

Effects of a high protein corn co-product as a replacement for soybean meal in calf starter grains

by

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Abstract

Provision of a nutritionally balanced starter grain to dairy calves within the first weeks of life is essential for setting calves up for a successful weaning transition. While conventional dried distillers' grains are an attractive protein source for most cattle diets, utilization in calf starter grains have yielded undesirable results. Evolutions in ethanol fractionation technology has brought about innovations in co-product feed ingredients which may be more applicable for use in calf starter grains. High protein corn co-products (HPCC), which are enriched with yeast bodies from fermentation, contain similar amounts of crude protein to soybean meal and may be a suitable option for calf starter grains. To assess HPCC as a substitute for soybean meal in starter grains, Holstein calves were fed starter grains from 14 to 84 d of age with either 0 (CTRL), 50 (50HPCC), or 100% (100HPCC) of the soybean meal replaced by HPCC. During the preweaning period (14 to 42 d of age), HPCC quadratically effected starter grain intake with 50HPCC calves consuming less grain than CTRL or 100HPCC calves. The HPCC inclusion increased bodyweight; however, treatment did not affect structural growth or feed efficiency. After weaning, effects of HPCC on starter grain intake were less discernable. Bodyweight and average daily gain increased with HPCC, but wither height was reduced in weaned 50HPCC calves. Males grew heavier and taller throughout the study. Plasma glucose and insulin concentrations increased with time and insulin concentration was increased by HPCC in weaned calves. There was no evidence of treatment effect on dry matter or crude protein apparent total tract digestibility in weaned calves. Males had greater apparent total tract digestibility of dry matter and crude protein of than females. Overall, these results suggest that HPCC is a suitable replacement for soybean meal in calf starter grains when supplemented with methionine and lysine.

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Dedication

*To Papa Wayne and Chuck Krushke,
two men whose passion for dairy cattle is udderly contagious.*

Chapter 1 - Review of Literature

Introduction

Rearing dairy replacement heifer calves is a large, but vital, investment in the future for dairy herds. Nutritional management of calves in the first couple months of life can have effects lasting into subsequent productive careers in the milking herd (Henrichs and Henrichs, 2011; Gelsinger et al., 2016). Calves are born without a functioning rumen and will rely largely on milk to satisfy their energy and nutrient needs for the first several weeks of life. Within these weeks it becomes critical that calves initiate rumen development to ensure successful adaptation to a solid diet at weaning. As a preruminant, the neonate calf is dependent on glucose from milk that's digested in the intestines for energy. However, mature ruminants rely on the volatile fatty acids (VFA) produced through ruminal fermentation for energy. Therefore, rumen development is marked not only by the physical growth and development of the rumen epithelium, but also by the systemic shift that occurs as the rumen microbial population is established and the epithelium becomes metabolically active (Baldwin et al., 2004; Laarman et al., 2012).

Foundational work in the 1950s and 60s elucidated that feeding calves milk alone is not adequate to develop the rumen. Rather, VFAs from fermentation are required to stimulate rumen epithelial papillary development and the presence of solid feed in the rumen encourages muscular growth (Warner et al., 1956; Tamate et al., 1962). Butyrate has been identified as the most effective VFA stimulator of rumen epithelial development with acetate being the least effective (Sander et al., 1959; Tamate et al., 1962). Ruminal production of butyrate is encouraged by feeding starches and sugars, which is why grain diets have been the traditional choice for introducing dairy calves to solid feed (Gorka et al., 2018). It is recommended that, in addition to feeding milk, calves should have access to starter grains within the first week of life

(NASEM, 2021). Starter grains should continue being fed through and after weaning before transitioning to a grower diet at about 10 to 14 wks of age (Drackely, 2008).

A well formulated starter grain will encourage dry matter intake (DMI) to sufficiently develop the rumen before weaning. Additionally, starters should supply adequate energy and protein to support growth during and after weaning. Increased metabolizable energy and protein intake from starter grain during and after weaning is associated with increased first lactation milk production (Raubal et al., 2019). The multitude of available feed ingredients, processing forms, and vast supplementation options make feeding starter grains somewhat complex. The purpose of this review is to explore the major components of starter grains intended for dairy replacement calves and their implications on calf growth and development

Energy Sources in Starter Grains

Energy requirements of the calf

Energy is the most basic requirement for the functionality of all living organisms. While preweaning calves satisfy a substantial amount of their energy needs through milk intake, weaned calves are often exclusively reliant on the energy provided by a starter grain. The NASEM (2021) estimates that weaned heifers weighing up to 125 kg require $0.0825 \text{ Mcal/kg BW}^{0.75}$ to satiate maintenance requirements. Additional energy is required to support growth. Supplying calves with adequate dietary energy in the form of fats or carbohydrates will spare proteins for growth purposes, rather than shunting them to energy production (Arieli et al., 1995). The NASEM (2021) suggests that the amount of energy retained per unit of metabolizable energy (**ME**) intake is $0.378 - 0.441$ for energy from starter grains. This is less efficient than energy obtained from milk which is often found to be 0.5 or higher. This disparity in efficiency

could be explained by increased energy demand of a more developed gastrointestinal tract present in calves consuming solid feed. Environmental conditions will also impact calves' energy requirements. If ambient temperatures are above or below the calf's thermoneutral zone, additional maintenance energy is required to support thermoregulation (Roland et al., 2016; NASEM, 2021).

Fats

Although protein does supply dietary energy, fats and carbohydrates are more efficient and economical energy sources in cattle diets. Fat is the most energy dense nutrient at 9 kcal/g compared to 4 kcal/g from carbohydrates or proteins (Rolls, 2009). While the fat content of milk fed to calves is often 20% or greater on a dry matter basis, starter grains should contain much less to avoid hindering rumen function. If feeding a starter containing pellets, a small amount of added fat is likely inevitable in the diet as fat aids in the pelleting process (Muramatsu et al., 2013). Beyond that, the literature is inconsistent in regard to how much fat can be added into starter grains. Several studies have successfully included fat at 5 to 10% of DM without impacting DMI or growth performance (Luchini et al., 1992; Sharma et al., 1986; Johnson et al., 1956). In Berends et al. (2018) researchers mixed increasing amounts of high fat pellets into calf starters to create diets ranging from 3.3–13.4% fat, or offered calves a single 11% fat pelleted starter for 84 d. Increasing the fat level in the starters increased calf ME intake with the exception of calves offered the single 11% fat pellet diet which had lower ME intake. Calves offered the 7% fat diet had the greatest starter intake while calves fed 11.0 to 13.4% fat diets had reduced intakes. The low starter intake likely resulted in the lower ME intake for the calves on the single high fat pellets. Body weight gain was also highest for calves on the 7% fat diet; however, feed efficiency did not differ between diets. On the contrary, other studies have

observed decreased performance when supplementing starter grains with fats. Miller et al. (1959) identified decreased starter intake and body weight gain when starters contained 10% hydrogenated cotton seed oil or brown grease. When Kuehn et al. (1994) fed calves a 3.7 or 7.3% fat starter, the 7.3% fat diet depressed DMI and ADG postweaning. As little as 2% supplemental fat in starter grains fed pre- or post- weaning depressed intake and body weight gain in Hill et al. (2015). A meta-analysis conducted by Hu et al. (2018) pulling from a dataset where starter fat content ranged from 3 to 7% found fat inclusion rate was negatively correlated with feed efficiency. Decreases in calf performance associated with feeding fat are linked to the inhibitory effects of fat on rumen microbial populations resulting in decreased fiber and overall diet digestibility (Palmquist and Jenkins, 1980; Amando et al., 2022). Therefore, added fats should not be used as the main energy source in starter grain, but including small amounts, 5% or less, is reasonable.

More recent research on the topic of fat content in starters has focused on increasing fat to sustain energy intake during periods of stress such as cold weather or weaning. Ghasemi et al. (2017) supplemented starters with no extra fat, 3% tallow, 3% soybean oil, or a 3% mixture of both to determine if increased energy from different types of fat would affect calf performance during temperatures that would induce cold stress. Their study identified no benefits to fat supplementation. The single fat source supplemented calves consumed the same amount of starter and had similar ADG and feed efficiency to non-supplemented calves. Khalilvandi-Behroozyar et al. (2023) however, observed increased starter intake and decreased inflammatory markers in the blood of calves fed starters supplemented with 3% soybean oil, 3% fish oil, or a 3% mixture of both during cold stress inducing temperatures. Calves on the fish oil supplemented starter had a higher weaning body weight than any of the other treatments

indicating that fat type may impact the effectiveness of fat supplementation. Weaning is a stressful transition during calfhood which removes milk as an energy source for calves often leading to a slump in energy consumption and growth (Terre et al., 2007; Sweeney et al., 2010). Amado et al. (2022) investigated whether supplementing starter grains with high fat pellets could help minimize energy intake deficits around weaning. Calves were fed a 3.1% fat control diet, or supplemented diets containing hydrogenated rapeseed triglycerides (6.7% fat) or hydrogenated palm free fatty acid (PFA; 7.1% fat). Calves offered the fat-supplemented diets consumed more starter DM throughout the study, with calves in the PFA group having higher ME intake than the other two groups during and after the weaning period. The increase in starter and energy intake translated into increased final body weight and higher ADG for calves on the PFA diet. The results of this study indicate that increasing fat content of starter during and after weaning can help sustain energy intake levels, however the benefits could be dependent on the fat source. Variation in results when different fat sources are supplemented could be explained by differences in fatty acid profiles. Previous work supplementing milk and starters with different fatty acid profiles resulted in effects on feed efficiency (Hill et al., 2007a) and immune functions (Karcher et al., 2014; Jasem et al., 2022).

Starches

Highly fermentable carbohydrates, namely starch, are the most important energy source in starter grains. Starter grain starch concentration has been positively correlated to starter intake before 8 wks of age, feed efficiency from 8 to 16 wks of age, and ADG and structural growth in calves up to 16 wks of age (Hu et al., 2018). Ruminal starch digestion is responsible not only for the butyrate production that is necessary for developing the rumen epithelium, but also propionate production (Huntington et al., 2006). Propionate is a major substrate for

gluconeogenesis, a vital process for functional ruminants who obtain only a small fraction of their glucose requirements directly from the diet (Yost et al., 1977). Ruminally digested starch also provides energy to support microbial cell protein production therefore contributing to the ruminant's protein supply (Storm et al., 1983; Gozho and Mutsvangwa, 2008). Starch is highly digestible in the rumen (Huntington et al., 2006), and rapid ruminal fermentation without proper buffering can result in low rumen pH which induces acidosis and reduces nutrient digestibility. Because of this, starter grain starch content should be regulated. The NASEM (2021) recognizes starters are typically 22 to 38% starch. Some studies have been able to feed starters containing more than 40% starch with positive growth effects and minimal signs of acidosis (Hill et al., 2008a; Satoh et al., 2023). However, it is important to note that in these studies calves had access to forages, either via feed or bedding. Gelsinger et al. (2020) modified the starch and fiber content of starter grains intending to induce acidosis (42.7% starch, 15% neutral detergent fiber (NDF)) or discourage acidosis (35.3% starch, 25.3% NDF) but found that calves on both diets spent an appreciable number of hours with a rumen pH below 5.6. Laarman et al. (2012) concluded that decreasing starter grain starch content was not an effective way to prevent low rumen pH after failing to see a difference in average rumen pH between diets that were 31.4 – 35.3% starch. Although, the less than 4% change in starch levels between experimental diets may not have been substantial enough to detect meaningful differences. Another study by Laarman and Oba (2011) monitored rumen pH during weaning for calves that had been fed milk and hay with or without access to a texturized starter before weaning. Their results showed no difference in rumen pH measurements but a negative correlation between hay intake and low rumen pH. Together these papers indicate that starter grain starch content may not be the sole culprit of low rumen pH in dairy calves.

Cereal grains such as corn, barley, wheat, oats, sorghum, and rice are the most common starch sources used in starter grains (Kahn et al., 2016). When comparing corn, barley, oats, and wheat in isonitrogenous and isostarch pelleted starter grains, corn-fed calves exhibited greater postweaning starter grain intake and body weight (Khan et al., 2007). Corn and wheat fed calves showed the most skeletal growth in the postweaning period and the highest feed efficiencies. Data published in a companion article observed that calves fed the corn and wheat diets had increased propionate production, total VFA production, and blood beta hydroxybutyrate (BHB) at 35, 50, and 70 d of age. However, the corn fed calves were able to maintain a higher rumen pH than wheat calves. Furthermore, when a subsample of calves was euthanized at 70 d of age, calves fed the corn and wheat diet had more advanced rumen development compared to calves on the barley or oat starters (Khan et al. 2008). Additional studies have reinforced that corn-based starters promote greater intake and body weight growth compared to other starch sources (Maiga et al., 1993; Sahib et al., 2023). It is important to note that it is very common for starter grains to contain multiple cereal grain products.

Numerous studies have investigated the impact of different processing techniques on grains included in calf starters, although results have been inconsistent. Feeding calves starters with steam flaked corn increased ADG and feed efficiency compared to ground corn in Eghtedari et al. (2024). However, Lesmeister and Heinrichs (2004) observed lower starter intake, reduced feed efficiency, and decreased hip width growth in calves fed steam flaked corn versus dry rolled corn. Rezapour et al. (2016) identified improved calf performance when corn and barley included in starters were dry rolled compared to ground. When comparing wheat processing techniques, Mirghaffari et al. (2012) determined calves fed popped wheat produced more ruminal VFAs and exhibited more characteristics of rumen maturation versus calves fed starters

with steam flaked, dry rolled, or ground wheat. Jarrah et al. (2013) identified no effects on starter intake, ADG, skeletal growth, or feed efficiency when comparing course ground, whole, steam rolled, and roasted barley, despite barley comprising 66% of the starter grain DM. Although authors admit that the small sample size (8 calves per treatment) may have limited their ability to detect differences. As a whole, the literature suggests that the effectiveness of processing likely interacts with other aspects of the diet such as grain type, particle size, and other ingredients within the starter.

Sugars

Sugars are also readily fermentable energy sources (Hanlon et al., 2023). Molasses, containing sucrose, glucose, and fructose (FoodData Central, 2019), is a common form of added sugar in starter grains. The addition of molasses provides not only nutritional value but also increases palatability, aids in binding ingredients and reduces feed dustiness (Morales et al., 1988). Molasses is typically included in starters at around 5%, as attempts to feed 10% or more have been unsuccessful. Lesmeister and Heinrichs (2005) observed that despite increased VFA production in calves fed a 12% molasses diet, their starter grain intake and heart girth growth decreased compared to calves fed a 5% molasses starter. Findings from Hill et al. (2008a) agreed with previous work, showing that calves fed a starter with 10% molasses or 5% molasses plus 1.5% granular sucrose had decreased ADG compared to calves fed a 5% molasses diet. Conversely Beiranvand et al. (2013) found that adding 5% granular sugar to starter grain had minimal effects on calf intake or performance. The lack of effects observed in their study may have been due to the fact their control diet did not contain any added sugar, so even the highest containing sugar diet, at 5%, was within the commonly advised range. Negative results were not recognized when up to 10% lactose was included in starter grains (Saegusa et al., 2017). Overall,

modest amounts of sugar are acceptable in calf starters. However, due to limitations on sugar inclusion level, starches remain the primary energy source for starters.

Including Fiber and Forage in Calf Diets

Mature dairy cattle diets require appreciable amounts of fiber and forage to support milk fat production, govern energy intake, and maintain rumen health. How to feed calves fiber remains a thoroughly studied yet unsettled topic. This is largely due to the limited digestive capabilities in young calves and concerns about energy displacement with fiber inclusion. The NASEM (2021) estimates that pre-weaned calves can digest only 32% of NDF. As calves begin consuming solid feed estimates of NDF digestibility increase to about 42% around weaning and are near 60% after weaning. Although, reductions in NDF digestibility may be observed as calves are fed increasing levels of starch which is common in starter grains (Chishti et al., 2021; Vagnoni et al., 2021). Based on these digestive limitations, recommendations for the NDF level in starters remain conservative at less than 13% (NASEM, 2021). Interestingly many studies examining the effects of fiber or forages in calf diets feed more than 13% NDF even in their lowest fiber diets. Hill et al. (2008a) fed post-weaned calves starter grains with 0, 14, 28, or 42% soyhulls to create diets ranging from 15 to 40% NDF and 15 to 43% starch. Their results showed a linear decrease in ADG as soyhull inclusion level increased. The decreased gain observed was likely explained by the calculated digestible energy content of their experimental starters decreasing from 3.56 mcal/kg to 3.29 mcal/kg as soyhull inclusion increased. Porter et al. (2007) reported lower DM and ME digestibility for 26.9 and 29.0% NDF starters compared to 16.9 and 20.2% NDF starters. However, in their study this did not translate into any effects on starter intake or calf growth performance. Vagnoni et al. (2021) was able to increase starter grain NDF

content from 16 to 20% with nonforage fiber sources while maintaining similar digestible energy levels and not affecting starter intake or calf performance. Together results from these studies support the idea that the negative effects observed when including high levels of fiber in starter grains can be at least partially attributed to the displacement of more digestible, higher energy ingredients within the diet.

One of the most common ways fiber is added to calf diets is through supplementation of roughages, a method which has been implemented with varying success. Several studies have identified increased DMI, ADG, and rumen pH for calves, especially postweaning, fed starter grains supplemented with forages (Khan et al., 2011; Castells et al., 2012; Hosseini et al., 2015; Aragona et al., 2020; Xiao et al., 2021). In an extreme case, calves fed a 100% high quality hay (21% crude protein (CP), 45.5% NDF, 11.2 MJ/kg DM of ME) diet for the first 14 wks of life performed comparably to calves fed a 30% hay and 70% concentrate diet (Terler et al., 2022). The high level of forage inclusion even in their starter based diet limits the implications of these results. Future research conducted evaluating hay only diets should focus on comparisons to more conventional starter only diets. Interestingly in the Terler et al. (2022) study, calves fed the exclusively hay diet had increased blood BHB levels at 77 and 91 d of age. Blood BHB levels in calves are used as an indicator of rumen epithelium metabolic activity (Deelen et al., 2016), indicating that calves fed exclusively hay may have had more developed rumens. Replication of this study with the addition of rumen VFA sampling and collection of rumen tissue would aid in understanding the implications of these findings. Terré et al. (2013) attempted evaluate whether advantages observed when feeding forages in calf diets were due to increased dietary NDF or because of properties of forage as an ingredient. Starter grain diets were designed in a 2x2 factorial as either low (18%) or high (36.7%) NDF with or without ad libitum chopped oat straw.

Their results showed that in the preweaning period calves fed forage or high fiber levels had decreased feed efficiency. However, in the postweaning period calves fed forage had greater starter intake and increased ADG independent of fiber level. Based on their results the authors suggested low fiber starters during the preweaning period with initiation of forage supplementation beginning around weaning. Other authors have identified forage inclusion in starter diets as detrimental to calf intake and growth performance. Stobo et al (1966) offered calves ad libitum access to hay but a restricted level of concentrates ranging from 0.45 – 2.27 kg/d. Results showed that calves offered more concentrate consumed less hay. Calf body weight and ADG linearly increased with increasing concentrate offering. Hill et al. (2012) observed that weaned calves fed a high starch (37%) texturized diet with 4% alfalfa-brome hay inclusion had higher DMI, ADG, hip width growth, and feed efficiency than calves fed 12% hay or a lower starch pelleted diet. Forage inclusion in calf diets has been associated with decreased digestibility of DM and CP which could contribute to decreases in calf performance when forages are fed at excess levels (Hill et al., 2019; Kargar et al., 2021).

The lack of consistency in the literature regarding whether or not forage provision is beneficial in calf diets indicates the complexities of the topic. A study comparing forage types fed calves starter grains supplemented with either no forage, alfalfa hay, rye grass, oat hay, barley straw, corn silage, or triticale silage (Castells et al., 2012). Starter grain intake was greater for calves offered triticale silage than calves offered no forage, alfalfa hay, and corn silage. Calves offered alfalfa and oat hay consumed the most forage. Total DMI was greatest for calves fed oat hay, barley straw, and triticale silage, clearly indicating that forage type impacts calf DMI. Average daily gain was also greater for calves fed oat hay, barley straw, and triticale silage than calves fed no forage or alfalfa hay. Feed efficiency, however, did not differ between any of

the tested forage types. Other studies that have investigated feeding corn silage to calves were able to replace 10, 50, or 100% of starter grain with corn silage without sacrificing intake, weight gain, skeletal growth or feed efficiency (Kanani et al., 2019; Kehoe et al., 2019). However, when calves were harvested at 8 wks of age in Kehoe et al. (2019), authors observed decreased gastrointestinal tract development in calves fed 100% corn silage. Therefore, it is advisable that although corn silage can be incorporated into starter diets, it should not replace starter grains altogether.

How forages are supplemented, either mixed into the starter grain or offered as a separate feed, has the potential to influence calves' intake behavior. Engelking et al. (2020) supplemented a texturized starter grain with 10% chopped grass hay fed to calves either from separate buckets or mixed together into one feed. Hay feeding method did not affect preweaning or weaning period DMI, but postweaning DMI was greater for calves fed the hay separately. Postweaned calves also had increased ADG and feed efficiency if hay was fed separately. Although authors did not report intakes of starter grain versus hay during the postweaning phase, calves fed hay separately increased starch intake compared to calves fed the mixed diet. The ability of calves fed hay separately to increase starch intake without increasing NDF intake likely explains the increased growth observed. Separate feeding of forages is also a practical on farm approach to introducing forages during and after weaning, when forage inclusion is most effective.

Another potentially influential aspect of forage provision to calves is forage particle size. Mirzaei et al. (2014) did not find any main effects on intake or calf growth performance when alfalfa hay was chopped at 2.92 versus 5.04 mm geometric mean particle size and supplemented in ground starter. However, particle size interacted with alfalfa feeding level after weaning as calves fed the shorter length alfalfa at 8% inclusion consumed less starter than calves fed the

longer length alfalfa at 8% or calves offered either length alfalfa at 16% inclusion. Comparisons of more dramatic differences in forage particle size, such as 0.82 mm versus 12.7 mm (Suarez-Mena et al., 2016) and 3 to 5 mm versus 10 to 12cm (Xiao et al.,2021), resulted in minimal effects on calf intake, performance, and digestive tract development. The lack of difference in fecal particle size in Suarez-Mena et al. (2016) indicated calves were able to break down the larger forage particles. Most of the aforementioned studies examining forage particle size fed ground or pelleted starter grains which can have inadequate particle size to support gastrointestinal tract development as is discussed further in a later section of this review. Therefore, the provision of forage of any reasonable size may have supported better rumen health and development. Overall, conservative forage provision to young calves is appropriate especially postweaning. However, situational context such as accompanying starter characteristics, forage type, forage particle size, and forage inclusion method greatly influence the success of feeding young calves forages.

Protein Sources in Starter Grains

Protein Requirements of the Calf

Raising heifers to reach breeding size in a timely manner allows for first calving between 22 and 24 months of age (for holsteins), maximizing production potential and minimizing heifer rearing costs (Steele, 2020; Bach et al., 2021). Thus, it is pivotal to supply replacement calves with adequate protein to support lean growth from a young age. The NASEM (2021) estimates ruminating calves convert CP to metabolizable protein with an efficiency of 0.70 which is lower than the 0.95 conversion rate for calves on milk alone. While early research determined calf growth performance could be supported by starters containing as little as 12% CP as-fed (Brown

et al., 1958; Thomas and Tinnimit, 1976), more current work suggests starters should be at least 18% CP on a DM basis. Akeyezu et al. (1994) observed linear increases in starter grain intake and ADG as starter grain CP increased from 15 to 22.4% of DM. The authors ultimately suggested that 19.6% CP was optimal, as no additional benefits were identified at 22.4% CP. Other studies have agreed with the finding that no additional advantages are gained when starters contain more than 18% CP (Hill et al., 2007b; Makzadeh et al., 2020). On the contrary, Kazemi-Bonchenari et al. (2022) reported increased preweaning starter grain intake and increased bodyweights in calves offered a 23% CP starter versus an 18% CP starter. Additional results from this 2x2 factorial study, which also fed a high (790 g/d) or low (526 g/d) level of milk, found an interaction when calves were fed the high milk level and 23% CP starter that promoted the greatest hip and wither height growth.

As accelerated, or intensified, milk feeding programs have gained traction in the industry it has been suggested that increasing the CP of starters beyond 18% to 22% or more may be advantageous (NASEM, 2021). It is important to acknowledge the role dietary energy supply plays in feeding protein efficiently. Feeding excess energy and inadequate protein results in growth that is proportionately higher in fat than if the dietary protein supply is nonlimiting (Donnelly and Hutton, 1976). And several studies have demonstrated that increasing protein levels increases growth performance and promotes protein deposition in the carcass rather than fat deposition (Brown and Lassiter, 1962; Brown et al., 2005; Stamey Lainer et al., 2021). Matching protein supply to the energy content of calf diets is vital to maximizing efficiency of desirable lean growth.

As calves' rumen fermentative capabilities develop, the rumen undegradable protein (RUP) content of the diet becomes more relevant. Benefits to weight gain and feed efficiency

have been realized in heifers weighing over 200kg when up to 55% of CP was supplied as RUP (Tomlinson et al., 1997; Silva et al., 2018). Somewhat lower levels of RUP have been studied in younger calves fed starter grains with varying success. Yousefinejad et al. (2021) observed increased postweaning ADG but no effects on starter intake, structural growth, or nitrogen flow in calves fed a 35 versus 25% of CP as RUP starter. Others have not detected differences in intake or growth performance when feeding starter grains ranging from 23 to 40% of CP as RUP (Hill et al., 2007b; Boorboor et al., 2020). It is possible that the lack of responses to increasing RUP levels in the preweaning period is attributed to limited ruminal digestive capabilities which may naturally allow higher amounts of protein to escape ruminal degradation. However, there is a need for additional literature elucidating how protein from starter grain is digested during the preweaning period when the rumen is in the initial stages of development. Modifying RUP levels in calf diets may have specific utility during periods of calf stress. Increasing RUP proportions from 39 to 49% of CP increased body weight gain and feed efficiency in three month old calves under heat stress inducing conditions (Bunting et al., 1996). Since these benefits were not observed in calves in thermoneutral environments, the authors suggested the observed benefits may be related to the effects of heat stress on passage rate. Heat stressed ruminants will reduce particle passage rate, therefore increasing ruminal protein degradation. Increasing the RUP supply to 49% for calves during heat stress likely increased the amount of amino acids (AA) reaching the intestines.

Amino Acid Requirements of the Calf

Optimizing the AA profile of feeds offered to cattle allows producers to feed lower amounts of protein while still meeting animal requirements and maximizing performance (Schwab, 2012). Methionine (MET) and lysine (LYS) have been identified as limiting AA as

early as the milk feeding period for dairy calves raised on milk replacers (Hill et al., 2008b; Chagas et al., 2018; Yu et al., 2018). A series of studies conducted in Japan found that MET was the first limiting AA followed by LYS in weaned calves under 11 wks of age fed a 15.3% CP corn and SBM-based pelleted starter (Abe et al., 1998). However, when a similar study was conducted on calves 13 to 16 wks of age fed the same base diet as Abe et al. (1998), MET and LYS were no longer limiting (Abe et al., 1999). Schwab et al. (1982) abomasally infused different combinations of AA into weaned calves fed a cereal byproduct-based pelleted starter. In their first trial calves infused with all 10 of the essential AA retained more nitrogen than calves infused with a combination of alanine, asparagine, glutamine, glycine, and serine.

In another trial from Schwab et al. (1982) calves were infused with 0, 0.3, 0.6, or 1.20 g/kg ration MET. A MET dose of 0.6 g/kg ration was deemed optimal, as there was an increasing quadratic effect of dose and calves on this treatment retained the most nitrogen. While on farm MET cannot be administered directly to the abomasum, rumen-protected MET and MET analogs offer other options to supply MET to calves. Mueller and Rodriguez (1974) fed calves up to 12 wks of age a 13% CP calf starter supplemented with 0, 0.25, or 0.5% of an unspecified methionine hydroxy analog (MHA). Dry matter intakes were greater for calves supplemented with 0 or .25% MHA than 0.5% MHA. Average daily gain and feed efficiency were greater for the non-supplemented calves. Interestingly serum MET concentrations numerically decreased with increasing MHA supplementation, but cysteine concentrations increased with supplementation indicating MET metabolism was occurring. In all, the authors concluded that decreases in calf performance when supplemented with 0.5% MHA may have been due to palatability issues or an extreme AA imbalance. More modern work by Molano et al. (2020) supplemented a 25% CP texturized starter with either rumen protected MET, 2-hydroxy-4-

(methylthio)-butanoic acid (HMTBa), or HMTBa isopropyl ester (HMBi). Results showed no differences in starter intake, but increased ADG for calves fed the HMBi versus HMTBa. Postweaning body weight was reduced by the inclusion of HMTBa versus calves on a non-supplemented control diet. There were no differences in feed efficiency or structural growth between treatments. Plasma MET concentration at weaning was greatest for rumen protected MET and HMBi fed calves. By the end of the study serum MET concentration for calves fed rumen protected MET was greater than calves fed HMBi and least for the control and HMTBa fed calves. Throughout this study calves fed the HMTBa diet performed similarly, or in some cases worse, than calves on the control diet. These findings agree with Mueller and Rodriguez (1974) that not all methionine hydroxy analogs are a suitable MET supplement for starter grains. However, authors in Molano et al. (2020) concluded that the general lack of performance effects suggests that their particular control diet (with 0.49% DM MET) may not have been deficient in MET.

Since LYS has been identified as the second limiting AA in calves it is not uncommon for MET and LYS to be supplemented together. Hill et al. (2016) fed 2 month old calves diets containing SBM only or SBM plus blood meal (a lysine source) and HMTBa. Addition of blood meal and HMTBa did not improve calf DMI, ADG, feed efficiency or skeletal growth. Although the actual MET and LYS content of the diets were not reported, the SBM only diets were approximately 18% CP which could have met calves' requirements without supplementation. Nirounmand et al. (2020) compared supplementing starter grains with differing forms of AA versus increasing the starter grain CP content. Diets offered were a control 18% CP starter with no added AA, 18% CP starter with rumen protected MET and LYS (RPLM), 18% CP starter with non-rumen protected MET and LYS (UPLM), or a 22% CP starter not supplemented with

AA. Calves offered the 22% CP starter had greater starter grain intake and ADG than calves on the control or RPLM starters. Calves on the RPLM starter were shorter in length at weaning and study conclusion, however no other skeletal growth differences were detected. Calves on the 22% CP starter had higher BUN at study conclusion but creatine and AAs were not different between treatments. These results indicate that providing AA in a rumen protected form was not advantageous compared to unprotected forms in the diets of young calves. Altogether, it is possible for calves to be deficient in certain essential AA. However, the lack of consistent improvements in performance when starter grains contain supplemental AA demonstrates that supplementation may not be necessary if the basal diet is adequate in protein quality and quantity.

Protein Sources in Starter Grains

Prized for its digestibility, palatability, and desirable AA profile, soybean meal is the gold standard protein source for starter grains (Miller-Cushon et al., 2014b; Khan et al., 2016; ZeidAli-Nejad et al., 2018). Other soy-based products have been evaluated as partial replacements for soybean meal with varying success. Substitutions of more than 25% of the conventional soybean meal either full fat extruded soybeans or xylose treated soybean meal has reduced starter grain intake previously (Kazemi-Bonchernari et al., 2015; ZeidAli-Nejad et al., 2018). Kazemi-Bonchernari et al. (2015) reported that calf ADG decreased with increasing levels of xylose treated soybean meal, however feed efficiency was improved at the 25% substitution level. Kim et al. (2010) observed no differences in feed efficiency and improved immune measures in calves fed starters where 32% of the conventional soybean meal was replaced by a fermented soybean meal. It appears that moderate amounts of other soy protein products can

replace conventional soybean meal in starters. However, consideration should be given to how replacement might modify starter fat and RUP levels.

Beyond soybean meal, other oil seed meals can be used as protein sources in starter grains. Canola and rapeseed meal have successfully replaced 60 to 100% of soybean meal in starter grains with minimal impacts on intake or growth in some studies (Claypool et al., 1985; Burakowska et al., 2021). Yet other studies have identified decreased digestibility, palatability, and calf performance associated with feeding canola or rapeseed meal based starters (Stake et al., 1976; Khorasani et al., 1990; Miller-Chushon et al., 2014b; Jafarpour et al., 2023). Jafarpour et al. (2023) identified interactions between protein source and processing of starch source. In their study starter grain intake increased for calves fed ground corn when canola meal was fed versus soybean meal. These findings suggest that performance of calves fed canola meal based starters may be dependent on the starch sources offered in conjunction. In Stake et al. (1976) calves offered a starter with sunflower meal had similar starter grain intake and ADG to calves offered a starter with soybean meal. Nine wk old weaned dairy calves offered diets supplemented for protein with either soybean meal, corn gluten meal, cottonseed meal, or fish meal consumed similar levels of feed regardless of protein source (Zerbini and Polan, 1984). Calves offered the fish meal diet had greater gains than calves on the corn gluten or cottonseed meal diets. The soybean meal and cottonseed meal fed calves had the highest ruminal ammonia concentration, with soybean meal fed calves producing the highest percentage of microbial protein in the abomasum. The fish meal fed calves, despite having the lowest ruminal ammonia production, exhibited the greatest amount of gain indicating that fish meal was a high quality RUP source for weaned calves.

While it's been established that animal derived proteins, such as blood and feather meal, are satisfactory protein sources in diets of older, larger, calves (Blasi et al., 1990; Klemsrud et al., 1998), literature regarding young dairy calves is scarce. Moss et al. (1995) replaced up to 75% of the CP from soybean meal with feather meal in starter grains and did not observe effects on starter grain intake or growth when fed to calves during the preweaning period. After weaning there were some differences in starter grain intake and feed efficiency between different levels of feather meal inclusion, however none of the treatments differed from the soybean meal only diet. Plasma urea nitrogen did not differ with substitution of soybean meal and although RUP content of the diets increased as soybean meal levels decreased, the observed responses were not linear. This led authors to conclude that results were likely influenced by something other than feather meal inclusion. Dried distillers' grains are sometimes used to modify RUP content or curb energy level in calf starters (Hill et al., 2007b; Hill et al., 2016). However when fed as a replacement for soybean meal in starter grains, decreases in ADG were observed in calves fed more than 20% diet as-fed dried distillers' grains (Suarez-Mena et al., 2011). The depressions in gain were likely caused by decreased diet digestibility with increasing dried distillers' grains inclusion. Urea should be avoided in starters fed to young calves as it requires a fully functional rumen to utilize properly. When Dayton and Morrill (1977) offered preweaned calves starter grain with 1.35% urea body weight gain was reduced. Throughout the reviewed literature soybean meal continues to excel as a protein source in calf starters.

Micronutrients

Vitamins

While there is considerable literature regarding vitamin and mineral supplementation in the milk portion of dairy calf diets, less work has been done regarding their addition to starter grains. Vitamins A, D, and E are the most commonly supplemented vitamins in calf diets, but there is very little modern literature addressing vitamin A and D in starter grains. Although the NASEM (2021) recommends that milk fed to calves be supplemented with choline and vitamin B, it states that supplementation is not necessary in starter grains because calves should be able to ruminally synthesize adequate quantities by weaning. This statement of course hinges on the assumption that producers wean calves with satisfactory rumen development. Liu et al. (2021) found that including 7.2 mg/kg DM of folic acid (vitamin B-9) in the diet of two month old weaned calves increased ADG, diet digestibility, rumen VFA concentration, and encouraged growth of cellulolytic microbial populations in the rumen. These results suggest that it may be advantageous to supplement at least certain B vitamins in starter grains. The NASEM (2021) recommends starter grains contain 3,700, 1,100, and 67 IU/kg DM of vitamins A, D, and E respectively. However, the literature reflects that these guidelines can be situational. For example, work with milk fed calves found that calves fed to achieve greater levels of growth (1.16 kg/d vs 0.11 or 0.58 kg/d) had reduced serum vitamin E concentration but similar vitamin A and D concentrations than slower growing calves (Nonnecke et al., 2010). Thus, vitamin E utilization appears to be dependent on growth rate in young calves and additional supplementation may be required in programs promoting aggressive growth. The amount of Vitamin E, also known as tocopherol, supplementation required in starter grains may also increase around the weaning period. In Lashkari et al. (2022), calves provided 200 mg/kg DM

(equivalent of 298 IU/kg) natural tocopherol in starter grain were able to maintain plasma tocopherol concentrations while calves offered 60 or 120 mg/kg DM had reduced concentrations during weaning. Quigley et al. (2021) also observed that bodyweight and feed efficiency linearly increased with increasing supplementation of tocopherols in milk and starter grains in the weeks surrounding weaning.

These studies have also suggested that vitamin form influences effectiveness of supplementation. In another trial from Lashkari et al. (2022), calves were orally dosed with 200 mg/d of RRR- α -tocopherol (natural form), RRR- α -tocopheryl acetate (natural acetylated form), or all-rac- α -tocopheryl acetate (synthetic form) for 5 wks surrounding weaning. Supplementation of the natural form tocopherol was the most effective, resulting in consistently increased plasma tocopherol concentrations compared to the other forms. Quigley et al. (2021) achieved greater concentrations of vitamin E in their calf diets by maintaining consistent α -tocopherol levels but increasing δ - and γ -tocopherols inclusion. Their results showed growth and oxidative status markers increased with increasing δ - and γ -tocopherols inclusion indicating that the δ - and γ -tocopherols are also effective forms of supplemental vitamin E. Pertaining to vitamin D, calcidiol was found to be more effective at increasing serum vitamin D concentrations compared to cholecalciferol when included in the milk and starter grain of calves through 96 d of age (Blakely et al., 2023). Seeing as much of the data used to set vitamin supplementation recommendations found in the NASEM (2021) is decades old or focused on milk feeding, additional literature examining modern starter grains and calf rearing programs would be beneficial.

Minerals

A variety of minerals are required in calf diets not only to support the functions of the calf herself but also the microbes in the developing rumen. The NASEM (2021) provides equations to calculate dietary adequate intakes in calf diets for calcium, phosphorus, magnesium, potassium, sodium, and trace minerals copper, iron, manganese, and zinc. Much of the current literature pertaining to calf mineral nutrition has focused on the form in which minerals are supplemented. Pino et al. (2018) conducted a study that supplied heifer calves with a mix of calcium, phosphorus, magnesium, potassium, sodium, iron, manganese, copper, zinc, and selenium in either an organic proteinate or inorganic sulfonated form. The assigned mineral form was present in all diets, including milk replacer and starter grain, offered to heifers from the last 60 d of fetal gestation (via dam supplementation) until 100 d into the heifer's first lactation. Mineral supplementation form had minimal effects on growth performance, although the inorganic mineral fed heifers had increased body weights at 22 and 24 months of age. Heifers offered organic minerals tended to calve 22 d earlier and had increased average milk production by the first 100 d in milk suggesting overall that organic form mineral supplementation may be advantageous. Utilizing 126 kg weaned beef calves Caramalac et al. (2017) identified that calves preferred supplemented copper, zinc, and manganese offered in hydroxychloride form over organic or sulfate form. Mousavi-Haghshenas et al. (2022) investigated whether mineral form interacted with calf birthweight to impact growth performance in a 2x2 factorial study that fed starter grains containing either inorganic trace minerals or a 50:50 blend of inorganic and advanced chelated trace minerals to Holstein calves of either low or normal birthweight. While trace mineral form did not impact ADG or feed efficiency, supplementing low birth weight calves with the chelated mineral blend increased their DMI so that it was not different from

normal birthweight calves. Feeding the chelated mineral blend also resulted in increased heart girth at weaning and increased hip width overall. Additionally, calves fed the chelated mineral blend showed signs of improved health status such as lower rectal temperature, lower heart rate, and more desirable nose and eye visual health scores. Altogether the literature reinforces the positive effects on immune function and growth performance when calves are provided sufficient mineral supplementation. Nutrient form has been identified as an important consideration for both certain vitamins and minerals.

Common Additives in Starter Grains

Ionophores and other Antimicrobials

Maintaining calf health is a multifactorial challenge with considerable financial ramifications for producers (McGuirk, 2008). Governing bodies around the world continue to crackdown on the use of non-therapeutic antibiotics in livestock production. The consequential restrictions on antibiotic inclusion in feeds has spurred research into alternative antimicrobials.

Ionophores, although classified as an antibiotic, are allowed for use in calf diets as a coccidiostat. While delivering ionophores to calves in the preweaning period is more effective via milk supplementation (Quigley et al., 1997), studies have recognized benefits when ionophores were included in starter grains as well. In a study utilizing calves naturally exposed to sporulated coccidia oocysts, supplementation of starter grains with lasalocid was more effective than unmedicated grain but less effective than decoquinate supplementation at reducing fecal coccidia oocysts and supporting calf growth (Heinrichs and Bush, 1991). Whether inclusion of ionophores in the diets of calves unchallenged by coccidiosis is beneficial or not is contended. Anderson et al. (1988) reported that supplementation of starter grain with lasalocid increased the

bodyweight and starter grain intake of healthy weaned calves compared to calves that received unmedicated feed. Authors were able to attribute improvements in performance to increased ruminal propionate concentrations, indicating that within weeks of weaning ionophores are able to modify the rumen function of calves in a similar fashion to that of mature ruminants. On the contrary Nussio et al. (2002) observed that administration of lasalocid or monensin in calf grains did not improve performance of healthy unchallenged calves. Provision of monensin in particular actually reduced heifer DMI and ADG throughout their 20 wk experiment. Wu et al. (2020) reported moderate benefits of ionophore supplementation via starter grains with a reduction of diarrhea occurrences and improved diet digestibility when fed to generally healthy calves. However, in their study monensin supplementation failed to improve growth performance or blood immune status markers.

More recent research has focused on the potential of plant-based compounds with known antimicrobial characteristics and their suitability in dairy calf diets to improve calf performance and health. In Wu et al. (2020) calves provided a supplement of oregano essential oil, probiotics, and cobalt lactate (EOC) exhibited greater performance than non-supplemented or monensin fed calves. Provision of starter grains with EOC increased ADG, feed efficiency, and abdominal girth growth in addition to reducing diarrhea occurrences and improving diet digestibility. Kazemi–Bonchenari et al. (2018) also concluded that inclusion of essential oils (a blend of thymol, eugenol, vanillin, limonene, and guaiacol) in calf starter grains was beneficial for promoting calf ADG. Both Wu et al. (2020) and Kazemi–Bonchenari et al. (2018) reported increased ruminal butyrate, propionate, and total VFA concentrations in calves supplemented with essential oil based products despite equivalent starter grain intakes to non-supplemented calves. Fennel powder and gallic acid (a phenolic compound found in plants) have also been

explored as antimicrobial additions in starter grains. Provision of starter grains with either ingredient increased starter grain intake and weight gain compared to non-supplemented calves (Saeedi et al., 2016; Xu et al., 2022). While the study examining fennel powder did not evaluate health related markers (Saeedi et al., 2016), gallic acid was found to improve certain blood antioxidant and inflammatory measure indicating improved health status (Xu et al., 2022). As a whole the literature suggests inclusion of antimicrobials in calf starter grains are beneficial to calf growth and health, although exact benefits appear to be somewhat dependent which specific antimicrobial is administered.

Yeast

Yeasts are the most researched microbial additive in calf starters with a variety of experiments investigating its effects on calf gut development, growth performance, and immunity. Yeasts are utilized in the diets of ruminants of all ages to modify the gut microbiome and therefore fermentation characteristics (Chaucheyras-Durand et al., 2008). Despite not yet possessing a fully developed rumen, modulation of ruminal fermentation with yeast supplementation has still been reported in the young calves. Studies have demonstrated that inclusion of yeast cultures, usually *Saccharomyces cerevisiae* (SC), increases ruminal acetate concentration and decreases ruminal lactate concentration (Quigley et al., 1992; Hučko et al., 2009). Despite reductions in ruminal lactate concentrations, supplementation of starter grains with yeast does not appear to modify ruminal pH (Quigley et al., 1992; Agarwal et al., 2002; Hučko et al., 2009). Acceleration of gastrointestinal tract development has also been observed with yeast supplementation in calf starter grains. Xiao et al. (2016) reported increased ruminal papillary surface area and increased intestinal villus length in calves fed a starter grain supplemented with a SC fermentation product. Inclusion of *Saccharomyces cerevisiae boulardii*

(SCB) in milk and starter grains fed to calves in Fomkey et al. (2017) resulted in changes to calf colon morphology consistent with more a mature gastrointestinal tract. Conversely some studies did not find evidence that yeast effects rumen development (Agarwal et al., 2002; Lesmeister et al., 2004). Supplementation of starter grains with 0, 1, or 2% DM inclusion of SC culture in Lesmeister et al. (2004) found that calves that received the 2% SC culture diet had improved ADG and greater skeletal growth than calves receiving non-supplemented starter grain. Galvão et al. (2005) and Khademi et al. (2022) also reported increased weight gain and improved feed efficiency when feeding SC products in calf starter grains, although it should be noted that the SC in Khademi et al. (2022) was delivered as part of a probiotic also containing also *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Bacillus*, and *Enterococcus* bacteria species. Improvements in calf growth performance are not universal with some studies failing to identity any effects of yeast supplementation on growth (Seymor et al., 1995; Hučko et al., 2009; Alugongo et al., 2017; Fomkey et al., 2017). Utilization of yeast products in starter grains may be particularly useful in the diets of high risk calves. Galvão et al. (2005) supplemented the milk and/or starter grain of calves that had failed to achieve passive transfer of immunity with either SC or SCB and found calves supplemented with either had reduced days with diarrhea. Furthermore, supplemented calves had greater starter grain intake through the trial. Fecal scores were also improved in calves fed starter grains containing 0.5 or 1% DM of a SC fermentation product compared to non-supplemented calves in Alugongo et al. (2017). Overall, the addition of yeasts and yeast products seem to yield favorable effects. However, results are inconsistent regarding which measures, whether health or developmental, are impacted by supplementation and whether or not those effects translate into improved growth and performance.

Exogenous Butyrate

A handful of studies have examined supplementing calf starter grains with exogenous forms of butyrate due to its key role in rumen development. Supplementation of butyrate via starter grains may have different effects on calf performance than supplementation via milk. In Górká et al. (2011) sodium butyrate (NaB) was included in the milk replacer and/or starter grains fed to Holstein and Holstein x Limousin calves for three weeks before slaughtering. Sodium butyrate inclusion in milk replacer produced calves that were heavier each week and had greater overall ADG. However, calves offered NaB in their starter grain had higher starter intake during the last week of study and a reduced number of days scoured. Regardless of delivery method, provision of NaB increased papillae length and width in the cranial dorsal sac and increased the size of the reticulorumen as percent of whole stomach weight indicating positive effects of NaB on rumen development. However, due to the abbreviated nature of the experiment it is not known if these developmental advantages would continue through and after weaning. In another study, supplementation of 1% rumen protected NaB in starter grains during and after weaning increased calf starter grain intake and ADG compared to non-supplemented calves (McCurdy et al., 2019). Additionally, calves consuming starter grain with NaB had increased plasma BHB levels during weaning and increased propionate as a percentage of ruminal VFA. Ślusarczyk et al. (2010) also observed that 1% NaB was an optimal dose, with calves in their study failing to increase starter grain intake when NaB was included at 3% compared to 0.3 or 1%. Authors suggested the reduction of intake was due to the strong odor given off by NaB. However, results also showed that feeding any of the NaB starters increased ADG in the preweaning period and for the whole study. Altogether supplementation of exogenous butyrate in starter grains does appear to promote calf gut development and growth.

Starter Grain Form

Calf starter grains are manufactured in a few common forms which can affect not only the feed palatability, but also rumen development. Feeding calves starters that are identical in ingredients but different in form can impact rumen pH, microbial population, and papillae development (Beharka et al., 1998; Pazoki et al., 2017). Ground or mash starters are the simplest and cheapest form to manufacture, as ingredients are simply combined and then ground to a similar particle size. Pelleted starters are produced by grinding and mixing all ingredients before processing them under heat, moisture, and pressure to press them into dense particles of a uniform size. The heat and pressure applied during pelleting can result in modifications to starch and protein digestibility (Thomas and Van Der Poel, 1995). Pelleting has been found to increase intake of forages, especially those of poor quality, which may be of particular use in calf diets (Moore et al., 1964). Texturized starters are presented as a mix of pelleted ingredients and processed grains.

Much research has been conducted regarding the effects of different starter forms on calf growth and performance. When comparing all three starter forms, Pazoki et al. (2017) reported starter intake, body weight, and ADG was higher for calves fed texturized starter than calves fed pelleted starter, but calves fed ground starter did not differ from either. Feed efficiency was not impacted by starter form. On the contrary, Bach et al. (2007) observed that feed efficiency was higher for calves fed pelleted starter, as calves consumed less starter but had the same ADG as their counterparts that were offered texturized starter. In a study that weaned calves once they reached a desired starter grain intake level, Franklin et al. (2003) found a similar pattern where calves on pelleted starter had lower starter grain intake and therefore took longer to wean than calves fed texturized starters. Hill et al. (2012) also found that texturized starter grains

encouraged the most intake versus pelleted or ground starter grains. In their study which compared texturized, pelleted, and ground starters, ADG was highest for calves fed texturized starter and lowest for calves fed ground starter, although feed efficiency did not differ for any of the three diets. The results of this study also suggest that particle size plays a role in the effectiveness of starter grain form, as calf performance was very similar for calves on the pelleted and ground starters which had similar geometric mean particle size (0.76 mm vs. 0.79 mm respectively). Results from Porter et al. (2007) also support the importance of particle size. In their study calves were fed either pelleted starters of smaller particle sizes (0.74 and 1.03mm) or ground starters of larger particle sizes (2.1 and 1.9mm). Calves fed the ground starter not only consumed more starter and had increased ADG, but also started ruminating sooner and for a higher percentage of the time. Additionally, calves fed the ground starter had numerically longer papillae (3.5 vs 2.9cm) and greater muscle/mucosa percentage (1.35 vs 1.30%). Based on results such as these reflected in the literature, the NASEM (2021) recommends a weighted average of at least 2.0 mm as adequate particle size for calf starters. The NASEM (2021) emphasizes the role particle size plays in maintaining rumen health and prevention of keratosis and bloat. One logical way to increase the particle size in starter grains might be to include chopped forages. A review and meta-analysis conducted by Ghaffari and Kertz (2021) suggests that ground starters supplemented with hay may be just as effective as texturized starters in stimulating starter intake. However, in live calf trials the interaction between forage and starter form has been inconsistent. Some studies have found that including chopped forage in pelleted or ground starter diets increased starter intake to levels greater than or equal to that of texturized starters (Terré et al., 2015; Pazoki et al., 2017). On the contrary, Omid-Mirzaei et al. (2018) identified no interactions between starter form and forage inclusion related to starter intake, or calf performance. Overall,

although no one starter grain form may be superior in every situation, it is clear that providing starter grains with adequate particle size is crucial. Calves fed starters of inadequate particle size consistently exhibited reduced starter grain intake and frequently gained less.

Starter Grain Palatability Considerations

Diet palatability is one of the many factors that influence feed intake in livestock. As with humans and other mammals, cattle have been observed modifying their feed intake based on olfactory and gustatory cues (Baile and Forbes, 1974). Therefore, palatability preferences should be considered when formulating a starter grain that will encourage calf intake. Sweet and salty tastes are known to stimulate intake (Goatcher and Church, 1970); a claim supported by Morill and Dayton (1978) who observed increased starter intake, especially around weaning, for calves fed a grain containing a sweet flavoring compared to calves offered an unsweetened grain. Using short-term exposure pairwise preference tests on weaned dairy calves, Miller-Cushon et al. (2014a) ranked the palatability of energy and protein sources commonly used in starter grains. Their results showed that calves preferred wheat meal the most, followed by sorghum meal, barley meal, and corn meal. Corn gluten feed and rice meal were the least preferred of the tested energy sources, although wheat middlings and oat meal were also nonpreferred. The nonfibrous carbohydrate content (NFC), which includes sweet tasting nutrients such as sugars, of each of the tested ingredients may have played a role in their preferability. Ingredients with the higher NFC content such as sorghum, corn, and wheat meal were generally more preferred than ingredients such as corn gluten feed and wheat midds that were lower in NFC. Regarding commonly used protein sources, calves showed the most preference toward soybean meal followed by dried distiller grains. Sunflower meal and pea meal garnered neutral preference, while rapeseed meal

and corn gluten meal were disliked. Protein source preferability may be more complex, as it did not appear to be as closely related to NFC content as the energy sources. However, it has been suggested that young ruminants may be capable of modifying their intake of protein based on their own nutrient requirements (Bach et al., 2012). In a longer term palatability study conducted over 6 wk, calves offered a soybean meal based pellet had increased intake compared to calves offered a canola meal based pellet despite similar nutrient compositions between pellets (Miller-Cushon et al., 2014b). In both of the Miller-Cushon et al. studies when calves were offered the tested ingredients as part of a larger mix, the initial preferences remained the same and calves exhibited preference related sorting. Although these data demonstrate calves' ability to differentiate between feed ingredients and their willingness to modify starter grain intake accordingly, the lack of data collected regarding calf growth and performance makes it difficult to quantify the magnitude of the effects in practical feeding applications. Terré et al. (2016) observed that when calves were not given a choice between ingredients known to differ in preferability, grain intake, ADG, and feed efficiency were not affected. Taken together these experiments suggest that although calves have feed preferences, as long as ingredients are generally acceptable to the palate adequate intake and growth performance can be reached.

Conclusion

A well formulated starter grain for dairy replacement heifer calves will not only provide adequate protein and energy for growth but will also be highly palatable and digestible to encourage intake and ruminal fermentation. Ingredients which are difficult to digest or offensive to the palate should be avoided. Other considerations regarding the calf's environment such as access to forage, bedding material, and external temperature should be factored in to feeding

decisions as well. The majority of existing literature pertaining to calf nutrition focuses on the milk feeding period only or extends just briefly beyond weaning. Additional work focusing on nutritional considerations of the post weaned calf would continue to improve effectiveness and efficiency of the dairy heifer rearing process.

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Chapter 2 - Effects of a high protein corn co-product as a replacement for soybean meal in calf starter grains

Abstract

Provision of a nutritionally balanced starter grain to dairy calves within the first weeks of life is essential for setting calves up for a successful weaning transition. While conventional dried distillers' grains are an attractive protein source for most cattle diets, utilization in calf starter grains have yielded undesirable results. Evolutions in ethanol fractionation technology has brought about innovations in co-product feed ingredients which may be more applicable for use in calf starter grains. High protein corn co-products (HPCC), which are enriched with yeast bodies from fermentation, contain similar amounts of crude protein to soybean meal and may be a suitable option for calf starter grains. To assess HPCC as a substitute for soybean meal in starter grains, Holstein calves were fed starter grains from 14 to 84 d of age with either 0 (CTRL), 50 (50HPCC), or 100% (100HPCC) of the soybean meal replaced by HPCC. During the preweaning period (14 to 42 d of age), HPCC quadratically effected starter grain intake with 50HPCC calves consuming less grain than CTRL or 100HPCC calves. The HPCC inclusion increased bodyweight; however, treatment did not affect structural growth or feed efficiency. After weaning, effects of HPCC on starter grain intake were less discernable. Bodyweight and average daily gain increased with HPCC, but wither height was reduced in weaned 50HPCC calves. Males grew heavier and taller throughout the study. Plasma glucose and insulin concentrations increased with time and insulin concentration was increased by HPCC in weaned calves. There was no evidence of treatment effect on DM or crude CP apparent total tract digestibility in weaned calves. Males had greater total tract apparent digestibility of DM and CP

than females. Overall, these results suggest that HPCC is a suitable replacement for soybean meal in calf starter grains when supplemented with methionine and lysine.

Introduction

Byproduct feeds from ethanol production are common on many dairy farms across the United States. Dried distillers' grains (**DG**) are an economical source of protein and energy for ruminants. Conventional DG are the nonfermentable solids remaining after the distillation of ground feedstock, typically corn or sorghum, and are usually marketed including the soluble fraction that is captured through evaporation of the thin stillage. Through this process the starch from the grain is removed but the protein, fat, and minerals are concentrated resulting in a feedstuff that is approximately 30% CP, 10% fat, and 30-40% NDF (Schingoethe et al., 2009; NASEM 2021). High RUP levels, 45 to 50% of CP, make DG an attractive ingredient for lactating cow diets, however DG can be successfully fed to growing and non-lactating cattle as well (Klienschmit et al., 2006; Martin et al., 2007; Schingoethe et al., 2009; Schroer et al., 2014). Limited work has previously evaluated the suitability of DG in dairy calf starter grains. Those data suggest DG inclusion of 20% or more in calf starter grains decrease calf growth performance and rumen development. (Thomas, 2006; Suarez-Mena et al., 2011). However, neither of these studies accounted for differences in limiting amino acid content between soy and corn protein sources.

Innovations in fractionation technologies within the ethanol industry have increased the availability of value-added corn co-products, such as high protein corn co-products (**HPCC**). To produce HPCC the corn bran is removed from the kernel through a variety of proprietary techniques. The resulting feedstuff is typically 45 to 50% CP. The majority of previous work investigating the use of HPCC in cattle diets has focused on lactating cow diets, with most studies finding that HPCC inclusion either did not affect or increased milk yield and component production (Hubbard et al., 2009; Kelzer et al., 2009; Christen et al., 2010; Carroll et al., 2023).

To date, no known literature was found regarding the use of HPCC in growing dairy heifers or calves. Calf starter grains typically utilize oilseed meals such soybean meal (**SBM**). However, the high CP of HPCC could be a promising alternative protein source in starter grains.

Therefore, the objective of this experiment was to evaluate the effects of substituting HPCC for SBM as a protein source in calf starter grains on dairy calf intake, growth performance, and blood metabolites. It was hypothesized that calves fed HPCC would perform similarly to calves fed SBM as a protein source when the feed was balanced for potentially limiting amino acids methionine and lysine.

Materials and Methods

Animals and diets

All animal use protocols for this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. Forty-two Holstein calves (21 male, 21 female) born at the Kansas State University Dairy Teaching and Research Center were enrolled at 14 d of age between June and August of 2023. Calves were from singleton births, weighed at least 34 kg at birth, and achieved a serum brix reading of 8% or greater at 3 to 5 days of age. Males were castrated with an elastic band within 7 days of birth, before treatment initiation. At the time of castration calves received meloxicam and a tetanus toxoid vaccine; a booster vaccine was administered 21 d later. Females received Nasalgen 3, Vision 7 and Vista 5 vaccinations at 7 wk of age (Merck Animal Health, Rahway, NJ). Calves were housed in individual hutches bedded with sawdust.

Calves were blocked by birth date and sex, then randomly assigned to 1 of 3 starter grain treatments so that each block contained 3 males and 3 females. Three experimental texturized

starter grains were offered, with either 0% (**CTRL**), 50% (**50HPCC**), or 100% (**100HPCC**) replacement of SBM with HPCC (Protomax, ICM, Inc.; Colwich, KS) on a DM basis. Assigned starter grain treatments were offered ad libitum beginning at 14 d of age till conclusion of the experiment at 84 d of age. Starter grain diet ingredients and nutrient composition are described in Table 2.1 and the composition of the HPCC is described in Table 2.2. The CTRL diet was formulated via commercial software (NDS Professional, RUM&N Sas, Reggio Emilia, Italy) to meet metabolizable energy and metabolizable protein requirements of a 110 kg calf according to the Cornell Net Carbohydrate and Protein System version 6.55. All diets were formulated to meet methionine and lysine requirements.

From birth through the first four weeks of the study, calves were fed pasteurized whole milk twice per day at 0600 and 1800h. The average composition of the milk provided can be found in Table 2.3. Milk allowance was determined according to birthweight. Calves weighing <36.3 kg at birth were offered 2.8 L of milk/d; calves weighing between 36.4 and 38.6 kg at birth were offered 3.8 L of milk/d; and calves weighing > 38.6 kg at birth were offered 5.7 L of milk/d. Step down weaning was initiated at 42 d of age with calves being offered half the original milk allowance once daily. Calves were weaned completely once they consumed 1.36 as fed-kg starter grain for three consecutive days or at 56 d of age, whichever came first. Fresh starter grain was offered daily at 0600 h and additional feed was offered at 1800 h as needed to maintain ad libitum access. Calves had ad libitum access to water at all times throughout the study.

Data and sample collection and analysis

Calf milk and starter grain intake was recorded daily. Daily orts for each calf were collected, subsampled, and composited by week. Fresh grain samples were obtained once a

week. All feed samples were frozen for subsequent analysis. Feed and orts samples were dried in a 55°C forced air oven for 48 hours to determine DM. Samples were ground through a 1mm screen with a Wiley mill (Thomas Scientific, Swedesboro, NJ). Then the dried and ground samples were analyzed for chemical composition at a commercial laboratory (Dairyland Laboratories, Inc.; Acadia, WI). Crude protein and acid detergent insoluble protein (**ADICP**) content were determined using the combustion method according to AOAC method 990.03 (AOAC International, 2012). Amylase-treated neutral detergent fiber (**aNDF**) was measured according to AOAC method 2002.04 (AOAC International, 2005) but was modified to use a sea sand filter aid and Whatman GF/C filter paper for residue collection, the results were then adjusted for organic matter (**aNDFom**). Starch was determined according to Starch Analysis in Animal Feed: Method workshop from 30th Annual MW AOACI Meeting and Exposition, with the modification of glucose analysis being completed on a YSI 2700 Select Biochemistry Analyzer (YSI, Yellow Springs, OH). An automated Soxtec 2047 (Foss analytics, Hilleroed, Denmark) machine and diethyl ether were used to determine ether extract (**EE**) according to AOAC method 920.39 (AOAC International, 2016). Dietary acid insoluble ash (**AIA**) was determined using methods from Van Keulen and Young (1977) and Kanani et al. (2015) for estimation of apparent total tract digestibility of the diets. A sample of the whole milk was collected once weekly and sent to MQT Laboratories (Kansas City, MO) for analysis of DM, fat, protein, and lactose content using mid-infrared spectroscopy (Bentley FTS; Bentley Instruments Inc., Chaska, MN).

Physical measurements and blood samples were obtained from each calf on study d 0, 14, 28, 42, 56, and 71 (+/- 1 d). Sampling occurred at 0600 h before milk and fresh grain were fed. Calves were measured for body weight (**BW**), wither height, hip height, and girth circumference.

Blood samples were drawn from the jugular vein into evacuated tubes. Two separate tubes coated in potassium EDTA and sodium fluoride/potassium oxalate were used for plasma collection. A third tube without additives was used for serum collection. Immediately after collection, tubes for plasma separation were placed on ice and the serum tubes were allowed to clot at room temperature. Samples were then centrifuged for 15 minutes at 1,900 x g for plasma and 2,700 x g for serum (AccuSpin FR; Beckman Coulter, Brea, CA) before aliquoting and freezing till analysis. A ChemWell-T autoanalyzer (Awareness Technology Inc., Palm City, FL) and Catachem reagent kits (Catachem Inc., Oxford, CT) were utilized for quantification of BHB (Catachem C442-0A; Martin et al., 2021 and Pralle et al., 2021), BUN (Catachem C263-03; Caputo Oliveira et al., 2020), glucose (Catachem C124-07; Pralle et al., 2021), and triglycerides (**TG**; Catachem C115-0A; Martin et al., 2021 and Pralle et al., 2021). Serum free fatty acid (**FFA**) concentration was determined via spectroscopy (BioTek PowerWave HT, Agilent, Santa Clara, CA) with a plate assay utilizing Catachem reagents (Catachem C514-0A) and a standard fatty acid solution (FUJIFILM, Lexington, MA) according to Martin et al., 2021 and Pralle et al., 2021. Plasma insulin concentrations were quantified with an ELISA assay (Mercodia, Uppsala, Sweden).

To determine apparent total tract diet digestibility, fecal grab samples were collected from each calf at 0600 and 1800 h on the final four days of the experiment and frozen for subsequent analysis. Fecal samples were composited by calf then dried in a 55°C forced air oven for 72 h to obtain DM values. The dried fecal samples were then ground through a 1mm screen with a Wiley mill and analyzed at a commercial laboratory (Dairyland Laboratories, Inc.; Acadia, WI) for CP, NDF, starch, EE, and AIA using methods discussed previously. Utilizing AIA as an internal marker, DM, CP, and starch apparent total tract digestibility was calculated

based on the ratio of nutrients in the feces to nutrients in the starters (Chochran and Galyean, 1994).

Statistical analysis

Preweaning and weaning period analysis

Data normality was assessed on visual inspection of studentized residuals plot, distribution histogram, and QQ plot and data was transformed as necessary. Models used the Kenward-Roger method for degrees of freedom and were adjusted for multiple comparisons with Tukey-Kramer tests when appropriate. All modeled outcomes were tested for preplanned contrasts of linear, quadratic effects of HPCC inclusion, and for CTRL vs. HPCC diets. Results are reported as least square means with 95% confidence intervals.

It was not determined until after the conclusion of the experiment that calves were being offered different amounts of milk by farm staff, and as such data from the preweaning and weaning phases were analyzed as a complete randomized design. There was one male and one female that received 2.8 L of milk/d, three males and 10 females received 3.8 L of milk/d, and 17 males and 10 females that received 5.7 L of milk/d. The two calves receiving 2.8 L of milk were excluded from the preweaning and weaning phase analysis. The resulting number of calves per treatment was n=13, n=14, n=13 for CTRL, 50HPCC, and 100HPCC respectively. For both the CTRL and 100HPCC starter treatment groups three calves received 3.8 L milk/d and 10 calves received 5.7 L milk/d. In the 50HPCC group seven calves received 3.8 L milk/d and seven calves received 5.7 L milk/d. Prewaning data (14-41 d of age) were analyzed using proc MIXED in SAS (version 9.4; SAS Institute Inc., Cary, NC). Treatment, sex, and week were used as fixed effects in models for intake, growth parameters, and blood metabolites. Feed efficiency and average daily gain (**ADG**) were modeled using the fixed effects of treatment and sex. Birthdate

and BW at birth were offered as covariates and retained in the model when $P < 0.10$. All preweaning models included milk allowance as a covariate regardless of significance.

Data from the weaning period (42-55 d of age) were also analyzed using proc MIXED. Dry matter intake models included treatment, sex, week, and their interactions as fixed effects. Birthdate, days the calf took to completely wean, initial BW, and milk allowance were all offered as a covariate and removed if $P > 0.10$ for weaning period models. Milk allowance was included as a covariate in the model for feed intake regardless of significance.

Postweaning analysis

Data for the postweaning period (56-84 d of age) included all 42 calves and was analyzed as a randomized complete block design with $n=14$ calves per treatment. Models were analyzed using proc MIXED with repeated measures when applicable. Models for analyzing postweaning intake, growth parameters and blood metabolites used fixed effects of treatment, sex, and week. A homogenous first order auto regressive variance structure was used for the covariance parameter estimates on the repeated subject of calf for physical growth and blood metabolite models. Block was used a random effect. Average daily gain, feed efficiency and apparent total tract digestibilities were modeled with fixed effects of treatments and sex, a heterogeneous autoregressive covariance structure and calf(block) as the random effect. Body weight (or height and girth measurements for respective models) at the end of the weaning period, initial BW, and milk allowance were tested as covariates and retained in the model when $P < 0.10$. Milk allowance was not significant for any of the growth or intake models.

Results

Prewaning

Starter grain intake on a kg/d basis and as a percentage of bodyweight increased with week ($P < 0.01$). There was an interaction between treatment and sex for daily starter grain intake ($P < 0.01$; Figure 2.1) whereby males consumed similar amounts of starter grain regardless of treatment but female 100HPCC calves consumed 0.26 and 0.33 kg DM more starter grain than CTRL and 50HPCC females, respectively. Similar results were observed for starter grain intake as a percentage of BW where treatment and sex interacted ($P < 0.01$; Figure 2.2). Female 100HPCC calves had greater starter grain intake relative to BW than 50HPCC females. Across both sexes, quadratic feed intake outcomes were observed from CTRL to 100HPCC, with the lowest intake for 50HPCC ($P < 0.01$; Figure 2.1; Figure 2.2).

High-protein corn coproduct inclusion in either diet increased BW compared to CTRL ($P < 0.02$; Table 2.4). Increasing HPCC inclusion linearly increased BW (50.5 95% CI [48.9, 52.1], 52.7 95% CI [51.1, 54.5], and 53.0 95% CI [51.3, 54.8] kg for CTRL, 50HPCC and 100HPCC, respectively; $P = 0.04$). Compared with females, males were heavier (54.1 kg 95% CI [52.6, 55.7] versus 50.1 kg 95% CI [48.8, 51.5]; $P < 0.01$; Table 2.4) taller at the wither (83.8 cm 95% CI [83.2, 84.4] versus 82.5 cm 95% CI [81.9, 83.1]; $P < 0.01$; Table 2.4) and hips (88.6 cm 95% CI [87.8, 89.3] versus 86.8 cm 95% CI [86.1, 87.5]; $P < 0.01$; Table 2.4), and had greater girth circumference (91.3 cm 95% CI [90.4, 92.1] versus 88.4 cm 95% CI [87.6, 89.2]; $P < 0.01$; Table 2.4). Males also gained 0.1 kg/d more than females ($P = 0.04$; Table 2.4) and tended to be more feed efficient (0.08 95% CI [0.07, 0.09] versus 0.07 95% CI [0.06, 0.08]; $P = 0.07$; Table 2.4). Bodyweights, heights, and girth measurements increased with time ($P < 0.01$; Table 2.4).

There was a tendency for a quadratic contrast for ADG ($P = 0.09$; Table 2.4) with 50HPCC calves gaining less than CTRL or 100HPCC calves.

Treatment, sex, and week interacted on plasma glucose ($P = 0.02$; Figure 2.4); however, treatment means did not separate when pairwise means were evaluated for this interaction ($P > 0.05$). Treatment and sex tended to interact for plasma BHB concentration ($P = 0.09$) whereby within females plasma BHB concentrations were numerically greater for 100HPCC, but within males plasma BHB were less for 100HPCC. Both plasma BHB and serum TG concentration increased with time ($P < 0.01$; Figure 2.5) while there was no evidence of time effects on other blood metabolites. Males had greater plasma BUN concentrations than females (14.7 mg/dL 95% CI [13.6,15.9] versus 12.9 mg/dL 95% CI [12.0, 13.8]; $P = 0.02$; Table 2.5) and tended to have lesser serum TG concentration than female calves (17.9 mg/dL 95% CI [15.8, 20.1] versus 21.2 mg/dL 95% CI [18.8, 23.8]; $P = 0.06$; Table 2.5). There was no evidence of treatment, sex or time effects on serum FFA concentrations ($P > 0.10$; Table 2.5).

Weaning

During the weaning period of 42 to 55 d of age, daily starter grain intakes increased with time ($P < 0.01$). A treatment by sex interaction was observed for starter grain intake as a percentage of BW ($P = 0.05$; Figure 2.6). Although means did not separate after adjustment for multiple comparisons, HPCC inclusion numerically increased intake relative to BW for females but decreased intake relative to BW for male calves. A tendency for a quadratic effect of increasing HPCC was detected on BW ($P = 0.08$; Table 2.6) with 50HPCC calves having lesser BW than CTRL or 100HPCC calves. There was no evidence of difference between treatment, sex or week for calf height and girth circumference measurements during the weaning period ($P > 0.10$; Table 2.6). Average daily gain tended to be greater in males than females (1.0 kg/d 95%

CI [0.93, 1.1] versus 0.91kg/d 95% CI [0.84, 0.98]; $P = 0.08$; Table 2.6). Treatment and sex interacted on feed efficiency ($P = 0.02$; Figure 2.7). Once again means did not separate, but numerically 50HPCC were the most feed efficient females and 100HPCC were the most feed efficient males.

Regarding blood metabolites, increasing HPCC inclusion linearly decreased plasma BHB concentration (0.26 95% CI [0.21, 0.31], 0.24 95% CI [0.20, 0.], and 0.18 mM/L 95% CI [0.15, 0.22] for CTRL, 50HPCC and 100HPCC, respectively; $P = 0.02$; Table 2.7). The calves fed CTRL tended to have greater plasma BHB concentration than calves fed HPCC treatments ($P = 0.08$; Table 2.7). Compared with females, males had 5.2 mg/dL greater serum TG concentration ($P = 0.04$; Table 2.7) and tended to have greater plasma BUN concentrations (19.2 mg/dL 95% CI [17.7, 20.7] versus 17.3 mg/dL 95% CI [15.8, 18.9]; $P = 0.09$; Table 2.7). Females tended to have greater plasma glucose concentrations than males (26.6 mg/dL 95% CI [23.1, 30.3] versus 21.4 mg/dL 95% CI [18.3, 24.8]; $P = 0.07$; Table 2.7). There was a tendency for a treatment by sex interaction for serum TG ($P = 0.08$) with CTRL males having numerically greater concentrations than 50HPCC or 100HPCC males. Additionally, feeding diets with HPCC tended to increase plasma insulin compared with CTRL ($P = 0.07$; Table 2.7). There was no evidence of difference of treatment, sex or time on serum FFA concentration during the weaning period ($P > 0.10$; Table 2.7).

Postweaning

Treatment, sex, and week interacted on daily starter grain intake in the postweaning period ($P = 0.03$; Figure 2.8). For males, 100HPCC calves increased daily grain intake more rapidly compared with the other treatments, but for females CTRL and 50HPCC increased their daily grain intake more rapidly than 100HPCC. High protein corn coproduct inclusion tended to

linearly increase daily grain intake ($P = 0.08$; Figure 2.8). There was an interaction of treatment, sex, and week for starter grain intake as a percentage of BW ($P < 0.02$; Figure 2.9). Male 100HPCC increased intake from week 10 to 12 more than CTRL and 50HPCC males, whereas in females 100HPCC intake increased at a lesser rate than the other treatments.

Weight, height and girth circumference increased with time ($P < 0.01$; Table 2.8). Increasing inclusion of HPCC in the diets linearly increased BW ($P = 0.05$; Table 2.8) and overall HPCC inclusion tended to increase BW compared to CTRL ($P = 0.06$; Table 2.8). Increasing HPCC inclusion linearly increased average daily gain ($P = 0.05$; Table 2.8) and calves fed diets with HPCC tended to have greater gain than those fed CTRL ($P = 0.08$; Table 2.8). Furthermore, males gained 0.14 kg/d more than females ($P < 0.01$; Table 2.8). Treatment and sex interacted on feed efficiency ($P = 0.04$; Figure 2.10) as 100HPCC females were 0.07 kg/kg DMI less feed efficient than their male counterparts, but there was no evidence of difference for feed efficiency across sex for the other treatments. There was a treatment effect on wither height ($P = 0.05$; Table 2.8) whereby 50HPCC decreased wither height compared with 100HPCC (92.4 cm 95% CI [91.2, 93.6] versus 93.7 cm 95% CI [92.5, 95.0]). Increasing HPCC treatment quadratically altered wither height ($P = 0.02$; Table 2.8); wither height was lower for 50HPCC compared with the other treatments. Males were taller at the hip compared with females (98.6 cm 95% CI [97.8, 99.4] versus 97.5 cm 95% CI [96.7, 98.3] cm; $P = 0.01$; Table 2.8). A treatment by sex tendency was observed for girth circumference whereby 50HPCC females had numerically greater girth than other females, but within males, 50HPCC had the smallest girth circumference ($P = 0.08$).

There was no evidence of difference for treatment, sex, or time on plasma BHB, serum FFA, or serum TG during the postweaning period ($P > 0.10$; Table 2.9). Including HPCC in the

starter grain increased plasma insulin concentration compared with CTRL ($P = 0.04$; Table 2.9) and increasing HPCC in the diet increased plasma insulin concentrations ($P = 0.04$ Table 2.9). Insulin concentrations also increased from 0.23 $\mu\text{g/L}$ 95% CI [0.17, 0.31] at wk 10 to 0.44 $\mu\text{g/L}$ 95% CI [0.33, 0.59] at wk 12 ($P < 0.01$). There was an interaction between treatment, sex, and week interaction on plasma glucose concentration ($P = 0.03$; Figure 2.11) whereby glucose concentrations increased from wk 10 to wk 12 in female CTRL calves but not for the other females, and glucose concentrations did not change with time for any of the males. Males had greater plasma BUN concentration than females (18.9 mg/dL 95% CI [18.0, 19.8] versus 17.4 mg/dL 95% CI [16.6, 18.3]; $P = 0.02$; Table 2.9).

Apparent total tract digestibility results are presented in Table 2.10. High protein corn coproduct inclusion tended to linearly increase CP digestibility ($P = 0.07$) and tended to increase DM ($P = 0.10$) and CP ($P = 0.06$) digestibility compared to CTRL. Males digested 4.5% more DM ($P = 0.02$) and 3.1% more CP ($P = 0.03$) than females. No evidence was found that treatment or sex affected starch digestibility.

Discussion

Starter grains in the present study were intentionally balanced for methionine and lysine, a unique feature compared to other studies evaluating corn distillers' products in calf starter grains (Thomas, 2006; Saurez-Mena et al., 2011). Methionine and lysine are the most common limiting amino acids in calf diets (Schwab et al., 1982; Abe et al., 1998), with DG-based diets being especially susceptible to lysine deficiencies (Kleinschmit et al., 2007). In most studies comparing corn distillers' products to SBM it is unknown to what extent the deviations in amino acid supply attribute to differences in animal performance. In lactating cow diets where

conventional DG displaced SBM, supplementation of rumen protected lysine and methionine improved milk yield and tended to improve energy corrected milk (Nichols et al., 1998).

Therefore, the current study was designed with supplemental methionine and lysine to minimize potential effects on growth and efficiency due to differences in limiting amino acid supply.

Prewaning and Weaning

Feed intake

A well-established connection has been made between milk allowance and starter grain intake in pre-weaned dairy calves, whereby calves decrease starter grain intake when offered increased amounts of milk (Huber et al., 1984; Jasper and Weary, 2002; Terre et al., 2007; Stamey et al., 2012). Since calves in the present study were fed differing amounts of milk during the preweaning and weaning period, intake data during these periods should be interpreted with care.

Conventional DG are a palatable protein source for starter grains (Miller-Cushon et al., 2014), with previous studies utilizing DG in starter grains reporting starter grain intakes of pre-weaned calves between 0.15 to 0.30 kg/d (Thomas, 2006; Saurez-Mena et al., 2011). Daily starter grain intakes of pre-weaned calves in the present study (around 0.30 kg/d) are similar or greater than those of pre-weaned calves fed DG starter grains (Thomas, 2006; Saurez-Mena et al., 2011), suggesting that despite additional processing during production, HPCC is still readily accepted by young calves. Previous work evaluating the replacement of SBM and corn in starter grains with conventional DG have not observed impacts on starter grain intake during the preweaning period (Thomas, 2006; Suarez-Mena et al., 2011). Thomas (2006) weaned calves at a similar age to calves in the present study and did not observe differences in starter grain intake during the preweaning period despite a DG inclusion of up to 56% of diet DM. Calves in Suarez-

Mena et al. (2011) were weaned two weeks earlier and had lesser preweaning starter grain intake than calves in the present study due to the earlier age at weaning, which may have limited the authors' ability to detect preweaning differences.

The reasons for the preweaning quadratic decrease in starter grain DMI for calves fed 50HPCC compared with the other treatments is not entirely clear but appears to be driven by the decrease in starter grain intake for HPCC within females (Figure 2.1; Figure 2.2). Feeding high protein (>18% CP) starter grains has been documented to increase starter grain intake in preweaned calves (Akeyezu et al., 1994; Kazemi-Bonchenari et al., 2022). Based on those previous studies, increasing the starter grain CP with HPCC inclusion from 23.7% CP in CTRL to 25.6% CP in 100HPCC in our study may have contributed to the increased starter grain intake for 100HPCC compared to CTRL or 50HPCC females (Figure 2.1); however, it is unclear why the same was not observed in males.

Many dairy calf nutrition studies do not report significant effects of calf sex on feed intake in preweaned calves (Lesmeister and Heinrichs, 2004; McCoard et al., 2019; Makizadeh et al., 2020; Jafapour et al., 2023). Preceding papers that have observed sex effects for starter intake are inconsistent with females consuming more grain in some studies (Kuehn et al., 1994) while males had greater intake in others (Sweeney et al., 2010). Morrill and Dayton (1978) detected a tendency for a sex by treatment interaction in a study that supplemented 10% CP starter grains with supplemental protein sources. However, their results suggested males on lower CP diets consumed greater amounts of starter, contrary to the present study where male's starter grain intake did not differ between treatments. Repetition of this study with a more consistent milk feeding design would help elucidate the effects of sex and HPCC inclusion on starter grain intake during the preweaning and weaning periods.

Growth and efficiency

During the preweaning period, inclusion HPCC in starter grains increased BW but did not affect structural growth or feed efficiency (Table 2.4). Considering over half the variation in calf BW at weaning can be accounted for by starter grain intake (Kertz et al., 1979), the linear increase in BW with HPCC inclusion is discordant with the quadratic effects observed for starter grain intake. It is unclear what is responsible for the observed disconnect.

Sex effects on growth measures were most pronounced during the preweaning period with male calves being heavier and exhibiting greater height, as expected. Holstein bull calves have previously been reported to be heavier and taller than their female counterparts in the preweaning period (El Bushra et al., 1989; Hopkins, 1997; Arrayet et al., 2001).

Blood metabolites

Inclusion of HPCC in starter grains fed during the preweaning period had minimal effects on blood metabolites possibly due to the low amount of starter intake. The three-way interaction between treatment, sex, and week observed for plasma glucose during this time is difficult to interpret. However, the numerical decreases in glucose concentration from wk 2 to wk 4 observed for most groups in the current study is in agreement with previous work which observed decreasing blood glucose within the first several weeks of life (Quigley et al., 1991; Sasaki et al., 2021). Quigley et al. (1991) interpreted the decline of blood glucose concentrations observed in their study as an indication of increasing rumen functionality, as it was accompanied by a simultaneous increase in blood BHB concentration. In the present study plasma BHB concentrations also increased with week, reflective of increasing starter intake and development of ruminal fermentative capabilities (Deelen et al., 2016). The subsequent linear decrease in plasma BHB concentration with increasing HPCC inclusion during the weaning period could not

be accounted for by differences in starter grain intake. Considering ruminal butyrate production is driven by fermentation of readily available carbohydrates (Gorka et al., 2018), the depression of plasma BHB may be attributed to the 2% decrease in starch content in the 100HPCC diet versus the CTRL diet.

Postweaning

Feed intake

As expected, starter grain intake continued to increase over time after calves were weaned. However, it is unclear why the rate of increase began to diverge with some groups of calves increasing their starter intake each week (100HPCC males, CTRL and 50HPCC females) while others maintained more consistent grain intake (CTRL and 50HPCC males, 100HPCC females; Figure 2.8; Figure 2.9). As a whole, during the postweaning period inclusion of HPCC tended to linearly increase calf DMI. Conversely, inclusion of conventional DG in calf starter grains did not affect DMI of weaned calves in Thomas (2006) and Suarez-Mena et al. (2011). Displacement of SBM with high protein DG (HPDG) or HPCC has been most studied in lactating cow diets. A study which substituted SBM with the same HPCC used in the current study (20% of dietary DM) in mid-lactation Holstein cow diets did not observe altered DMI (Zynda et al., 2022). Additional studies evaluating other HPDG or HPCC as replacements for SBM in cow diets have also reported no evidence of effects on DMI (Hubbard et al., 2009; Kelzer et al., 2009; Christen et al., 2010; Brown and Bradford 2020). However, due to the drastic differences in the composition of diets fed to lactating cows and weaned calves, direct comparisons are difficult to make.

Growth and efficiency

The postweaning ADG in the current study (0.97 -1.1 kg/d; Table 2.8) was greater than that of calves in other studies feeding conventional DG in starter grains of post-weaned calves which ranged from 0.64 to 0.83 kg/d (Thomas, 2006; Suarez-Mena et al., 2011). A meta-analysis by Zanton and Heinrichs (2005) found that prepubertal ADG is quadratically related to first lactation milk production, with production decreasing when ADG exceeds 0.9 kg/d. Therefore, the NASEM (2021) advises an ADG of 0.9 kg/d for prepubertal Holstein heifers, although authors admit greater ADG may be acceptable if gain is lean and not deposited as excess adipose tissue. Body condition scores were not collected on calves in the current study but could have clarified if the observed ADG was appropriate or excessive.

Although both BW and ADG linearly increased with HPCC inclusion, no significant differences were detected for feed efficiency likely because DMI also tended to linearly increase with HPCC. Both Thomas (2006) and Suarez-Mena et al. (2011) reported reductions in feed efficiency with conventional DG inclusion in their calf grains during the postweaning period. The reduction in feed efficiency in Thomas (2006) was driven by numerically greater starter grain intake with DG inclusion, however in Suarez-Mena et al. (2011) the reduction in feed efficiency was a function of DG decreasing ADG. Distillers' grains and HPCC contain greater concentrations of NDF than SBM. Therefore, substitution of SBM with DG or HPCC can not only dilute the energy density of diets but also exacerbate gut filling properties of diets, both factors that can potentially reduce feed efficiency. In the current study limitations due to gut fill or energy displacement with HPCC inclusion were not clearly evident, with starter grain intake and feed efficiency being mostly unchanged by HPCC inclusion. However, only a modest

amount (8.3 to 16.6% of DM) of HPCC was used in these diets and it is unknown if greater levels of inclusion would increase the gut filling effects of the diet.

Calf sex only affected hip height during the postweaning period, with males exceeding hip height compared with females, but did not effect BW or wither height. The only effect of HPCC on structural growth was for wither height where 100HPCC calves grew taller than 50HPCC calves. Similar to the present study, Thomas (2006) observed taller wither heights in weaned calves fed a 56% DG diet compared to a 28% DG diet. Other studies replacing SBM with alternative protein sources in starter grains have observed structural growth to be reduced (Jafapour et al., 2023), increased (Rastgoo et al., 2020), or unaffected (Suarez-Mena et al., 2011). It has been previously postulated that wither height is highly correlated with BW (Heinrichs et al., 1992; Thomas and Timmit, 1976), but this is not always the case (Rastagoo et al., 2020; Jafapour et al., 2023). We observed a different relationship between height and BW in the present study, as wither height quadratically decreased from CTRL to 50HPCC and increased from 50HPCC to 100HPCC, but BW increased linearly with HPCC (Table 2.8). Nonetheless, the decreased wither height observed in 50HPCC calves could be undesirable as reduced calfhood height is associated with decreased milk production potential (Van De Stroet et al., 2016). Furthermore, since wither height growth rate is maximized and the feed cost per cm of growth is minimized during the first six months of life (Kertz et al., 1998), the increased wither height observed in 100HPCC calves is preferable.

Blood metabolites

Plasma glucose concentrations remained elevated after weaning (≥ 87 mg/dL), with concentrations of plasma glucose continuing to increase over time in CTRL females (Figure 2.11). This is unusual as plasma glucose concentrations in calves are expected to drop closer to

concentrations expected of mature ruminants after weaning (Quigley et al., 1991). Overall insulin concentration also increased with time during the postweaning period. Blood insulin concentration is known to increase with BW as demonstrated in six-month-old Holstein and wagyu steers (Matsuzaki et al., 1997). The linear increase of plasma insulin concentrations in weaned calves fed HPCC is likely related to the linear increase in BW and ADG with HPCC inclusion. Studies utilizing growing beef steers have demonstrated that plasma glucose and insulin levels increase as animals are given access to diets containing increasing amounts of energy (Ellenberger et al., 1989; Schoonmaker et al., 2003). Therefore, the sustained elevated blood glucose and insulin concentrations coupled with the aggressive ADG observed in the present study suggests that starter grains exceeded calves' energy requirements in the postweaning period.

Blood urea nitrogen concentrations were greater in males than females during the postweaning period. Other studies have reported heifers with greater plasma BUN concentrations (Walker et al., 2010; Brown et al., 2023) or no effect of sex on plasma or serum BUN concentrations in growing lambs and cattle (von Keyserlingk and Mathison 1993; Bailey et al., 2008; Qui et al., 2022). While differences in plasma BUN concentrations can be reflective of differences in nitrogen intake (Quigley and Bernard, 1991; Zanton and Heinrichs, 2008), the lack of sex effects on DMI during the postweaning period in the present study is likely not representative of the sex differences we observed on plasma BUN.

Alternatively, plasma BUN can also reflect nitrogen use efficiency. Khon et al. (2005) demonstrated that plasma BUN is positively correlated with nitrogen clearance rate in both growing and mature ruminants, reflecting nitrogen that is not utilized by peripheral tissues and excreted in urine. Because androgens, such as testosterone, are responsible for increasing muscle

mass (Dubois et al., 2012), it is understood that males take up and deposit greater amounts of protein during growth versus females (Fortin et al., 1981; Geay, 1984) with a concomitant decrease in plasma BUN concentration (Walker et al., 2010). However, castration of males, which was performed prior to treatment initiation in the present study, decreases androgen levels. As a consequence, suppression of protein deposition due to castration increases nitrogen excretion (Biagini and Lazzaroni, 2011). Nonetheless, steers still deposit more protein tissue than heifers (Fortin et al., 1981) so this does not completely explain why males had greater BUN than females in the present study.

Increases in plasma BUN concentrations in young ruminants also indicates more advanced rumen development (Quigley and Bernard, 1991) as dietary proteins are degraded by microbes which produce ammonia with excess amino acids. The ammonia is then absorbed by the rumen epithelium and converted to urea in the liver. Calves with more developed rumens are able to degrade protein more extensively and release amino acids for production of ruminal ammonia (Quigley and Bernard, 1991). Interestingly, in the current study males had greater starter grain intake during the preweaning period. Since starter grain intake is a key factor in rumen development (Baldwin et al., 2004), the greater starter grain intake of males at a younger age could have resulted in more advanced rumen development and fermentative capabilities than their female counterparts. This idea is further supported by the increased apparent total tract digestibility observed in males which is discussed further in the following section. However, it is important to note that BHB, the blood metabolite typically measured to indicate rumen development (Deelen et al., 2016), was not different between sexes. Hence, additional measurements of nitrogen flow and rumen development would be needed to further clarify why BUN differed between sexes in this study.

Apparent Total Tract Digestibility

The apparent total tract digestibility of DM (80 to 84%), CP (85 to 89%), and starch (96 %) reported in the present study are consistent with findings from other studies utilizing weaned dairy calves on a concentrate-based diet (Hill et al., 2016a; Hill et al., 2016b; Makizadeh et al., 2020; Rastgoo et al., 2020). Apparent total tract digestibility of starch was unaffected by HPCC inclusion, as was expected considering starch sources and concentration remained mostly consistent between diets. The tendency for improved apparent total tract digestibility of DM and CP with the inclusion of HPCC is somewhat unexpected as it suggests that HPCC could possibly be equally or more digestible than SBM. Soybean meal is known for being highly digestible by ruminants, with a total digestible dietary protein (**TDDP**) value greater than 90% of CP (Kleinschmit et al., 2007). Laboratory analysis provided by the manufacturer estimated the current HPCC to be 80% of CP TDDP (using the methods of Ross and Van Amburgh, 2013). This value is similar to TDDP values observed in conventional DG which range from 70 to 85% of CP (Kleinschmidt et al., 2007). Therefore, it would be expected that CP apparent total tract digestibility would be reduced by substituting HPCC or DG for SBM in starter grains. However, inclusion of 49% conventional DG in starter grains did not affect CP apparent total tract digestibility in weaned calves (Saurez-Mena et al., 2011). Replacement of soy-based proteins with HPCC or HPDG is most studied in lactating cow diets where OM or CP digestibility has either been reduced or unaffected by HPCC or HPDG (Kelzer et al., 2009; Brown and Bradford, 2020). When the same HPCC used in the present study replaced the soy-based proteins in the diet of mid lactation Holstein cows, DM and CP apparent total tract diet digestibility was unaffected (Zynda et al., 2022).

Digestibility of the current HPCC has been evaluated more thoroughly in swine where it was found that HPCC had equal or greater ileal digestibility of CP, essential amino acids, and non-essential amino acids compared to conventional DG (Yang et al., 2021). However, this is of limited applicability in regard to ruminants, as the nature of the rumen undegraded protein fraction of this HPCC has not yet been evaluated. Further research focusing on the nature of HPCC digestion in ruminants could help explain why CP apparent total tract digestibility was greater than expected in the HPCC diets of the current study.

In the present study, increased apparent total tract digestibility of both DM and CP were observed in males compared with females. The effect of sex on diet digestibility in young artificially reared calves is not well defined in the literature. While some studies utilizing post-pubertal sheep and goats have reported sex effects on digestibility, results have been inconsistent regarding which sex has greater digestive capabilities (Aregheore, 1995; Aregheore, 1997; Pereira et al., 2018). The observed increase in DM apparent total tract digestibility for males in the present study could possibly be related to more advanced rumen development with greater capability to break down feeds via fermentation (Quigley et al., 2018). However, this is difficult to verify without the collection of rumen samples.

Other considerations

Despite not containing the solubles from distillation, the particular HPCC used in the present study was enhanced with yeast bodies obtained during the fractionation process at an inclusion rate of approximately 25% (as-fed basis). While it is possible the yeast content of the HPCC contributed to improved BW and ADG of HPCC fed calves in the current study, it is infeasible to distinguish between effects of protein content versus yeast content.

Yeasts may be used in cattle diets to improve rumen function through stabilization of pH, and increased fiber digestion in mature ruminants or acceleration of rumen maturation in young ruminants (Chaucheyras-Durand et al., 2008). Utilizing the current HPCC with or without the yeast component in lactating cow diets, Zynda et al. (2022) found no evidence that the addition of the yeast affected DMI, milk production, diet digestibility, nitrogen utilization, or manure characteristics. Authors speculated that the majority of the yeast added to the HPCC may have been deactivated during the additional processes required for HPCC production versus conventional DG production.

Previous work supplementing calf starter grains with yeast has found positive effects on growth and or gastrointestinal tract development, however, outcomes affected vary by study. Some studies have reported that supplementing starter grains with yeast increases starter grain intake (Galvão et al., 2005) and BW or ADG (Lesmeister et al., 2004; Galvão et al., 2005), while others have found no evidence that yeast supplementation enhances calf growth (Hučko et al., 2009; Alugongo et al., 2017). Average daily gain and BW of weaned calves in the current study was greater with increasing inclusion of HPCC (Table 2.8), but again, given the design of this study it is not possible to separate effects of CP content from effects of yeast content. Reports of increased ruminal papillary and intestinal villi development (Xiao et al., 2016) and improved fiber digestion (Hučko et al., 2009) in calves fed yeast supplemented starter grains indicate benefits to gastrointestinal tract development. Contrary to this, Lesmeister et al. (2004) found that yeast supplementation did not affect rumen development. The overall inconsistency of specific outcomes between studies supplementing calf diets with yeast suggests that type of yeast, quantity supplemented, and method of supplementation are all important factors influencing efficacy.

Benefits to the health and immune status of calves fed starter grains supplemented with yeast are commonly related to improved fecal scores and reduced incidences of diarrhea (Galvão et al., 2005; Magalhães et al., 2008; Alugongo et al., 2017). Including 2% yeast culture in the starter grains of Magalhães et al. (2008) tended to reduce incidence of fever and diarrhea resulting in yeast supplemented calves being treated less, for shorter amounts of time, and having lower overall mortality. Calves in the present study were low risk and overall healthy so it is unknown whether the yeast content of the HPCC would be beneficial during health challenges.

Conclusion

Substituting HPCC for SBM as a protein source in starter grains balanced for methionine and lysine improved calf gain during the first three months of life. Increased gains were obtained without sacrificing feed efficiency in the preweaning period, however effects on feed efficiency in weaned calves were less clear. Increasing HPCC inclusion tended to improve diet apparent total tract digestibility in weaned calves. Further studies with the ability to evaluate rumen function would be valuable in understanding how HPCC affected rumen development. Based on calf growth and feed efficiency findings, HPCC is a suitable replacement for SBM in starter grains when supplemented with potentially limiting amino acids.

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Tables

Table 2.1. Starter grain ingredients and analyzed nutrient composition

Item	Treatment		
	CTRL	50HPCC	100HPCC
Ingredient, % DM			
Not in pellet			
Cracked corn	20.9	20.9	20.9
Soybean meal, 44%	16.6	8.3	-
High protein corn co-product ¹	-	8.3	16.4
Oats	11.1	11.1	11.1
Molasses	10.5	10.5	10.5
Ajipro-L ²	-	0.05	0.27
Within pellet			
Wheat middlings	24.6	24.6	24.6
Blood meal	7.0	7.0	7.0
MKC B1800 ³	3.7	3.7	3.7
Ground corn	3.5	3.5	3.5
Calcium carbonate	1.8	1.8	1.8
Vitamin E	0.05	0.05	0.05
Vitamin D	0.10	0.10	0.10
MetaSmart ⁴	0.06	0.05	0.04
Nutrient, % DM			
DM	90.5	90.9	91.0
CP	23.7	24.3	25.6
AD-ICP	0.52	0.73	1.02
aNDFom	21.2	19.6	21.5
Starch	26.5	25.6	24.6
EE	4.0	4.0	4.3

¹Protomax (ICM Inc., Colwich, KS)

²Ajinomoto Health & Nutrition North America, Chicago, IL

³Trace Mineral Premix (MKC Cooperative, Moundridge, KS); Guaranteed analysis: 2.0 g/kg lasalocid; 220,500.0 I.U./kg vitamin A; 22,050.0 I.U./kg vitamin D; 275.6 I.U./kg vitamin E; 11.0 ppm selenium; 25% min calcium; 16% min sodium chloride; 1% min magnesium

⁴Adisseo Inc., Antony, France

Table 2.2. Chemical composition of a high protein corn co-product fed to calves¹

Nutrient, % DM	
DM	95.1
CP	47.74
AD-ICP	4.14
aNDFom	24.22
Starch	8.08
EE	11.2

¹Protomax (ICM Inc., Colwich, KS)

Table 2.3. Chemical composition of pasteurized waste milk fed to calves

Item	Average $\pm$ SD
DM, %	12.0 $\pm$ 0.4
Fat	3.0 $\pm$ 0.3
Protein	3.4 $\pm$ 0.3
Lactose	4.8 $\pm$ 0.1

Table 2.4. Growth, dimensions and feed efficiency of Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		P-values			Contrasts ²
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Week	
Body weight, kg	50.5 [48.9, 52.1]	52.7 [51.1, 54.5]	53.0 [51.3, 54.8]	50.1 [48.8, 51.5]	54.1 [52.6, 55.7]	0.07	<0.01	<0.01	L, CvH
Wither height, cm	82.9 [82.2, 83.6]	83.0 [82.3, 83.8]	83.5 [82.8, 84.3]	82.5 [81.9, 83.1]	83.8 [83.2, 84.4]	0.46	<0.01	<0.01	
Hip height, cm	87.2 [86.3, 88.0]	88.0 [87.1, 88.9]	87.8 [86.9, 88.7]	86.8 [86.1, 87.5]	88.6 [87.8, 89.3]	0.37	<0.01	<0.01	
Girth, cm	89.4 [88.4, 90.4]	89.9 [88.9, 90.9]	90.2 [89.2, 91.2]	88.4 [87.6, 89.2]	91.3 [90.4, 92.1]	0.58	<0.01	<0.01	
ADG, kg/d	0.63 [0.56, 0.71]	0.56 [0.49, 0.63]	0.64 [0.56, 0.72]	0.56 [0.50, 0.62]	0.66 [0.59, 0.72]	0.22	0.04	-	q
Feed efficiency ³	0.08 [0.07, 0.09]	0.07 [0.06, 0.09]	0.07 [0.06, 0.08]	0.07 [0.06, 0.08]	0.08 [0.07, 0.09]	0.35	0.07	-	

¹Data presented as least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

³Feed efficiency calculated as g of gain/g of feed

Table 2.5. Blood urea nitrogen (BUN), free fatty acid (FFA), and insulin concentrations Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		<i>P</i> -values		
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Week
BUN, mg/dL	14.5 [13.3, 16.0]	13.4 [12.4, 14.6]	13.3 [12.3, 14.4]	12.9 [12.0, 13.8]	14.7 [13.6, 15.9]	0.33	0.02	0.90
FFA, mM/L	0.17 [0.15, 0.19]	0.17 [0.15, 0.20]	0.17 [0.14, 0.19]	0.16 [0.14, 0.19]	0.18 [0.15, 0.20]	0.89	0.46	0.47
Insulin, µg/L	0.10 [0.07, 0.13]	0.10 [0.08, 0.14]	0.10 [0.08, 0.14]	0.10 [0.08, 0.13]	0.10 [0.08, 0.13]	0.90	0.95	0.24

¹Data presented at least square means with 95% confidence intervals

Table 2.6. Growth and dimensions of Holstein dairy calves during the weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		P-values			Contrasts ²
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Week	
Daily starter grain									
DMI, kg	1.7 [1.6, 1.8]	1.7 [1.6, 1.8]	1.7 [1.6, 1.9]	1.7 [1.6, 1.8]	1.7 [1.6, 1.9]	0.77	0.43	<0.01	
Body weight, kg	76.7 [74.5, 79.0]	74.1 [72.1, 76.2]	76.1 [73.9, 78.4]	74.5 [72.6, 76.4]	76.8 [74.8, 78.8]	0.21	0.12	-	q
Wither height, cm	89.0 [88.1, 90.0]	88.2 [87.3, 89.1]	88.6 [87.6, 89.5]	88.3 [87.5, 89.1]	88.9 [88.0, 89.7]	0.48	0.37	-	
Hip height, cm	93.9 [92.9, 95.0]	93.0 [92.1, 94.0]	93.3 [92.3, 94.3]	93.6 [92.8, 94.5]	93.2 [92.3, 94.1]	0.43	0.52	-	
Girth, cm	99.9 [98.8, 101]	99.3 [98.2, 100]	98.6 [97.5, 99.8]	98.8 [97.8, 99.8]	99.8 [98.8, 101]	0.28	0.21	-	
ADG, kg/d	0.95 [0.87, 1.0]	0.95 [0.87, 1.0]	0.96 [0.87, 1.0]	0.91 [0.84, 0.98]	1.0 [0.93, 1.1]	0.99	0.08	-	

¹Data presented at least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

Table 2.7. Blood urea nitrogen (BUN), free fatty acid (FFA), and insulin concentrations Holstein dairy calves during the weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		P-values		Contrasts ²
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	
BHB, mM/L	0.26 ^a [0.21, 0.31]	0.24 ^{ab} [0.20, 0.28]	0.18 ^b [0.15, 0.22]	0.22 [0.19, 0.26]	0.22 [0.19, 0.26]	0.05	0.99	L, cvh
BUN, mg/dL	18.3 [16.5, 20.2]	19.4 [17.6, 21.2]	17.1 [15.2, 19.0]	17.3 [15.8, 18.9]	19.2 [17.7, 20.7]	0.22	0.09	
Glucose, mg/dL	76.6 [69.6, 83.7]	79.2 [72.5, 86.0]	81.6 [74.5, 88.6]	82.8 [77.1, 88.5]	75.5 [69.8, 81.2]	0.60	0.07	
Insulin, µg/L	0.15 [0.10, 0.22]	0.25 [0.17, 0.37]	0.22 [0.14, 0.32]	0.21 [0.15, 0.28]	0.19 [0.14, 0.27]	0.16	0.79	cvh
FFA, mM/L	0.08 [0.07, 0.10]	0.07 [0.06, 0.08]	0.08 [0.06, 0.09]	0.08 [0.07, 0.10]	0.07 [0.06, 0.08]	0.43	0.11	
TG, mg/dL	25.4 [21.2, 30.0]	23.8 [19.9, 28.0]	22.7 [18.7, 27.0]	26.6 [23.1, 30.3]	21.4 [18.3, 24.8]	0.65	0.04	

¹Data presented at least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

Table 2.8. Growth and dimensions of Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		P-value			Contrasts ²
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Week	
Body weight, kg	94.3 [91.6, 97.1]	96.3 [93.6, 99.1]	97.1 [94.4, 99.9]	94.6 [92.0, 97.2]	97.2 [94.6, 99.9]	0.14	0.06	<0.01	L, cvh
Wither height, cm	93.5 ^{ab} [92.2, 94.8]	92.4 ^a [91.2, 93.6]	93.7 ^b [92.5, 95.0]	93.3 [92.1, 94.6]	93.1 [91.9, 94.3]	0.05	0.62	<0.01	q
Hip height, cm	98.0 [97.1, 98.9]	97.8 [97.0, 98.7]	98.3 [97.4, 99.1]	97.5 [96.7, 98.3]	98.6 [97.8, 99.4]	0.71	0.02	<0.01	
Girth, cm	106.4 [104.9, 107.9]	107.2 [105.7, 108.7]	107.4 [105.9, 108.9]	106.3 [104.9, 107.8]	107.6 [106.1, 109.1]	0.32	0.07	<0.01	
ADG, kg/d	0.97 [0.87, 1.1]	1.0 [0.95, 1.1]	1.1 [1.0, 1.2]	0.96 [0.88, 1.0]	1.1 [1.0, 1.2]	0.14	<0.01	-	L, cvh

¹Data presented at least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

Table 2.9. Blood β -Hydroxybutyrate (BHB), urea nitrogen (BUN), free fatty acid (FFA), triglyceride (TG), and insulin concentrations Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).¹

Item	Treatment			Sex		P-values			Contrasts ²
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Week	
BHB, mM/L	0.25 [0.22, 0.27]	0.25 [0.22, 0.28]	0.25 [0.23, 0.28]	0.25 [0.23, 0.27]	0.25 [0.23, 0.27]	0.93	0.99	0.56	
BUN, mg/dL	17.9 [16.8, 19.0]	18.7 [17.7, 19.8]	17.9 [16.8, 18.9]	17.4 [16.6, 18.3]	18.9 [18.0, 19.8]	0.40	0.02	0.94	
FFA, mM/L	0.089 [0.081, 0.098]	0.094 [0.085, 0.103]	0.088 [0.078, 0.097]	0.090 [0.082, 0.097]	0.091 [0.083, 0.100]	0.58	0.74	0.68	
TG, mg/dL	26.8 [23.5, 30.6]	23.7 [20.8, 27.1]	24.6 [21.6, 28.0]	24.8 [22.2, 27.7]	25.2 [22.6, 28.2]	0.41	0.82	0.87	
Insulin, μ g/L	0.25 [0.18, 0.34]	0.34 [0.24, 0.46]	0.39 [0.28, 0.53]	0.32 [0.24, 0.43]	0.31 [0.24, 0.42]	0.10	0.86	<0.01	CvH, L

¹Data presented at least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

Table 2.10. Apparent total tract digestibility of dry matter, crude protein, and starch in starter grains fed to weaned calves ¹

Item	Treatment			Sex		<i>P</i> -value ²			
	CTRL	50HPCC	100HPCC	Female	Male	Trt	Sex	Trt*sex	Contrasts ²
Apparent total tract digestibility, %									
DM	80.1 [76.8, 83.3]	83.4 [80.2, 86.6]	83.4 [80.0, 86.8]	80.0 [77.3, 82.7]	84.5 [81.8, 87.1]	0.25	0.02	0.40	cvh
CP	85.8 [83.4, 88.2]	88.2 [85.8, 90.5]	89.0 [86.5, 91.5]	86.1 [84.1, 88.1]	89.2 [87.3, 91.1]	0.15	0.03	0.49	cvh, l
Starch	96.6 [95.3, 97.8]	96.2 [95.0, 97.5]	96.5 [95.2, 97.7]	96.1 [95.1, 97.1]	96.7 [95.7, 97.8]	0.93	0.40	0.15	

¹Data presented at least square means with 95% confidence intervals

²L: linear dose of HPCC ($P \leq 0.05$); l: linear dose of HPCC ($0.05 \leq P \leq 0.10$); Q: quadratic dose of HPCC ($P \leq 0.05$); q: quadratic dose of HPCC tendency ($0.05 \leq P \leq 0.10$); CvH: CTRL vs HPCC ($P \leq 0.05$); cvh: CTRL vs HPCC tendency ($0.05 \leq P \leq 0.10$)

Figures

Figure 2.1. Daily starter grain intake of Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).

Interaction of treatment and sex on daily starter grain intake during the preweaning period ($P < 0.01$) presented as least square means with 95% CI. Bars within sex without common superscript differ ($P \leq 0.05$). A quadratic effect of dose was detected ($P < 0.01$).

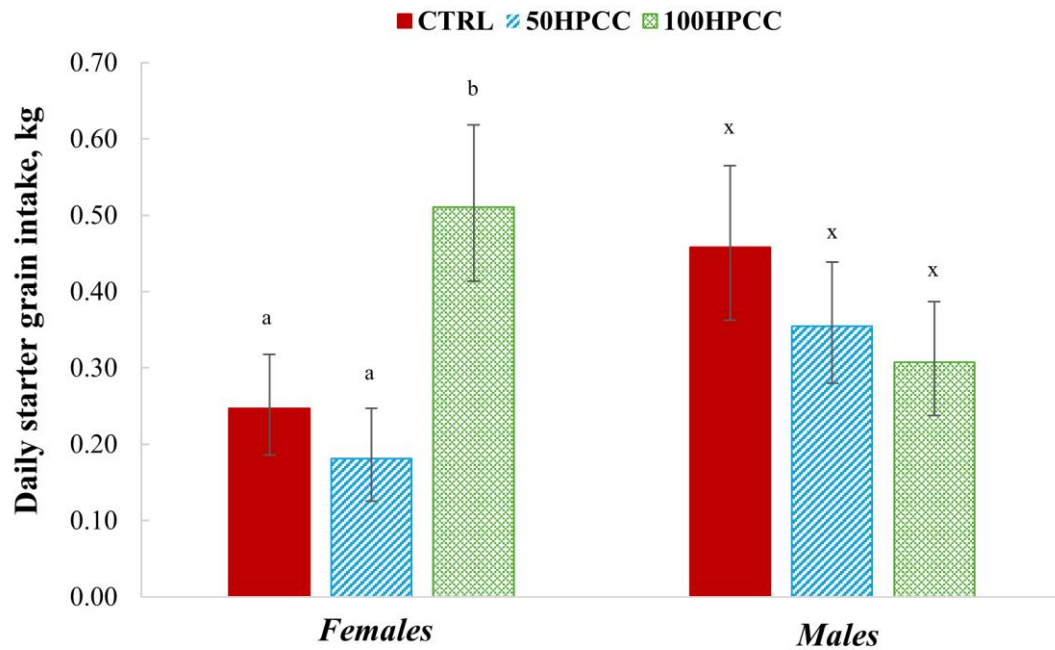


Figure 2.2. Starter grain as a percentage of bodyweight of Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).

Interaction of treatment and sex on starter grain intake as a percentage of bodyweight during the preweaning period ($P < 0.01$) presented as least square means with 95% CI. Bars within sex without common superscript differ ($P \leq 0.05$). A quadratic effect of dose was detected ($P < 0.01$).

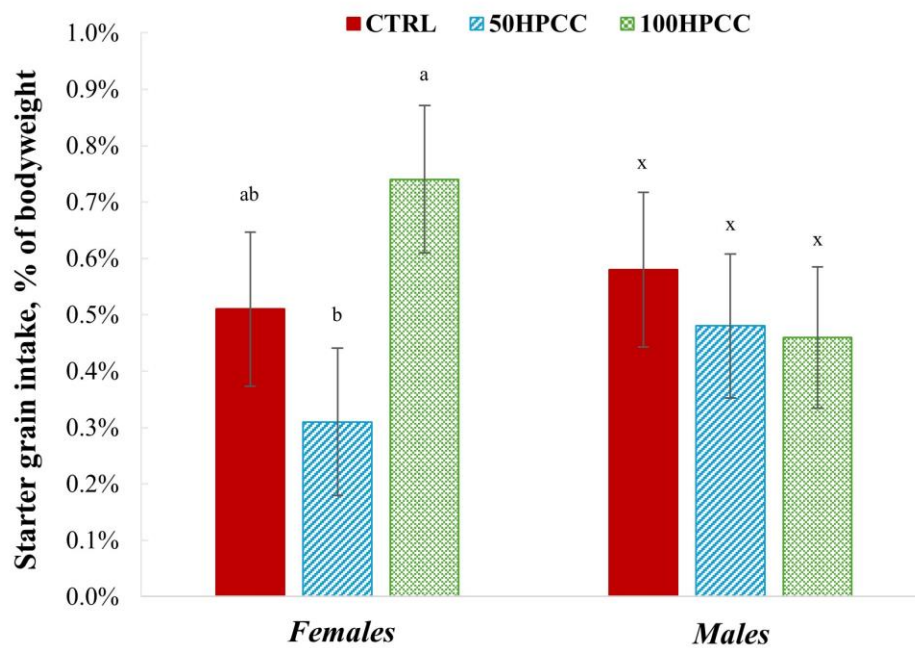


Figure 2.3. Blood glucose concentrations of Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).

Interaction of treatment, sex and time on plasma glucose concentrations collected from calves during the preweaning period ($P = 0.02$) presented as least square means with 95% CI. Means did not separate after adjustment for multiple comparisons.

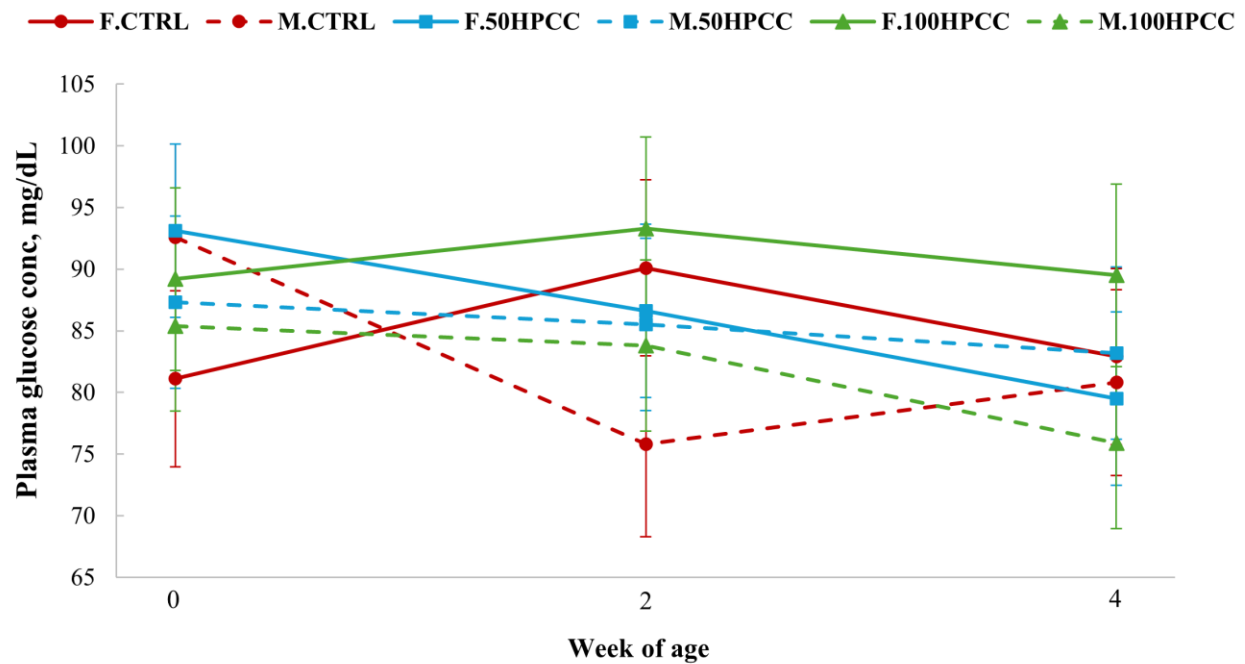
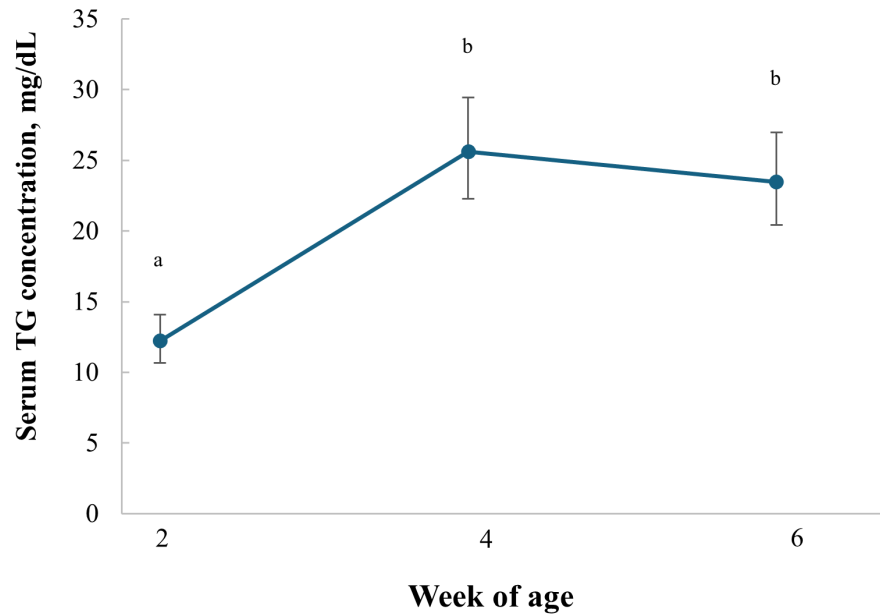


Figure 2.4. The effect of week on blood triglyceride and β -hydroxybutyrate concentrations of Holstein dairy calves during the preweaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).

Week effect on serum TG ($P < 0.01$; Panel A) and plasma BHB concentration ($P < 0.01$; Panel B) during the preweaning period presented as least square means with 95% CI. Points that do not share letters differ ($P \leq 0.05$).

A)



B)

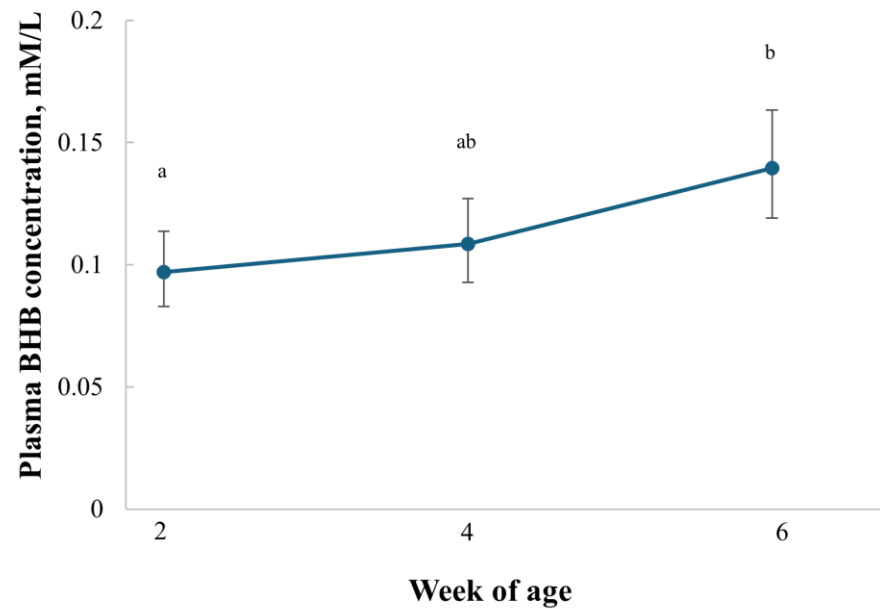


Figure 2.5. Starter grain intake as a percentage of bodyweight of Holstein dairy calves during the weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC).

Interaction of treatment and sex on starter grain intake as a percentage of bodyweight during the weaning period ($P < 0.05$) presented as least square means with 95% CI. Means did not separate after adjustment for multiple comparisons.

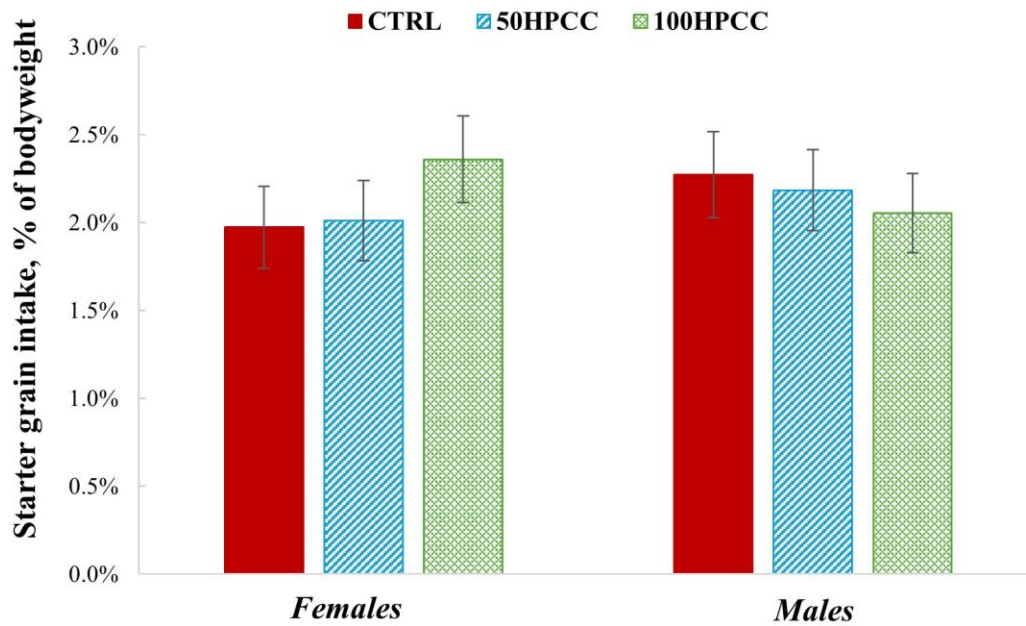


Figure 2.6. Feed efficiency of Holstein dairy calves during the weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC)

Interaction of treatment and sex on calf feed efficiency during the weaning period ($P < 0.02$) presented as least square means with 95% CI. Means did not separate after adjustment for multiple comparisons.

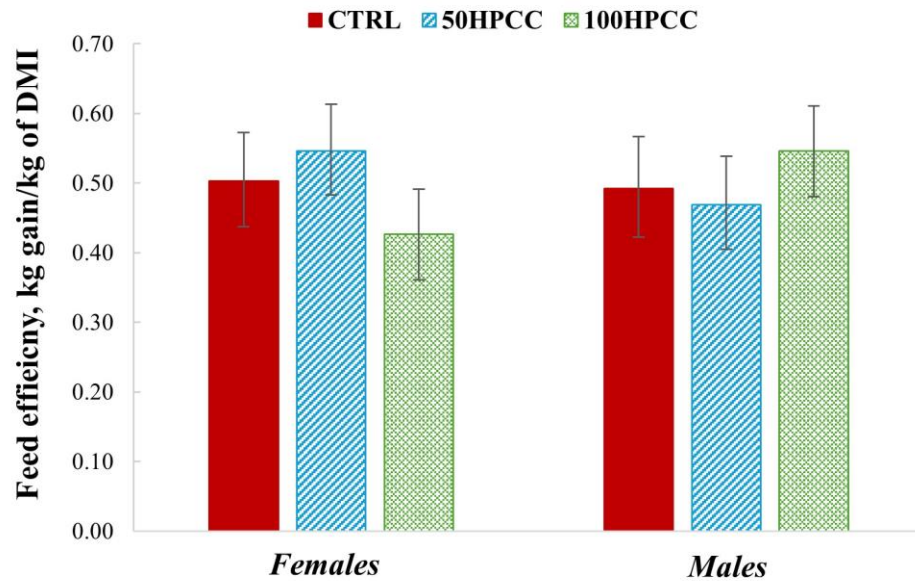


Figure 2.7. Daily starter grain intake of Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC)

Interaction of treatment, sex, and time on daily starter grain intake during the postweaning period ($P = 0.03$) presented as least square means with 95% CI. Means did not differ within week. A linear tendency of dose was detected ($P = 0.08$).

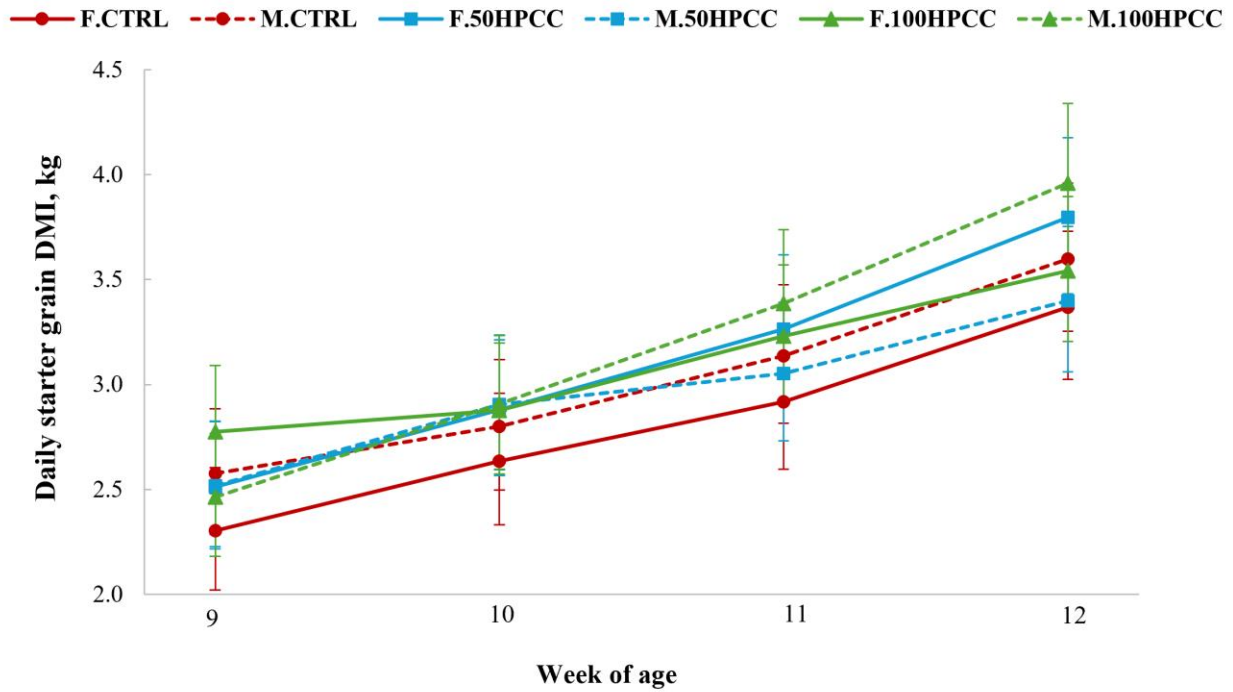


Figure 2.8. Starter grain intake as a percentage of bodyweight of Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC)

Interaction of treatment, sex, and time on starter intake as a percentage of bodyweight during the postweaning period ($P = 0.02$) presented as least square means with 95% CI. Means did not differ within week. A quadratic effect of dose was detected ($P < 0.01$).

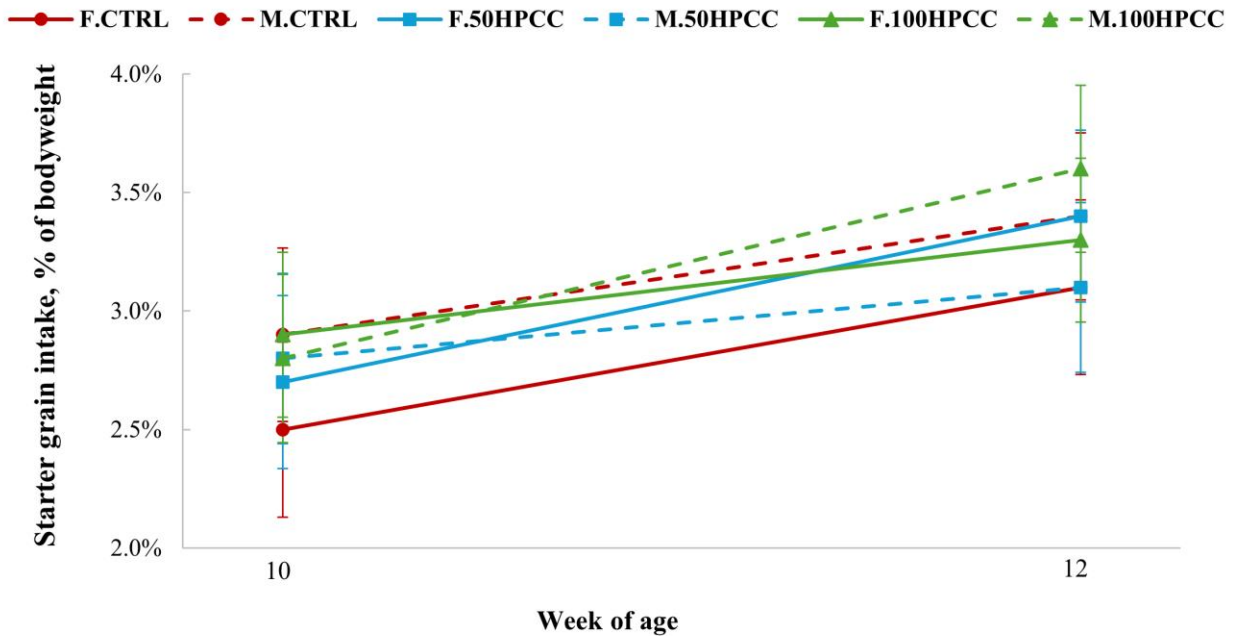


Figure 2.9. Feed efficiency of Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC)

Interaction of treatment and sex on feed efficiency during the postweaning period ($P < 0.04$) presented as least square means with 95% CI. Bars within sex without common superscript differ ($P \leq 0.05$).

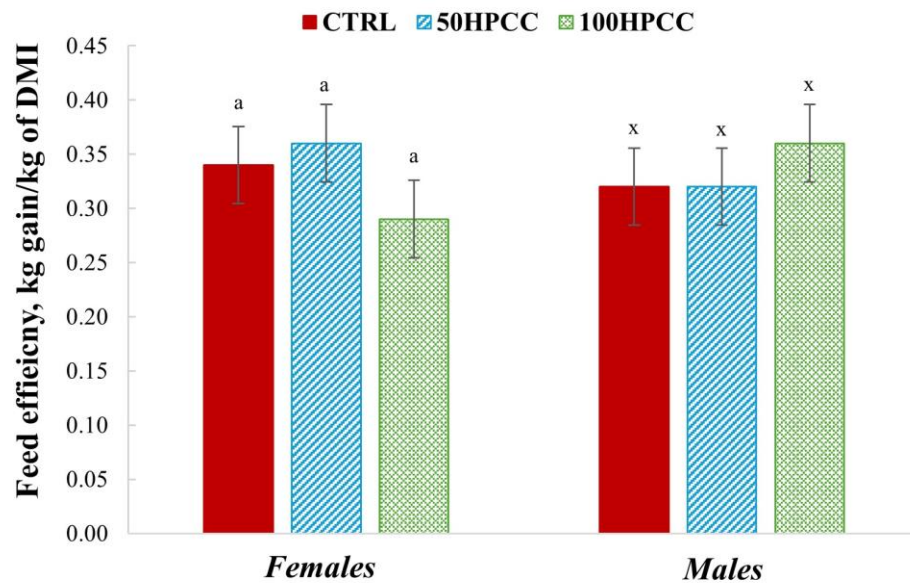


Figure 2.10. Blood glucose concentrations of Holstein dairy calves during the post weaning period fed starter grains with increasing replacement of soybean meal with a high-protein corn product (HPCC)

Interaction of treatment, sex, and time on plasma glucose concentration collected from calves during the postweaning period ($P = 0.03$) presented as least square means with 95% CI. Means did not separate within week.

