	10.0	7.05	0.24	0.43	0.05	<0.01
E balance[8]	40.4	46.4	34.9	41.8	3.5	47.2	34.3	4.17	0.04	0.01	0.09
% unless otherwise specified											
Fecal E	26.8	26.5	28.8	27.2	0.88	27.3	27.3	0.95	0.05	0.16	0.98
DE	73.2	73.5	71.2	72.8	0.88	72.7	72.7	0.95	0.05	0.16	0.98
Urinary E	3.10	2.48	3.17	2.87	0.22	2.71	3.09	0.18	0.29	0.04	0.20
CH_4 E	5.86	5.33	6.09	5.37	0.39	5.73	5.60	0.47	0.57	0.01	0.85
ME	64.2	65.7	62.0	64.5	1.25	64.2	64.0	1.42	0.06	0.03	0.91
Milk NE_L	22.3	21.2	22.9	22.6	1.03	22.0	22.5	1.18	0.16	0.35	0.76
Maint. E	9.13	8.38	9.60	8.95	0.32	9.10	8.88	0.36	0.04	<0.01	0.62
E balance	41.9	44.5	39.1	41.9	2.6	42.2	41.5	2.7	0.06	0.06	0.83

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]None of the interaction effects were significant (data not shown).

[3]GE_I: Gross Energy Intake.

[4]DE: Digestible Energy = GE_I - Fecal E.

[5]ME: Metabolizable Energy = DE – (Urinary E + CH_4 E).

[6]Milk NE_L based on Eq. 2-15 of NRC (2001).

[7]Maint. E: Maintenance Energy = 0.080 Mcal/kg $BW^{0.75}$ (NRC, 2001).

[8]Energy balance = GE_I – (Fecal E + Urinary E + CH_4 E + Maintenance E + milk NE_L).

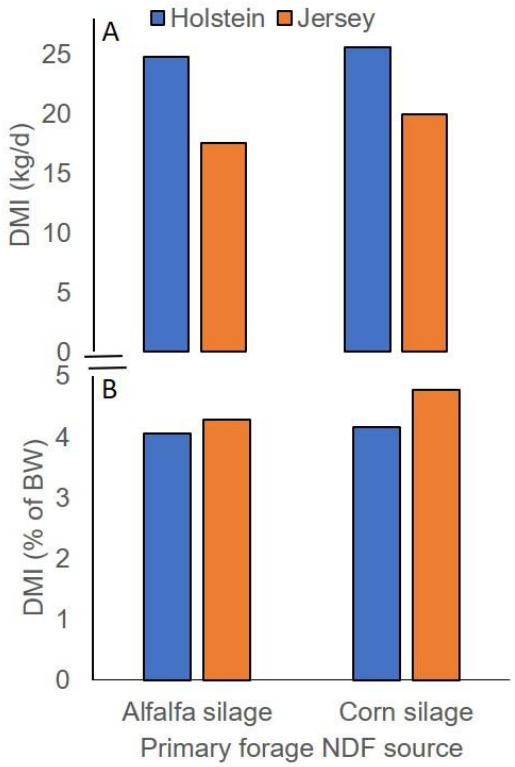

Figure 1. Breed × forage NDF source interaction on DMI when expressed as kg/d (A; $P = 0.04$), and % of BW (B; $P < 0.01$).

CHAPTER 3. Effect of dairy cow breed, and diet on greenhouse gas emissions from manure during storage and after field application

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HIGHLIGHTS

- Breed and diet effects on manure greenhouse gas (GHG) emission were assessed.
- Greater loss of volatile C than N increased manure C:N ratio during storage.
- Methane loss was 25 times greater during manure storage than after field-applied.
- But nitrous oxide loss was 19 times lower during storage than after field-applied.
- 100-d GHG (50-d storage + 50-d field) was greater for low than high forage-fed cows.
- Neither cow breed nor forage source affected 100-d GHG emissions.

ABSTRACT

On-farm greenhouse gas (**GHG**) emissions from cows, manure, and fields (to produce feed) comprise more than 72 % of the United States milk carbon footprint. Recent studies examined the impact of dietary strategies on enteric methane (**CH_4**) emissions, however, tradeoffs between enteric CH_4 and manure related GHG emissions have not been determined. Thus, the objective of this study was to determine the carry-over effects of dairy cow breed and diet on manure composition and manure GHG emissions during storage and after field application. Feces and urine from eight primiparous, mid-lactation cows (four Holstein and four Jersey) were collected from a companion study over three days (d). Four cows in each breed were fed four different diets which were arranged as 2×2 factorial with two levels of forage neutral detergent fiber [**FNDF**, 19 (low FNDF, **LF**) vs. 24% (high FNDF, **HF**) of dry matter] and two sources of FNDF [alfalfa silage (**AS**) vs. corn silage (**CS**); either at 70:30 or 30:70 ratio, on a dry matter basis]. The crude protein, neutral detergent fiber, starch and energy content were similar across diets. Carbon di-oxide (**CO_2**), CH_4, and nitrous oxide (**N_2O**) were measured using a static chamber method over 50 d of storage and 50 d of field measurement (30 d in the fall and 20 d in the following spring) after land application of manure. None of the interactions among treatment factors were significant ($P > 0.10$). Cow breed did not affect manure composition, which was affected by both FNDF level and FNDF source. Manure pH was lower, but acid detergent fiber and starch concentrations were greater for LF-fed than HF-fed cows. Compared to AS, CS-fed cows had greater manure organic matter, total carbon, neutral detergent fiber and acid detergent fiber concentrations. All the variables for manure composition measured changed over time from the beginning to the end of storage period except starch. Treatments did not affect either hourly CO_2, CH_4 and N_2O fluxes, nor cumulative emissions (over 50-d of storage or over 50-d after land

application), except a tendency significant ($P < 0.10$). to emit 22% lower manure CO_2 by HF-fed cows than LF-fed cows. Cumulative CH_4 and N_2O emissions were respectively 25 times greater and 19 times lower during the 50-d manure storage than the subsequent 50-d after field application. Cumulative field N_2O emission was 17 times greater during spring than fall. In this study, manure of LF-fed cows tended to emit 51 to 72% greater combined (storage plus field) non-CO_2 GHG emissions than HF-fed cows (depending on mode of expressions). However, neither cow breed nor FNDF source affected combined non-CO_2 GHG emissions.

Keywords. alfalfa silage, corn silage, forage level, forage source, Holstein, Jersey

1. INTRODUCTION

By the end of the first decade of this century, the United States dairy sector contributed nearly 2% of the total US greenhouse gas (**GHG**) emissions (Thoma et al., 2013). The three major GHG emitted from dairy production systems are carbon dioxide (**CO_2**), methane (**CH_4**) and nitrous oxide (**N_2O**) (Owen and Silver, 2015). Enteric fermentation (cow emission), the emissions during collection, processing and storage of manure and field emissions associated with feed production are the three main sources of on-farm GHGs contributing roughly 72% of the milk carbon footprint (**CF)** in the United States (Thoma et al., 2013). Manure CH_4 emission contributed about 43% of the total dairy CH_4 emissions in the United States (USDA, 2011). Emissions of CH_4 and N_2O varied considerably depending on the manure management (modes of collection, processing, storage, and field application) and in particular duration of storage and method of processing (Aguirre-Villegas and Larson, 2017). Thus, GHG mitigation practices in the manure chain has potential to reduce the CF of milk and overall GHG emissions from dairy production systems.

Among enteric emission mitigation strategies, feeding and nutritional management has been considered one of the best short-term strategies because of ease of application, cost-

effectiveness and farmer adoption potential (Hristov et al., 2013; Knapp et al., 2014). Replacing neutral detergent fiber (**NDF**; mainly forage) with starch (mainly corn gain) in the diet of dairy cows reduced enteric CH_4 production (g/d) and intensity (g/ kg of milk) (Aguerre et al., 2011). Also, the substitution between corn silage (**CS**) and alfalfa silage (**AS**) in the forage portion of the diet had significant quadratic effect on enteric CH_4 emission with the greatest emissions were reported when, on a dry matter (**DM**) basis, the AS to CS ratio was roughly 50:50 (Arndt et al., 2015; Hassanat et al., 2013). These studies also reported lower manure nitrogen (**N**) excretion (the major source of N_2O from manure) and greater NDF excretion (a major source of CH_4 from manure) when cows were fed more CS than AS. Therefore, increasing CS at the expense of AS in dairy cow diet might decrease manure N_2O and increase manure CH_4 emissions (Hassanat et al., 2013). Few studies, however, have combined measurements of CH_4 and N_2O emissions during manure storage and after land application. Nevertheless recent studies have emphasized the importance of evaluating nutritional mitigation strategies on whole-farm GHG emissions in order to account for possible interactions or tradeoffs that may exist among sub-systems (Hristov et al., 2013; Montes et al., 2013; Olijhoek et al., 2018; Wattiaux et al., 2019). Thus, one needs to study dietary effects not only on enteric emissions but also the carry-over effects on manure GHG emissions during storage and after land application.

Possible differences between Holstein and Jersey breed has called the attention of researchers. For example, Aikman et al., (2008) reported that Jersey cows had greater NDF digestibility (59.5 vs. 56.5 %) and lower urinary N excretion (163 vs. 211 g/d per cow) than Holstein cows fed the same diet, and Capper and Cady (2012) constructed a model that predicted that the production of 50,000 tons of cheddar cheese with Jerseys would reduce N excretion (18%) and CF (20%) compared to Holsteins. As the Jersey breed is gradually becoming more popular in

the US due to smaller body size, greater adaptability to adverse condition, and lower fertility problems than Holstein (Hoards Dairyman, 2015), the full extent of the effect of breed on milk CF remains to be fully assessed.

To the best of our knowledge, no study has examined the main effects and interactions among dairy cow breed and dietary composition on GHG emissions measured from the cow, the manure and the field. Measures of cow performances, efficiencies, and enteric CH_4 emission in response to these effects have been reported in a companion study (Uddin et al., 2019). The objective of the study reported here was to determine the effects of dairy cow breed, dietary FNDF level and FNDF source on manure composition, GHG emissions during manure storage and after land application of manure.

2. MATERIALS AND METHODS

The University of Wisconsin-Madison Institutional Animal Care and Use Committee approved the protocols used in this study and those used in the companion study to feed the cows and collect the manure. This study was completed with two parts conducted consecutively. In part I, we measured emissions during manure storage (September – October 2017) and in part II we measured emissions after land application (November – December during fall of 2017, and April - May during spring of 2018).

2.1 Part I: Emissions during manure storage

Cow feces and urine were collected during a companion study designed as a split-plot, triplicated 4×4 Latin square, in which cow breed (Holstein and Jersey) formed the main plot and diet formed the subplots (Uddin et al., 2019). Four dietary treatments were arranged as 2×2 factorial where factors were level of FNDF [19 (**LF**) vs. 24% (**HF**), DM basis] and source of FNDF (70:30 vs. 30:70 ratio of AS NDF:CS NDF).

2.1.1. Experimental design

Although 24 cows (12 Holstein and 12 Jersey) were used in the companion study, urine and feces were collected from eight cows (one Latin square from each breed) during the last three days of the third period. Cows in individual tie-stall were catheterized to collect urine in unacidified carboys whereas the feces were collected on a stainless-steel pan fitted in the gutter. During collection, no bedding material was used in the stall. Fecal material was scraped in the gutter as needed to keep cows clean and improve accuracy of collection. Although there was no attempt to alter barn temperature or ventilation, the loss of carbon (**C**) and N was expected to be minimal during collection because of the separation of urine from feces (Vaddella et al., 2010).

For this study, manure (feces + urine) was reconstituted in triplicate 16-gallon plastic barrels by mixing feces and urine in the ratio (wet weight basis) produced from each cow. All 24 barrels (two cow breeds × four dietary treatments × three replications) were kept at room temperature ranging from 20 to 23°C (Livestock Laboratory, University of Wisconsin-Madison). Each barrel contained approximately 54 kg (as-is) of manure leaving approximately 15 cm headspace to allow for GHG flux measurements. Feces and urine were initially stirred for 1 min, mixed, and stirred again for 3 min using a drill mixer set at 400 rpm (to avoid a vortex and yield homogenous contents). During mixing, a sample of about 700 g was collected from each barrel. The pH (accumet^{TM} AP85 portable pH meter) was measured prior to refrigeration of the sample at 4° C for shipment to the university of Wisconsin Soil and Forage Analysis Laboratory (Marshfield, WI, USA) where samples were analyzed within three days of sampling for DM, total N, ammoniacal-N, Acid detergent fiber (**ADF**), NDF, starch, ash, organic matter (**OM**), total C, and C:N ratio. At the end of 50 days of storage, the manure content of each barrel was weighed, and

mixed using drill mixer for 5 min at 400 rpm and a sample was collected for the same analyses as described above for initial sample.

2.1.2. GHG measurements

The manure CO_2, CH_4 and N_2O fluxes were measured using a static chamber method as described by Collier et al. (2014) and Oates et al. (2016) in accordance with the US Department of Agriculture-Agricultural Research Service's GHG reduction through agricultural carbon enhancement network (GRACEnet) protocol (Parkin and Venterea, 2010). Measurements were on the following eight days (d) of storage: d 3, d 5, d 10, d 17, d 24, d 31, d 38 and d 50 (between September 19th and November 6). For each sampling event, a lid was sealed at the top of the barrel to convert the headspace in a gas accumulation chamber. A 30 mL air sample was drawn immediately from the chamber using a 30 mL nylon syringe (BD slip tip sterile syringe) fitted with a 23-gauge needle and two-way stopcock. Subsequently, three more samples were drawn at 16 min intervals over a 48 min period. Samples were then transferred to a pre-labelled 5.9-ml Exetainer vials (Labco Limited, Buckinghamshire, UK) piercing septum with flushing method as described by Oates et al. (2016). All samples were taken between 1000 and 1500 h local time. After sampling, lids were removed, and barrels were left open until the next sampling event. Gas samples were then analyzed for GHG concentrations using gas chromatography (IRGA, LiCor 820, Lincoln, NE, USA) fitted with particular gas detector (CO_2- infrared gas analyzer, IRGA, LiCor 820, Lincoln, NE, USA; CH_4- electron capture detector, micro-ECD, Agilent 7890A gas chromatography System, Santa Clara, CA, USA; N_2O- flame ionization detector, FID, Agilent 7890A). Calibration of gas chromatography was conducted using standard CO_2, CH_4 and N_2O gas with the concentrations of 400, 1 and 1 ppm, respectively.

2.2. Part II: Emission after land application

Field measurements of manure GHG emissions were conducted at Agronomy field laboratory in three 42.5×27.5 m^2 main plots (plots # G1-101, G1-212 and G1-309), Arlington Agricultural Research Station, University of Wisconsin-Madison. No tillage, continuous corn had been growing on these plots for 8 to 10 years. The soil type of these plots was classified as Plano silt-loam (Jokela et al., 2011).

2.2.1. Experimental design

Manure from eight barrels (two breeds × four diets) from each replication was randomly applied to eight sub-plots in one of the main plots. Eight sub-plots of 0.91×0.91 m^2 were located within the main plots. Main plots G1-101, G1-212 and G1-309 randomly received the replication I, III and II, respectively. The slurry was surface-applied manually to each sub-plot in amounts (ranging from an equivalent of 5638 to 8351gallon ha^{-1}) calculated to yield 180 kg available N ha^{-1} based on compositional analysis performed at the beginning of the storage. This N application rate is typical for loamy soil of Wisconsin (Laboski and Peters, 2012).

2.2.2. GHG measurements

Emission measurements were performed using cylindrical stainless-steel chambers. For field emissions, six measurements were taken between November 5th and December 5th 2017 on d 53, d 56, d 59, d 66, d 73 and d 80 relative to the day of manure reconstitution. Sampling ceased due to permanent snow cover but was reinitiated in the spring with four measurements conducted between 25 April and 14 May 2018 (d 221, d 228, d 234 and d 240 after manure reconstitution). Sampling and analytical procedures were as described above for the manure storage.

2.3. Accessory measurements and records

Ambient temperature and relative humidity were recorded at three time points in each sampling day during both part I and II using a digital indoor-outdoor hygrometer (Thermopro, TP60S). Soil temperature was also recorded in part II for each chamber separately using a soil temperature probe (Checktemp 1C, Hanna Instruments, Smithfield, RI, USA). Height of barrel's headspace in part I and height of cylindrical steel chamber in part II were measured and recorded on every sampling day. Any weather-related event such as snowfall or rainfall during the field sampling period were also recorded.

2.4. Calculation of GHG fluxes

Four gas concentrations against respective time points were fitted to a linear regression model. The slope of the regression was used to calculate gas flux as per Collier et al. (2014) as follows:

$$F = S \times V \times A^{-1}$$

Where, F = GHG gas flux (mg/h per kg raw manure), S = slope of the regression (mg/h per m^3), V = chamber volume (m^3), and A = chamber surface area (m^2).

Approximately, 2% of the calculated fluxes were discarded when visual observation of regression plot indicated a measurement problem (e.g., leakage). The missing fluxes were calculated using linear interpolations whenever possible. During manure storage, N_2O fluxes from day 3 and 5 were discarded because emission values were negligible, noisy and sometimes negative. The calculated hourly flux was then converted into daily aggregate fluxes assuming the estimated flux was the average flux of the sampling day. The cumulative (over 50-day of storage or 50-day of field) emission for each gas and each chamber for whole experimental period were calculated by

trapezoid area under the curve method. The 100-d cumulative (storage + field) emission was calculated for both CH_4 and N_2O. Combined GHG emissions expressed as CO_2-equivalent (CO_2-e) were calculated by summing cumulative emissions where CH_4 and N_2O were multiplied by their global warming potential of 28 and 298, respectively (Myhre et al. 2013).

2.5. Data Analysis

The hourly GHG fluxes (mg/h per kg raw manure) were analyzed with the lme function of lme4 package in R version 3.5.3 using the repeated measure mixed effects model where fixed effects were cow breed, FNDF level, FNDF source, day of GHG measurement, and all possible interactions among FNDF level, FNDF source and day of GHG measurement. The cow was fitted as random effect, and the auto-correlation covariance structure with a continuous day as covariate was fitted using corCAR1 function.

Cumulative storage (50-d) and cumulative field (50-d) emissions, as well as cumulative total (storage plus field) emissions of each GHG, combined GHG and manure composition were analyzed using a simpler non-repeated mixed model containing fixed effect of cow breed, FNDF level, FNDF source, interaction between FNDF level and FNDF source; and random effect of cow.

Due to presence of interactions between treatment factors × day of measurement, the contrasts of cumulative manure GHG emissions during storage vs. field and fall-field vs. spring-field measurements were tested using unequal variance t-test. Additionally, a two tailed paired t-test was performed to assess the difference between initial and final manure composition during storage. Significance level and tendency were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively. Non-significant interactions effects are not reported.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of manure

Effects of cow breed and dietary treatments on initial manure composition are shown in Table 1. Breed did not affect chemical parameters of manure except for DM and starch content. The lower DM content of Jersey manure compared to Holstein aligned with their lower feces to urine ratio as determined in the companion study (1.77 vs. 2.08; Uddin et al., 2019). Compared to HF, LF-fed cows had 0.18 pH unit lower manure pH but 2.20 and 0.54 percentage unit greater ADF and starch content, respectively. In contrast to our findings, Aguerre et al. (2011) reported no change in manure pH when forage level increased from 47 to 68% of dietary DM (in comparison to 54 and 68 % of dietary DM in our LF and HF diet, respectively). Compared to AS, CS-fed cows had 2.25, 1.30, 1.30, and 4.00 percentage unit greater manure OM, total C, ADF and NDF content, respectively. This difference may be associated with a greater digestibility of the fiber (NDF) in CS than AS as observed in companion study, but may also be due to the composition of the AS and CS based diet, in particular the fact that the CS diet contained 6.6 percentage unit greater soyhull than the AS diets (7.9 vs 14.5%, respectively; Uddin et al., 2019).

Effects of treatments on manure composition either persisted after 50 days of storage (e.g., the effect of breed on moisture content, the effect of FNDF level on pH, or the effect of FNDF source on NDF content), or no longer existed (e.g., the effect of FNDF level on moisture and starch content or the effect of FNDF source on ADF content; Table 1). The overall chemical composition of manure after 50 days of storage differed substantially from the manure entering storage (Table 2). During storage, DM content increased, however, OM, total C, ADF and NDF content decreased reflecting continued anaerobic fermentation of dietary fiber with production and emission of gaseous C. Similarly, the 42% increase in ammoniacal-N content reflected substantial degradation

of N-containing organic compounds during storage, but the 44% increase in total N content reflected most likely a comparatively much lower loss of volatile N compared to volatile C. The substantially (32%) reduction in C:N ratio after 50 days of storage suggested that stored manure may behave differently than un-stored manure after application.

3.2. Manure GHG during storage (Part I) and after field application (Part II)

Results will be presented focusing first on the hourly pattern of GHG fluxes as illustrated in Figures 1, 2, and 3, for CO_2, CH_4 and N_2O, respectively followed by an analysis of Table 3 showing the results of the cumulative emission during the 50-d of storage, during the 50-d of measurement in the field and the 100-d emission for each GHG. The comparison between 50-d storage vs. 50-d field emissions and between 30-d fall vs. 20-d spring field emissions are shown in Table 4. The field measurement site average annual rainfall and snowfall were 870 mm and 99 cm, respectively (US climate data, 2019). The average ambient temperature for fall and spring during GHG measurement period were 6.2 and 32.0°C, respectively (Figure 4). Whereas the average soil temperature for fall and spring were 1.8 and 8.7°C, respectively (Figure 4).

3.2.1 CO_2 flux and cumulative CO_2 emissions

Emission of CO_2 from enteric fermentation and from manure are not accounted for in determining CF of milk using life cycle assessment (Baldini et al., 2018; Thoma et al., 2013) because cattle consume forages that removed atmospheric CO_2 through photosynthesis. Nevertheless, CO_2 contributes substantially to C-cycle of dairy systems. In our study, breed and FNDF source did not affect manure CO_2 flux (Figure 1) or cumulative CO_2 emissions (Table 3). However, manure from LF-fed cows tended to have 9.20 percentage unit (or 29%) greater cumulative CO_2 emission than HF-fed cows during the 50-d field measurement, which was a difference observed in the fall (27% greater cumulative CO_2 emissions during fall for LF-fed cows

than HF-fed cows) but not the spring. Furthermore, there was a tendency for greater CO_2 flux during storage than after field application (i.e., interaction between FNDF level × day of measurement (Panel B in Figure 1). The cumulative CO_2 emission was 2.9 times greater during the 50-d storage than during the 50-d after manure application (Table 4). Likewise, cumulative CO_2 emission was 1.2 times greater during fall than spring measurements (Table 4). Notably, CO_2 fluxes were much more variable in the spring, as indicated by a 50% increase in the SEM, compared to the fall (Table 3). Other studies reported substantial CO_2 emission during storage of manure (Aguerre et al., 2012; Holly et al., 2017) and the CO_2 flux in our study was comparable with those reported by Aguerre et al., (2012). However, 100-d cumulative CO_2 (storage plus field) emissions (g/kg raw manure) was 7.8 times greater in our study than the values reported by Holly et al. (2017) who measured CO_2 fluxes even longer period (182-d storage and 126-d after land application). This discrepancy may be due to multiple reasons. First, analytical measurement of CO_2 differed between the two studies. Second, we used reconstituted manure whereas Holly et al. (2017) used manure which included bedding materials and waste-water. Third, manure C content in our study was much greater (47875 vs. 22833 mg/kg raw manure) than reported in Holly et al. (2017). Fourth, temperature during storage ranged from 20 to 23 °C in our study but from 1 to 18 °C in Holly et al. (2017). Fifth, the formation of an organic crust (which occurred in Holly et al. (2017) but not in our study) that may contribute to a reduction in CO_2 and CH_4 emissions but an increase in N_2O emission (Aguerre et al., 2012).

3.2.2 CH_4 flux and cumulative CH_4 emissions

During storage, CH_4 fluxes tended to be greater for LF than HF-fed cows and for CS than AS-fed cows (Figure 2). However, these differences did not persist during the field measurement period (i.e., significant interactions between FNDF level × day of measurement, and FNDF source

× day of measurement). Methane fluxes were noticeably greater during storage than during the field measurement period. The peak in CH_4 flux observed approximately at week 2 of storage followed by a gradual drop afterward (Figure 2) was a pattern reported also in Aguerre et al. (2012). Three possible processes have been identified as possibly contributing to a decrease in CH_4 flux in the latter part of storage. First, a decrease in pH may reduce methanogenesis. Second, the formation of a crust, when it happens, may act as physical barrier to gaseous emissions that originate from the anaerobic environment of the stored manure (VanderZaag et al., 2008). Third, the micro-environment of the stored manure may favor oxidative bacteria that convert CH_4 to CO_2 (Peterson et al., 2005). During field measurement, CH_4 emission values were almost zero and approximately 19 % CH_4 fluxes in our study were negative particularly during field measurements. Since most field measurements were conducted during the fall (November to December) when both ambient and soil temperature were very low which might have contributed to the negative CH_4 fluxes through favoring the consumption of CH_4 and converting it into CO_2 by oxidative bacteria (Figure 4; Butterbach-Bahl et al., 2013; Conrad, 2007; Gao et al., 2014; Peterson et al., 2005).

Compared to HF, LF-fed cows manure tended to emit greater CH_4 during storage (3.65 vs. 2.24 g CH_4/kg of manure) resulting in greater 100-d cumulative CH_4 emissions (3.78 vs. 2.35 g CH_4/kg of manure; Table 3). This effect might be due to greater availability of fibrous substrate in the manure of cows fed LF diet compared to those fed the HF diet (e.g. ADF and NDF; Table 1). Although CH_4 emission during field measurements were less than 5% of the 100-d cumulative emissions, we found that more CH_4 was emitted from the field when the manure was from Holstein compared to Jersey, a difficult-to-explain difference that existed in the fall but not in the spring (Table 3). Overall, cumulative CH_4 emission was 25 times greater during the 50-d storage than 50-

d of field measurements, which in turn was five times greater in the fall than in the spring (Table 4). Cumulative total CH_4 emissions (g/kg raw manure) in our study was 13 times greater than the values reported by Holly et al. (2017). The five reasons mentioned above to explain the discrepancy for CO_2 emissions between the two studies might also apply to explain the discrepancy in CH_4 emission. Yet, manure processing can help to reduce C loss from manure during storage and after field application. For instance, Holly et al. (2017) reported that compared to raw manure, anaerobically digested manure had 44% less C loss during storage and from field mainly due to reduction of CH_4 emission.

3.2.3 N_2O flux and cumulative N_2O emissions

None of the treatment factors of this study affected N_2O flux which was affected only by the day of measurement (Figure 3). The N_2O fluxes were almost nil during manure storage and during fall measurement in the field but there was a sharp spike for N_2O flux during spring measurement in the field (Figure 3). Approximately, 9% N_2O fluxes were negative particularly fluxes measured during first week of manure storage. Research showed that negative N_2O flux could happen due to direct consumptions of N_2O (Wen et al., 2016). However, most of the negative N_2O fluxes were likely due to methodological limit for detections (Cowan et al., 2014).

However, cumulative N_2O was 19 times greater during field emission than during storage (Table 4). Although, a similar pattern was reported by Aguerre et al. (2012) and Holly et al. (2017), the 100-d cumulative N_2O emission was 2.3 times greater in our study than in Holly et al. (2017). Three reasons may explain this difference. First, initial manure N content was greater in our study than in Holly et al. (2017) (3560 vs. 2205 mg/kg raw manure). Second, differences in soil type and weather conditions. Third, the use of different measurement techniques. In our study, field N_2O emission was 17 times greater during 20-d spring than 30-d fall measurement periods (Table 4).

The substantially greater field N_2O emission during spring that the fall was most likely associated with the difference in temperatures (Figure 4) since increasing temperature is known to increase N_2O emission (Butterbach-Bahl et al., 2013), and greater water filled pore-spaced in spring than in the fall due to melting covered snow and event of rainfall as observed in this study. As suggested by our data and other research (Sun et al., 2016; Wattiaux et al. 2019), N_2O emissions could be reduced by avoiding application of manure or N-fertilizer during or around heavy precipitations.

3.3 Storage plus field non-CO_2 GHG emissions expressed as CO_2-e

Taking into account fat-and protein corrected milk (**FPCM**) yield and manure production differences for four dietary and two breed scenarios, additional calculation was performed to determine storage plus field non-CO_2 (CH_4 plus N_2O) GHG emissions over 100-d period expressed as CO_2-e (g/kg manure, g/kg FPCM, and kg/cow).

These GHG emissions tended to be greater for LF than HF diet when expressed as g CO_2-e/kg of manure (56% greater, Figure 5),expressed as g CO_2-e/kg of FPCM (72% greater, Figure 6), and expressed as kg CO_2-e/cow (51% greater, Figure 7). As illustrated in these Figures the greater emissions for manure of LF-fed cows than HF-fed cows were associated mainly with greater emission during storage rather than after field application. As discussed above, manure of LF-fed cows had greater availability of substrates to form GHG than manure of HF-fed cows. The share of CH_4 and N_2O to total non-CO_2-e of GHG were 88.6 and 11.4%, respectively. These percentages of CH_4 and N_2O were not affected by treatment factors (data not shown).

4. CONCLUSIONS

Cow breed and FNDF source did not affect any of the measured CO_2, CH_4, and N_2O fluxes or cumulative GHG emissions determined in this study. However, there was tendency to emit greater cumulative manure CO_2 during field and cumulative CH_4 during storage measurements for LF fed cows than HF-fed cows. Cumulative manure CH_4 emissions was 25 times greater during 50-d storage than 50-d field emissions but cumulative manure N_2O emissions was 19 times lower during 50-d storage than 50-day field emissions. In addition, cumulative field N_2O emission was 17 times greater during spring than fall. In this study, compared to HF, LF-fed cows tended to emit substantially greater amount of non-CO_2 GHG expressed as CO_2-e either per kg manure or per kg FPCM or per cow basis.

ACKNOWLEDGEMENTS

We would like to acknowledge the advice and assistance for fieldwork and sampling from fellow lab members Omar Ivan Santana, Tanner Wears, Fernanda De Kassia Gomes, Fei Sun and Benito Albarran Portillo. We are also thankful to Greg Sanford and Larence Oates who are scientist at Wisconsin Energy Institute, University of Wisconsin-Madison. Authors also express their gratitude to statistical consultant Tedward Erker for his help with statistical analysis.

FUNDING

This project was financially supported by the National Institute of Food and Agriculture, US Department of Agriculture Hatch Multi-state research formula fund (# WIS01941, and USDA-NIFA # 2013-68002-20525).

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Table 1. Effects of cow breed, forage NDF level and forage NDF source on chemical composition of reconstituted manure at the beginning (day 0) and end (day 50) of storage.

	Dietary Treatments[1]					Breed			*P* value		
Item	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Day 0 manure composition, % of dry matter (DM) unless otherwise specified											
pH	8.00	7.60	7.86	8.10	0.19	7.85	7.91	0.14	< 0.05	NS[2]	NS
DM, % of fresh	10.6	10.4	10.5	9.7	0.52	10.8	9.8	0.36	< 0.10	< 0.10	< 0.01
OM	79.0	82.4	79.1	80.2	1.80	80.4	79.9	1.25	NS	< 0.05	NS
Total C	45.8	47.8	45.9	46.5	1.00	46.7	46.3	0.72	NS	< 0.05	NS
Total N	3.60	3.30	3.30	3.80	0.29	3.42	3.52	0.21	NS	NS	NS
NH_4-N	2.00	1.83	1.85	2.20	0.26	1.94	2.0	0.18	NS	NS	NS
ADF	32.7	34.2	30.7	31.8	1.00	32.4	32.3	0.71	< 0.01	< 0.05	NS
NDF	45.7	50.5	45.0	48.2	1.77	47.9	46.9	1.25	< 0.10	< 0.01	NS
Starch	1.34	1.54	0.96	0.85	0.20	1.44	0.90	0.14	< 0.01	NS	< 0.01
C:N	12.8	14.7	14.0	12.8	1.19	13.7	13.5	0.84	NS	NS	NS
Day 50 manure composition, % of dry matter (DM) unless otherwise specified											
pH	7.19	6.71	7.14	7.19	0.07	7.05	7.06	0.05	< 0.05	<0.05	NS
DM, % of fresh	11.6	11.1	11.6	10.7	0.42	11.9	10.6	0.30	NS	NS	< 0.01
OM	77.1	79.3	77.7	78.6	0.30	78.3	78.0	0.22	NS	< 0.05	NS
Total C	44.7	46.0	45.1	45.6	0.17	45.4	45.3	0.12	NS	< 0.01	NS
Total N	5.24	4.88	4.83	5.22	0.21	4.96	5.13	0.15	NS	NS	NS
NH_4-N	2.85	2.70	2.55	3.12	0.18	2.67	2.94	0.13	NS	NS	NS
ADF	29.3	29.0	30.6	29.8	0.37	29.5	29.8	0.27	< 0.05	NS	NS
NDF	38.4	39.6	39.1	41.6	0.47	39.6	39.7	0.34	< 0.05	< 0.01	NS
Starch	1.36	1.12	1.13	0.97	0.13	1.24	1.06	0.09	NS	NS	NS
C:N	8.59	9.50	9.50	9.17	0.40	9.25	9.13	0.28	NS	NS	NS

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]NS: Non-significant with $P > 0.1$.

Table 2. Comparison of chemical composition of manure entering storage (initial) and removed from storage (final) 50 days later.

Parameters	Initial composition (mean ± SD)	Final composition (mean ± SD)	Paired t-test *P*-value
pH	7.88 ± 0.05	7.05 ± 0.05	< 0.01
DM, % of fresh	10.29 ± 0.17	11.28 ± 0.25	< 0.01
OM[1]	80.2 ± 0.48	78.2 ± 0.22	< 0.01
Total Carbon[1]	46.5 ± 0.28	45.4 ± 0.13	< 0.01
Total N[1]	3.47 ± 0.07	5.00 ± 0.10	< 0.01
NH_4-N[1]	1.97 ± 0.06	2.80 ± 0.10	< 0.01
ADF[1]	32.3 ± 0.34	29.67 ± 0.21	< 0.01
NDF[1]	47.4 ± 0.59	39.7 ± 0.33	< 0.01
Starch[1]	1.17 ± 0.10	1.14 ± 0.07	NS[2]
C:N	13.6 ± 0.30	9.20 ± 0.19	< 0.01

[1] % of dry matter.

[2]NS: Non-significant with $P > 0.1$.

Table 3. Effects of cow breed, dietary forage NDF level and forage NDF source on cumulative CO_2, N_2O and CH_4 emissions of manure during storage and after field application.

Item	Dietary Treatments[1]					Breed			*P* value		
	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Cumulative CO_2 emission, g/kg raw manure unless otherwise specified											
100-d total	154	177	118	121	20.7	148	137	14.6	NS[2]	NS	NS
50-d storage	114	134	87.9	86.9	19.3	109	103	13.7	NS	NS	NS
50-d field	40.2	42.4	30.5	33.7	3.79	39.1	34.2	2.68	< 0.10	NS	NS
30-d fall	21.8	22.9	15.9	19.4	1.59	21.7	18.3	1.13	< 0.10	NS	NS
20-d spring	18.4	19.5	14.5	14.3	2.39	17.4	15.9	1.69	NS	NS	NS
Cumulative CH_4 emission, g/kg raw manure unless otherwise specified											
Total	2.84	4.72	2.46	2.23	0.58	3.52	2.60	0.41	< 0.10	NS	NS
Storage	2.69	4.61	2.34	2.14	0.57	3.36	2.53	0.40	< 0.10	NS	NS
Field	0.148	0.116	0.120	0.091	0.029	0.167	0.071	0.021	NS	NS	<0.05
Field-Fall	0.130	0.104	0.098	0.065	0.035	0.142	0.056	0.024	NS	NS	<0.10
Field-Spring	0.017	0.011	0.022	0.027	0.008	0.025	0.014	0.005	NS	NS	NS
Cumulative N_2O emission, mg/kg raw manure unless otherwise specified											
Total	40.1	46.3	33.9	35	4.99	41.8	35.9	3.53	NS	NS	NS
Storage	2.78	2.12	1.11	1.80	0.59	2.45	1.46	0.42	NS	NS	NS
Field	37.4	44.2	32.8	33.2	5.09	39.3	34.4	3.60	NS	NS	NS
Field-Fall	1.64	3.15	1.49	1.74	0.55	2.10	1.92	0.39	NS	NS	NS
Field-Spring	35.7	41.1	31.3	31.4	5.05	37.2	32.5	3.57	NS	NS	NS

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]NS: Non-significant with $P > 0.1$.

Table 4. Comparison of cumulative (mean ± SE) CO_2, CH_4 and N_2O emissions during 50 days of storage vs. 50 days of manure applied soil measurement and fall-field vs. spring-field measurements.

GHG	Storage	Field	Field-fall	Field-spring	Contrasts[1] *P*-value	
					I	II
CO_2 g/kg manure	106 ± 6.15	37.0 ± 1.54	20 ± 0.95	17.0 ± 0.81	< 0.01	< 0.05
CH_4 g/kg manure	2.94 ± 0.26	0.118 ± 0.01	0.099 ± 0.015	0.019 ± 0.004	< 0.01	< 0.01
N_2O, mg/kg Manure	1.95 ± 0.32	36.9 ± 2.55	2.00 ± 0.29	34.9 ± 2.49	< 0.01	< 0.01

[1]Contrasts: I = storage vs. field, II= fall-field vs. spring-field measurements.

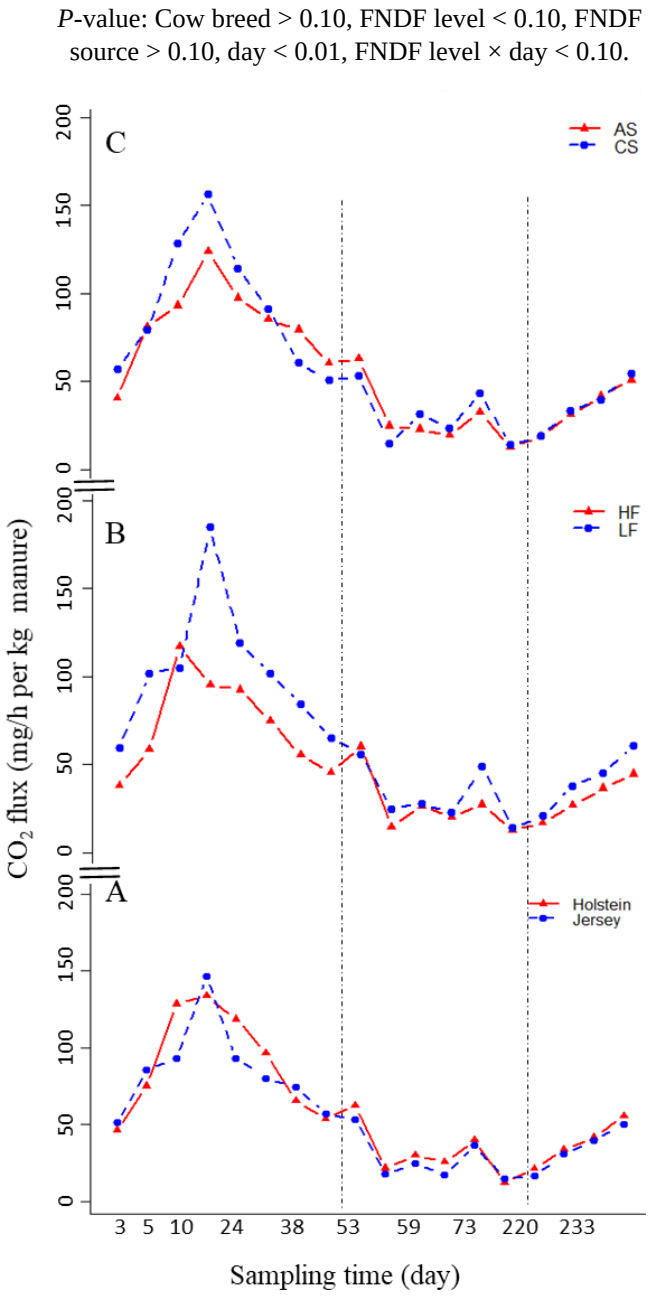

Figure 1. Hourly CO_2 fluxes (mg/kg of raw manure) as affected by cow breed (panel A), dietary forage NDF level (panel B) and forage NDF source (panel C). In each panel, data on the left side of the dotted vertical line were from 50-d manure storage (September to November 2017), data between two vertical lines were from 30-d of field measurements during fall (November to December, 2017) and data on the right side of hatched vertical line were from 20-d of field measurements during spring (April to May, 2018).

P-value: Cow breed > 0.10, FNDF level < 0.10, FNDF source > 0.10, day < 0.01, FNDF level × day < 0.01, FNDF source × day < 0.05.

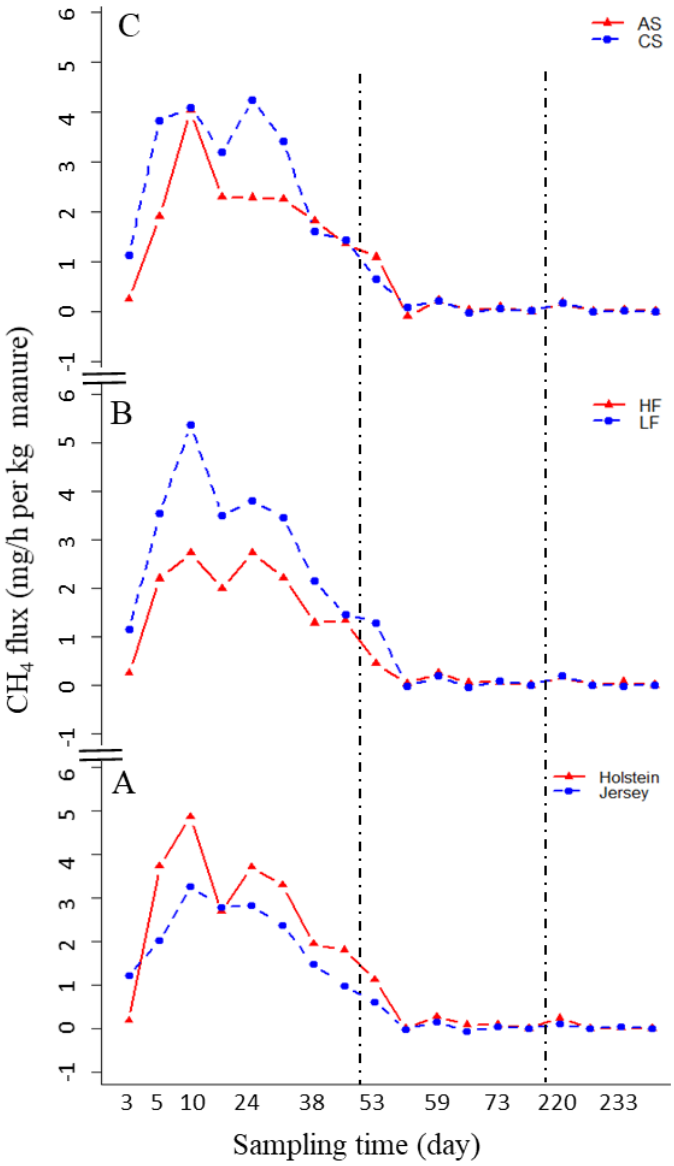

Figure 2. Hourly CH_4 fluxes (mg/kg of raw manure) as affected by cow breed (A), dietary forage NDF level (B) and forage NDF source (C). In each panel, data on the left side of the dotted vertical line were from 50-d manure storage (September to November 2017), data between two vertical lines were from 30-d of field measurements during fall (November to December, 2017) and data on the right side of hatched vertical line were from 20-d of field measurements during spring (April to May, 2018).

P-value: Cow breed > 0.10, FNDF level > 0.10, FNDF source > 0.10, day < 0.01, FNDF level × day < 0.01.

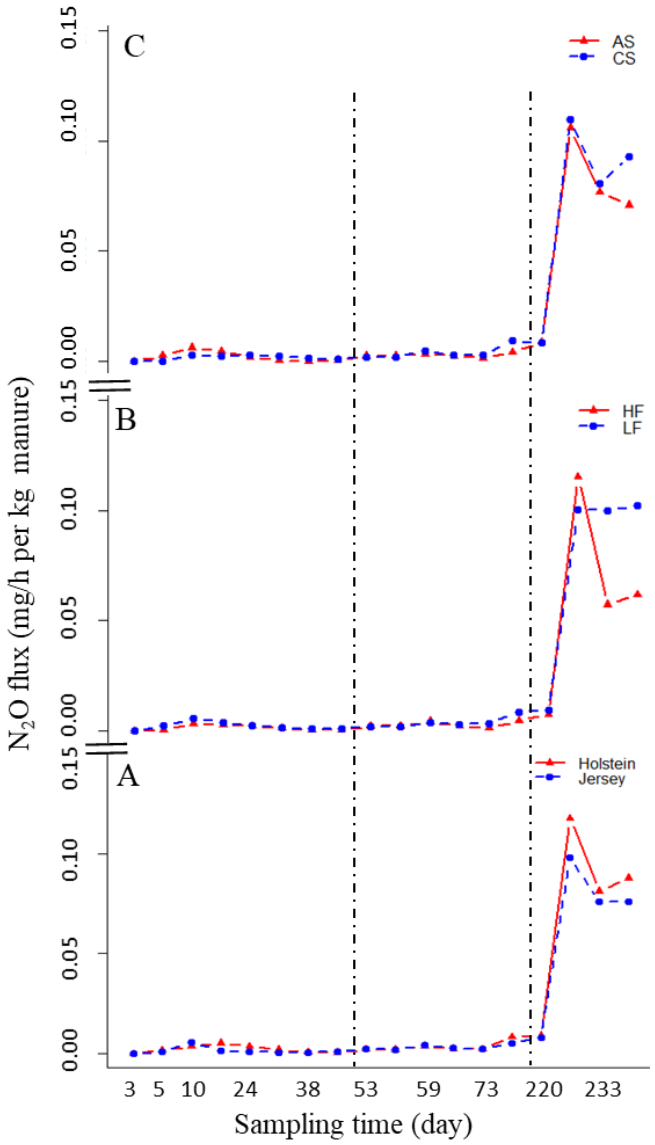

Figure 3. Hourly N_2O fluxes (mg/kg of raw manure) as affected by cow breed (A), dietary forage NDF level (B) and forage NDF source (C). In each panel, data on the left side of the dotted vertical line were from 50-d manure storage (September to November 2017), data between two vertical lines were from 30-d of field measurements during fall (November to December, 2017) and data on the right side of hatched vertical line were from 20-d of field measurements during spring (April to May, 2018).

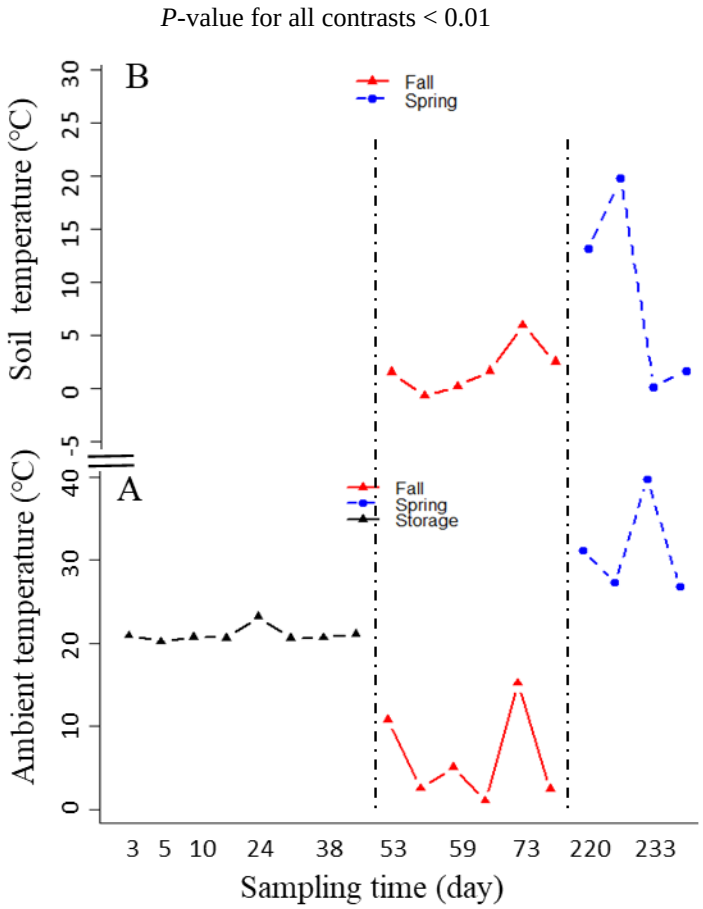

Figure 4. Ambient and soil temperature (℃) over the experimental period. In each panel of A (ambient temperature) and B (soil temperature); data on the left side of the dotted vertical line were from 50-d manure storage (September to November 2017), data between two vertical lines were from 30-d of field measurements during fall (November to December, 2017) and data on the right side of hatched vertical line were from 20-d of field measurements during spring (April to May, 2018).

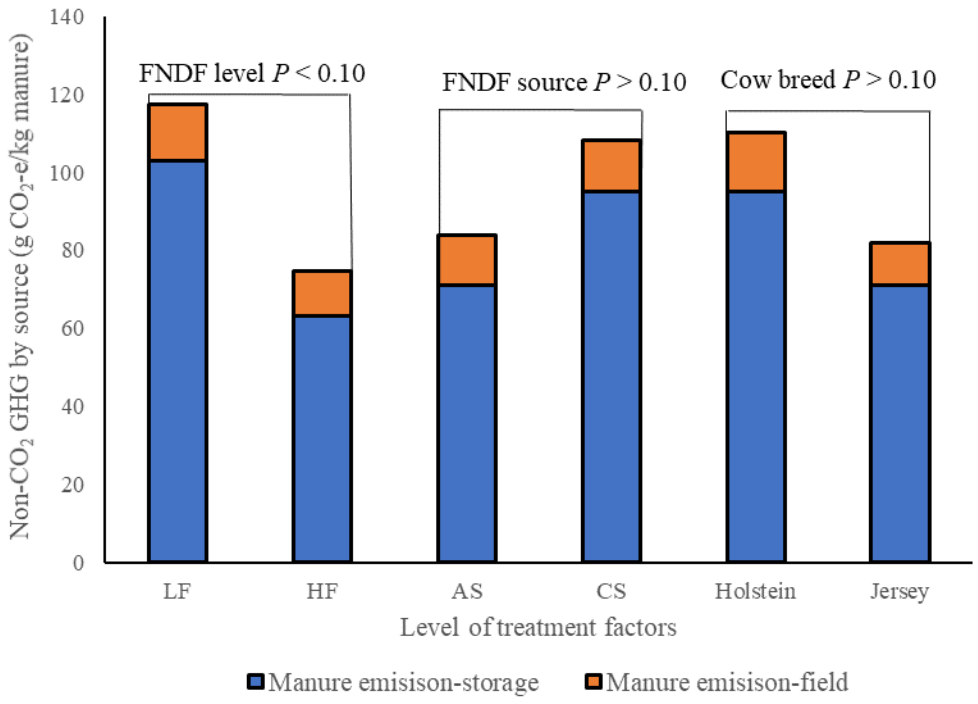

Figure 5. Effects of cow breed, dietary forage NDF level and forage NDF source on cumulative total of non-CO_2 greenhouse gas (CH_4 and N_2O) emissions expressed as CO_2-e of g/kg raw manure.

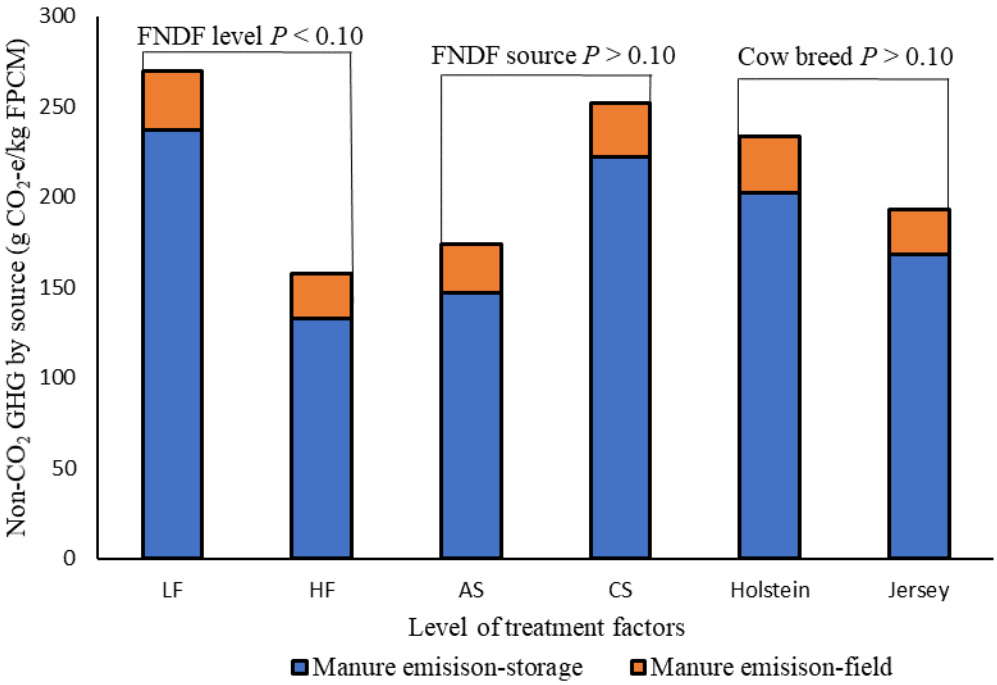

Figure 6. Effects of cow breed, dietary forage NDF level and forage NDF source on cumulative total of non-CO_2 greenhouse gas (CH_4 and N_2O) emissions expressed as CO_2-e of g/kg FPCM.

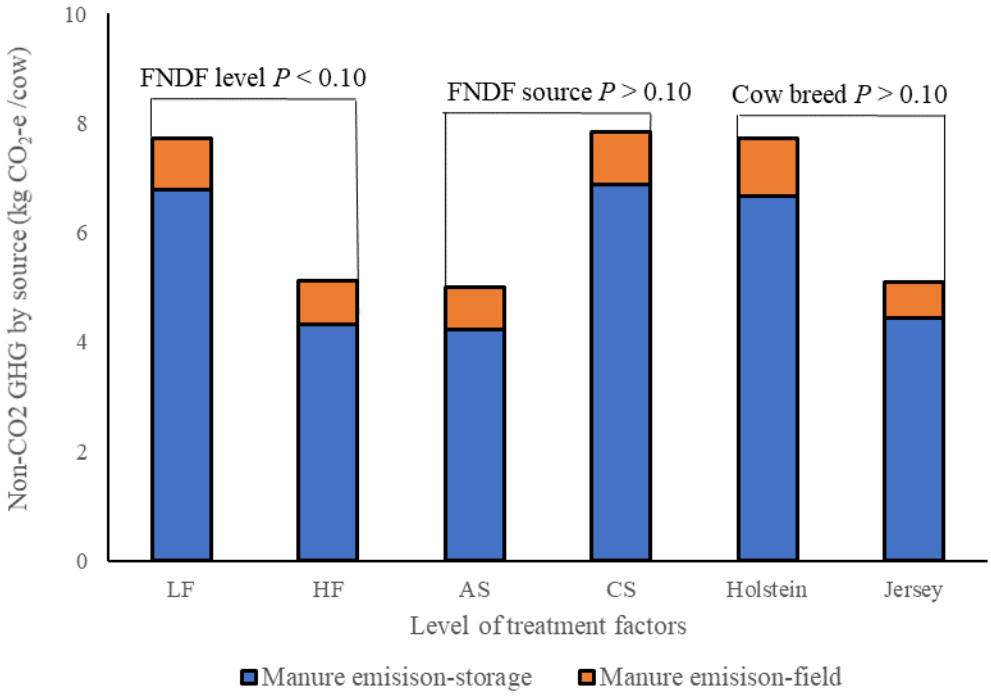

Figure 7. Effects of cow breed, dietary forage NDF level and forage NDF source on cumulative total of non-CO_2 greenhouse gas (CH_4 and N_2O) emissions expressed as CO_2-e of kg/cow.

CHAPTER 4. *Short Communication*: Effects of forage level and forage source on in-vitro ammonia emission from manure of Holstein and Jersey cows

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INTERPRETIVE SUMMARY

***Short Communication*: Effects of forage level and forage source on in-vitro ammonia emissions from manure of Holstein and Jersey cows** By *Uddin et al. 2019.* Our objective was to determine the carry-over effects of cow breed, dietary forage level and forage source on manure ammonia emissions. Low forage fed cows emitted less ammonia expressed as per cow, per kg of manure or percentage of manure N than high forage fed cows. Although, forage source did not affect ammonia emissions, cow breed did impact ammonia emissions. Compared to Holstein, Jersey emitted 17% less ammonia per cow but tended to emit 15% greater ammonia as percentage of manure N. However, none of the treatment factors affected ammonia emissions expressed per kg of fat-and protein corrected milk.

ABSTRACT

One of the major forms of manure N loss to the atmosphere is ammonia (**NH_3**) which causes human health problem, acidification, eutrophication, biodiversity loss and indirect emissions of nitrous oxide, a potent greenhouse gas. Thus, the objective of this study was to determine the effects of replacing alfalfa silage (**AS**) neutral detergent fiber (**NDF**) with corn silage (**CS**) NDF at two levels of forage NDF (**FNDF**) on in-vitro NH_3 emissions of manure collected from Holstein and Jersey cows. Total collection of feces and urine was conducted over 3 d using 8 primiparous, mid-lactation cows (4 Holstein and 4 Jersey). Four cows of each breed were fed 4 different diets arranged as 2×2 factorial with 2 levels of FNDF [19 (low FNDF, **LF**) vs. 24% of dry matter (high FNDF, **HF**)] and 2 sources of FNDF (70:30 vs. 30:70 ratio of AS NDF: CS NDF). Manure NH_3-N was determined in triplicate using lab-scale chamber where 16 g reconstituted manure (urine + feces) was incubated for 48h at 15℃ with sampling at 1, 3, 6, 12, 24, 36 and 48h. Hourly NH_3-N emissions data were analyzed using a repeated measure mixed model in R. The fixed effects were breed, FNDF level, FNDF source, time of measurement, all possible interactions among FNDF level × FNDF source × time; cow was included as a random term. The cumulative NH_3-N emissions over 48h was analyzed using a non-repeated mixed model with the same fixed and random effect terms without including time. None of the treatment factors affected hourly manure NH_3-N emissions except a tendency ($P < 0.10$) of lower NH_3-N for LF-fed cows than HF-fed cows which was dependent on sampling time (FNDF level × time interaction). Similarly, cumulative NH_3-N emissions was not affected by cow breed and FNDF level but manure from CS-fed cows tended to emit less NH_3-N than manure of AS-fed cows. After accounting for manure excretion and milk yield differences, compared to HF, LF-fed cows emitted respectively 20, 15 and 18% less NH_3-N expressed as per cow, per kg manure and percentage of manure N.

Furthermore, compared to Holstein, Jersey emitted 17% less NH_3-N per cow due to lower manure excretion but tended to emit 15% more NH_3-N when expressed as percentage of manure N. None of the treatments affected manure NH_3-N expressed per unit of fat-and protein corrected milk.

Key words: alfalfa silage, corn silage, cow breed, manure ammonia, neutral detergent fiber

Short Communication

Efficiency of converting feed nitrogen (**N**) into milk N is low in dairy cattle and typically it ranges from 25 to 35% (Hassanat et al., 2013; Arndt et al., 2015a; Arndt et al., 2015b). The remaining feed N is excreted almost equally via feces and urine although the proportion mostly depends on dietary CP level (Olmos-Colmenero and Broderick, 2006). The excreted N is lost at different stages of manure management chain (collection, storage and after land application) in several forms namely ammonia (**NH_3**), nitrous oxide, and nitrate (Wattiaux et al., 2019). Ammonia volatilization is the major form of N loss which accounts for 15 to 50 % of the excreted manure N (Powell et al., 2011b; Velthof et al., 2012). Livestock contribute more than 70% of the total anthropogenic NH_3 emissions to atmosphere, most of which (50% of the total atmospheric NH_3 emissions) originate from the manure of dairy and beef cattle (Velthof et al., 2012, EPA 2016). Ammonia released into the atmosphere form particulate matter less than 2 microns that affect human health (Erisman and Schaap, 2003; Moldanová et al., 2011). Upon redeposition, NH_3 can also cause acid rain and soil acidification (Van Breemen et al., 1983), eutrophication of aquatic ecosystems and biodiversity loss (Bobbink et al., 1998). Additionally, NH_3 is an indirect source of nitrous oxide which is a potential greenhouse gas (Schreiber et al., 2012; Wattiaux et al., 2019). Furthermore, NH_3 emission is a loss of manure N which could otherwise be available to crops upon land application. Therefore, it is of utmost importance to reduce manure NH_3 emission.

Manure NH_3 emissions from dairy cows vary widely between production systems (e.g., 12.2 vs. 25.2 g NH_3/d per cow respectively for stanchion barn in Wisconsin vs. stanchion barn in the Netherlands) and housing systems (e.g., 12.2 vs. 64 g NH_3/d per cow respectively for stanchion barn vs. freestall barn in Wisconsin) due to differential manure handling system (Powell et al., 2011b). Under farm-like conditions, ammonia-N loss averaged 110 g/day per lactating cow, but ranged from 64 g/day to 178 g/day with no clear seasonal pattern but a 34 g/d reduction (from 127 to 93 g/d) associated with reducing dietary CP from (DM basis) 18.2 to 16.7% (Aguerre et al. 2010). Ammonia emissions can be reduced through reducing urinary urea N excretion which is the major substrate for NH_3 formation. Reducing dietary CP level, increasing level of concentrate or starch in the diet (or decreasing forage) and decreasing RDP to RUP ratio could reduce urinary urea N relative to total urinary N excretion (Davidson et al., 2003; Olmos Colmenero and Broderick, 2006; Sun et al., 2019). Increasing proportion of corn silage (**CS**) at the expense of alfalfa silage (**AS**) in the forage portion of the diet linearly decreased manure N excretions and increased feces to urine ratio (Hassanat et al., 2013; Arndt et al., 2015). In these two studies, replacement of AS with CS in the diet also increased dietary starch concentrations which might have contributed to decrease manure N excretions. But, the shift in forage source also shifted the CP source in the diets with most of the CP originating from soybean meal when cows were fed CS based diet but from AS when cows were fed AS based diet. Thus, the effects of dietary treatments in these two studies on manure N excretions was confounded between starch level and CP source in the diet. However, our companion study also reported lower manure N excretions when AS was replaced with CS keeping dietary starch, CP, RDP and RUP constant, but changing levels of dietary non-forage NDF (Uddin et al., 2019). This latter study also reported 17% lower urinary N excretions (urinary N / N intake of 21.6 vs 26.0 for cows fed the low forage NDF (**FNDF; LF**)

diets than the high FNDF (**HF**) diets. Furthermore our companion study indicated that cows fed CS-based diets had a 16% lower urine excretion (20.3 vs. 24.0 kg/d, $P<0.01$) and a 34% reduction in the feces to urine excretion (2.21, vs. 1.65 kg/kg, $P<0.01$) than cows fed the AS-based diets. Therefore, we hypothesized that manure NH_3 emissions would be reduced when cows were fed the LF diets compared to the HF diets the extent of which may depend on dietary forage source (AS vs. CS). An additional hypothesis focused on differential manure NH_3 emissions between Holstein and Jersey cows because of differential nutrient digestibility (Aikman et al., 2008), and the tendency to have differential N utilization efficiency (Kauffman and St-Pierre, 2001). Our companion study indicated also a greater ratio of fecal to urine excretion for Holstein compared to Jersey (Uddin et al., 2019). Therefore, the objective of this study was to determine the effect of iso-nitrogenous and iso-starch diets with varying level and source of FNDF including their interactions on in-vitro NH_3 emissions from manure of Holstein and Jersey cows.

The manure used in this study was collected from a companion study described in detail elsewhere (Uddin et al. 2019). Institutional Animal Care and Use Committee approved protocol was followed for animal use and care during manure collection, which was conducted at the Dairy Cattle Center, University of Wisconsin-Madison. Briefly, in this companion study, 12 primiparous Holstein and 12 primiparous Jersey cows were fed 4 diets in a 2×2 factorial arrangement as split-plot, triplicated 4×4 Latin square design with breed as main plot and diets as sub-plots. The dietary factors were FNDF level [19.0 (LF) and 24.0 % (HF), DM basis] and FNDF source (70:30 and 30:70 ratio of AS NDF:CS NDF). The 4 dietary treatments were offered as TMR. Each Latin square period lasted 4 wk in length including last 2 wk for sampling. For this NH_3 experiment, total collection of feces and urine was conducted on selected 8 cows (one Latin square from each breed) at the end of period 3. During a 3-d total collection, the weight of feces and urine were

recorded and approximately 500g feces and 100 ml urine sample were collected every 8 h following hand mixing procedure. The 9 fecal and urine samples collected from each cow were composited and stored separately at -20℃ until further analysis**.**

Ammonia emission was determined with a lab-scale ventilated chambers for which construction design and procedure have been described elsewhere (Misselbrook et al., 2005; Misselbrook and Powell, 2005; Powell et al., 2011a). In brief, plastic drainage pipe (10 cm diameter and 19 cm height) was used to construct the chamber. The base of the pipe was capped permanently with glue and top lid was made in such a way so that it can be fitted with silicone grease to seal properly. Each lid had four inlet and outlet ports for ensuring proper air mixing inside the chamber. One acid trap (0.075 L, 0.02 mol L^{-1} of orthophosphoric acid) was connected to inlet to remove any NH_3 coming through inlet air and a second acid trap was connected to outlet to collect NH_3 during incubation of manure sample. The whole set-up containing 6 chambers was installed in a large incubator maintaining a temperature of 15℃continuously. In each chamber, approximately 16 g of reconstituted manure was incubated on a petri-dish. The reconstitution was done to maintain the same feces and urine ratio as produced by each cow (wet weight basis), which on average were 2.08 vs 1.77 for Holstein and Jersey (P = 007) and 2.21 vs 1.65 for CS vs AS based diet (P<0.01) (Uddin et al., 2019). At first, required amount of feces (upon thawing and proper mixing) was measured and put in a petri-dish which was covered with parafilm until the addition of urine. The measured amount of urine was poured on petri-dish and mixed properly. Then, petri-dish containing manure was put in chamber immediately and covered with greased lid. Chamber was then connected with inlet and outlet ports. The airflow was maintained at 4 $Lmin^{-1}$. Each run lasted for 48h and sampling (changing outlet acid trap containing NH_3) was performed at 1, 3, 6, 12, 24 and 36h and last measurement was at 48h. The outlet acid was diluted to 0.1 L

with deionized water at each sampling time for all treatments and replications. Then, the diluted solution was analyzed for NH_4^+ with flow injection analyzer (QuickChem Methods 12–107–06–2-A; Lachat Instruments, 1996). The hourly NH_3-N (mg) flux, which was determined at each sampling point, is the product of NH_3-N concentrations in acid trap solution (mg L^{-1}) and the volume of acid trap solution (0.01 L). The cumulative NH_3-N emission for each treatment at each run was calculated by summing emission of all time points measured over 48h period.

As observed in the companion study, dietary FNDF level, FNDF source and cow breed also affected fat-and protein corrected milk (**FPCM**) yield, manure yield and manure characteristics (Appendix I). Therefore, cumulative manure NH_3-N measured over 48h incubation period for 16 g manure sample was scaled-up with some additional calculations adjusting for daily manure volume and FPCM yield differences, and the scaled-up variables were expressed in the following ways: g NH_3-N/d per cow, g NH_3-N/kg FPCM, g NH_3-N/kg raw manure, and NH_3-N as % of total manure N excreted. The manure NH_3-N per cow (cumulative NH_3-N emission per 16 g manure sample × total volume of manure) provides the cow to cow variability information. The NH_3-N per kg FPCM (total NH_3-N emissions per cow/total FPCM yield per cow) is an important expression since total emission is the function of total milk production and FPCM is often considered as the functional unit for milk carbon footprint. The manure NH_3-N expressed either per kg raw manure (total NH_3-N emissions per cow/total manure yield per cow) or percentage of manure N excreted (total NH_3-N emissions per cow/total manure N excreted per cow) indicates the vulnerability of manure N which is lost in the form of volatilized NH_3.

Hourly NH_3-N emission fluxes were analyzed with lme function of lme4 package in R version 3.5.3 using the repeated measure mixed effects model containing fixed effects of cow breed, FNDF level, FNDF source, hour of measurement; and all two and three way interactions among FNDF

level, FNDF source, and hour of measurement. The cow was fitted as random effect and the auto-correlation covariance structure, with hour of measurement as continuous covariate was fitted using corCAR1 function. Cumulative NH_3-N emission and scaled-up NH_3-N emission variables calculated for 48h period of incubation were analyzed using a simple non-repeated mixed model containing fixed effect of cow breed, FNDF level, FNDF source, interaction between forage NDF level × forage NDF source; and random effect of cow. Effects were reported as significant or as tendency for $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

The hourly NH_3-N emission result is presented in Figure 1. The hourly NH_3-N emission followed a pattern similar to previously published studies in which same NH_3 measurement protocol was followed to study the effect of dietary tannin (Powell et al., 2010) and the effect of dietary CP and tannin levels (Powell et al., 2011a). The peak emission for hourly NH_3-N occurred at around 24 h after starting incubation and declined thereafter to return to the initial (1-hr) value after 48 hours of incubation. In this study, hourly manure NH_3-N emission did not differ between cow breeds (Holstein vs. Jersey) or between FNDF sources (AS vs. CS). However, compared to HF, LF-fed cows manure tended ($P < 0.10$) to emit less NH_3 particularly around peak emission hour (i.e., significant interaction between FNDF level × sampling hour, Figure 1). Although the hourly manure NH_3-N emissions were similar for the first 6 hr of incubation, the subsequent emissions were consistently higher for the manure from the HF-fed cows than the LF-fed cows. This increment in manure NH_3-N emissions for HF-fed cows compared to LF-fed cows was most likely associated with greater excretions of urinary N for HF than LF-fed cows (149 vs. 132 g urinary N/d per cow) as observed in companion study (Appendix I, Uddin et al., 2019). Because most of the urinary N excreted is in the form of urea which is very labile to be volatilized (James et al., 1999). Thus, increasing level of concentrate in dairy cows' diet (or increasing non-forage

fiber mainly from soyhulls in this case) not only helped to reduce N excretion through manure but it can potentially reduce manure NH_3-N emissions. Also, in agreement with Powell et al. (2011a), our results suggest that measures (e.g. acidification of manure or solids-to-liquid separation of manure or incorporation of manure to soil during land application) must be taken immediately to avoid losses of NH_3-N since most of the NH_3-N was lost within 36h after mixing feces with urine (Kai et al., 2017; Holly et al., 2017).

Cumulative NH_3-N emissions over 48h period of incubation did not differ between cow breeds and FNDF levels (Figure 2). However, CS-fed cows tended to emit less cumulative manure NH_3-N than AS-fed cows (Figure 2). The greater feces to urine ratio (2.04 vs. 1.40) for CS-fed cows than AS-fed cows might have contributed to this difference (Uddin et al., 2019) because urinary N (mostly in the form of urea) is very prone to be volatilized as NH_3 (James et al., 1999). Nevertheless, cumulative NH_3-N emission pattern and magnitude were comparable with the values reported by Powell et al. (2010) for 16.8 % CP diet which is very similar to the average CP content of our dietary treatments (i.e., 17% CP, DM basis).

Results of the analysis of the scaled-up variables (g NH_3-N/d per cow, g NH_3-N/kg FPCM, g NH_3-N/kg raw manure, and NH_3-N as % of total manure N excreted) are presented in Table 1. Compared to HF, LF-fed cows emitted 20% less NH_3-N (g/d per cow) from manure incubated over 48 h period. The excretion of lower amount of total urinary N (g/d per cow) might have contributed to this reduction in NH_3-N emissions since volume of feces and urine plus fecal N excretions between LF and HF-fed cows were similar (Appendix I; Uddin et al., 2019). Furthermore, urinary N has greater potential to be volatilized than fecal N (James et al., 1999). This greater proportion of urinary N excretions relative to fecal N also increased N volatilization for HF than LF-fed cows' manure expressed either per kg manure or as percentage of manure N

basis (Table 1). However, the NH_3-N emission intensity (g NH_3-N/kg FPCM) was similar between LF and HF-fed cows' manure. Nevertheless, FNDF source did not affect these scaled-up NH_3-N emission variables (Table 1) except a tendency for greater NH_3-N (% of manure N) for AS-fed cows than CS-fed cows. In the case of breed, Jersey expectedly emitted 17% less NH_3-N (g/d per cow) than Holstein mainly due to lower manure volume for Jersey than Holstein (56 vs. 72 kg manure/d per cow; Uddin et al., 2019). However, Jersey tended to emit 15% greater NH_3-N over 48h incubation period when expressed as percentage of manure N basis and this increment in NH_3-N (% of manure N excretions) was associated with lower feces to urine ratio for Jersey than Holstein as reported in companion study by Uddin et al., (2019). Breeds had similar manure NH_3-N intensity (g NH_3-N/kg of FPCM).

In this study, feces and urine were collected separately and then reconstituted to manure by properly mixing them without inclusion of bedding materials or lime or waste-water. Therefore, our study conditions do not fully mimic the typical dairy manure handling scenario where manure is mixed with bedding material, lime used in stall and subsequently with waste-water. Our study condition could be considered as a limitation because other factors (e.g., addition of lime or bedding materials, mixing of feces with urine depending on barn facilities) might have affected manure NH_3 emissions differently. On contrary, our study condition could also be considered as a strength because it allowed us to capture the carry-over effects of cow breed and diets on manure NH_3 emissions with minimal confounding effects. In our study condition, hourly and cumulative NH_3-N emissions from 16 g manure sample incubated over 48h period were not affected by any of the treatment factors. Total manure NH_3-N emissions (g/d per cow) measured over 48 h incubation period were affected by cow breed and FNDF level but not with FNDF source. Only FNDF level affected manure NH_3-N emissions expressed either per kg manure or as percentage of

manure N which was not affected by cow breed and FNDF source. Manure NH_3-N emissions intensity (g NH_3-N/kg FPCM) measured over 48h incubation period was similar across dietary treatments and between cow breeds.

ACKNOWLEDGEMENTS

This project was financially supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture Hatch Multi-state research formula fund (# WIS01941, and USDA-NIFA # 2013-68002-20525). Authors are happy to acknowledge the help by Katherine G. Wells during sampling period of this ammonia measurement experiment. Authors also express their gratitude to statistical consultant (CALS, UW-Madison), Tedward Erker for his help with the statistical analysis.

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Table 1. Effects of cow breed, forage NDF level and forage NDF source on in-vitro manure NH_3-N emissions measured over 48 h period and expressed as per cow, per kg FPCM, per kg manure and as percentage of manure N basis after adjusting for milk and manure yield differences.

	Dietary Treatments[1]					Breed			P value		
Item	LFAS	LFCS	HFAS	HFCS	SEM	Holstein	Jersey	SEM	FNDF Level	FNDF Source	Breed
Total NH_3-N emissions over 48 h period of incubation											
g/d per cow	134	143	174	173	7.86	170	141	5.56	<0.05	0.60	<0.05
g/kg FPCM	5.14	5.24	6.05	5.30	0.36	5.10	5.80	0.25	0.25	0.45	0.15
g/kg Manure	2.30	2.20	2.80	2.50	0.10	2.40	2.49	0.07	<0.05	0.11	0.45
% of Manure N	49.5	42.0	58.6	52.3	2.80	47.0	54.2	1.96	<0.05	0.08	0.08

[1]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

P-value: Cow breed > 0.10, FNDF level < 0.10, FNDF source > 0.10, hour < 0.01, FNDF level × FNDF source > 0.1, FNDF level × hour < 0.01, FNDF source × hour < 0.10, FNDF level × FNDF source × hour < 0.01.

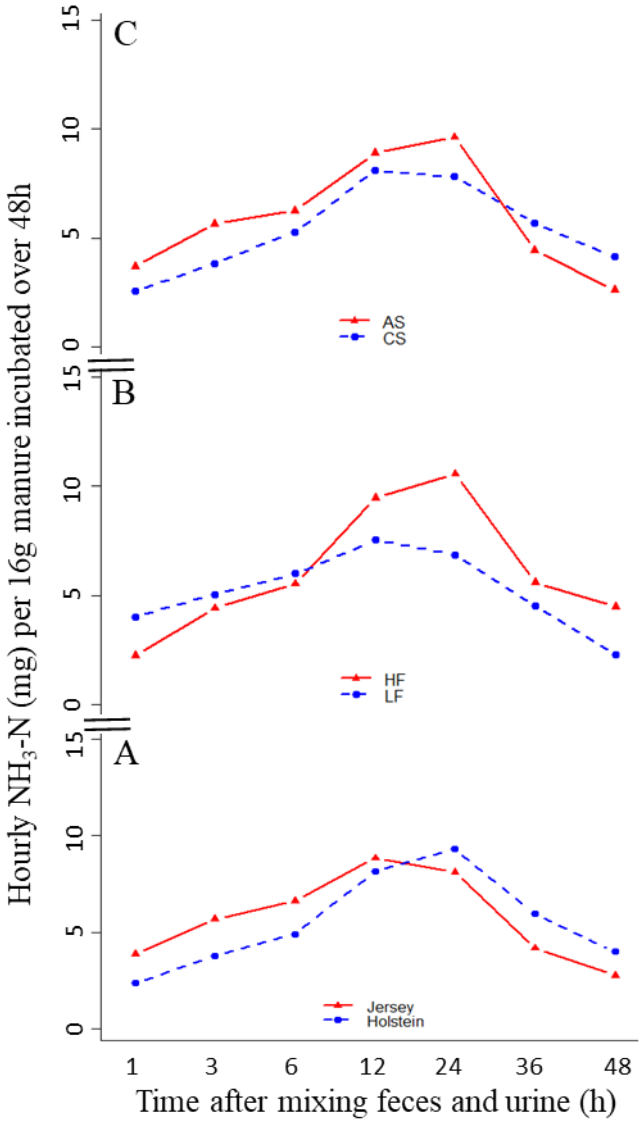

Figure 1. Hourly NH_3-N (mg) emissions from 16 g manure incubated over 48 h period as affected by cow breed (A), dietary forage NDF level (B) and forage NDF source (C).

P-value: Cow breed > 0.10, FNDF level > 0.10, FNDF source < 0.1, hour < 0.01, FNDF level × FNDF source > 0.1, FNDF level × hour < 0.01, FNDF source × hour < 0.05, FNDF level × FNDF source × hour > 0.10.

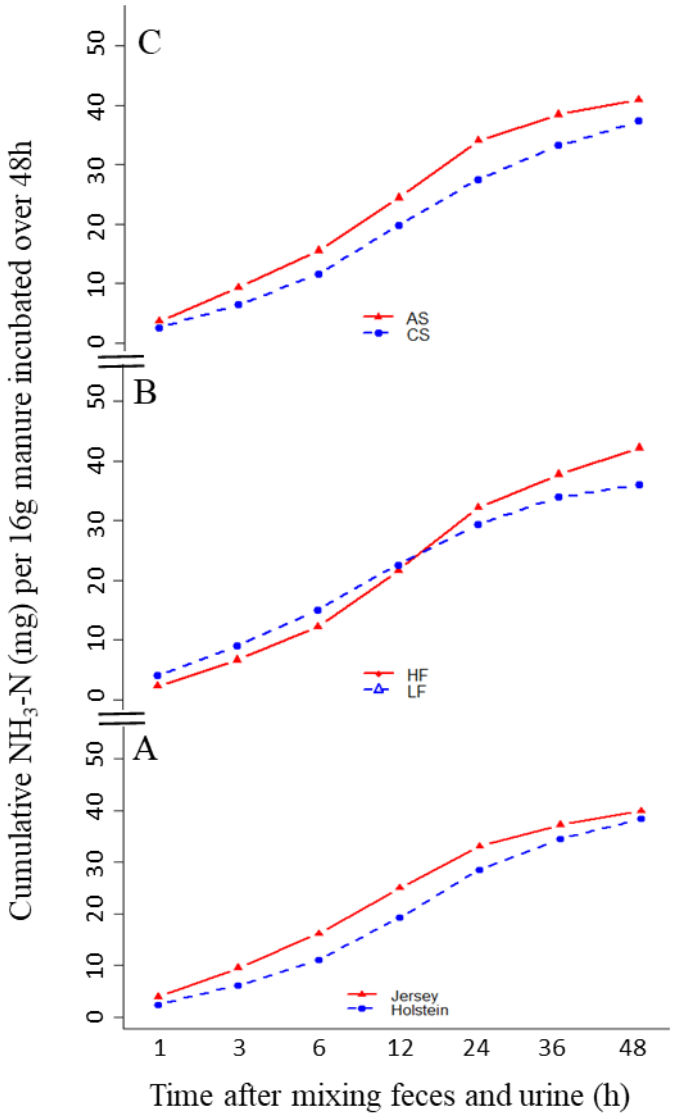

Figure 2. Cumulative NH_3-N (mg) emissions from 16 g manure incubated over 48 h period as affected by cow breed (A), dietary forage NDF level (B) and forage NDF source (C).

Appendix I. Dry matter intake, fat- and protein corrected milk (FPCM) yield, feces and urine excretions, fecal and urinary N excretions, and manure N and ammoniacal-N concentrations as affected by cow breed, forage NDF level and forage NDF source[1].

Item	Dietary Treatments[2]				Breed	
	LFAS	LFCS	HFAS	HFCS	Holstein	Jersey
DMI (kg/d)	22.5	23.6	19.9	21.9	25.2	18.8
FPCM (kg/d)	29.2	29.9	27.4	29.0	32.7	24.9
Feces as-is (kg/d)	38.1	43.9	39.8	45.1	48.0	35.4
Urine as-is (kg/d)	24.1	19.3	23.9	21.2	23.6	20.7
Feces/Urine	1.62	2.26	1.67	2.15	2.08	1.77
Fecal N (g/d per cow)	165	170	165	156	190	139
Urinary N (g/d per cow)	135	129	144	154	164	118
Total manure N (% DM)	3.60	3.30	3.30	3.80	3.42	3.52
Manure NH_4-N (% DM)	2.00	1.83	1.85	2.20	1.94	2.0

[1]These data are adapted from Uddin et al., (2019) and Uddin (2019) where one can find the *P*-value for the statistical differences.

[2]Dietary Treatments: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

CHAPTER 5. Analysis of greenhouse gas emissions from dairy systems using a cradle-to-gate life cycle assessment integrating measured enteric and manure management greenhouse gas emissions for different diets and breeds

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ABSTRACT

The objective of this study was to determine and compare the carbon footprint (**CF**) of milk production for four lactating cows' dietary and two breed (Holstein and Jersey) scenarios using measured enteric methane and greenhouse gas (**GHG**) emissions during manure storage and after field application. The diets were formulated as 2×2 factorial with two levels (19 and 24 % of forage neutral detergent fiber (dry matter basis) referred as low forage and high forage diets) and two sources [70:30 or 30:70 ratio of alfalfa silage (**AS**) and corn silage (**CS**)] of forage neutral detergent fiber. Emissions and animal performances were collected in companion studies. Scenarios were incorporated in a modelled averaged size Wisconsin dairy farm consisting of 122 lactating cows (all primiparous), 22 dry cows, 56 heifers over one year of age and 63 heifers under one year of age. We assumed that manure is field applied according to a nutrient management plan and the farm cropland is used to produce forages (AS and CS) and corn grains fed to the cows. Purchased inputs included other concentrate feed to balance rations of the cows and the chemical fertilizer necessary to fertilize the crops as recommended. The 'cradle-to-gate' life cycle assessment was performed with SimaPro using fat-and-protein corrected milk (**FPCM**) as the functional unit. Emissions were allocated between milk and meat using an economic allocation and we performed a sensitivity analysis using no allocation and mass allocation. Low forage-fed cows had 11% greater CF than high forage-fed cows (1.57 vs. 1.42 kg CO_2-e/kg FPCM) most likely due to containing greater amount of highly digestible soyhull in the low forage than high forage diets. Whereas both forage sources (AS and CS) had very similar CF (averaged to 1.50 kg CO_2-e/kg FPCM). The milk CF for Holsteins was 5% greater than for Jerseys (1.55 vs. 1.48 kg CO_2-e/kg FPCM) possibly due to structural differences of methane forming substrates in manure of Holsteins and Jerseys because rate of passage for ruminal contents for the Jersey is greater than

the Holstein. Overall, the CF when an economic allocation was used was 1.5 kg CO_2-e/kg FPCM. However, CF was reduced to 1.42 kg CO_2-e/kg FPCM when a sensitivity analysis was performed using mass allocation instead of economic allocation. Additionally, the CF was further reduced by 7.5% when a sensitivity analysis was performed for the Holstein breed scenario which included a combination of primiparous and multiparous cows in the herd instead of using primiparous cows only. GHG mitigation strategies (choice of cow breed or diet) evaluated at the animal scale indicated different results than that of whole-farm scale evaluations, highlighting the need to assess these practices on a whole-farm basis using measurements specific to the production system under consideration.

Key Words. alfalfa silage, corn silage, forage level, forage source, Holstein, Jersey

1. INTRODUCTION

Life cycle assessment (**LCA**), a standardized and widely used holistic approach to determine environmental impacts of a product or a process, has been used in recent decades in dairy production systems to determine the carbon footprint (**CF**) of milk production (O'Brien et al., 2014). The CF of milk production in the intensive systems common in the developed world is much lower than the extensive system used primarily in the developing word (1.3 vs. 7.5 kg CO_2-e/kg of fat-and-protein-corrected milk, **FPCM)** suggesting a greater potential to reduce CF of milk in extensive system **(**FAO, 2010; Knapp et al., 2014). Even within intensive system of developed world, the CF of milk production may vary widely depending on choice of functional unit, system boundary, LCA methodology (attributional vs. consequential), methods of calculation, methods of co-products allocation (e.g., economic vs. mass) and other assumptions (e.g., warming potential of greenhouse gases, **GHG**) (Baldini et al., 2018; Zanten et al., 2018; Wattiaux et al., 2019). For

instance, CF of milk across 10 studies ranged from 0.84 to 1.5 kg CO_2-e/kg FPCM in intensive system as per 'cradle-to-gate' LCA methodology (Wattiaux et al., 2019) whereas, a 'cradle-to-grave' LCA conducted in the US reported that the CF of US milk production is 2.04 kg CO_2-e/kg FPCM (Thoma et al., 2013). The latter study also identified enteric methane (**CH_4**), manure management, and feed production as three hotspots contributing to approximately 70% of the total GHG emissions from dairy production systems (Thoma et al., 2013). Thus, mitigation strategies emphasizing these three GHG emissions hotspots may help to further reduce the CF of milk production.

Mitigation strategies to reduce enteric CH_4 emission can be classified as feeding and nutritional management, breeding and combinations of herd management strategies (e.g., disease management, improving feed efficiency and life-time productivity). Among mitigation strategies, feeding and nutritional strategies have been extensively studied because of their immediate response (short-term impact) and ease of applicability **(**Knapp et al., 2014**).** Some of the recently studied and potential nutritional strategies for mitigating enteric CH_4 emission include replacement of alfalfa silage (**AS**) with corn silage (**CS;** Hassanat et al., 2013**;** Arndt, 2015), replacement of AS with timothy silage (Hassanat et al., 2014), replacement of CS with barley silage (Benchaar et al., 2014), manipulating forage to concentrate ratio (Aguerre et al., 2011), and replacement of starch with dextrose (Sun et al., 2019). Several studies also pointed out that there might be a carry-over effects of diet on manure GHG emissions due to changing manure composition (e.g., organic matter content, form and content of nitrogen, carbon to nitrogen ratio; Hassanat et al., 2013; Uddin et al., 2019b; Wattiaux et al., 2019). For instance, Kulling et al. (2001) reported a decrease in manure nitrous oxide (**N_2O**) but increase in manure CH_4 emission when manure originated from a diet with a reduced level of dietary crude protein. Thus, a holistic evaluation of dietary strategies

on whole-farm GHG emissions is important and necessary to capture and assess the potential tradeoffs and interactions between sub-systems of dairy production system such as the tradeoff between enteric emission and manure management related GHG emissions (Montes et al., 2013**;** Knapp et al., 2014**;** Kebreab et al., 2019; Wattiaux et al., 2019).

Midwestern dairy producers are shifting toward producing and feeding greater amount of CS at the expense of AS mainly to support increasing herd size without concomitant change in land-base (Martin et al., 2017). Effects of the changes in dietary forages and associated change in land use (cropping system) on CF of milk have been modeled at the system level using LCA (Little et al., 2017), but to our knowledge, no studies have been conducted to generate empirical data combining in a single experiment measurement of enteric emissions and subsequent emissions from manure during storage and after field application (Uddin, 2019).

Separately, differences in dairy breed have been of interest. Simulation studies showed a greater CF expressed per unit of FPCM or cheese production for Holstein than Jersey associated with either smaller body size or greater fertility in the latter compared to the former (Capper and Cady, 2012; Riva et al. 2014). However, both breeds had similar enteric CH_4 intensity (g/kg FPCM) as per findings of experimental studies (Olijhoek et al., 2018; Uddin et al. 2019a).

Furthermore, Baldini et al., (2018) reported an under-estimation of GHG emissions (21%) when Intergovernmental Panel on Climate Change (**IPCC**) based emission factors (commonly used in LCA study) was used instead of measured emissions for manure management related GHG emissions hence IPPC recommendations might not best represent actual emissions for a particular production system or capture the differences in regional characteristics or farm management practices (Owen and Silver, 2015).

Aiming to evaluate the effects of different dietary scenarios on whole-farm GHG emissions, we previously conducted one experiment subdivided in three consecutive studies to evaluate the effects of four dietary treatment combinations [(two forage neutral detergent fiber (**FNDF**) level × two forage source) and two breed (namely Holstein and Jersey)] on enteric CH_4 emission, lactation performances, and manure yield (Uddin et al. 2019a); manure composition and manure GHG emissions during storage followed by a land application of manure to crop-field (Uddin et a. 2019b); and manure ammonia (**NH_3**) emission (Uddin, 2020). Therefore, the objective of this study was to determine and compare the CF of milk production in Wisconsin using a cradle-to-gate LCA approach for four dietary scenarios of lactating Holstein and Jersey cows using our measured GHG emissions associated with enteric fermentation, during manure storage, and after field-application of manure.

2. MATERIALS AND METHODS

2.1. Description of scenarios included in this study

In this study, an average size Wisconsin dairy herd consisting of 122 lactating cows, 22 dry cows, 56 growing heifers over one year of age and 63 growing heifers under one year of age was modelled (USDA-NASS, 2017; Rotz et al., 2018). A similar herd structure was assumed for both Holstein and Jersey breeds and that all lactating cows in the herd were mid to late lactation primiparous cows. The latter assumption does not mimic actual herd structure, which typically consists of both primiparous and multiparous cows. However, our goal was to determine and compare CF of milk production based on measured emissions for four dietary and two cow breeds scenarios and measured enteric emissions were available for primiparous cows only. A sensitivity test was also performed to determine the effect of inclusion of multiparous cows (accounting for only milk production differences between primiparous and multiparous) in the herd structure on

CF. Body weight, dry matter intake (**DMI**), FPCM and manure yield information of lactating cows for four dietary and two breed scenarios were collected from companion studies shown in Table 1 (Uddin et al., 2019a). Whereas, the non-lactating animals' body weight and DMI information were literature based (Table 1; Akins, 2016; NRC, 2001).

The four dietary treatment combinations for lactating cows were arranged as 2 × 2 factorial with two level of FNDF from either one of the two forage sources. The FNDF level were 19 and 24% (dry matter basis) respectively referred as low FNDF (**LF**) and high FNDF (**HF**) (Table 2). The sources of FNDF were either AS or CS (i.e., 70:30 or 30:70 ratio of AS NDF:CS NDF). The details of the diets including chemical composition have been described in Uddin et al. (2019a). Non-lactating animals' diets were formulated using the DMI and body weight information from Akins (2016) and NRC (2001) to meet the NRC (2001) recommended dietary guideline for specific group of non-lactating animals (Table 2).

2.2 Description of the LCA method

An attributional LCA was performed using SimaPro 9.0 as per ISO 14040 and 14044 standards (ISO, 2006a, 2006b).

2.3 Functional unit

The functional unit used for this study was 1 kg FPCM calculated based on the International Dairy Federation guidelines (IDF, 2010) using the milk composition for different scenarios as reported in Uddin et al. (2019a) adjusted to 4.0% milk fat and 3.3% milk protein. The CF result was expressed as kg CO_2-e per kg FPCM.

2.4 Co-products allocation and system boundaries

The production of dairy products results in the production of a co-product, meat. The LCA method accounts for this co-product and divides the inputs and outputs of the system into the

multiple products of that system. This division can be done by system expansion, system subdivision, or allocation. In this study, an attributional allocation method was adopted based on the economic relationship (price of co-products) between milk and meat (Table 3). The economic allocation between milk and meat was calculated based on the income (as percentage of total income) generated by each co-product (USDA-NASS, 2017). A sensitivity analysis was performed to determine the effect of using no allocation (i.e., 100% emission was allocated to milk) or mass allocation (IDF 2015) instead of economic allocation.

In this study, the system boundary included emissions from enteric fermentation, manure management (from barn to field including indirect N_2O emissions from ammonia volatilization), on-farm feed production, and purchased feed production including energy and materials used plus transportation, Figure 1. Measured and estimated or literature-based emission factors are also shown in Figure 1 using different color code. Capital goods (e.g., buildings, machinery) production related emissions were not accounted for in this study. Biotic CO_2 was not included in this study as it is assumed to be recycled back through plant photosynthesis during feed production. The CO_2 emissions from burning fossil fuels for farm electricity to run milking machine, feeder, cooling fan and manure handling was included in this study.

2.5 LCA inventory data

2.5.1 Forage production and purchased feed related emissions

All forage production (CS and AS) and corn grain were assumed to be produced on-farm, a common practices in Wisconsin, and all other concentrate feed ingredients were purchased from local sources. The total land required for on-farm AS, CS and corn grains production under different dietary and breed scenarios were calculated based on DM yield of AS (6,263 kg DM/ha), CS (16,858 kg DM/ha) and corn grain (9,768 kg DM/ha) as per USDA-NASS (2017). AS and CS

chemical composition, animal intake and dietary composition were taken from Uddin et al. (2019a) (Table 4). All manure was assumed to be recycled back to on-farm forage and grain production assuming 80% availability of manure K and manure P, and 50% availability of manure N to crops (Laboski and Peter, 2012). The amount of N, P and K supplied by manure were calculated using manure yield information and manure N content (Uddin et al. 2019b), plus P and K concentrations (Laboski and Peter, 2012). The rest of the total required fertilizer for on-farm feed production were assumed to be purchased as chemical fertilizer.

The emissions for on-farm feed production were calculated based on off-farm inputs (e.g., chemical fertilizer, herbicide, pesticide etc.) needed for AS, CS and corn grain production as shown in Table 5 (Ecoinvent version 3.0). The emission factors for soybean meal, molasses, blood meal, and vitamin and minerals premix were taken from Adom et al., (2012), and Adom et al. (2013) reported for the Midwestern region (Table 6). The feed ingredients were assumed to be transported for 24.8 km as per Kannan et al. (2016) since most of the feed were assumed to be sourced locally. The emissions from the return of an empty feed truck were not accounted for. The transportation emissions for a 32-metric ton size freight truck was based on Ecoinvent database (version 3.0).

2.5.2 Enteric CH_4 and manure chain GHG

Enteric CH_4 of lactating cows were measured in Uddin et al. (2019a) whereas enteric CH_4 for non-lactating animals was predicted using DMI and dietary NDF information with the equation developed by Niu et al. (2018). The CH_4 and N_2O emissions during storage and after land application of manure were measured in Uddin et al. (2019b) with the assumption that the chemical composition of non-lactating and lactating animals' manure is similar.

2.5.3 Farm fossil energy

The average annual electricity consumption for a lactating cow in Wisconsin is 1,000 kWh (Scott Sanford, UW-Extension specialist). The same study also indicated that the 46% of this energy is used for milk harvesting and cooling, another 46% is used for farm lighting and ventilation and the rest (6%) is used for feeding, manure handling and miscellaneous. Based on this information, we calculated that the farm electricity consumption for each lactating and non-lactating animal would be 2.74 and 1.00 kWh/d, respectively.

2.6 Impact Assessment

The mid-point impact assessment was calculated based on IPCC 2013 single issue method for GHG emissions (Myhre et al., 2013). The global warming potential used for converting CH_4 and N_2O to CO_2 were 28 and 265, respectively (IPCC, 2013) over 100 years (Myhre et al., 2013).

3. RESULTS AND DISCUSSION

3.1 Carbon footprint for four dietary scenarios

The CF of different dietary scenarios including the contribution of each process on overall CF is shown in Table 8 and Figure 2. Overall, the CF for LF-fed cows was 11% greater than HF-fed cows (1.57 vs. 1.42 kg CO_2-e/kg FPCM). This difference was due to proportionately greater manure management related GHG emissions for LF fed-cows than HF-fed cows particularly when FNDF was mainly from CS (i.e., LFCS diet). This greater emissions of CH_4 during storage of manure for LF than HF-fed cows were most likely due to greater availability of substrates (e.g., ADF and NDF) for CH_4 formation (Uddin et al. 2019b; Benchaar and Hassanat, 2019) because in this study, LFCS diet contained substantially greater amount of highly digestible soyhulls compared to other three diets (Uddin et al. 2019a). Soyhull fiber contains less lignin than forage fiber and therefore, it is likely that less lignified (high digestible) soyhull yielded more CH_4 during

longer term anaerobic fermentation of CH_4 forming substrates in manure. In agreement to our hypothesis, Benchaar and Hassanat (2019) also reported a 55% greater CH_4 emissions during manure storage for cows fed highly digestible brown mid-rib CS compared to conventional CS. The contribution of enteric CH_4 to overall CF was approximately 5 percentage unit lower for LF than HF-fed cows whereas the contribution of other processes on overall CF (e.g., feed production related emission, fossil fuel and transportation) were similar for LF and HF-fed cows (Figure 2). Alfalfa silage and CS-fed cows had very similar CF (1.52 vs. 1.48 kg CO_2-e/kg FPCM, for CS and AS-fed cows, respectively), but being slightly greater for CS (~2.5%) than AS-fed cows. Compared to AS, CS-fed cows had proportionally 13% greater manure management related GHG emission particularly CH_4 emission during storage of manure (0.52 vs. 0.45 kg CO_2-e/kg FPCM for CS and AS, respectively), which was partially compensated by 7% lower GHG emissions for feed production (0.22 vs. 0.24 kg CO_2-e/kg FPCM for CS and AS, respectively). Little et al. (2017) also reported similar CF for AS and CS-fed cows in Canadian production system although average CF in their study was 25% lower than our study (1.12 vs. 1.50 kg CO_2-e/kg FPCM, respectively). In our study, we used measured emissions whereas Little et al. (2017) used IPCC based emission factors which might have contributed for this difference. Baldini et al. (2018) also reported a 21% greater CF for milk production when they used measured emission for manure management instead of IPCC estimates. This greater CF in our study can also be explained by the greater proportion of emissions allocated to milk in our study (93% in this study and 89% in Little et al. 2017).

3.1 Carbon footprint for Holstein and Jersey breed scenarios

The CF of milk production for Holstein and Jersey breed scenarios including the contribution of each process is shown in Table 9 and Figure 3. Overall, the CF of milk production for Holsteins was 5% greater than Jerseys (1.55 vs. 1.48 kg CO_2-e/kg FPCM). This greater CF for

Holstein than Jersey was mainly due to 29% greater manure management related GHG emissions (0.54 vs. 0.42 kg CO_2-e/kg FPCM, respectively) although the enteric CH_4 was 7% lower for the Holstein than Jersey (0.67 vs. 0.72 kg CO_2-e/kg FPCM, respectively; Figure 3). This greater CH_4 emission during storage of manure from Holsteins than Jerseys was most probably due to the differences in manure yield, and structural differences of CH_4 forming substrate between breeds. This structural differences could be the differential proportion of forage and non-forage sourced ADF and NDF in manure resulting from differential rate of passage for ruminal contents between breeds since Jersey is known to have greater rate of passage than Holstein. Lower enteric CH_4 for Holsteins than Jerseys was most likely associated with greater FPCM for the former than the latter. Riva et al. (2014) reported the similar trend but a greater difference (20%) between Holstein-Friesian and Jersey cow's milk CF than our study. Moreover, the CF reported by Riva et al. (2014) was lower than the CF of this study (0.88 vs. 1.52 kg CO_2-e/kg FPCM, respectively). In another study comparing dual purpose Fleckveigh with Holstein-Frisian cattle breed, the magnitude of difference in CF was 8% which was comparable to our study (Zehetmeier et al., 2014). Furthermore, the latter study also reported a wide range of CF for both Fleckveigh (0.9 to 1.25 kg CO_2-e/kg FPCM) and Holstein-Friesian (0.79 to 1.20 kg CO_2-e/kg FPCM) indicating the large variability in CF across and within breeds. Comparison of results among LCA studies is challenging and caution should be adopted before making any conclusions. Because, LCA results vary significantly across studies due to variability during goal and scope definition, allocation among co-products, boundary of systems and source of emission data (Wattiaux et al. 2019). For instance, Riva et al. (2014) allocated emission between milk and meat using biological allocation (on an average, 76% emission was allocated to milk) whereas we used economic allocation (93% emission was allocated to milk). Most importantly, all three studies cited above used mainly IPCC

based EF for inventory whereas we used measured EF for enteric CH_4 and manure management related emissions. Additionally, the three major sources of GHG for both breeds were enteric CH_4 followed by manure management and feed production related GHG emissions which comprised around 92% GHG emissions for milk CF for both breeds (Figure 3).

3.1 Sensitivity analysis

3.1.1 Effects of allocation methods on milk carbon footprint of four dietary and two cow breed scenarios

Overall, the average CF across dietary and breed scenarios was 1.50 kg CO_2/kg FPCM regardless of allocation methods. The CF result in our study was greater than the average CF value reported by Wattiaux et al. (2019) across continents (1.50 vs. 1.0 kg CO_2/kg FPCM) but our CF value falls within the range of literature values reported for North America and Europe (1.10 to 1.66 CO_2/kg FPCM; Thoma et al., 2013; Battini et al., 2016; Capper, 2011; and FAO, 2010). This greater CF value in our study was most likely due to the differences in allocation methods Thus, a sensitivity test for different allocation methods (no allocation, mass allocation and economic allocation) revealed the large variability of CF in our study (Figure 4 and 5). The 100, 93 and 88% emission was allocated to milk respectively for no allocation, economic allocation and mass allocation methods compared in this study (Table 3). Depending on the allocation method, the CF of milk production varied widely across dietary scenarios ranging from 1.31 to 1.76 kg CO_2-e/kg FPCM (Figure 4). As expected, the CF was greatest (1.60 kg CO_2-e/kg FPCM) when 100% emissions were allocated to milk whereas the CF was lowest (1.42 kg CO_2-e/kg FPCM) when emissions were allocated to milk and meat using mass allocation as per IDF (2015) default. Battini et al. (2016) also reported the similar magnitude and trend of CF when same three allocation methods were compared. Contrary to other studies reported here, our assumption for herd structure

(e.g., primiparous cows only) might have also contributed to this difference since primiparous cows have lower milk production than a typical herd consists of both primiparous and multiparous cows.

In terms of FNDF level, LF-fed cows had greater CF than HF-fed cows irrespective of allocation. Similarly, AS-fed cows had slightly lower (2.5%) CF than CS-fed cows regardless of allocation methods. In case of breed, the CF for milk production ranged from 1.41 to 1.66 kg CO_2-e/kg FPCM. Jersey had nearly 5% lower CF than Holstein regardless of the allocation method (Figure 5). Again, the CF was lowest for mass allocation (1.44 kg CO_2-e/kg FPCM) and the greatest when 100% emission was allocated to milk (1.63 kg CO_2-e/kg FPCM).

3.1.1 Carbon footprint of milk production between herd composed of either only primiparous cows or mixture of primiparous and multiparous cows

In this study, we assumed that all the lactating cows in the herd were primiparous. This assumption was one of the limitations in our study because this assumption does not mimic real scenario since herd is typically composed of both primiparous and multiparous cows. Therefore, we performed a sensitivity test for only the Holstein breed scenario to determine the effect of inclusion of multiparous cows in the herd on CF through accounting milk production differences between primiparous and multiparous cows. Because, primiparous cows produce 22 to 27 % lower milk than multiparous cows (Siewert et al., 2019; Toledo et al., 2017; Lea and Kim 2006) which might have impact on overall CF. Our sensitivity results showed that regardless of allocation method, Holstein herd composed of only primiparous lactating cows had approximately 7.5% greater CF than the Holstein herd composed of both primiparous and multiparous cows (1.56 vs. 1.44 kg CO_2-e/kg FPCM, Figure 6). This reduction of 0.12 kg CO_2-e/kg FPCM was mainly due to greater milk production of multiparous cows than primiparous cows. A meta-analysis using 30

published LCA studies also found a reduction of 0.0617 kg CO_2-e/kg FPCM when milk yield was increased by 10% (Lorenz et al., 2019). Therefore, this difference needs to be accounted for while comparing our CF results with other studies that mimic actual herd structure consisting of both primiparous and multiparous cows.

4. CONCLUSIONS

In conclusions, the average CF across four dietary and two breed scenarios was 1.50 kg CO_2-e/kg FPCM which falls at the higher end of CF values reported in literature. In this study, we used measured emissions instead of using IPCC based emission factors and we also assumed that dairy herd is composed of only primiparous cows since empirical data were only available for primiparous cows. These two factors might have contributed to greater CF results in our study compared to previously published results. The CF value was reduced from 1.50 to 1.42 kg CO_2-e/kg FPCM when mass allocation was used instead of economic allocation. Sensitivity analysis for Holstein breed scenario revealed that inclusion of multiparous cows in the herd further reduced CF by 7.5% due to mainly greater milk production of multiparous cows. Thus, we found a CF value of 1.31 kg CO_2-e/kg FPCM when emission was allocated between milk and meat using mass allocation for Holstein breed scenario consisting of both primiparous and multiparous cows. Our findings demonstrated that LF-fed cows had approximately 11% greater CF than HF-fed cows. On contrary, the CF between AS and CS-fed cows was very similar. Thus, under the condition of this study, the type of forage produced on-farm (AS vs CS) may have a much lower effect on milk CF than the level of forage in the diet. Likewise, the CF for Holstein was only 5% greater than Jersey. Compared to the findings of this study, evaluating the same treatment factors on either animal-scale GHG emissions (enteric CH_4) or manure management related GHG emissions indicated

contradictory results. Therefore, GHG mitigation strategies (choice of cow breed or diet) should be evaluated holistically to avoid wrong conclusions.

FUNDING

This project was financially supported by the National Institute of Food and Agriculture, US Department of Agriculture Hatch Multi-state research formula fund (# WIS01941, and USDA-NIFA # 2013-68002-20525).

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Table 1. Body weight (BW), dry matter intake (DMI), fat-and protein corrected milk (FPCM) and manure yield of different animal groups.

Item (kg/d)	Lactating animals' information[1]					
	Four dietary group of cows[2]				Breeds	
	LFAS	LFCS	HFAS	HFCS	Holstein	Jersey
BW (kg)	512	518	512	514	613	415
DMI	22.5	23.6	19.9	21.9	25.2	18.8
FPCM[3]	29.2	29.9	27.4	29.0	32.7	24.9
Manure	67.2	65.8	65.8	67.0	73.9	58.3
	Non-lactating animals' information[4]					
	Holstein			Jersey		
Animal group	Dry cows	Heifer (>1yr)	Heifer (< 1yr)	Dry cows	Heifer (> 1yr)	Heifer (< 1yr)
BW (kg)	600	450	225	400	300	150
DMI	13.0	11.3	5.7	9.7	8.4	4.2
Manure[5]	31.8	22.1	22.1	25.1	17.4	17.4

[1]Lactating Holstein and Jersey cows' information were collected from companion study (Uddin et al. 2019a).

[2]Lactating cows' diets: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[3]FPCM: Fat-protein corrected milk which was calculated as per IDF (2010) formula.

[4]Nonlactating Holstein and Jersey animals' information were adapted from Akins (2016) and NRC (2001), respectively.

[5]Non-lactating animals' manure excretion were predicted using their DMI information with the equation developed using lactating Holstein and Jersey cows manure excretion.

Table 2. Summary of four dietary treatments for lactating cows and three group of non-lactating animals' diet.

Ingredients (% DM[1])	Lactating cows' diets[2]				Non-lactating animals' diets[3]		
	LFAS	LFCS	HFAS	HFCS	Dry cows	Heifer (> 1 yr)	Heifer (< 1 yr)
Alfalfa silage	36.60	15.70	45.73	19.60	47.00	44.20	40.00
Corn silage	17.00	39.60	21.23	49.55	47.00	54.80	35.00
Corn grain	23.20	10.70	21.20	5.70	-	-	13
Soybean meal	7.40	12.20	5.80	11.90	2.25	-	11
Soy hulls	12.70	18.75	3.00	10.20	2.5	-	-
Molasses	0.60	0.60	0.60	0.60	-	-	-
Blood meal	0.70	0.70	0.70	0.70	-		-
Vitamin & minerals	1.8	1.8	1.8	1.8	1.3	1.0	1.0

[1]DM: Dry matter.

[2]Lactating cows' diets: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[3]Heifers and dry cows' diets were formulated with NRC (2001) using information from NRC (2001) and Akins (2016).

Table 3. Calculation of allocation for different co-products.

Co-products	Allocation method	
	Economic	Mass
Milk:meat	93:7	88:12[1]

[1]As per IDF (2015) default value for mass allocation between milk and meat for intensive system.

Table 4. The required land for on-farm feed production under different dietary and breed scenarios with same herd size.

Item	Land required for on-farm forage production (ha/farm[1])					
	Four dietary group of cows[2]				Breeds	
	LFAS	LFCS	HFAS	HFCS	Holstein	Jersey
Alfalfa silage	83.0	52.0	89.0	56.0	81.0	61.0
Corn silage	22.8	37.0	24.0	41.0	35.0	26.0
Corn grain	23.6	11.7	19.0	6.0	17.5	13.1
Total forage	128.6	100.7	132.0	103.0	133.5	100.0

[1]A farm consists of 122 lactating cows, 22 dry cows, 56 growing heifers over one year of age and 63 growing heifers under one year of age.

[2]Lactating cows' diets: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

Table 5. Inputs required for on-farm feed production [adapted from Aguirre-Villegas et al. (2015) with additional calculation taking into account for recent AS, CS and corn grain DM yield/ha of land as per USDA-NASS (2017)].

	Per ton of DM[1] yield		
	Alfalfa silage	Corn silage	Corn grain
Seeds (kg)	0.9	1.7	1.8
Herbicide (kg)	0.42	-	0.32
Pesticide (kg)	0.1	0.3	0.3
Lime (kg)	162	30	8
Diesel (L)	25	19	8
Gasoline (L)	-	-	1.7
Petrolium gas (L)	-	-	2.5
Electricity (kWh)	-	-	4.5
Natural gas (m^3)	-	-	1.7
Machinery (kg)	5.5	5.5	1.2
Nitrogen (kg)	0.00	11	19
Phosphorus (kg)	4.7	2.0	2.5
Potassium (kg)	40	9	4

[1]DM: Dry matter.

Table 6. The emission factors for purchased feed ingredients.

Feed ingredients	Emission factors (kg CO_2-e/ton DM)
Soybean meal[1]	400
Soyhulls[2]	410
Molasses[2]	562
Blood meal[2]	70
Vitamin and mineral premix[2,3]	890

[1]Adapted from Adom et al. (2012).

[1]Adapted from Adom et al. (2013).

[3]The EF for vitamin and mineral premix is the average EF weighted for each component of the premix as per ratio used in the diets described by Uddin et al. (2019a).

Table 7. Emission sources including their respective emission factor for enteric CH_4 and manure management emissions.

Emission Sources	Four dietary Scenarios[1]				Two breed Scenarios		References/ Comments
	LFAS	LFCS	HFAS	HFCS	Holstein	Jersey	
	Enteric CH_4 by animal type (kg/d per animal)						
Lactating cows	0.440	0.439	0.413	0.418	0.471	0.385	Uddin 2019a
Dry cows[2]	0.264	0.264	0.264	0.264	0.284	0.243	Niu et al. 2018
Heifers (> 1yr)[2]	0.245	0.245	0.245	0.245	0.304	0.228	Niu et al. 2018
Heifers (< 1yr)[2]	0.185	0.185	0.185	0.185	0.194	0.176	Niu et al. 2018
	Manure CH_4 (g/kg manure) and manure N_2O emission (mg/kg manure)						
Storage[3] CH_4	2.69	4.61	2.34	2.14	3.36	2.53	Uddin 2019b
Field[4] CH_4	0.814	0.638	0.660	0.501	0.919	0.391	Uddin 2019b
Storage N_2O	2.78	2.12	1.11	1.80	2.45	1.46	Uddin 2019b
Field N_2O	206	243	180	183	216	189	Uddin 2019b
	Indirect N_2O (kg/farm) due to NH_3 volatilization and NO_X leaching						
Indirect N_2O[5]	0.387	0.386	0.481	0.453	0.472	0.379	Due to NH_3
Indirect N_2O[5]	0.002	0.006	0.002	0.007	0.005	0.004	Due to NO_x

[1]Lactating cows' diets: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

[2]Since dry cows, growing heifers (> 1year of age) and growing heifer (< 1 year of age) were assumed to feed same diet under different dietary scenarios, so the emission factors for non-lactating animal groups were similar across dietary scenarios.

[3]Manure was stored over 50 days before land application and thus, storage emission factors were for 50 days period.

[4]Field emissions was conducted over 50 days both in fall and spring followed by land application (surface application) and then, the field emission factors were extrapolated to annual emissions.

[5]Calculation of indirect N_2O due to NH_3 volatilization and NO_X leaching were calculated as per De vries et al (2003) and IPCC (2006a, 2006b) equations. All possible NH_3 and NO_x sources (during manure storage, land application of N both from manure and chemical fertilizer) were included in this calculation. However, NH_3 emission occurs at barn was not accounted for since we did not have barn NH_3 emission in our study condition due to direct collection of feces and urine separately (details can be found in Uddin et al. 2020).

Table 8. Contribution of each process on carbon footprint of milk production (kg CO_2-e/kg FPCM) determined using economic allocation for different dietary scenarios.

Emission Sources	CF of four dietary scenarios (kg CO_2-e/kg FPCM)			
	LFAS	LFCS	HFAS	HFCS
Enteric CH_4	0.68	0.67	0.70	0.67
Manure chain GHG	0.47	0.64	0.43	0.39
During storage	0.24	0.40	0.22	0.20
CH_4	0.23	0.39	0.22	0.20
N_2O	0.01	0.01	0.00	0.00
During field	0.23	0.24	0.21	0.19
CH_4	0.07	0.06	0.06	0.05
N_2O	0.16	0.18	0.15	0.14
Feed production	0.24	0.23	0.23	0.21
Forage production	0.09	0.10	0.10	0.09
Purchased feed	0.14	0.13	0.12	0.12
Feed transportation	0.01	0.00	0.01	0.00
Indirect N_2O	0.03	0.03	0.02	0.03
Farm fossil energy	0.08	0.08	0.09	0.08
Total Carbon Footprint	1.49	1.65	1.46	1.38

[1]Lactating cows' diets: LFAS = Low (19.0%) Forage NDF with a 70:30 ratio of alfalfa silage (AS) NDF:corn silage (CS) NDF; LFCS = Low (19.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF; HFAS = High (24.0%) Forage NDF with a 70:30 ratio of AS NDF:CS NDF; HFCS = high (24.0%) Forage NDF with a 30:70 ratio of AS NDF:CS NDF.

Table 9. Contribution of each process to carbon footprint of milk production (kg CO_2-e/kg FPCM) determined using economic allocation for Holstein and Jersey breed scenarios.

Emission Sources	CF of cow breed (kg CO_2-e/kg FPCM)	
	Holstein	Jersey
Enteric CH_4	0.67	0.72
Manure chain GHG	0.54	0.42
During storage	0.30	0.23
CH_4	0.30	0.23
N_2O	0.00	0.00
During field	0.24	0.19
CH_4	0.07	0.04
N_2O	0.17	0.15
Feed production	0.23	0.22
Forage production	0.10	0.10
Purchased feed	0.12	0.12
Feed transportation	0.01	0.00
Indirect N_2O	0.03	0.03
Farm fossil energy	0.08	0.09
Total Carbon Footprint	1.55	1.48

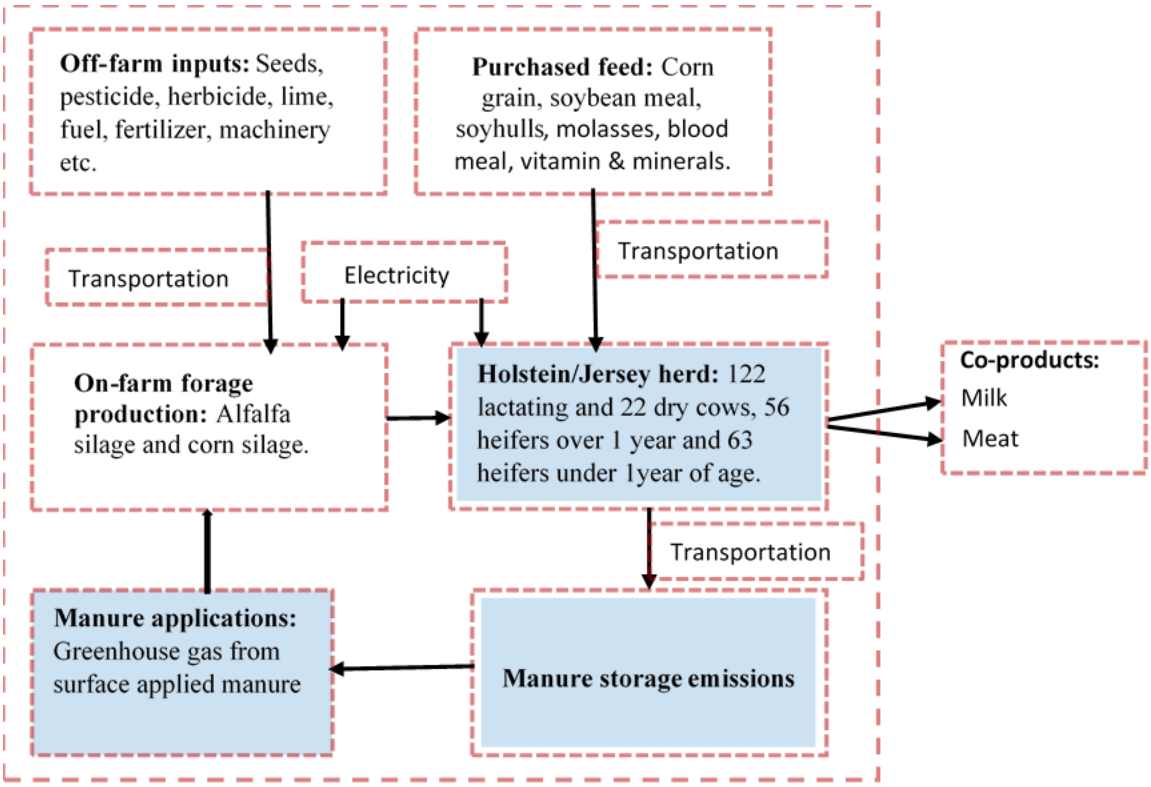

Figure 1. Boundary of the systems included in this life cycle assessment study (boxes with blue and white background are showing measured and estimated emission factors, respectively).

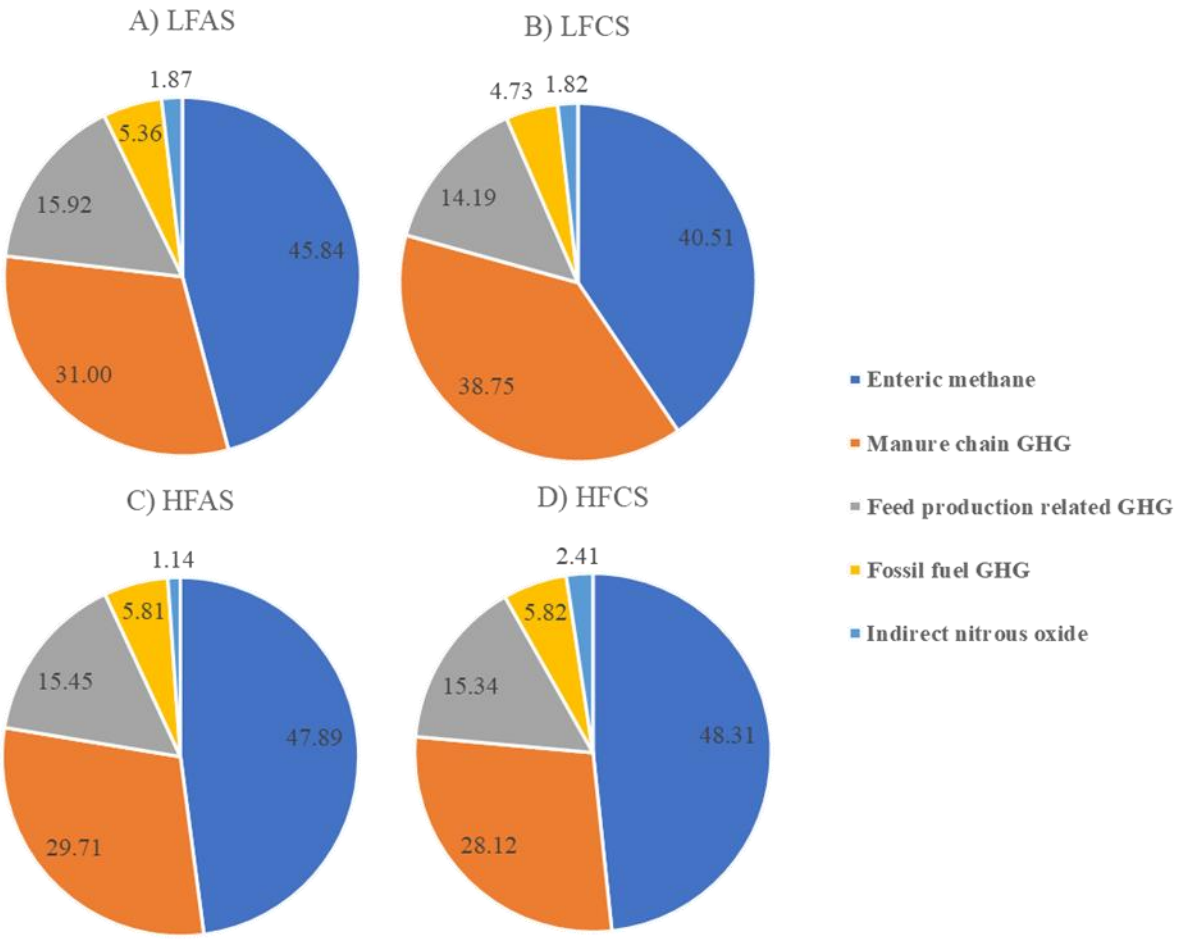

Figure 2. The percentage contribution of different GHG sources to total milk carbon footprint for different dietary scenarios using economic allocation.

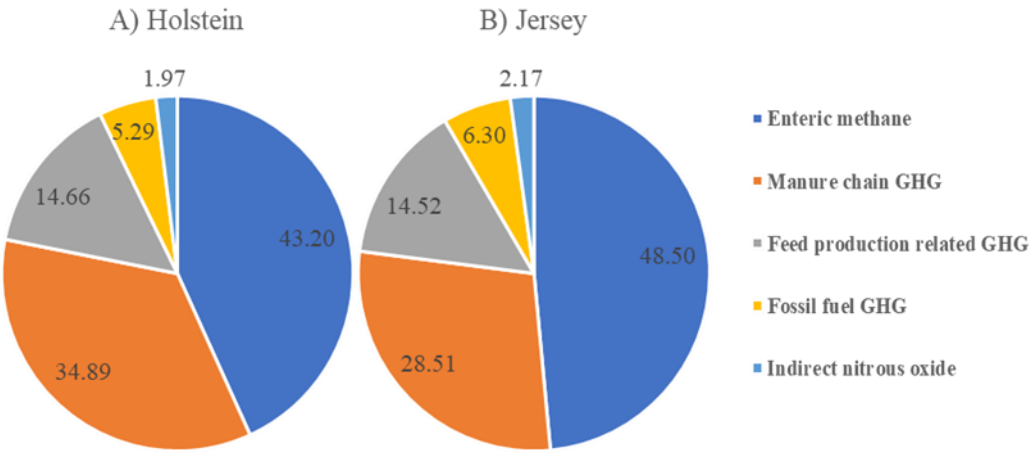

Figure 3. The percentage contribution of different GHG sources to total carbon footprint of milk production for Holstein and Jersey breeds using economic allocation.

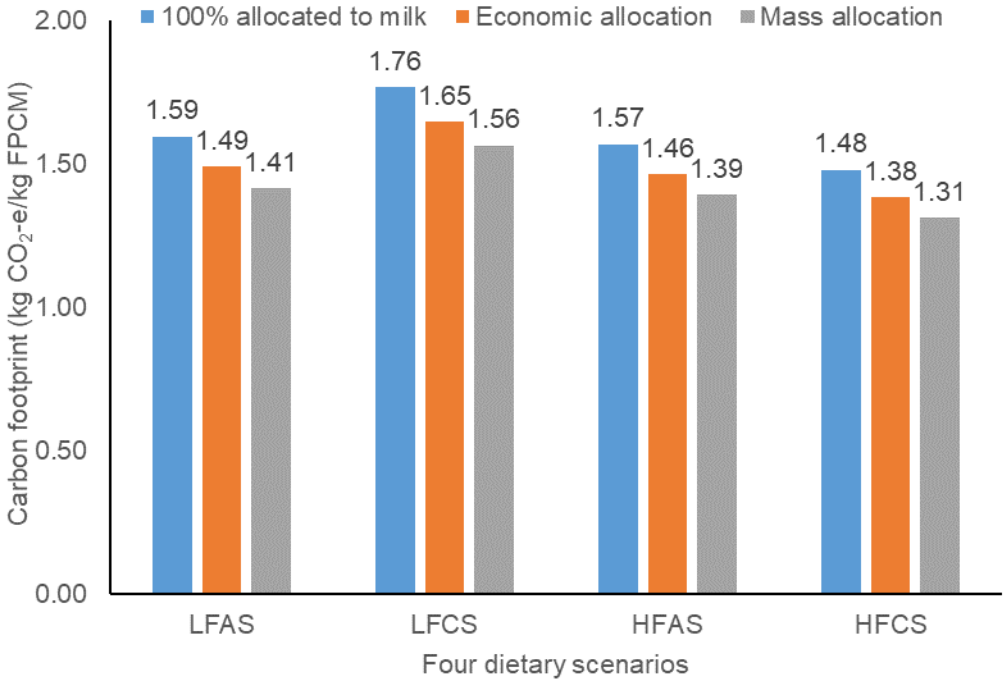

Figure 4. Carbon footprint of milk production (kg CO_2-e/kg FPCM) for four different dietary treatment scenarios as affected by choice of co-products allocation.

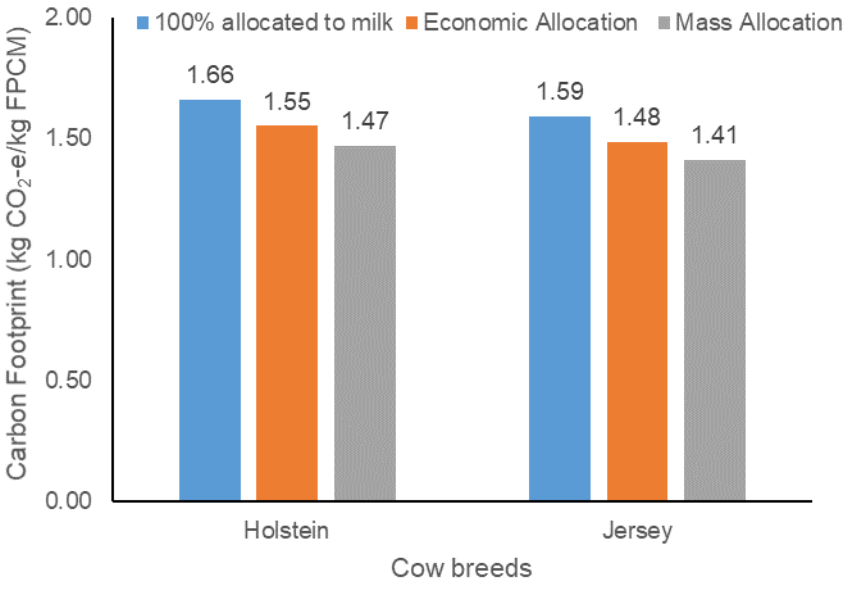

Figure 5. Carbon footprint of milk production (kg CO_2-e/kg FPCM) for two cow breed scenarios as affected by choice of co-products allocation.

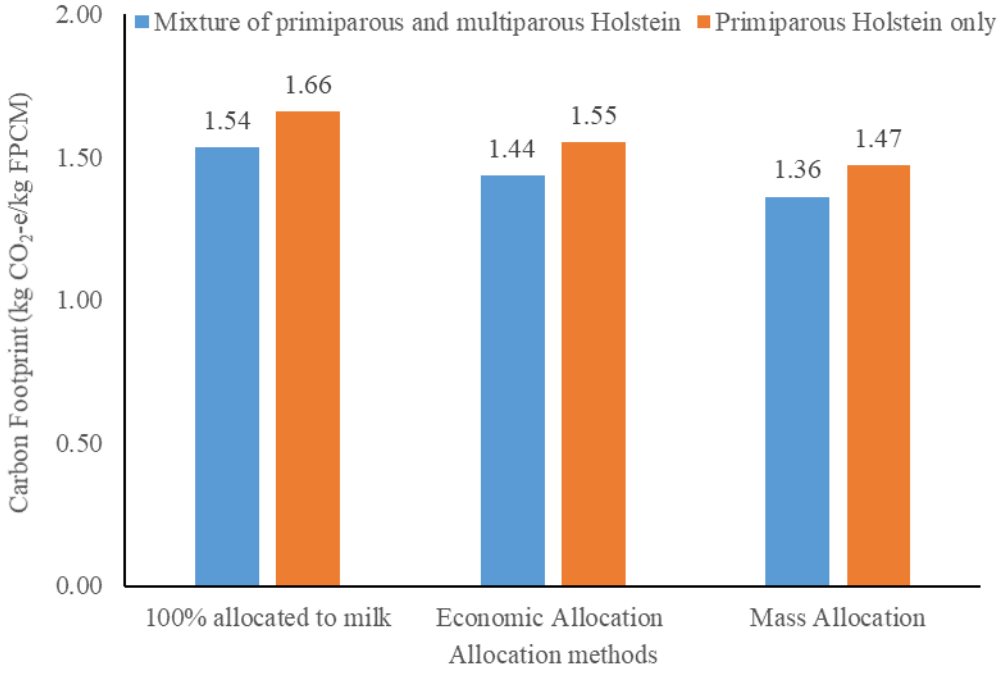

Figure 6. Comparison of carbon footprint of milk production (kg CO_2-e/kg FPCM) for Holstein breed scenario when herd consisted of only primiparous cows vs. mixture of primiparous and multiparous cows.

CHAPTER 6. Future Research

In our first experiment (Chapter 2), we used a 2x2 factorial arrangement of treatments to examine the effects of forage neutral detergent fiber (**FNDF**) when incorporated in dietary dry matter (DM) at a level of either 19% (low FNDF, **LFNDF**) or 24% (high FNDF, **HFNDF**) with a ratio of alfalfa silage (**AS**) NDF to corn silage (**CS**) NDF of either 70:30 or 30:70 on enteric methane (**CH_4**) emissions, lactation performances, feed efficiency, energy and N balances of Holstein and Jersey cows. In this study, dry matter intake (**DMI**) was highly and positively correlated with the level of non-forage fiber incorporated from soyhulls (Figure 1) as the level of FNDF was reduced in the diet. Forage source had no effect on CH_4 production (g/d per cow), but we found that LFNDF-fed cows had a 6% greater enteric CH_4 production (g/d per cow) than HFNDF-fed cows. These results led us to the conclusion that methanogenesis was not altered as much by the difference in chemical composition of the NDF between AS and CS but rather by the level of soyhull in the diet (Figure 2), an effect mediated by the impact of soyhulls on DMI (Figure 1). Both LFNDF- and HFNDF-fed cows had a similar CH_4 yield (g/kg DMI) and CH_4 intensity (g/kg FPCM).

Compared to HFNDF, LFNDF-fed cows had a lower CH_4 yield expressed as g/kg NDF intake but a greater CH_4 yield expressed as g/kg FNDF intake (Figure 3). These results led us to the conclusion that methane yield is greater for soyhull (non-forage fiber) NDF than for the forage NDF. Compared to HFNDF, LFNDF-diets contained a greater proportion of non-forage NDF, particularly from highly digestible soyhulls that was added to their diet to keep the NDF content similar across diets. This is also one of the typical limitations of nutritional replacement studies - changing one chemical parameter in the diet could bring a change to other chemical parameters due to differential chemical composition of different feed ingredients. Although researchers

attempt to avoid confounding effects by keeping most things constant except for the treatment factors, most replacement studies (e.g., replacing one ingredient with another) nonetheless create a plausible confounding effect. One avenue for future research is to focus on methane per unit of digestible energy available from a feed. Therefore, future research should investigate the effects of soyhulls in diets with varying forage to concentrate ratios specifically on ruminal parameters and enteric CH_4 emissions in dairy cows. The hypothesis to test is that soyhull NDF yield substantially more methane than forage NDF because (a) it is more digestible than forage NDF (a direct effect) and (b) because it increases DMI by increasing rate of passage and thus alleviating rumen fill compare to forage NDF.

Forage source (AS and CS) did not affect CH_4 production but CS-fed cows had lower CH_4 yield than AS-fed cows irrespective of calculation modes (Figure 4). Arndt et al. (2015) also reported no difference in enteric CH_4 (g/d per cow) production and yield (g/kg DMI) when 20:30 vs. 80:20 ratio of AS:CS was compared. Disagreement with our findings, Arndt et al. (2015) reported a lower CH_4 yield expressed either as g/kg NDF intake or g/kg digested NDF for AS-fed cows than CS-fed cows. Arndt et al. (2015) formulated the diets in a way where both FNDF source and starch concentrations varied which might have created confounding effects between them.

In our second experiment (chapter 3), we investigated the carry-over effects of the same three treatment factors as mentioned for the first experiment (FNDF level, FNDF source and cow breed) on greenhouse gas (**GHG**) emissions, namely carbon dioxide (**CO_2**), CH_4 and nitrous oxide (**N_2O**) measured during storage and after subsequent land application of manure. We measured GHG for 50 days during storage and 50 days after subsequent land application (30 days during fall and 20 days during spring). The manure was stored at lab conditions, which was a temperature-controlled environment. Thus, our storage condition does not fully mimic the typical manure

storage scenario because farmers do not store manure in controlled environments. Typically, manure storage periods vary from 0 (small farm) to 6 (large farm) months (Aguirre-Villegas and Larson, 2017). Thus, the manure storage period and storage environment should be carefully considered while designing any future research on manure storage GHG emissions. Our field measurements also do not represent full fall and spring season scenarios since our measurements were short term in late fall and early spring only. Our goal was not to determine seasonal effects on GHG emissions but rather to determine the carry-over effects of cow breed and diet on manure GHG emissions after field application. Compared to HFNDF, LFNDF-fed cows manure had greater emissions of 100-day (storage plus field) non-CO_2 GHG which were not affected either by forage source (AS and CS) or by cow breed (Holstein and Jersey). We found a huge spike in N_2O emissions during spring but not in the fall measurement. This difference might be due to differences in ambient temperature, soil temperature and soil humidity, which were greater during spring (April to May) than in the fall (November to December) measurement period in our study.

Our final goal was to evaluate the effects of dairy cow breeds and dietary treatments on whole-farm GHG emissions. Thus, we took a holistic approach to determine whole-farm GHG emissions as affected by aforementioned treatment factors. We conducted an attributional life cycle assessment (**LCA**) to compare the carbon footprint (**CF**) of all scenarios. Our findings showed that CF was similar across forage source and two breed scenarios, but LFNDF-fed cows had approximately 11% more CF than HFNDF-fed cows. Attributional LCA is the first step which is less complicated, and less data demanding way to determine and compare CF of different scenarios. However, consequential LCA might lead to different conclusions because attributional LCA does not account for all the consequences that may happen due to replacing one ingredient in the diet with another. Furthermore, the methodological differences between attributional and

consequential LCA were well captured in a study conducted by Zanten et al., (2018). They concluded that choice of LCA methodology (attributional vs. consequential) might direct to contradictory conclusions. They compared the effect of replacing soybean meal with rapeseed meal on changes in land use via both attributional and consequential LCA. They found a 14% decrease in agricultural land use when they used attributional LCA and a 10% increase in land use when they used consequential LCA (Figure 5). Therefore, future research might focus on determining the CF of the same scenarios studied here using a consequential LCA approach. Like other LCA studies, we did not account for soil organic carbon (**SOC**) stock change due to unavailability of data of SOC change. However, different forage production (AS or CS) might affect SOC stock differentially. For example, over the first 20 years the Wisconsin Integrated Cropping Systems Trial found a loss of SOC with annual row crop while perennial crop was a sink of C (Sanford et al., 2012). Thus, quantification of SOC stock change for AS and CS cropping system over longer period is necessary and SOC change need to be accounted for in future LCA study evaluating the effects of forage source on CF.

It is well established that the CF of milk production in extension systems of developing countries is much greater than in the intensive system found typically in developed world. For instance, CF of milk production in Sub-Saharan Africa was 5.8 and 3.6 times greater than US and world milk CF, respectively (Figure 6; FAO, 2010) because cows in an extensive system produce much lower amount of milk than in an intensive system. However, these cows in developing countries do not only produce milk, meat and manure, but they also provide additional benefits to the farmers (Herrero et al., 2013). For example, cows in developing countries may provide (a non-fossil fuel) source of power for tillage or transportation. They are considered as financial assets and provide a form of food security insurance (e.g., source of nutrient for the family members of

the farmer). These cows also provide other non-monetary benefits to the farmers, such as social standing. When multifunctionality of cows was accounted for and emission was allocated to all functionality, the CF for milk production in extensive systems was very similar to that in intensive systems (Figure 7; Weiler et al., 2014). Similarly, milk production from cattle and buffaloes in South-Asian countries (e.g., India, Bangladesh) also reported to have substantially greater CF than world average (5.0 vs. 2.1 kg CO_2-e/kg FPCM; FAO, 2010). These cattle and buffaloes also possess multifunctionality. Therefore, future research should focus on determination of CF of milk production from cattle and buffaloes in South-Asian countries accounting for multifunctionality.

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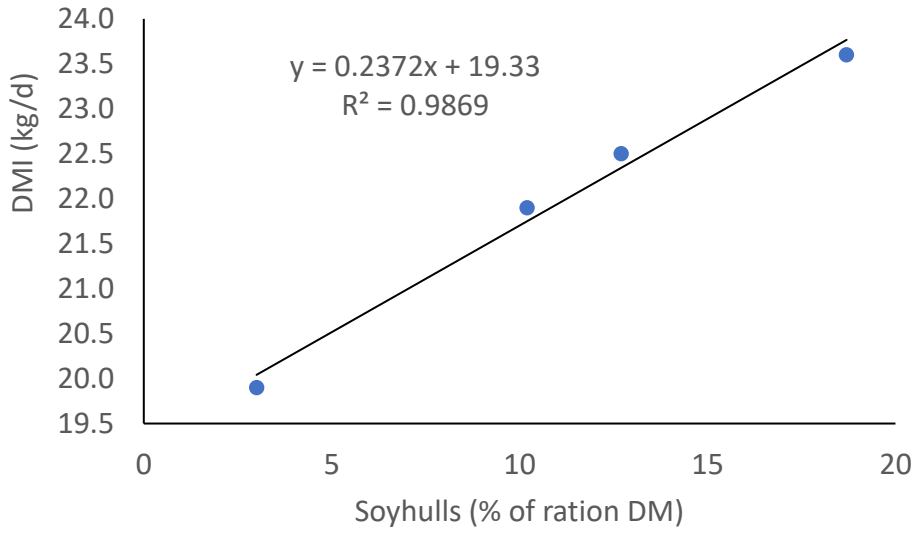

Figure 1. Relationship between DMI (kg/d) and Level of Soyhulls in dietary dry matter (DM); From left to right on the x axis, the data points corresponded to levels of 3.0, 10.2, 12.7 and 18.7 in the HFAS, HFCS, LFAS, and LFCS ration, respectively.

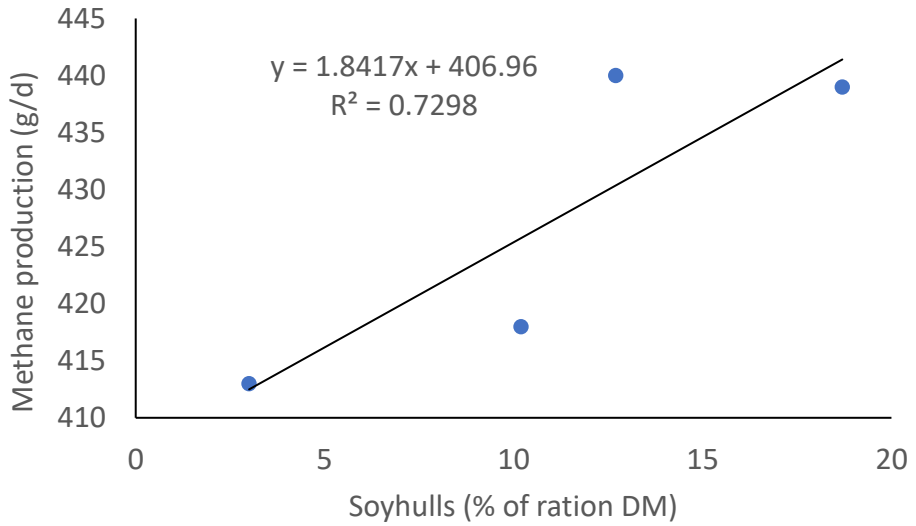

Figure 2. Relationship between enteric methane production (g/d) and level of Soyhulls in ration dry matter (DM); From left to right on the x axis, the data points corresponded to levels of 3.0, 10.2, 12.7 and 18.7 in the HFAS, HFCS, LFAS, and LFCS ration, respectively.

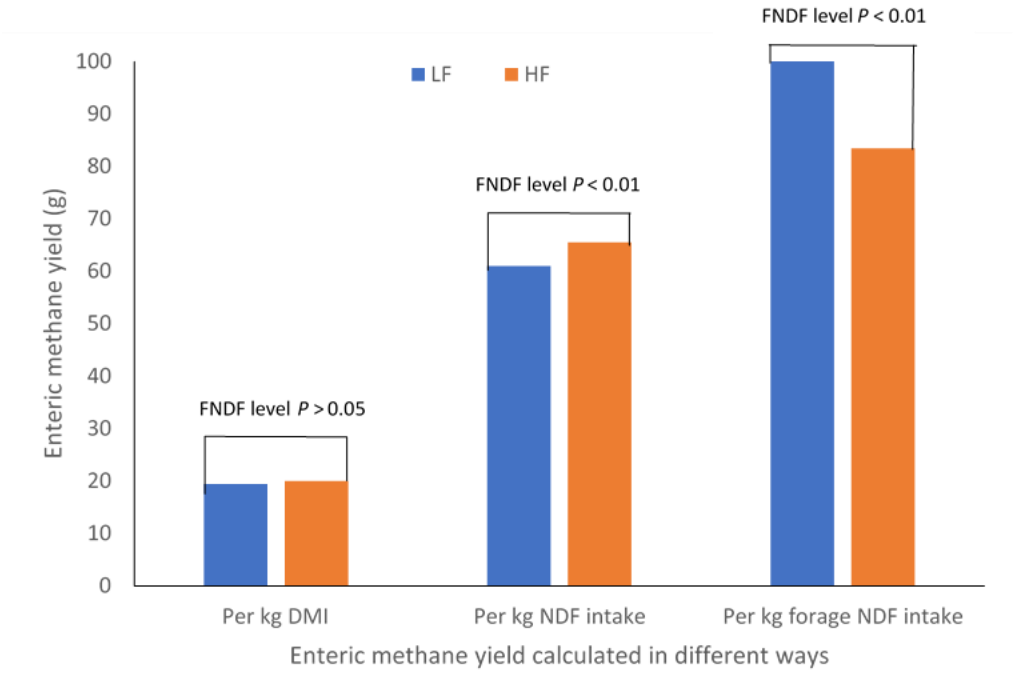

Figure 3. Effects of forage neutral detergent fiber level on enteric CH_4 yield depended on mode of calculation.

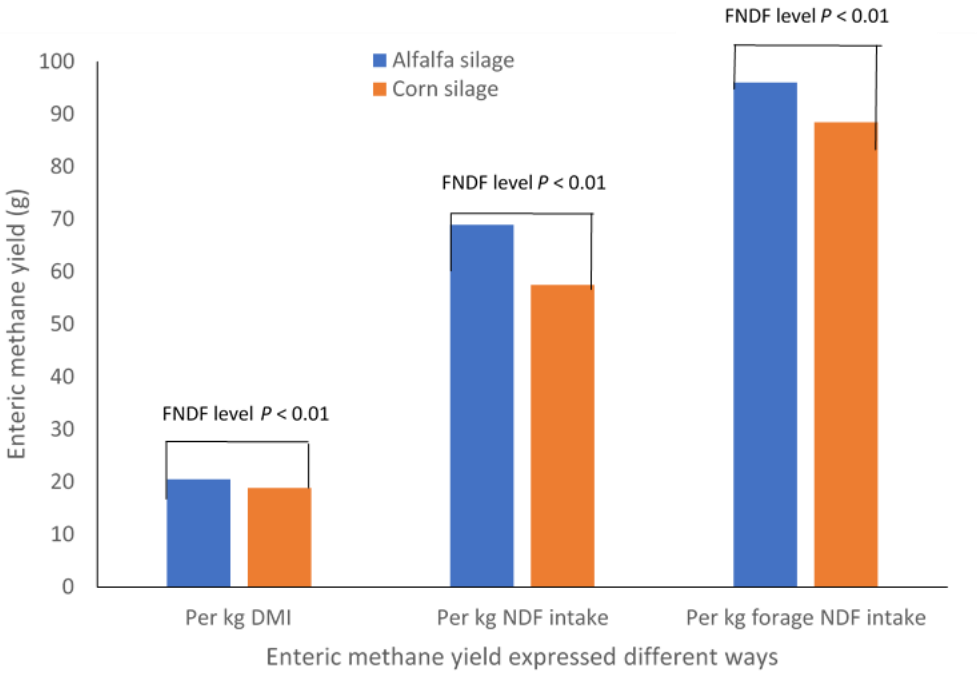

Figure 4. Effects of forage neutral detergent fiber source on enteric CH_4 yield expressed in different ways.

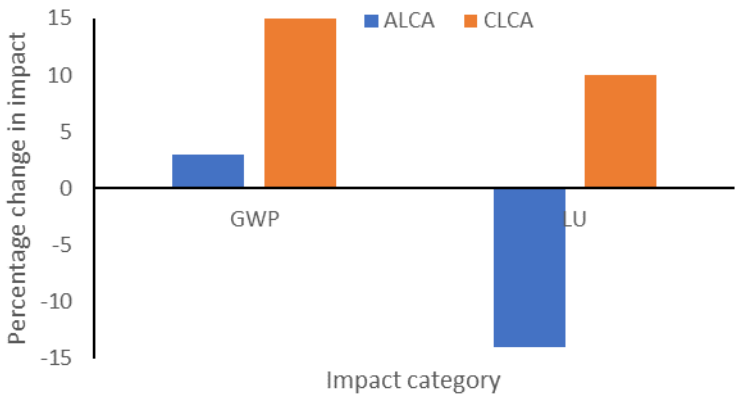

Figure 5. Effect of LCA methodology on global warming potential (GWP) and land use (LU) expressed as percentage change (adapted from Zanten et al., 2018).

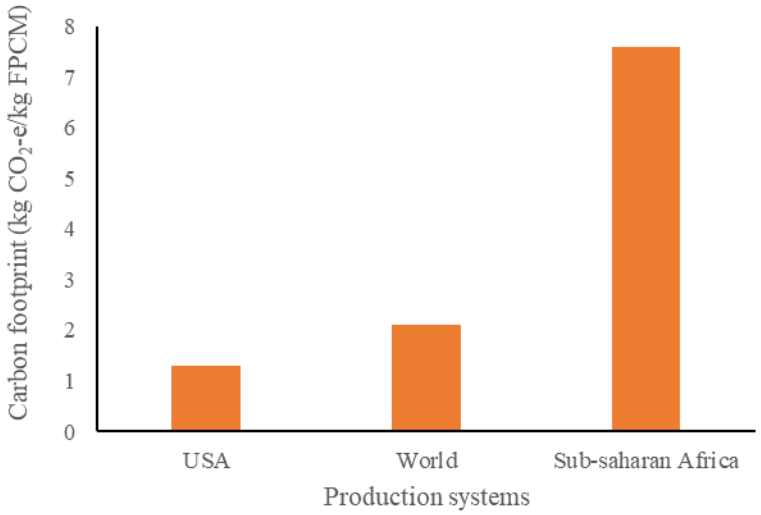

Figure 6. Carbon footprint of milk for different production systems without accounting for multifunctionality of cows (FAO, 2010).

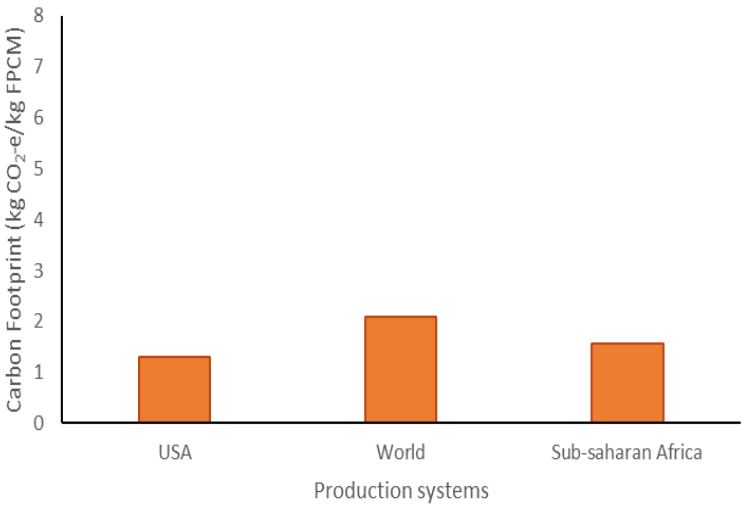

Figure 7. Carbon footprint of milk for different production systems accounting for multifunctionality of cows and emission was allocated to different functionality of cows (FAO, 2010; Weiler et al., 2014).