

ENSILING AND PROCESSING OF CORN SILAGE AND HIGH MOISTURE CORNS
AND LABORATORY METHOD COMPARISON OF STARCH DIGESTION IN
RUMINANTS

by

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CHAPTER I: Review of the Literature

Introduction

In lactating dairy cows, starch can comprise 20% - 40% of ration dry matter intake (DMI) depending on the level of milk production, stage of lactation, ration formulation strategies or models, and ingredient availability and prices. The interest in improving starch digestibility in ruminant diets has been stimulated by a recent rise in the price of high-starch cereal grains (Fredin et al., 2013). Corn and corn silages are an important dietary ingredient for lactating dairy cows, as well as many other ruminants, and starch supplied through these are a vital source of dietary energy. Ruminal and total tract starch digestibility can be highly variable across forages and grains (Orskov et al., 1986). Site of starch digestion alters the amount and nature of nutrients delivered to the animal and further affects ruminant metabolic efficiency. The rate at which starch is degraded in the rumen affects rumen fermentation and fiber digestibility in dairy cattle (Orskov et al., 1986).

Through extensive and (or) rapid rumen starch digestion, potentially due to greater degree of corn processing, extended silo fermentation or a flourier endosperm, negative associative effects on rumen fermentation can occur. Excessive rapidly degradable starch can result in greater fluctuation in production of a potent acid within the rumen and reduce rumen pH. Rumen acidosis (pH < 5.5) can decrease rumen fiber digestibility, milk fat, and (or) DMI, negating improvements in ruminal and total tract starch digestibility (Firkins et al., 2001). It has been found that propionic acid produced

from high starch diets can cause problems in milk production. If the quantity of propionic acid absorbed exceeds the capacity of the liver to remove it from the blood, it will stimulate insulin production. This will result in an increased uptake of nutrients by tissues and a reduction in lipolysis and cause a reduction in milk yield and milk fat (Orskov et al., 1986).

A high concentration of starch may alter ruminal fiber digestion by negatively affecting the ruminal pH. Grant and Mertens (1992) observed a negative effect of starch on fiber digestion in vitro when the pH was controlled pH of 6.2 and lowered to 5.8. A similar effect was observed in Mertens and Loften (1978) where cornstarch was added to fibrous forages and fiber digestion kinetics was determined in vitro. The addition of starch results in a linear increase in lag time of fiber digestion and a decrease of the extent of fiber digestion. Lopes et al. (2009) also fed corn differing in endosperm type. It was found that feeding a less vitreous corn to dairy cows increased starch digestion, but decreased fiber digestion.

Measuring nutrient digestibilities is challenging for both individual nutrients as well as total mixed rations. In commercial forage testing laboratories in vitro and in situ techniques are most often utilized, but cannot be related to dairy cattle performance. In vivo apparent total-tract nutrient digestibilities using markers is common among researchers, but is time and cost prohibitive on a commercial level (Schalla et al., 2012). Other methods of determining in vivo ruminal starch digestion include marker techniques, duodenal cannula sampling, omasal sampling, as well as rumen evacuation (Huhtanen et al., 2006).

Total tract starch digestibility can increase milk and milk protein yield and improve feed efficiency (Firkins et al., 2001). In dairy cows, total tract starch digestibility can range from 70% - 100% (Firkins et al., 2001; Ferraretto et al., 2013). Fredin et al. (2014) found that fecal starch concentration in lactating dairy cows is closely and linearly related to total-tract starch digestibility ($R^2 = 0.94$). The equation is as follows:

$$\text{Total-tract starch digestibility, \%} = 100\% - (1.25 \times \text{fecal starch\%})$$

Considerable variation in the site and extent of starch digestion remains to be quantified for predictive purposes in models and ration evaluation software used for lactating dairy cows diets. Therefore, accurately determining rumen starch digestion is essential. Variation in extent and site of starch digestion affect the quality and the quantity of the nutrients delivered to the animal.

Determining starch digestibility is important for proper ration carbohydrate formulation. Adequate energy must be supplied to support continued lactation performance gains, however, excessive starch loads must be avoided. Assays are capable of determining starch digestion across laboratories, while in agreement with in vivo results, and have become imperative to the field of ruminant nutrition. The objective of this literature review evaluate techniques that may be related to in vivo digestion.

1.0 Corn grain and whole-plant corn silage physical and chemical characteristic effects upon starch digestion

1.1 Kernel endosperm properties

Corn is comprised of three basic parts: pericarp, germ, and endosperm. The endosperm represents approximately 75%-80% of the corn grain (Huntington, 1997; Correa et al., 2002). The endosperm also contains hydrophobic proteins that encase the starch granules. These non-starchy, hydrophobic components limit starch availability to ruminal microorganisms for digestion (McAllister et al, 1993). Unlike floury endosperm, which contains microfissures or void spaces, starch granules in vitreous endosperm are enclosed in a continuous protein matrix (Philippeau and Michalet-Doreau, 1998). Kernel vitreousness is the ration of vitreous to floury endosperm (Lopes et al., 2009).

Endosperm development can be evaluated by the light box method (Felker and Paulis, 1993), manual dissection (Dombrink-Kurtzman and Bietz, 1993) or Stenvert kernel hardness (Pomeranz et al., 1985). The light box method technique provides a mean of estimating vitreosity of corn kernels by quantifying the brightness of the corn kernel images obtained by viewing kernels on a light box. It is subjective to visual classification of kernel vitreosity and not very reproducible. This method depends heavily on skill, experience, and visual acuity of the rater. Proportions of hard, vitreous kernels based on this measurement correlated well with visually determined proportions (Felker and Paulis, 1993). Dombrink-Kurtzman and Bietz (1993) described another method of manual dissection of the kernels to obtain horny and floury endosperm fractions for comparison. This method is more labor intensive, but does not rely of subjectivity like the light box method does. Stenvert Hardness Test as described in Pomeranz et al. (1985) utilizes grinding resistance, column height of

freshly ground grain, and the ration of coarse to fine particles to determine endosperm composition.

Philippeau et al. (1999) evaluated genetic variation by comparing flint and dent corns and found that flint corn has a greater proportion of vitreous endosperm than dent corn. The effective rumen starch degradability was higher for dent than for flint corns, averaging 61.9% and 46.2% of starch, respectively. These two corn types differed in vitreousness, averaging 51.4% and 71.8%, respectively.

Kernel vitreousness may be a useful parameter for which to select corn hybrids for high ruminal starch availability. Correa et al. (2002) found correlations between kernel vitreousness and ruminal starch availability (-0.93). With advancing maturity, kernel vitreousness increased while ruminal starch digestion decreased. To test maturity levels, corn was harvested at three different maturity levels: half milk line (HM), black layer (BL), and mature (MT) stages of maturity. Starch remained unchanged throughout the maturity levels (79.3%, 80.1%, and 79.9%, respectively) but vitreousness increased with maturity (42.8%, 46.1%, and 48.2%, respectively).

Philippeau et al., 1999 stated that ruminal corn grain starch degradability could be accurately predicted using grain vitreousness and rapidly measurable physical traits. Better understanding the relationship between kernel vitreousness and starch digestibility may improve corn hybrid selection for silage and grain production and possibly result in improved corn-based diet performance by ruminants (Correa et al., 2002).

1.2 Kernel particle size and processing

Chemical and physical treatments applied to corn grain and whole-plant silages are commonly meant to increase starch degradability. This can be accomplished through particle size reduction, gelatinization and the disruption of the protein matrix encapsulating the starch granules. These processing treatments increase digestibility by providing opportunity for bacterial attachment to starch granules (Huntington, 1997). The starch granules in corn that are encapsulated by prolamin-starch matrix and have been recognized to be negatively related to starch degradability (Philippeau et al., 2000). These physical treatments increase the starch availability to both rumen microbes and digestive enzymes or work to solubilize the starch-protein matrix. Gelatinization can lead to chemical and physical changes in the starch granule. Disrupting prolamin hydrogen bonds and absorbing water facilitate microbial or enzyme attachment and degradation of the starch granule (Offner et al., 2003).

Ruminal and total tract starch digestibilities are inversely related to particle size for dry corns (Remond et al., 2004; Theurer et al., 1999; and Dhiman et al., 2002). Remond et al. (2004) observed a 23% increase in ruminal starch digestion when microns size was 3668 microns compared to 730 microns (35.5% vs. 58.6%). Similarly, Dhiman et al. (2002) saw an increase of total tract starch digestibility from fine ground corn (1130 microns; 93.6%) to course ground corn (1650 microns; 96.1%).

Dhiman et al. (2002) compared not only coarse ground corn (MPS) and fine ground corn (MPS), but included steam flaked corn (flaked density) as well and observed a total tract starch digestibility of 99.1%. In the process of steam flaking grains, grain is steamed for 30 to 60 minutes in a vertical, stainless steel chamber to increase moisture to 18%-20%, then flaked between preheated large rollers (Theurer et al., 1999). This is followed by grain compression of the starch between rollers to rupture the starch granules and damage the protein matrix; it is important to hydrate the starch with moist heat to gelatinize the starch granules.

Cows fed steam flaked corn excreted 410 grams per day less starch than coarse ground corn and 245 grams per day less starch than fine ground corn. These results suggest that corn grain steam flaking reduced starch excretion in the feces compared with coarse ground corn or fine ground corn. Theurer et al. (1999) observed similar results seeing a 19% increase in total tract starch digestibility between dry rolled corn and steam flaked corn.

1.3 Ensiling

The fermentation process in the ensiling high moisture corn grains and silages result in a partial solubilization of the hydrophobic starch-protein matrix in the corn endosperm (Baron et al., 1986, Philippeau et al., 1997). Moreover, the hydrophobic starch-protein matrix encapsulating the starch granules breaks down during ensiling (Philippeau et al., 1999). Increased ruminal starch degradability after ensiling is likely a result of degraded endosperm proteins (Philippeau et al., 1997).

Greater ruminal and total-tract starch digestibility is well established in dairy cows fed high-moisture corn compared with dry corn (Firkins et al., 2001). Ensiling high moisture corn grain increased the mean in vivo ruminal dry matter and starch degradability by 5.6% and 5.9%, respectively, (Philippeau et al., 1997). Ferraretto et al. (2014) assessed ensiling impact by surveying results over time. The authors used the month of the samples submission as an indicator of length of ensiling period. The data set included samples submitted from March 2011 to May 2013. An observation of 9% unit in vitro starch digestion increase from October to August of the following year. An increase of ammonia-N and soluble crude protein concentrations was also observed (155% and 49%, respectively). Positive relationships between in vitro starch digestion and ammonia-N ($R^2 = 0.61$) and soluble crude protein ($R^2 = 0.55$) were detected (Ferraretto et al., 2014). Hoffman et al. (2011) also observed an increase in ammonia-N and soluble crude protein, appearing to be a function of the extent of fermentation.

Hoffman et al. (2011) confirmed the prolamin proteolysis, finding that the reduced zein protein subunits that cross-link the starch granules were degraded by proteolytic activity over a 240 d extended ensiling period through electron micrographs.

2.0 Analytical measures that may be related to in vivo rumen starch

2.1 Prolamin

Corn prolamin proteins, often referred to as zein, are essentially insoluble in the rumen due to their hydrophobic nature (Philippeau et al., 2000). A modified

turbidimetric zein method (Larson and Hoffman, 2008) may be utilized to determine zein content in corn grain based on previous methods that divide corn endosperm proteins into multiple protein fractions (Landry et al., 2000).

Larson and Hoffman (2008) defined the rapid turbidimetric (rTM) laboratory procedure to quantify prolamin proteins in dry corns. This assay was defined as dried ground corn that is defatted using acetone, filtered, and dried and acetone-insoluble dry matter is retained. There was a close agreement ($R^2 = 0.88$) between alcohol soluble protein of acetone insoluble dry matter (Landry et al., 2000) and zein contents determined in Larson and Hoffman (2008). In determining prolamin proteins (zein) the rTM method was found to be reasonable if distinguishing variable endosperm types in dry corns. This assay was moderately precise, but Larson et al., 2008 concluded that improvements to this assay to increase the accuracy should be pursued.

Nellis et al. (2013) evaluated this procedure for high moisture corns as well. Modifications were made to improve biological function in determining prolamin: defatting the sample with acetone was replaced with a borate phosphate buffer extraction to remove buffer-soluble proteins, *tert*-butanol was used to more efficiently extract the prolamins, and since the use of *tert*-butanol without the defatting using acetone would influence turbidity measurements, turbidity was abandoned and replaced with a Bradford protein assay (rBM) (Bradford, 1974). Dry corns mean prolamin concentrations estimated by rTM and rBM were similar (3.65% vs. 3.66%, respectively). Prolamin concentrations estimated by rTM yielded a higher mean than rBM in high moisture corns (4.19% vs. 3.24%, respectively) suggesting that the rTM

method previously described by Larson and Hoffman (2008) may over estimate prolamins in fermented feeds such as high moisture corn. (Nellis et al., 2008). Both dry corn and high moisture corn were negatively related to peak absolute rates of in vitro gas production (Nellis et al., 2008; Hoffman et al., 2012).

2.2 Ammonia-N and Soluble-CP

Ammonia-N has been suggested in combination with mean particle size (MPS), dry matter content, and length of silage fermentation time in determining ruminal starch digestion (Hoffman et al., 2012). Soluble crude protein (CP) is analyzed by the borate-phosphate buffer extraction as described in Krishnamoorthy et al. (1983). Borate-phosphate buffer had a correlation coefficient of 0.92 with insoluble nitrogen obtained with autoclaved rumen fluid (Krishnamoorthy et al., 1983). Hoffman et al., (2011) observed an increase of soluble CP in high moisture corns from day 0 (1.5% - 2%) to day 240 (>4%) of fermentation. Observations of higher ammonia-N results from day 0 to day 240 were reported as well. Ferraretto et al., 2014 observed positive relationships between in vitro starch digestibility and ammonia-N ($R^2 = 0.61$; $P = 0.001$) and soluble CP ($R^2 = 0.55$; $P = 0.001$) that may suggest that ammonia-N or soluble CP are an indicator of starch digestion in HMC. Ammonia-N and soluble CP may be valuable measurements that will be useful in predicting animal responses once validated again in vivo measurements.

2.3 Particle Size

Assessing dry or HM corn particle size is typically done using MPS (Hoffman et al., 2012). The American Society of Agricultural Engineers (ASAE) defined procedure is based on a log-normal distribution of the ground particles (S319.3). Within this technique a complete sieve analysis is used to determine particle size and distribution in feed materials, including dry and high moisture corns. The ASAE method uses 14 test sieves and vigorously sifts feed material through them. The fraction of each sieve is weighed and used to compute a geometric mean particle size. The MPS refers to the mid point of the distribution (mean), where 50% of the material is coarser by weight, and 50% of the material by weight is finer. The standard sieve sizes by nominal opening are 4.76, 3.36, 2.38, 1.68, 1.191, 0.841, 0.594, 0.420, 0.297, 0.212, 0.150, 0.103, 0.073, and 0.053 millimeters.

While gross differences can be evident, MPS is challenging to use within a feed or ration evaluation system because starch digestibility is significantly different for dry corns compared to high moisture corns. Meaning the same MPS corns may in fact have different digestibility values. Therefore an index system based on MPS and nutritional chemistries was developed to better predict the fermentation potential of corns termed effective mean particle size (eMPS) (Hoffman et al., 2012). Adjustments are made to the MPS based upon additional nutritive measures to better reflect the grain energy potential to develop eMPS.

To calculate eMPS the following equation is used: $eMPS = MPS - MPS \times rrMPS_{dry}$ or $eMPS = MPS - MPS \times rrMPS_{HM}$ for dry and high moisture corns, respectively, where

$rrMPS_{dry} = 0.58 - 0.15 \times (\text{prolamin protein})$ and $rrMPS_{HM} = 0.21 + 0.08 \times (\text{NH}_3\text{-N, \% of N})$. Starch digestion variation between corns was better explained with eMPS than using only MPS ($R^2 = 0.84$ vs $R^2 = 0.50$, respectively) (Hoffman et al., 2012).

2.4 Kernel Processing Score

Determining the available energy in whole plant corn silages can be difficult due to varying physical form of the grain and stover. Each part can differ in chemical composition presenting unique challenges (Ferreira and Mertens, 2005). There has been an increased interest in kernel processing of whole plant corn silages using harvesters equipped with onboard processing rolls (Cooke and Bernard, 2004). Cooke and Bernard (2004) observed greater apparent digestibility of starch for diets contain 2mm roller opening vs. 8mm roller opening processed silage (85.4% vs. 75.6%).

Ferreira and Mertens (2005) developed a system using sieves with apertures $>4.75\text{mm}$ that separate intact and large kernel fragments. These portions are used to measure minimally fragmented starch ($\text{Starch}_{>4.75}$). The concentration of minimally fragmented starch was found to be highly variable ($CV = 50\%$). The proportion of minimally fragmented starch is related to the in vitro disappearance of whole silages ($R^2 = 0.67$). This suggests that $\text{Starch}_{>4.75}$ may provide a classification as processed or unprocessed to describe the impact on starch digestibility.

The percentage of starch minimally fragments was calculated by dividing $\text{Starch}_{>4.75}$ by total starch in silage DM ($\text{Starch}_{>4.75}/\text{Total}$). $\text{Starch}_{>4.75}/\text{Total}$ was positively correlated with mean particle size ($r = 0.46$). They proposed that the inverse of

Starch_{>4.75/Total} be used as a corn silage fragmentation index (CSFI) (Ferreira and Mertens, 2005):

$$\text{CSFI} = 100 - \text{Starch}_{>4.75/\text{Total}}$$

The proportion of minimally fragmented starch provides an index for whole plant corn silage this is related to in vitro digestion of whole silages. When it is validated by in vivo trials it may be a useful substitute for processing adjustment factor (Ferreira and Mertens, 2005).

2.5 Degree of Starch Access

A laboratory method for degree of starch gelatinization was modified for the evaluation of starch recovery in corn grain and silages. The modifications included using an undried and unground sample to account for the differences in particle size, dry matter content, and endosperm type. The sample amount was increased to ensure a representative sample of corn grains and corn silages that have not been dried or ground. This assay is used to evaluate starch recovery by enzymatic hydrolysis. The amended assay was renamed the degree of starch access (DSA) to describe the degree of enzyme access to starch after gelatinization of undried and unground feeds (Blasel et al., 2006).

DSA is described as

$$\text{DSA (g/kg starch)} = \frac{[\text{recovered starch : undried/unground}]}{\text{Total starch}} \times 1000$$

Total starch

With corn grains, particle size was related to DSA (Blasel et al., 2006). The DSA was reduced 2.7% for each increase of 100 μm in particle size. Dry matter content of corn grains was also related DSA. For each 1.0% increase in dry matter content the DSA decreased 2.0% (Blasel et al., 2006).

To test the effects related to kernel vitreousness, samples were ground through an 8-mm screen. Even when samples were all ground through the same size screen, floury samples were, on average, 200 μm less in MPS than flint or highly vitreous corn grain samples. This is due to samples with greater vitreous endosperm having a higher kernel density, which could influence post-grinding particle size. The DSA assay accounted for about 60% of the variation of endosperm type, and suggested that ruminal starch degradation is higher in corn grain samples with a low vitreous endosperm.

Particle size, dry matter, and vitreousness have been related to starch digestion. In describing DSA relationships with each of these parameters, one can hypothesize that DSA may be useful to measure the potential of starch digestion in corn grain samples. However DSA has not yet been directly related to starch digestion.

Whole plant silage, however, is more difficult to analyze due to its heterogeneous nature of the stalk, cob and leaves. The starch fraction cannot be readily separated from the forage portion. In corn silages, both the dry matter and starch content were negatively related to the DSA. The MPS of corn silage was negatively correlated to the DSA assay ($P < 0.003$, $r = -0.67$). This implies that as the particle size of starch decreases the DSA increases.

The standard deviation for total starch averaged 1.8%, the recovered starch and DSA were less precise averaging 2.0% and 3.8%, respectively. Poor precision for the DSA is expected because of the heterogeneous nature of undried and unground samples (Blasel et al., 2006).

The DSA assay is relatively simple to perform in a laboratory setting and may be related to starch digestion in corn grains and corn silages, however limitations include poor repeatability and the assay still requires evaluations to relate it to in vivo starch digestion to acquire the full potential of the measure.

2.6 In vitro rumen starch digestibility

Rumen in vitro digestion techniques are meant to simulate in vivo digestion. Starch degradation is commonly measured in vitro by directly measuring starch disappearance after incubation for various time intervals (Menke et al., 1979). The premise to this technique is to assess starch level both before and after simulated rumen digestion (Richards et al., 1995). Determination of starch residues requires a complete hydrolysis of starch to glucose, but only starch degraded when it is completely degraded into glucose is considered (Huhtanen et al., 2006).

The intention of in vitro systems is to evaluate the intrinsic characteristics of the feed, a substrate-limited system should be created, with optimal conditions for ruminal microbial activity regarding pH, anaerobiosis, microbial numbers, and essential nutrients (Huhtanen et al., 2006). Other factors affecting in vitro starch digestion measures include: source of rumen fluid (animal), donor animal diet, ratio of rumen fluid to

artificial saliva, and grinder type. In vitro starch digestion results should be compared within an in vitro run because of variable rumen fluid nature from run to run; sample rankings can be compared within or between runs (Richards et al. 1995). The in vitro system estimated degradability are on average about one third lower than in situ values, and are also lower than what would be expected in vivo (Offner and Sauvant, 2004).

2.7 In vitro rumen gas production

Gas production techniques are similar to in vitro rumen starch digestion except that gas production is continuously measured over time, starch level is not determined post digestion and gas pressure is assumed related to carbohydrate metabolization. Cumulative in vitro gas production pressures of all samples are recorded, with adjustment for the gas production of the blank sample (Hoffman et al., 2012). Gas production pressure readings are converted to gas volume and expressed as mL/0.2 grams of dry matter. This results in an expression of gas production volumes between 0 and 100 mL (Parissi et al., 2005). Gas production based techniques can yield hundreds or thousands of data points and have allowed researchers to develop elaborate digestion kinetic models for different feedstuff nutritive components.

Digestion information provides relevant nutritional values for feedstuffs, the response by the animal, as well as some impacts to the environment (Krishnamoorthy et al., 2005). Tahir et al. (2013) predicted ruminal starch digestion using gas production data closely matching in vivo data ($R^2 = 0.81$) in starch-rich feedstuffs.

2.8 In situ rumen starch digestibility

Determining rumen starch digestion by the in situ technique may be a better approach to simulating the rumen environment due to exposing feedstuff to living rumen, without a given feeding regimen (Huhuanen et al., 2006). Under this approach, the feed is directly suspended into the rumen of a cow that is fit with a rumen fistula. This allows immediate contact of the test feed with the rumen environment. The test feed though, is not subject to the total ruminal experience including mastication, rumination and passage (Nocek et al., 1988).

Samples are weighed into a nylon bag (Ankom Co, Fairport, NY; pore size: 53 μ m; internal dimensions: 5x10 cm) and introduced in to the rumen for various times, with no less than 3 replications per sample (Offner et al., 2003). Bags are then removed after incubation periods and rinsed in cold water, frozen, and washed until water is clear. Sample bags are dried for 48 hours in an 80 degrees Celsius oven and weighed. Starch content is determined on the incubation residues (Philippeau et al., 1997, Philippeau et al., 1999, and Remond et al., 2004).

Although the in situ technique directly uses the rumen environment, this technique may have limitations. Offner et al. (2003) confirmed there are large differences due to intrinsic properties of the feed. There is a strong influence of processing, especially on feedstuffs containing slowly degrading starch. Using a porous bag to contain the feed has been debated. Bag porosity is a compromise between limiting influx of rumen contents not associated with the test feed and allowing influx of

microbial populations to degrade the test feed, while at the same time limiting the efflux of undegradable feed particles.

Feed particles or starch may be solubilized and lost from the bag prior to ruminal incubation. Feedstuff particles lost through pores but not due to rumen digestion can be considered soluble. The actual digestibility of the soluble fraction is not quantifiable through the in situ technique but is often assumed to be rapidly and completely degraded within the rumen (NRC, 2001). However others have proposed that the soluble or washout fraction is not completely degraded and in fact may flow quickly through the rumen with liquid phase material. The loss of fine particles through the nylon bag under this theory would then correspond to overestimating the starch degradability (Michalet-Doreau and Cerneau, 1991, Dewhurst et al., 1995). As a result of debate, the in situ rumen technique may not be ideal for finely ground.

Researchers have also opted to use a pre-incubation wash to quantify and remove this questionable fraction, as well as to pre-wet the sample to mimic salivation (Nocek et al., 1988). The proportion of starch and DM that passes through the pores of the bag without being degraded can be determined by weighing sample into nylon bags, immersed in 250 ml of a buffer solution at pH 6.9 and agitated for 2 hours in a 39 degrees Celsius water bath. After removal, the bags were rapidly washed with distilled water. Lost particles were recovered from the solution by filtration. The filters were dried at 80 degrees Celsius for 48 hours and weighed, and the starch content was determined (Philippeau et al., 1999).

Nylon bags can have confining conditions that could lead to the microbial population inside the bag differing from that of the surrounding rumen environment. This could lead to quantity and activity differences of the microorganisms, which may lead to some negative digestive interactions (Nocek et al., 1988, Michalet-Doureu and Ould-Bah, 1992, Sauvant et al., 1994). Microbial matter that includes starch may remain in the bag residue. Typically contamination of microbial content in starch digestion is ignored (Michalet-Doreau and Cerneau, 1991) or assumed completely removed by vigorous cold water rinsing (Nocek., et al 1988).

2.9 In vivo rumen starch digestion

To determine ruminal starch digestion by in vivo techniques separation of digestion between the different stomach compartments of the digestive tract are needed (Huhuanen et al., 2006). This is accomplished by the collection of digesta samples through a duodenal cannula (Harmond and Richards, 1997), sampling through the omasal (Huhtanen et al., 1997) or the use of digestibility markers (Owens and Hanson, 1992).

The duodenal cannula assume that there is a representative sample within each digesta phase, which is often criticized because they allow separation of fluid and particles relative to true digestia (Huhtanen et al., 2006). Omasal sampling utilizes a device inserted into the omasum via the ruminal cannula and tubing that connects the device to the ruminal cannula. This procedure requires less surgical intervention than the traditional methods using duodenal cannulas as sampling sites to study fore

stomach digestion and avoids potentially confounding endogenous secretions of the abomasum (Huhtanen, et al., 1997). Although this method has some advantages, it is more laborious compared with duodenal sampling and it has not been widely tested for the determination of ruminal starch digestibility (Huhtanen et al., 2006). A marker is not always suitable as well; possible problems that can occur can include marker migration, phase separation, inhibition of digestion and osmotic effects within the gut (Owens and Hanson, 1992).

Conclusions

Starch digestion in ruminants is an ever-expanding research area. Several approaches to forecast in vivo rumen starch digestion have been described here. There are numerous treatments available that can be applied to corn grains and whole plant corn silages to improve starch degradation and utilization in the rumen, however, accurately assessing starch potential remains challenging.

There has been considerable research evaluating starch digestion techniques, however, the industry continues searching for a practical, accurate and precise assay that is applicable across feed types and laboratories. The aim of this research is to evaluate effects of starch digestibility on dairy farms as well as gauge similarities between two common starch digestion assays in commercial laboratories, the in vitro and in situ method.

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CHAPTER II: Survey of Starch Digestibility on Wisconsin Dairy Farms across winter months

Abstract

A field survey was conducted on 30 commercial Wisconsin dairy farms to estimate variations in starch digestion from harvest (November 2011) through the winter (April 2012). Commercial dairy farms that participated milked 223 +/- 206 cows with bulk tank averages of 32.7 +/- 5.5 kg/day per cow. Whole plant corn silage (WPCS), high moisture corn (HMC) and dry corn (DC) that would be fed in the fall into the spring were collected from each farm. Each sample was analyzed for dry matter content, starch content, particle size and 7-hour ruminal in vitro starch digestibility (IVSD; % of starch). A composite manure sample was also collected from each farm by combining fecal grab samples from 10 cows within the herd between 45-120 days in milk (DIM). Each sample was analyzed for starch content, which was then used to calculate total tract starch digestibility (TTSD). For WPCS the dry matter content (DM; 35.0% +/- 4.5 vs. 36.2% +/- 5.1), starch content (34.7% +/- 4.8 vs. 34.1% +/- 4.8), and kernel processing score (KPS; 57% +/- 11.1 vs. 61.1% +/- 12.4) were similar for fall (Nov. 2011) and spring (Apr. 2012) sampling periods, respectively. For WPCS the IVSD was 6.6% units greater for spring than fall collected samples (90.3% +/- 3.7 vs. 83.7% +/- 7.5). For HMC the DM content (72.0% +/- 7.2 vs. 74.8% +/- 5.9), mean particle size (MPS; 1725 μ +/- 562 vs. 1548 μ +/- 626), and IVSD (75.7% +/- 8.2 vs. 74.5% +/- 7.2) were similar for fall (Nov. 2011) and spring (Apr. 2012) sampling periods, respectively.

The fecal starch content (3.3% +/- 3.0 vs. 4.1% +/- 4.0) and calculated TTSD (95.9% +/- 3.7 vs. 94.9% +/- 5.0) were similar for the fall and spring sampling periods, respectively. Results suggest that improved control of WPCS and HMC harvest and processing practices and longer WPCS ensiling times would increase starch digestibility in dairy cows.

Introduction

The predominant forage used by the dairy industry today in the United States is WPCS (Johnson et al., 1999). Corn that is harvested for silage differs from other forage sources because of the presence of grain, which represents about 45% of the whole-plant DM (Philippeau & Michalet-Doreau et al., 1998). Dairy producers often are forced to feed forages, including WPCS that have only been ensiled for a few days or weeks to due low inventory (Young et al., 2012). Approximately 50% of the WPCS energy value is derived from starch. By being able to improve WPCS starch digestibility (starchD) it could improve lactation performance in dairy cattle.

Starch digestibility can be influenced by WPCS harvest maturity, kernel processing (Johnson et al., 1999) and length of ensiling (Young et al., 2012). Ferraretto et al., (2012) reported that DM content of WPCS increases with advancing maturity. Ferreira and Mertens (2005) defined kernel processing based on the amount of material that passes through the 4.75 mm sieve resulting in a kernel processing score (KPS). Scores greater than 70%, 50-69%, and less than 50% are considered to be optimal, adequate, and poor kernel processing, respectively. Young et al., (2012) found an increased starchD as time of ensiling increased in a controlled lab setting from fresh, 45-day, and 150-day of 66.3%, 75.2%, and 80.7%, respectively.

Corns are typically processed before feeding to enhance their nutritional value for lactating dairy cows (Hoffman et al., 2012). The extent of processing for corns is typically measure by mean particle size (MPS). Starch digestibility can be improved by lowering the MPS and in HMC ensiling time. Hoffman et al (2011) reported that ensiling

HMC for 240-days reduced the zein-protein subunits that cross-link starch granules and suggested that the starch-protein matrix was degraded over the long ensiling period.

Similar results were reported in Ferraretto et al (2014) where a 9% unit increase of IVSD was observed in samples submitted in October of 2011 versus August 2012.

The objective of this survey was to better understand the effects of ensiling WPCS and corns over an extended ensiling period has on IVSD would allow dairy farmers and nutritionist to optimize diets.

Materials and Methods

Farms included for this survey needed to plan to feed 2011 WPCS and corns in the fall and that would still be feeding the same WPCS and corns in the spring. Farms were invited to participate in the survey by their local county-based agriculture agent. There were thirty farms that participated that were located in the following Wisconsin counties: Buffalo, Calumet, Chippewa, Clark, Fond du Lac, Jackson, Monroe, Oconto, Outagamie, Price, Shawano, Sheboygan, and Waupaca. Farms in the study milked on average 223 cows, herd size ranged from 38 to 1,000 milking cows. Bulk tank milk yield average was 32.7 kg per cow and ranged from 20.4 – 42.2 kg per cow per day.

Samples of WPCS, HMC, and DC were collected in November 2011 and April 2012 to determine for DM content, starch content, particle size and 7 hour IVSD. A fecal sample was also collected from each farm through a recal grab done by a county agent. The fecal sample was a composite of fecal grab samples of ten cows that were

between 45 and 120 days in milk (DIM). The fecal was analyzed for starch content and estimated for TTSD.

Samples were dried to a constant weight at 105°C to determine DM content. Starch content on the samples was determined and corrected for free glucose according to the procedures described by Hall (2009), with modifications for samples to be analyzed on a YSI Biochemistry Analyzer (YSI Inc, Yellow Springs, OH). Fecal starch was analyzed on the manure samples and used to determine TTSD using the following equation: $TTSD\% = (100 * (0.9997 - 0.0125 * \text{fecal starch, \% of DM}))$; $R^2 = 0.94$ (Ferraretto, L. and R. D. Shaver., et al 2012).

Kernel Processing Score (KPS) was determined by drying approximately 150 grams of sample in a 50 degree Celsius oven for 24 hours. Samples were sieved through 8 screens (19, 13.2, 9.5, 6.7, 4.75, 2.36, 1.18, 0.50 mm) by aggressively shaking on a vertical shaker for ten minutes. The amount of material that passed through the 4.75 mm sieve was analyzed for the starch content, this in turn becomes the processing score based on Ferreira and Mertens (2005) found that the percentage of starch greater than 4.75 mm (minimally fragmented) was positively correlated to mean particle size.

The particle size of HMC and DC was determined using the American Society of Agricultural Engineers (ASAE) defined procedure based on a lognormal distribution of the ground particles. This technique utilizes a complete sieve analysis to determine particle size and distribution in the corns. The 7 sieves used for this trial had nominal openings of 2, 1, 0.850, 0.500, 0.250, 0.150, 0.100 millimeters.

Samples were dried in a 50° Celsius forced air oven for 24 hours. Dried samples are poured into the tower of sieves and allowed to sift through the sieves by vigorously shaking the sieves for ten minutes on a vertical shaker. The fraction of each sieve was weighed and used to compute the MPS, which refers to the mid point where 50% of the grain is coarser by weight and 50% of the grain is finer by weight.

Ruminal 7-hr IVSD was analyzed according to Richards (et al., 1995), with modified rumen fluid collection according to the Goeser and Combs (2009) technique.

Data evaluating the effects of ensiling over winter months was analyzed using SAS JMP version 11.0. This was a completely randomized experimental design, with season of sample submission as a fixed effect. Sample means were first regressed against model parameters using backwards elimination through JMP mixed modeling. Parameters previously outlined as well as two-way interactions were accessed within the model. The final model for IVSD was:

$$Y_{ab} = \mu + T_a + S_b + TS_{ab} + e_{ab}$$

Where Y_{ab} = IVSD, response variable, μ = population mean, T_a = class effect of sample type, S_b = fixed effect of season of sample submission, TS_{ab} = type and season interaction, and e_{ab} = random residual error, assumed to be normally distributed.

The final model for starch (%DM), DM%, and MPS was:

$$Y_{ab} = \mu + T_a + e_a$$

Where Y_{ab} = starch (%DM), DM%, or MPS, response variable, μ = population mean, T_a = class effect of sample type, and e_a = random residual error, assumed to be normally distributed.

The final model for KPS and TTSD was:

$$Y_{ab} = \mu + S_b + e_b$$

Where Y_{ab} = KPS or TTSD, response variable, μ = population mean, S_b = class effect of sample type, and e_b = random residual error, assumed to be normally distributed.

Results and Discussion

Descriptive statistics for WPCS, HMC, DC, and fecal samples by season of sample submission are in Tables 1 (Fall) and 2 (Spring). Dry matter content in WPCS ranged approximately 25% units for the total survey. This suggests there could be more opportunity to control the maturity at harvest. In this survey there were about 20% of the farms with WPCS with greater than 40% DM (Figure 1). Ferraretto and Shaver (2012) found that WPCS with greater than 40% DM content could reduce digestibility. The DM content means for DC, HMC, and CS are in Table 3; there was no statistical difference between season of sample submission.

The KPS score, determined by Ferreira and Mertens (2005), reports that the degrees of kernel damage in WPCS (percent of starch passing through 4.75 mm screen) is related to in vitro starch digestibility. There was a slight linear increase in IVSD by KPS, but no statistical relationship ($p = 0.395$) found in this study (Figure 2). Measurements of KPS for fall and spring samples are described in Table 4. Although on average the WPCS were adequately processed, approximately 25% of the samples were below 50% KPS falling into the poor quality (Figure 3).

On average, spring WPCS samples were approximately 7% units greater compared to the fall samples for IVSD (Table 5), though this was not a completely controlled study, as not the exact same WPCS were analyzed after short and long ensiling periods, these results do suggest that the starch digestibility of WPCS increased over an extended ensiling period (Figure 4). These observations are in agreement with truly controlled experiments done by Young (et al., 2012) that evaluated length of silo fermentation effects of starch digestibility in WPCS.

Results of IVSD between season for HMC are in conflict with the hypothesis that starch digestibility increasing with lengthened ensiling periods. It also did not agree with previous research (Hoffman et al., 2011) with controlled research trials.

Unlike WPCS, in HMC the IVSD was not statistically different between fall and spring samples (Table 5). In this trial cold ambient temperatures at harvest and throughout storage may slow the silo fermentation for HMC. The fermentation may not speed up until the ambient temperature rises throughout the end of spring to early summer. With sample collections of November and April this may have not been a long enough time period to see a significant increase in starch digestibility.

Another factor could have been the samples DM. On average the samples were 74% dry in the fall and spring collected samples (Table 3). Approximately 45% of the samples were above 74% DM (Figure 5). A high DM content can limit the extent of fermentation.

The HMC samples collected averaged approximately 1635 microns (Table 6), but about 35% of the samples had a MPS of greater than 2000 microns (Figure 6). Starch

digestibility is found to be inversely related with MPS (Hoffman et al., 2012). With greater moisture contents, warmer ambient temperatures at harvest and sampling later than April, we may have been able to observe an increase in starch digestibility with a lengthened ensiling period (Figure 7).

There were very few DC samples submitted for this trial. The samples were on average finely ground (550 microns, Table 6) which lead to an average of 71.73% and 74.30% IVSD for spring and fall samples (Table 5).

The average starch content for the fecal samples was 3.68% (DM basis) for fall and spring samples (Table 7). The TTSD resulted in an average of 94.8% and 95.9% for fall and spring samples (Table 8), with a maximum of 99% for fall and spring (Figure 8). Higher fecal starch contents from 15% to 20% resulted in the lower TTSD samples shown in Figure 8.

Differences in the WPCS IVSD that were observed between the sampling periods were likely not great enough to be able to detect a difference in TTSD. Post-ruminal digestion, DIM, and differences in rations may be factors in the lack of difference in fecal starch and TTSD between the fall and spring sampling periods.

Conclusion

With fermented feeds, especially WPCS, our data suggests that a longer storage time can make a significant difference in starch digestibility. It would be beneficial for farms to ensile feeds for a longer period of time before feeding out. In many cases this is not possible due to the inventory available, but there are other ways to improve

starch digestibility. Such as harvesting WPCS and HMC at lower DM contents to enhance fermentation and greater kernel processing at harvest. Fecal sampling is an easy and inexpensive approach to gauge starch digestibility on farm, and make any necessary adjustments to harvesting and processing in the fall and (or) ration formulation throughout the year.

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Table 1. Survey data of WPCS, HMC, DC and fecal samples from commercial dairies submitted in the fall season

Type	Analyte	Average	n	Stdev	Min	Max
WPCS	Starch % DM	34.7	30	5.4	16.0	44.1
HMSC	Starch % DM	72.1	19	6.2	56.9	81.8
DC	Starch % DM	74.9	6	6.4	70.6	89.8
Fecal	Starch % DM	3.3	29	3.0	0.4	15.2
WPCS	IVSD (7hr)	83.7	29	7.4	58.1	93.9
HMSC	IVSD (7hr)	75.7	19	8.2	65.4	89.6
DC	IVSD (7hr)	73.5	6	4.1	70.0	81.5
WPCS	DM %	35.0	31	5.7	28.7	55.1
HMSC	DM %	72.0	20	7.3	51.4	82.4
DC	DM %	84.1	6	3.7	76.3	86.2
WPCS	KPS, %	57.0	29	11.0	34.9	74.3
HMSC	MPS, microns	1725.0	20	585.7	780.0	2710.0
DC	MPS, microns	550.0	5	66.5	461.0	619.0
Fecal	TTSD, %	95.9	29	3.71	80.9	99.5

Type: WPCS = Whole plant corn silage; HMC = High moisture corn; DC = Dry corn

Analytic: IVSD = In vitro starch digestion, %; DM = Dry matter, %; KPS = Kernel processing score, % starch >4.75mm; TTSD = Total tract starch digestion, %

Table 2. Survey data of WPCS, HMC, DC and fecal samples from commercial dairies submitted in the spring season

Type	Analyte	Average	n	Stdev	Min	Max
WPCS	Starch % DM	34.1	35	4.8	23.9	41.9
HMSC	Starch % DM	68.3	23	9.3	48.3	79.1
DC	Starch % DM	74.9	3	2.6	75.1	80.2
Fecal	Starch % DM	4.1	30	4.0	0.6	19.6
WPCS	IVSD (7hr)	90.3	35	3.7	82.5	96.2
HMSC	IVSD (7hr)	74.5	23	7.2	61.6	85.8
DC	IVSD (7hr)	73.5	3	4.4	68.6	76.8
WPCS	DM %	36.2	32	5.2	28.1	50.5
HMSC	DM %	74.8	23	5.9	60.1	86.6
DC	DM %	84.1	3	2.3	85.9	90.0
WPCS	KPS, %	61.1	35	12.4	38.6	88.7
HMSC	MPS, microns	1548.0	23	625.8	539.0	2684.0
DC	MPS, microns	550.0	3	58.5	530.0	635.0
Fecal	TTSD, %	94.9	30	5.0	75.5	99.2

Type: WPCS = Whole plant corn silage; HMC = High moisture corn; DC = Dry corn

Analytic: IVSD = In vitro starch digestion, %; DM = Dry matter, %; KPS = Kernel processing score, % starch >4.75mm; TTSD = Total tract starch digestion, %

Table 3. WPCS, HMC, and DC dry matter least square means submitted from commercial dairy farms

Type	DM,% ¹	Std Error
DC	84.1 ^a	1.93
HMC	73.75 ^b	0.86
WPCS	35.92 ^c	0.73

Type: WPCS = Whole plant corn silage; HMC = High moisture corn; DC = Dry corn

¹ = Least square means

Least square means with differing superscript differ at P < 0.05

Table 4. WPCS kernel processing score least square means submitted from commercial dairy farms

Season	KPS,% ¹	Std Error
Spring	61.08 ^a	2.2
Fall	56.97 ^a	1.99

WPCS = Whole plant corn silage; KPS = Kernel processing score,% starch <4.75mm

¹ = Least square means

Least square means with differing superscript differ at P < 0.05

Table 5. Ruminal in vitro starch digestion (7hr) means by sample type and season of submission for commercial dairy farms

Type*Season	IVSD,% ¹	Std Error
WPCS*spring	90.34 ^a	1.09
WPCS*fall	83.72 ^b	1.20
HMC*spring	74.53 ^c	1.34
HMC*fall	75.67 ^c	1.47
DC*spring	71.73 ^c	3.72
DC*fall	74.30 ^c	2.63

Type: WPCS = Whole plant corn silage; HMC = High moisture corn; DC = Dry corn; IVSD = In vitro starch digestion, %

¹ = Least square means

Least square means with differing superscript differ at P < 0.05

Table 6. Mean particle size means by feed type submitted from commercial dairy farms

Type	MPS, microns ¹	Std Error
HMC	1652.16 ^a	86.32
DC	550.362 ^b	200.11

HMC = High moisture corn; DC = Dry corn; MPS = Mean particle size

¹ = Least square means

Least square means with differing superscript differ at $P < 0.05$

Table 7. Starch (%DM) means by sample type submitted from commercial dairy farms

Type	Starch,% ¹	Std Error
DC	74.86 ^a	2.47
HMC	69.97 ^a	0.85
WPCS	34.36 ^b	0.69
Fecal	3.68 ^c	0.72

Type: WPCS = Whole plant corn silage; HMC = High moisture corn; DC = Dry corn

¹ = Least square means

Least square means with differing superscript differ at $P < 0.05$

Table 8. TTSD (%) averaged by season based on fecal starch

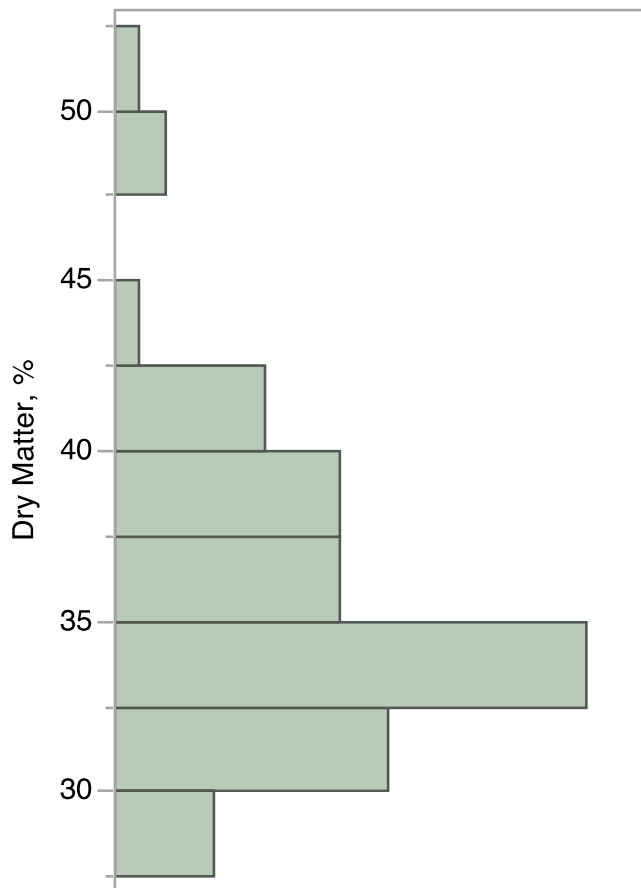
Season	TTSD,% ¹	Std Error
Spring	95.88 ^a	0.8
Fall	94.87 ^a	0.81

TTSD = Total tract starch digestibility

¹ = Least square means

Least square means with differing superscript differ at $P < 0.05$

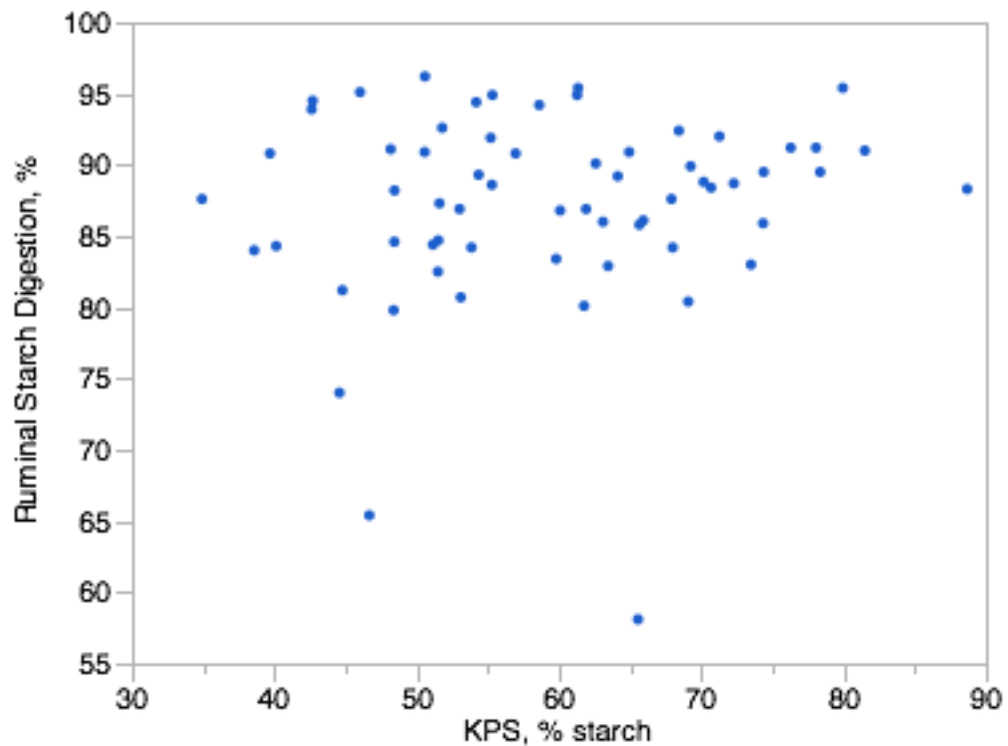
Figure 1: Sample DM% for WPCS submitted in the survey from commercial dairy farms



Type: WPCS = Whole plant corn silage

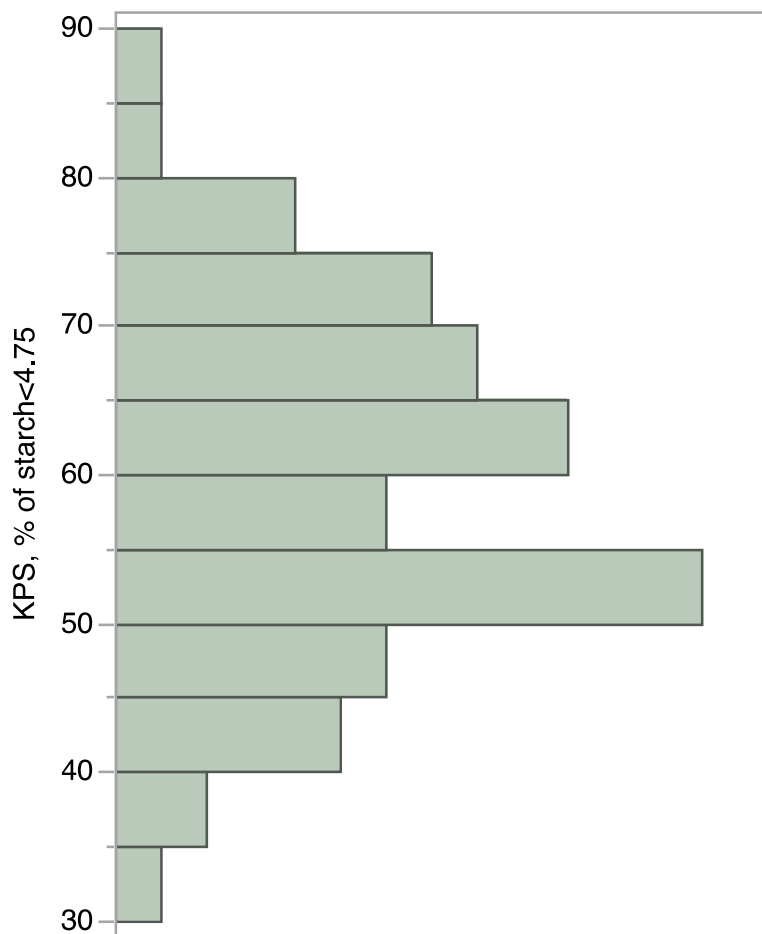
Longer bars indicate a greater relative number of results

Figure 2: Relationship between ruminal starch digestion (7h) and KPS in WPCS submitted from commercial dairy farms



WPCS = Whole plant corn silage; IVSD = In vitro starch digestion; KPS = Kernel Processing Score, % of starch <4.75mm

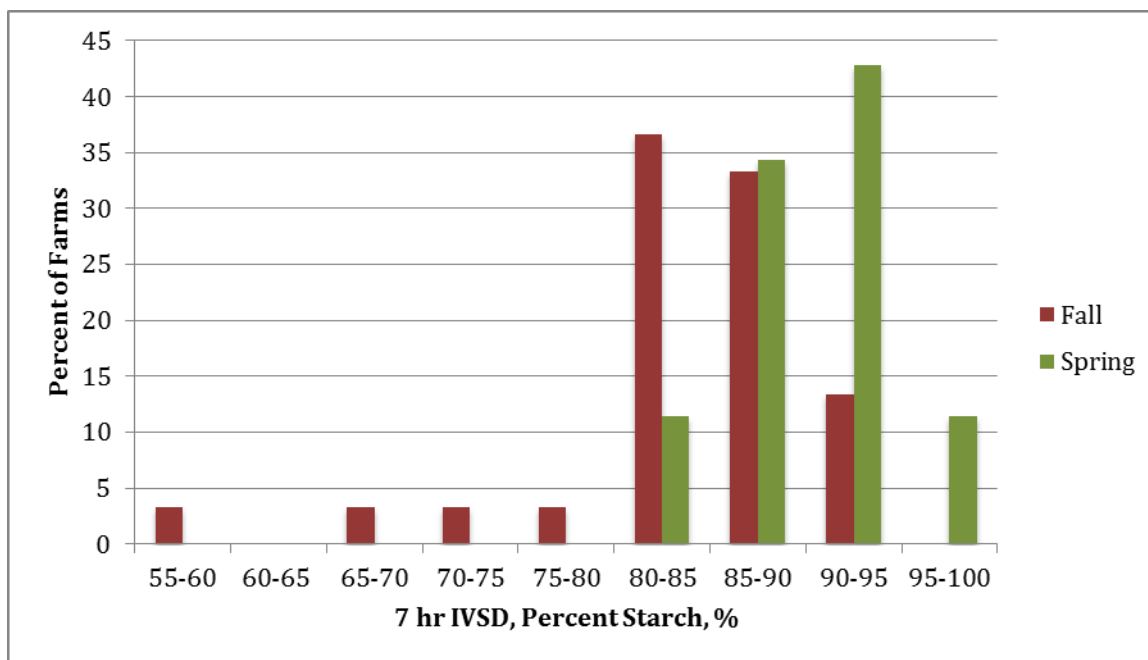
Figure 3: Distribution of WPCS KPS% for all samples submitted from commercial dairy farms



WPCS = Whole plant corn silage; KPS = Kernel Processing Score

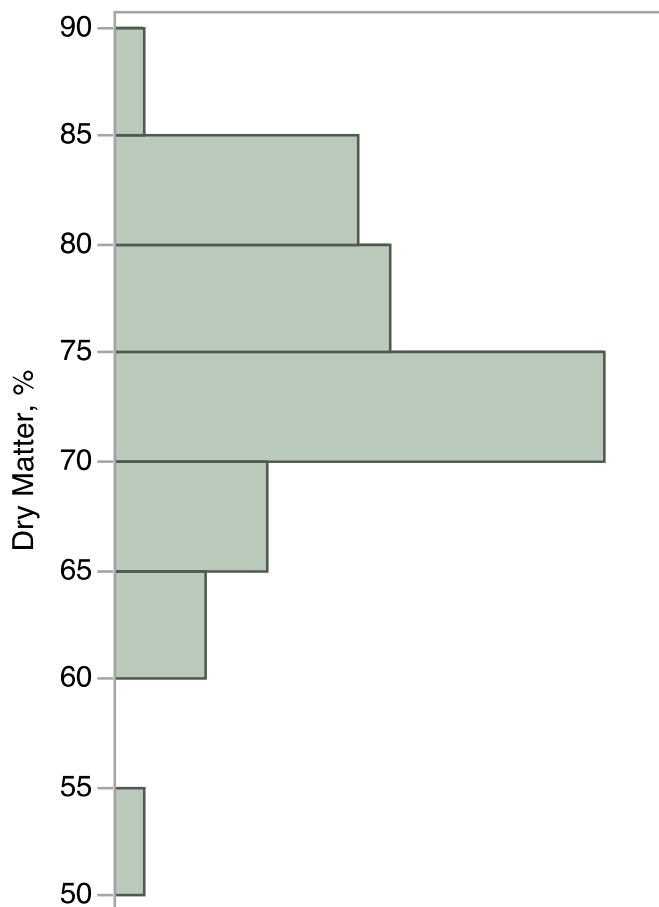
Longer bars indicate a greater relative number of results

Figure 4: WPCS ruminal in vitro starch digestion, % results by season of submission from commercial dairy farms



WPCS = Whole plant corn silage; IVSD = In vitro starch digestion, %

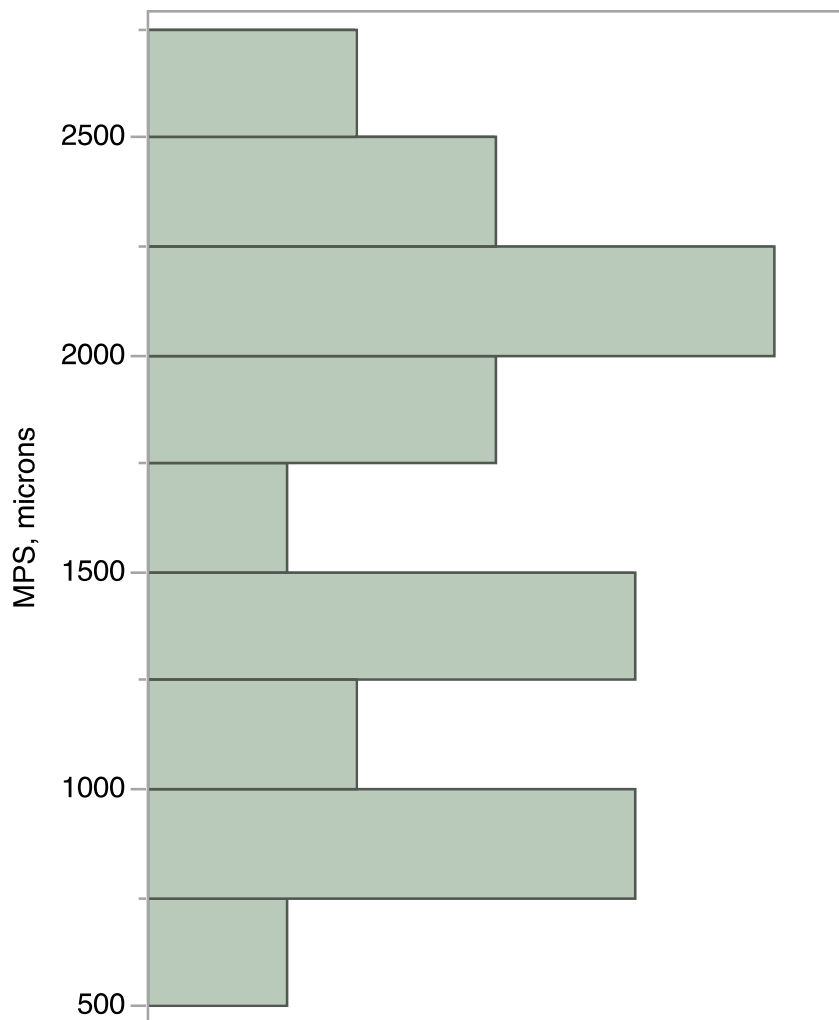
Figure 5: Distribution of HMC DM% for all samples submitted from commercial dairy farms



HMC = High moisture corn; DM = Dry Matter, %

Longer bars indicate a greater relative number of results

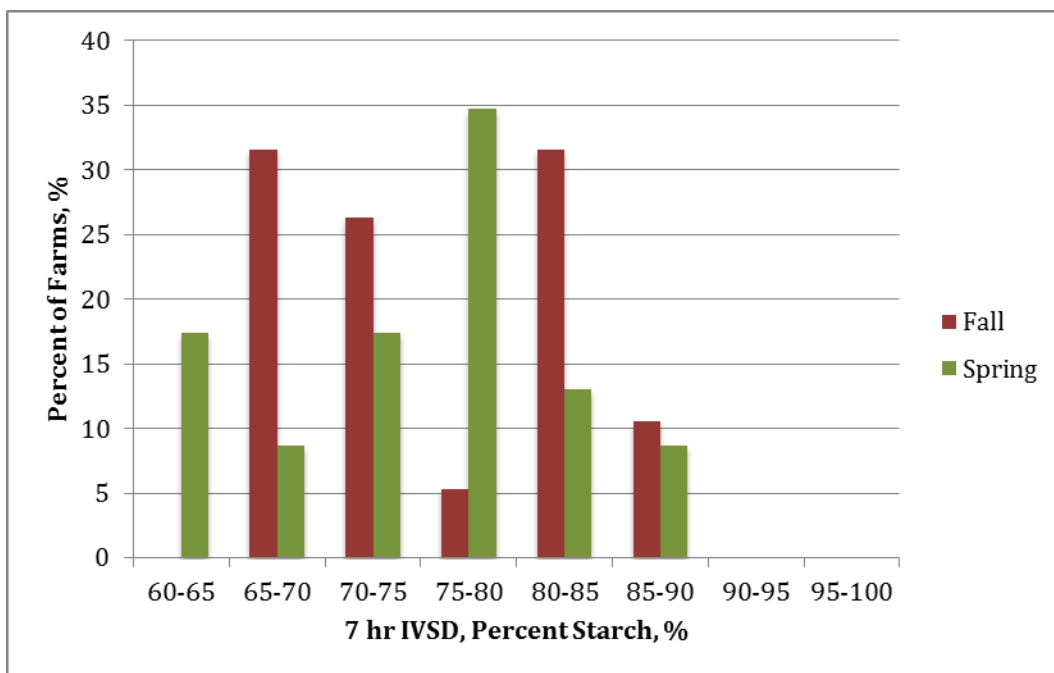
Figure 6: Distribution of HMC MPS for all samples submitted from commercial dairy farms



HMC = High moisture corn; MPS = Mean particle size

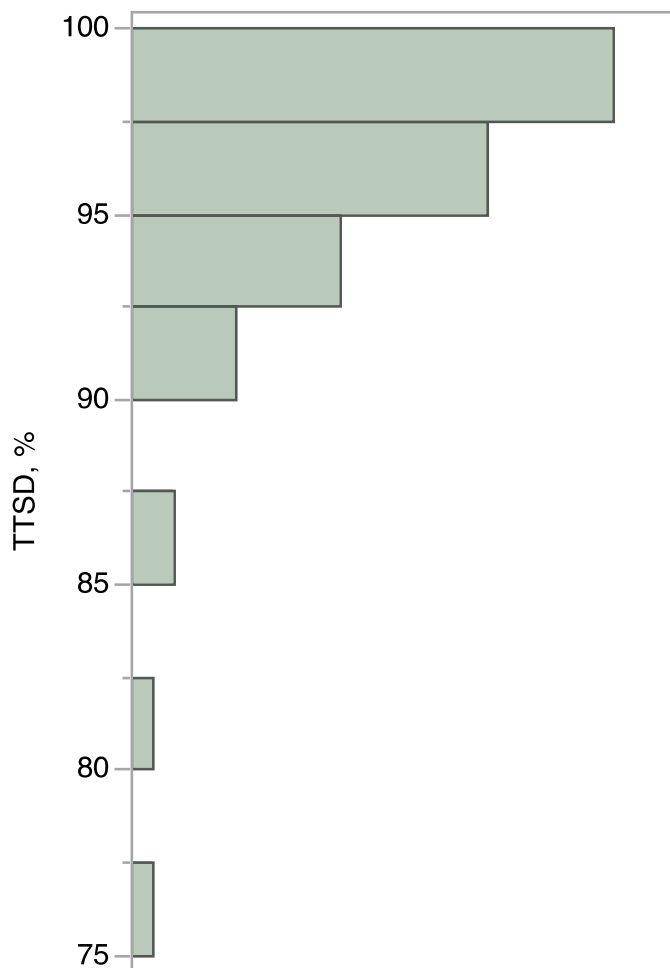
Longer bars indicate a greater relative number of results

Figure 7: HMC ruminal starch digestion, % results by season of submission from commercial dairy farms



HMC = High moisture corn; IVSD = In vitro starch digestion, %

Figure 8: Distribution of TMR predicted total tract starch digestion (TTSD,%). TTSD% was predicted using the following equation: $TTSD\% = (100 * (0.9997 - 0.0125 * \text{fecal starch, \% of DM}))$; $R^2 = 0.94$ (Ferraretto, L. and R. D. Shaver., et al 2012).



TTSD = Total tract starch digestibility, %

Longer bars indicate a greater relative number of results

CHAPTER III: In vitro starch digestion methods compared to in situ starch digestion

Abstract

Ruminal starch digestibility is highly variable across and within feed types. Factors such as particle size, genetics and ensiling can contribute to the ruminal starch digestion variability in different feedstuffs. Accurately determining rumen starch digestion is important to continue advancing ruminant nutrition and dairy performance and minimize wasted nutrients. Two experiments were conducted to determine if an in vitro rumen starch digestion method yielded comparable results to an in situ rumen starch digestion technique. Whole plant corn silage (WPCS), dry corn grain (DCG), and high moisture corn (HMC) samples were collected from a commercial feed analysis laboratory (Rock River Laboratory, Inc in Watertown, WI). In experiment 1 two standard samples were collected (DCG n=1 and WPCS n=1) and expected to differ in starch digestibility. Four different in vitro treatments were compared to the in situ method for 3h and 7h incubations. The treatments were as followed: SLP = standardized rumen fluid – low pH (5.0); SHP = standardized rumen fluid – high pH (7.0); NLP = non-standardized rumen fluid – low pH (5.0); NHP = non-standardized rumen fluid – high pH (7.0). Goeser et al. (2009) observed improved repeatability in fiber digestion with a standardized priming technique. This technique was evaluated to assess for determination of rumen in vitro starch digestion. The pH of the in vitro rumen solution after digestion was evaluated at two levels. Experiment 1 suggested

NLP was most closely related to rumen in situ starch digestion based on a non-statistical difference between NLP and in situ results.

Experiment 2 then further tested the relationship between NLP and IS on a larger set of samples (WPCS n= 10, HMC n=10, and DCG n= 4). Samples were analyzed using both NLP and IS for rumen starch digestion. Experiment 2 resulted in a statistical difference between the NLP and rumen in situ starch digestions. When investigating the difference in results from Experiment 1, we hypothesized that environmental factors affected the experiment. As a result, the rumen in vitro technique was reanalyzed. Rumen in vitro results were biologically acceptable, yet still were significantly different from the in situ results, contradicting results observed in experiment 1. Results should be interpreted with caution due to the several month difference in time between NLP and in situ techniques. The rumen cannulated cows experienced differing diets and environments, which likely affected results. Experiment 2 suggests further research is warranted under a more controlled setting.

Following experiment 2, a larger set of WPCS, HMC, and DCG (n = 75, n = 75, and n = 25, respectively) were incubated in situ for 3h and 7h to describe feedstuff population statistics. We observed substantial variability in rumen in situ starch digestion for WPCS, HMC, and DCG. The coefficient of variation was greater than 18% for all sample types and both time lengths. Sample types ranked by most digestible to least were WPCS > HMC > DCG.

Introduction

Starch in the kernels of whole plant corn silage (WPCS), high moisture corn (HMC), and dry corn (DC) provide substantial energy to diets fed to lactating dairy cows. However, the site of starch digestion determines whether starch serves as an energy source for ruminal microbes or becomes available in the intestines (van Zweieten et al., 2008). In a review of lactation studies, Theurer et al. (1999) concluded that greater ruminal starch digestion lead to improvements in performance by lactating dairy cows.

Accurately estimating starch digestibility for feedstuffs in the rumen is valuable to forecast performance changes resulting when switching feeds within these feed types. There have been many academic and practical approaches evaluated as measures for predicting starch digestion, each differing in the using the actual rumen environment or a simulated environment. The two techniques used in this study were rumen in situ and in vitro digestion procedures. Both rumen in vitro (IV) and rumen in situ (IS) starch digestion techniques are commonly used in commercial feed analysis laboratories around the country and world to estimate ruminal NDF and starch digestibility.

The rumen IS procedure used in estimating starch degradation is often justified because it involves the actual rumen environment (Huhtanen and Sveinbjornsson, 2006). This is a possible advantage over any IV technique, as the only other measures that utilize the actual rumen are in vivo measures (Kitessa et al. 1999), however, these

are invasive and extremely time consuming. The IS technique is a simple and direct method (Offner et al., 2003); nutrient (including starch) digestion can be estimated by incubating feeds in porous nylon bags in the rumen for various lengths of time. While *in situ* offers potential advantages in accuracy relative to *in vivo* over *in vitro*, grind size used during the IS technique can affect the degradability significantly. Michalet-Doreau and Cerneau (1991) compared three grind sizes, 0.8 mm, 3.0 mm, and 6.0 mm for dry matter degradation when washed in water for 15 minutes, and observed 14.6%, 8.9% and 1.8% degradability for corn grain. The NRC (2001) considers these small particles lost through the pores of the bag to be immediately degraded in the rumen. Using IS technique on very fine feedstuff particles is not recommended (Offner et al., 2003), due to potentially greater losses through the bag pores. Another advantage with rumen IS starch digestion measures is that multiple time periods can be assessed to model starch digestion kinetics (Orskov and McDonald, 1979). Digestion parameters can then be used in diet formulation software.

In a quantitative review of the IS method by Offner et al. (2003), they observed that there was not a significant interaction between laboratory and sample type. This observation suggests that there was not difference in the ranking of results from different laboratories. Offner and Sauvant (2004) observed that estimates of IS starch degradability were comparable to numerous observations for starch digestibility *in vivo*. Results showed that the IS technique accurately predicted starch digestion in the rumen and the total tract. Offner et al. (2003) also suggests that no less than 3 replicates should be used when utilizing the IS technique to account for variation.

However, starch digestion for feedstuffs with slowly degrading starch was underestimated, whereas starch digestion was overestimated with rapidly degradable starch when analyzed by the IS method. Underestimation of starch digestion in nylon bags can be explained by high proportions of vitreous endosperm (van Zwieten et al, 2008), while overestimation could be due to the nylon bag pore size relative to sample grind size.

Hindle et al. (2005) suggested that IS techniques should be performed in animals that have been adapted to a starch source similar to that of interest to provide a more accurate simulation of the in vivo system. While potentially more accurate and informative rumen IS techniques can be expensive and less efficient than IV techniques.

Rumen IV digestions can be characterized as being completely removed from the animal and carried out in a simulated environment. Many factors affect rumen in vitro incubations: ratio of feed to rumen fluid, ratio of rumen fluid to IV media, composition of the IV media, and type of mill used for grinding, grinder screen size and diet of the donor animal (Huhtanen and Sveinbjornsson, 2006). Repeatability from one run to the next has challenged IV techniques, however, Goeser et al. (2009) observed a reduction in inter-assay error in fiber digestion when the rumen fluid inoculum was primed with a simulated total mixed ration to standardize the activity of the rumen fluid digesting bacteria before inoculation. Results suggest that by using this method, in vitro fiber digestion estimates are less likely to be affected by varying rumen fluid inoculum activity (Goeser et al., 2009). This method has yet to be assessed for ruminal in vitro starch digestion assays.

Our objective was to determine if an IV rumen starch digestion method yielded comparable results to rumen IS starch digestion technique. This could result in a more cost effective assay for dairy nutritionists and producers to utilize to determine ruminal starch digestion.

Materials and Methods

Experiment 1

Two samples were collected from a commercial feed analysis laboratory (Rock River Laboratory, Inc. in Watertown, WI); DC (n=1) and WPCS (n=1) to test high and low digestible starch samples. Samples were dried and ground to pass a 1 mm Udy cyclone mill (Udy Corporation, Fort Collins, CO) for dry matter and starch analysis, 4 mm Wiley mill (Thomas Scientific, Swedesboro, NJ) for IV methods, and 6 mm Wiley mill (Thomas Scientific, Swedesboro, NJ) for IS methods. Treatments were set up in a factorial arrangement to test pH and standardization of rumen fluid in relation to IS. Four IV treatments (trt) were compared to the rumen IS starch digestion method. SLP = standardized rumen fluid – low pH (5.0); SHP = standardized rumen fluid – high pH (7.0); NLP = non-standardized rumen fluid – low pH (5.0); NHP = non-standardized rumen fluid – high pH (7.0).

Samples were dried at 105°C to determine DM content for 3 h. Starch content was determined and corrected for free glucose according to the procedures described by Hall (2008), with modifications to allow for sample analysis on a YSI Biochemistry

Analyzer (YSI Inc, Yellow Springs, OH). Starch was calculated as $100 \times [(volume/WT.) \times (glucose) \times (0.9)/1000]$.

For the IV methods, samples were weighed (0.5 g +/- 0.05 g) into 125 ml Erlenmeyer flasks. Samples were then digested in triplicate for 3h and 7h time points. For SLP and SHP, rumen fluid inoculum collection and standardization was done according to Goeser et al. (2009). For NHP and NHP rumen fluid collection was done according to Richards et al. (1995). Rumen fluid inoculum was collected and added to the samples at approximately 8:30 am. After incubation, 15 mL of 0.1 M sodium acetate buffer (pH 5.0) was added to stop digestion, samples in SLP and NLP were titrated to a pH of 5.0 – 5.5 using 0.5 M hydrochloric acid. The in vitro solution pH was assessed after the sample was digested. In the starch assay (Hall, 2008) the sodium acetate buffer solution is described to be at a pH of 5.0. Yet Hoffman (personal communication, 2013) helped identify that solution pH may not be at 5.0. We tested the in vitro solution pH after the sodium acetate buffer was included per the Richards et al. (1995) IV technique and found the pH to average 7.0. Solution pH is critical because amyloglucosidase, which is used to solubilize the starch in the Hall (2008) procedure, has a specific activity at pH of 4.5 – 5.5 (Megazyme, Bray, Ireland).

Samples analyzed using IS were weighed (6 g per bag) in triplicate into 5 cm x 10 cm nylon bags with 50 micron porosity (Ankom Technology, Macedon, NY). One replicate was placed in each of three different ruminally-cannulated lactating dairy cows consuming a 58% forage diet with a 50:50 ratio of WPCS to legume (DM basis) for 7 h and 3 h. Rumen IS bags were introduced to the rumen at 900h for 7 h analysis,

1300h for 3 h analysis, and all residue bags were removed together at 1600h. Bags were placed immediately into ice water to terminate microbial digestion. Bags containing digested residue were rinsed until effluent was clear by hand washing to remove all microbial protein. Rinsed bags were dried in a 50 degrees Celsius forced air oven for 24 hours and weighed to determine sample dry matter digestion. Residue samples were composited and ground to 1 mm to determine starch content.

Starch content in the residues was then determined as previously described. Rumen starch digestion was calculated as $100 \times [(Starch_{original} - Starch_{residue}) / (Starch_{original})]$.

Data were analyzed using SAS JMP version 11.0. Sample type, hour, and treatment were considered to be fixed effects. Week was considered to be a random effect. Backwards elimination was used to determine the final model. A lower AIC and BIC was considered superior and effects were removed from the model accordingly. Least square means were provided for all fixed and random effects through SAS JMP version 11.0. The final model included:

$$Y_{ijkl} = \mu + T_i + H_j + R_k + TR_{ik} + TW_{il} + HR_{jk} + RW_{kl} + e_{ijkl}$$

Where Y_{ijkl} = starch digestion, response variable, μ = population mean, T_i = sample type, H_j = time (hour), R_k = treatment, TR_{ik} = sample type and treatment interaction, TW_{il} = sample type and week interaction, HR_{jk} = hour and treatment interaction, RW_{kl} = treatment and week interaction, and e_{ijkl} = random residual error, assumed to be normally distributed. Main effects considered significant at $P < 0.05$.

Experiment 2

Following experiment 1, WPCS (n=10), HMSC (n=10), and DC (n=4) were obtained from a commercial feed analysis laboratory (Rock River Laboratory, Inc. in Watertown, WI) from August 2013 to December 2013. Samples were selected to be diverse in chemical and physical characteristics. Samples were analyzed on a near infrared spectroscopy instrument, samples varying in starch (% of dry matter) and soluble protein (% of crude protein) were chosen to capture samples that we assumed would vary in starch digestibility. Sample preparation and the rumen IS technique was the same as previously described.

For the IV methods, samples were weighed (0.5 g +/- 0.05 g) into 125 ml Erlenmeyer flasks. Samples were then digested in triplicate for 3h and 7h time points. Rumen fluid collection was done according to Richards et al. (1995). Rumen fluid inoculum was collected and used to inoculate samples at approximately 830 h. After incubation, 15 mL of 0.1 M sodium acetate buffer (pH 5.0) was added to terminate digestion. Samples were titrated to a pH of 5.0 – 5.5 using 0.5 M hydrochloric acid. In vitro solution pH was determined after the sample was digested.

Data were analyzed using SAS JMP version 11.0. Sample type, hour, and treatment were considered fixed effects. The final model was:

$$Y_{ijk} = \mu + T_i + H_j + R_k + RH_{ij} + e_{ijkl}$$

Where Y_{ijkl} = starch digestion, response variable, μ = population mean, T_i = sample type, H_j = time (hour), R_k = treatment, RH_{ij} = treatment and hour interaction, and e_{ijkl} = random residual error, assumed to be normally distributed. Main effects were considered significant at $P < 0.05$.

Following Experiment 2, a larger set of samples were analyzed for rumen starch digestion through the IS approach. These samples were chosen at random from a commercial feed analysis laboratory (Rock River Laboratory, Inc. in Watertown, WI). These were included to further explain descriptive statistics for rumen starch digestion in WPCS, HMC, and DG. An additional WPCS ($n = 65$), HMC ($n = 65$), and DCG ($n = 21$) were included. This resulted in a total set of WPCS ($n = 75$), HMC ($n = 75$) and DCG ($n = 25$) analyzed as previously described in Experiment 1 for the rumen IS starch digestion.

Results and Discussion

Experiment 1

Experiment 1 aimed to evaluate rumen IV starch digestion techniques relative to a standard rumen IS starch digestion approach. We observed statistically insignificant differences between trt NLP and IS, yielding similar starch digestion means when averaged across the 3h and 7 h incubation time points (73.53% vs. 73.52%, respectively) suggesting these two techniques were comparable. Equivalence test was performed to analyze the of means between the NLP and IS treatments. A difference of 4.5 still did not yield a significant p-value, means are to be considered equivalent.

All other treatments were significantly different in starch digestion relative to the IS technique as shown in Table 1. Treatment means, treatment means by sample type, treatment means by length of digestion, and treatments by week due to interactions are in Table 1.

For SHP and NHP, where the pH remained 7.0 due to heavily buffered rumen IV media, the results were not significantly different. This suggests that if the pH is held near 7.0, the rumen fluid standardization technique described by Goeser et al. (2009) had no effect. But for SLP and NLP where the pH was lowered to 5.0 – 5.5, there were lower values with standardized rumen fluid for SLP (69.36% vs. 73.53%).

In Figure 1 is the interaction between sample type and treatment. The IS showed a greater difference between DC and WPCS than any of the IV methods (SLP, SHP, NLP and NHP), suggesting that the IS technique captures greater variation in ruminal starch digestion. For IV with DCG, the Goeser et al. (2009) procedure for rumen primed fluid showed no difference, but in WPCS the non-standardized fluid yielded higher digestibility results than the standardized; treatment means shown in Table 1. This contradicts what Goeser et al. (2009) found that there was no impact of priming rumen fluid on neutral detergent fiber digestibility. We hypothesize that amylase activity in the rumen fluid may decrease while the rumen fluid is allowed to standardize.

The treatments with a pH of 7 (SHP and NHP) also yielded greater values for both DC and WPCS. This could be explained by the starch remaining in the IV solution not being completely hydrolyzed, because the amyloglucosidase pH specific activity level is

4.5 – 5.5 according to Megazyme (Megazyme International, Bray, Ireland). A more basic pH (> 5.5) would lead to decreased enzyme activity, which could result in lowered recovered starch thereby leading to higher starch digestion values.

Treatment week was considered to be a random variable, yet in Figure 2 the treatments were separated by week to demonstrate an interaction. Each of the IV methods increase in starch digestibility from week 1 to week 2, while the IS method stayed relatively constant between week 1 and week 2 (74.0% vs. 73.6%, respectively). These results suggest that the IS method may be more consistent across weeks than the in vitro method, although additional work is warranted. A possible explanation could be that IV methods are completely removed from the animal and environmental effects may have a greater impact on IV relative to IS methods where the direct ruminal environment is utilized. Table 2 demonstrates the sample type means by week of digestion. We observed an increase in rumen starch digestion in both WPCS and DCG from week 1 to week 2 (12.6% and 7.3%, respectively), due to the increases in all of the IV treatments.

In Figure 3 is the between 3h and 7h digestion across techniques and treatments. Rumen IS technique detected the widest range between 3h and 7h incubation time points. Higher 3h digestion values were observed compared to 7h digestion in SHP which is not possible (Figure 3). The pH appeared to have the largest affect on DCG and WPCS starch digestion. Samples with a post digestion pH of 7.0 were greater at both time points than samples that were pH 5.0 – 5.5. We believe this is due to greater amyloglucosidase activity during the starch assay, causing greater residual

starch recovered after digestion than samples with a pH of 7.0 where starch residue determination is theorized to be incomplete. When all of the starch in the residual samples is not hydrolyzed and recovered, this results in higher ruminal starch digestion results. We observed that using non-standardized rumen fluid and adjusting the pH to between 5.0 – 5.5 may be comparable to IS ruminal starch digestion results.

Experiment 2

In Experiment 2, results observed in Experiment 1 were to be verified on a larger sample set. However, we observed NLP yielded lower starch digestion than IS (Table 3), which contradicts results in Experiment 1. There was a statistical difference in rumen starch digestion when a larger sample set was analyzed for the IV NLP technique and IS technique.

In Figure 4 is the effect of digestion method and treatment hour on starch digestibility. The IV method yielded lower results for both 3h and 7h than the IS technique. There was a greater difference between the IV 3h and 7h than the IS 3h and 7h (20.7%-units vs. 11.0%-units). This experiment was run in the winter months, where it was unseasonably cold. Due to this complication, the IV technique was repeated in the following months. This delay in analyzing ruminal IV starch digestion could have affected our results due to diet and environmental changes that the donor cows endured. These inconclusive results suggest that the IV and IS technique do not produce comparable results.

After we observed contradictory results, additional samples were analyzed using rumen in situ starch digestion technique to further describe population statistics. In Table 4 are the population statistics for WPCS, HMC, and DCG by time of incubation. The coefficient of variation for rumen starch digestion was greater than 18% for all sample types and digestion times, suggesting great variability within feed sample type. In Figure 5 is the distribution of all sample types by length of digestion. However, the rumen in situ starch digestions were assessed for normality using Shapiro-Wilk (1965) goodness of fit test and were found to not be normal. Suggesting these samples do not represent a normal population.

The average for all corn types (HMC and DCG) for 7h ruminal IS starch digestion in this experiment was similar to the results of Patton et al. (2012) in a literature review of in vivo ruminal starch digestion for corn (53.2% vs. 54.6%). Firkins et al. (2001) found similar results in DCG for rumen starch digestibility compared to results we observed (47.0% vs. 49.9%, respectively). A similar range of rumen IS starch digestion for DCG was observed by Correa et al. (2002), where kernel virtuosity was tested. They observed a range of 34.9% to 62.3% with a mean of 48.2%, similar to our results that ranged 31.7% to 74.3% with a mean of 49.9%. We observed that HMC had a greater ruminal starch digestibility than DCG, which agrees with previous research (Firkins et al., 2001; Hoffman et al., 2012). Our observations, in collaboration with cited work, suggest that the IS technique may be a more suitable technique if it can be practically applied.

Conclusion

Results observed in Experiment 1 suggested that using non-standardized rumen fluid and adjusting the pH to between 5.0 – 5.5 may yield comparable to IS ruminal starch digestion results. When evaluating additional samples to further test this hypothesis in Experiment 2, there was a statistical difference between the in vitro starch digestion and in situ starch digestion results.

These could have been caused by many varying factors that could have led to these differing outcomes. In Experiment 1 the in vitro and in situ samples were run in the same week. Due to complications with cold weather temperatures during Experiment 2 we had to repeat the in vitro starch digestions a few months later. The change in season and in diets could have affected the rumen fluid collected for in the in vitro digestions. This trial warrants further research testing the in situ and in vitro techniques in a more controlled setting, as well as the effects of differing rumen fluid due to diet and temperature on starch digestion.

Additional samples analyzed for rumen IS starch digestion suggests that there is large variability in rumen starch digestion within feed type for WPCS, HMC, and DCG.

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Table 1. Experiment 1 ruminal starch digestion (% of starch) results by treatment

	Treatments ¹					SE ³	P <
	SLP	SHP	NLP	NHP	IS		
Type							
DCG	67.5 ^c	84.6 ^{a,b}	67.9 ^c	84.2 ^{a,b}	60.4 ^d	5.2	0.0001
WPCS	71.2 ^c	85.3 ^{a,b}	79.5 ^b	88.4 ^a	87.2 ^a		
Time (hour)							
3	68.2 ^e	86.6 ^a	71.3 ^{d,e}	84.9 ^{a,b}	69.2 ^{d,e}	5.2	0.001
7	70.5 ^{d,e}	83.3 ^{a,b}	75.7 ^{c,d}	87.8 ^a	78.4 ^{b,c}		
Week							
1	62.6 ^d	78.7 ^b	67.7 ^{c,d}	80.2 ^b	74.0 ^{b,c}	1.5	0.0001
2	76.2 ^b	91.1 ^a	79.4 ^b	92.5 ^a	73.6 ^{b,c}		
Treatment ²	69.4 ^a	85.0 ^b	73.5 ^c	86.3 ^b	73.8 ^c	5.0	0.0001

¹ Treatments: SLP = Standardized rumen fluid - low pH; SHP = Standardized rumen fluid - high pH; NLP = Non-standardized rumen fluid - low pH; NHP = Non-standardized rumen fluid - high pH; IS = In situ

² Treatment means for 3h and 7h starch digestion

³SE = Standard Error

a, b, c, d & e not connected by the same letter in Type, Time, Week and Treatments are significantly different (P < 0.05)

Treatment by type (P < 0.001), treatment by time (P < 0.01), and treatment by week (P < 0.001) interactions.

Type: DCG = Dry corn grain, WPCS = Whole plant corn silage

Table 2. Experiment 1 rumen starch digestion (% of starch) means for two different feed types and two weeks

Type	Week ¹		SE ²	P <
	1	2		
DCG	69.2 ^c	76.5 ^b	0.94	0.01
WPCS	76.0 ^b	88.6 ^a		

¹ Sample week means for 3h and 7h starch digestion

² SE = Standard Error

Type: DCG = Dry corn grain, WPCS = Whole plant corn silage

a, b, & c not connected are significantly different (P < 0.05)

Table 3. Experiment 2 ruminal starch digestion (% of starch) means for multiple feed types and digestion time lengths

	Mean	SE ⁴	P <
Type ²			
HMC	49.6 ^b	1.4	0.01
WPCS	55.6 ^a	1.4	
DCG	47.6 ^b	2.2	
Time (hour)			
3	43.0 ^a	1.4	0.0001
7	58.9 ^b	1.4	
Treatment ^{1,2}			
IS	56.9 ^a	1.4	0.0001
NLP	44.8 ^b	1.4	

¹ Treatments: NLP = Non-standardized rumen fluid - low pH; IS = In situ

² Means for 3h & 7h starch digestion

⁴SE = Standard Error

^a & ^b not connected by the same letter in Type, Time, and Treatments are significantly different (P < 0.05)

Type: DCG = Dry corn grain, WPCS = Whole plant corn silage, HMC = High moisture corn

Table 4. Rumen in situ 3h and 7h starch digestion (% of starch) descriptive statistics

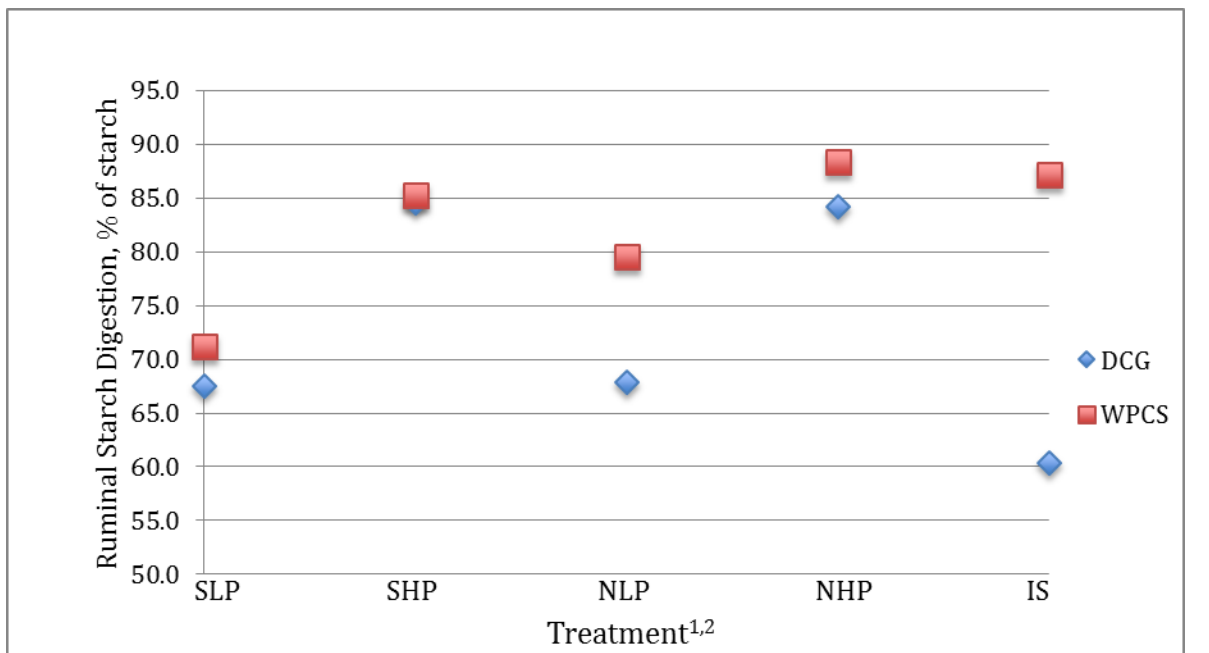
Type	Mean	St.dev. ¹	Minimum	Maximum	C.V ⁴
WPCS					
3	61.0	19.8	0.9	88.2	32.5
7	75.2	13.8	16.9	93.8	18.4
HMSC					
3	46.0	13.7	24.4	91.4	29.8
7	56.4	12.8	32.3	91.3	22.7
DCG					
3	38.1	13.7	20.1	67.7	36.0
7	49.9	11.2	31.7	74.3	22.4

Type: WPCS = Whole plant corn silage, HMC = High moisture corn, DCG = Dry corn grain

¹ Standard Deviation

⁴ Coefficient of Variation

Figure 1. Experiment 1 rumen starch digestion, averaged across 3 h and 7 h, for 5 different techniques and treatments and two feed types

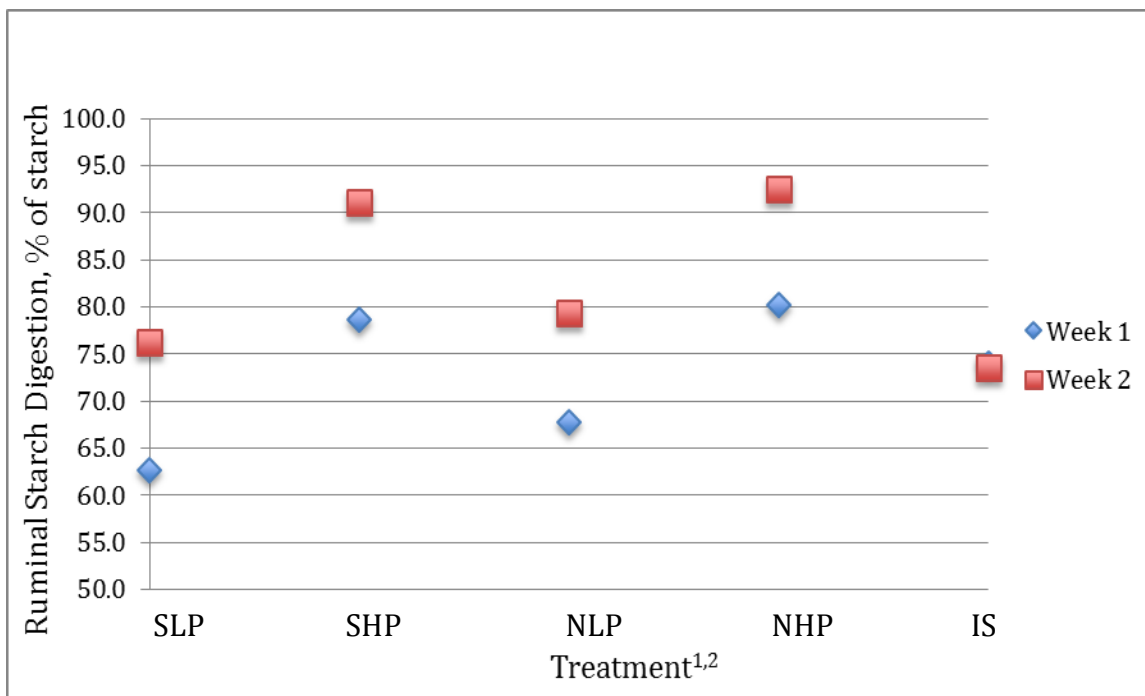


¹Treatments: SLP = Standardized rumen fluid - low pH; SHP = Standardized rumen fluid - high pH; NLP = Non-standardized rumen fluid - low pH; NHP = Non-standardized rumen fluid - high pH; IS = In situ

² Treatment means for 3h & 7h starch digestion

Treatment * type interaction ($P < 0.001$)

Figure 2. Experiment 1 rumen starch digestion, averaged across 3 h and 7 h for 5 different techniques and treatments and two weeks

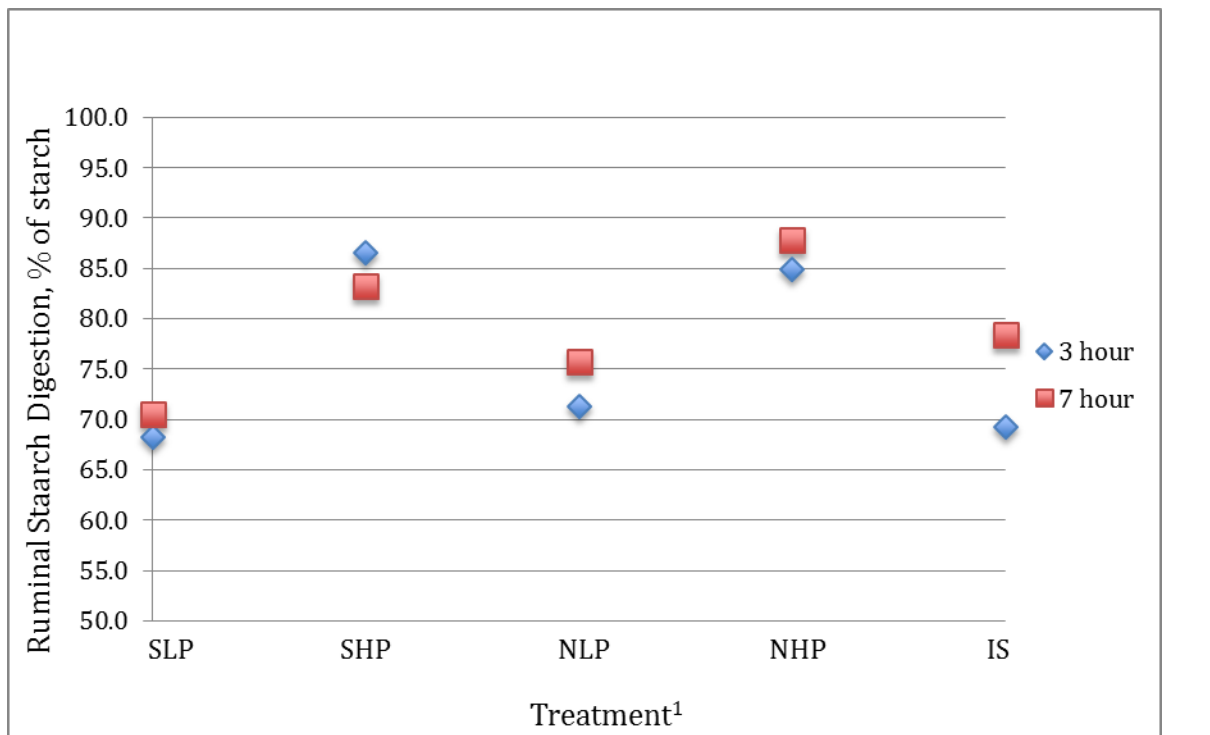


¹ Treatments: SLP = Standardized rumen fluid - low pH; SHP = Standardized rumen fluid - high pH; NLP = Non-standardized rumen fluid - low pH; NHP = Non-standardized rumen fluid - high pH; IS = In situ

² Treatment means for 3h & 7h starch digestion

Treatment by week interaction ($P < 0.001$)

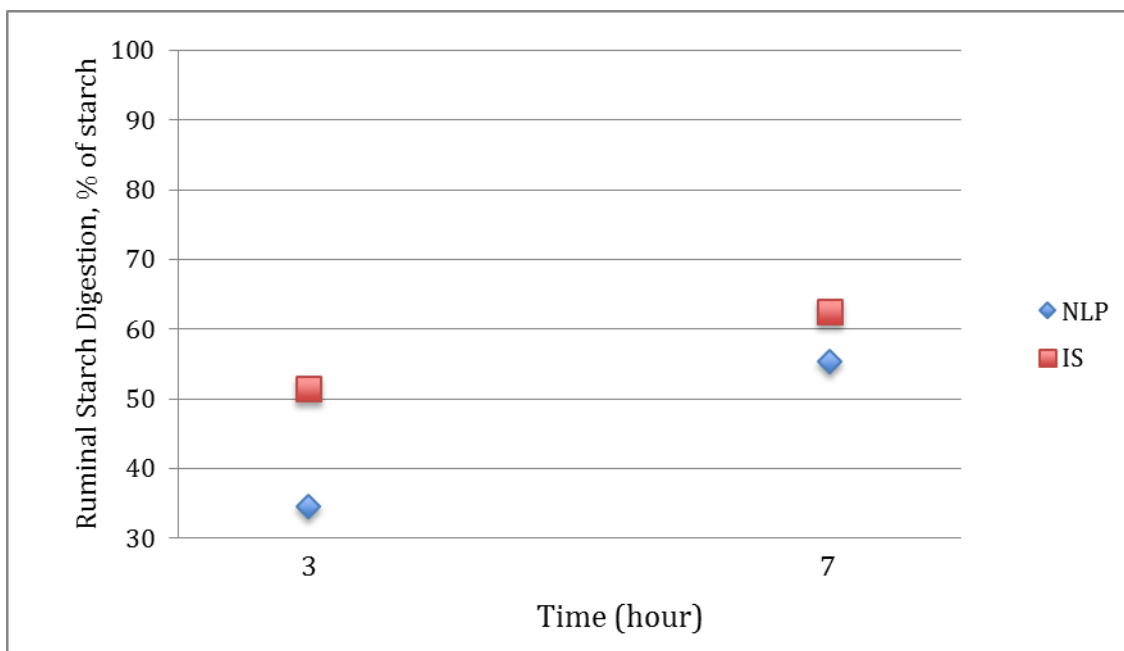
Figure 3. Experiment 1 rumen starch digestion, for 5 different techniques and treatments



¹Treatments: SLP = Standardized rumen fluid - low pH; SHP = Standardized rumen fluid - high pH; NLP = Non-standardized rumen fluid - low pH; NHP = Non-standardized rumen fluid - high pH; IS = In situ

Treatment by time interaction ($P < 0.01$)

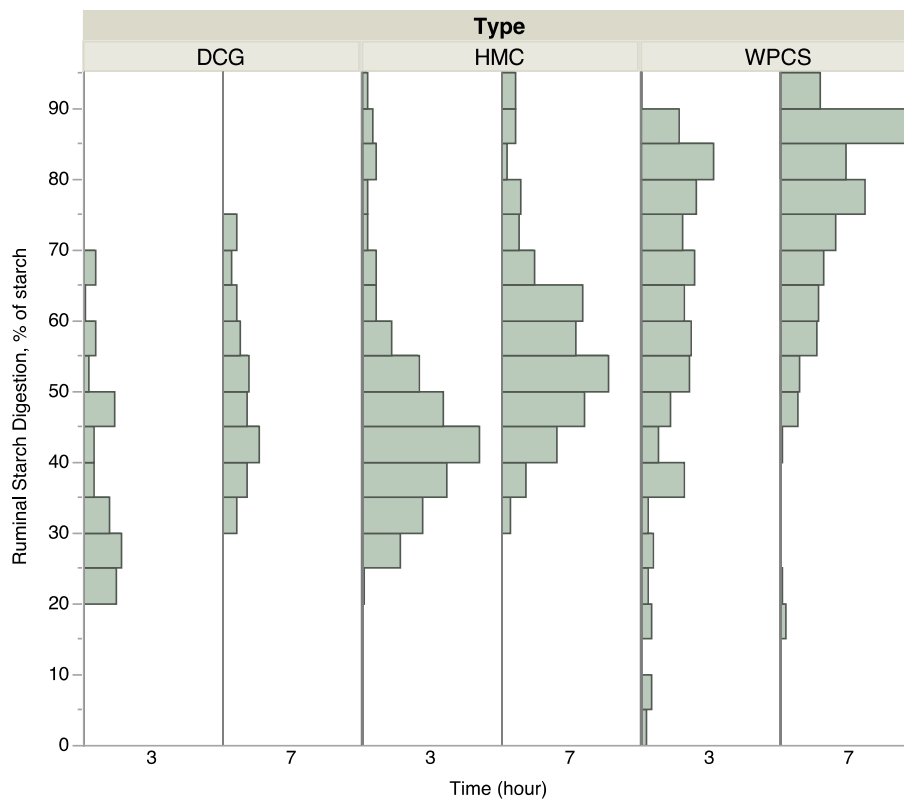
Figure 4. Experiment 2 effect of time of digestion by treatment on ruminal starch digestibility, %



Treatments: NLP = Non-standardized rumen fluid - low pH; IS = In situ

Treatment by time interaction ($P < 0.01$)

Figure 5. Rumen in situ 3h and 7h starch digestion distributions for DCG, HMC, and WPCS



Type: DCG = Dry corn grain, HMC = High moisture corn, WPCS = Whole plant corn silage