Making sense of starch by NDF interactions

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Introduction

Associative effects of feeds, nutrients, diets and dry matter intake (**DMI**) influence the digestibility of nutrients in vivo. However, associative effects are largely ignored with commercial-lab in vitro or in situ digestibility measurements.

Presented in Table 1 are the findings of a survey performed by the authors of websites and sample reports of 4 major dairy feed testing labs in USA for analyses related to starch and NDF digestibilities. Dairy nutritionists have a seemingly endless stream of assays, and calculations from these assays, available for characterizing feed ingredients and diets. The inclusion of biological assays, e.g. digestibility in rumen fluid, to go along with chemical assays, e.g. NDF, lignin, starch, etc., in the commercial feed analysis system has been a major step forward for the industry to characterize feed ingredients and diets according to their nutritive value.

However, when attempting to interpret and translate, to the farm, from the myriad of assays and calculations listed in Table 1, the inherent flaws of rumen in vitro and in situ measurements relative to in vivo digestibility results should be kept in mind. A partial list is as follows:

- Measurements relative to ingredient and nutrient composition and physical form of diet fed to donor or incubation cows (Cone et al., 1989; Mertens et al., 1996) rather than client farms where results will be used, e.g. effects of variable diet starch content and source on ruminal amylase activity and in vivo

starch digestibility; effects on in vivo fiber digestibility of fluctuations in ruminal pH via production, buffering, absorption and passage of volatile fatty acids; effects of variation in rumen degradable protein on in vivo fiber and starch digestibility; etc.

- Measurements relative to DMI of donor or incubation cows rather than client farms with highly variable milk yield and hence DMI levels. Determination of digestion rates (k_d) allows this discrepancy to be partly corrected for by using rate of passage (k_p) assumptions. However, DMI may influence rumen pH (Shaver et al., 1986) and hence k_d; this effect would not be accounted for with k_p assumptions in the $k_d/(k_d+k_p)$ calculations of digestibility.
- Fine grinding of incubation samples, to pass through a 1-2 mm screen, results in measurement of maximal rates and extents of NDF digestibility, while grinding incubation samples to pass through a 4-6 mm screen may mask the effects of test feed particle size on starch digestibility.
- Ruminal in vitro and in situ techniques ignore post-ruminal starch and NDF digestion. The proportion of starch digested post-ruminally can be significant (Ferraretto et al., 2013).

Therefore, for the most part, the assays or calculations from these assays, listed in Table 1, should be viewed as relative index values for comparison among feeds/diets or over time within feeds/diets, rather than as predictors of in vivo digestibility results. The obvious exceptions include: 1) determination of fecal starch concentrations to estimate in vivo total tract starch digestibility (**TTSD**) for diets (Fredin et al., 2014; Owens et al., 2015), and 2) determination of concentrations of fecal and diet undigested NDF (**uNDF** at 120-288 h) along

with the nutrients of interest, in both fecal and diet samples, to determine in vivo total tract nutrient digestibility for diets (Schalla et al., 2012; Krizsan and Huhtanen, 2013). It is noted, however, that these results provide no information about site of digestion and pertain only to the diet fed rather than specific feed ingredients included within the diet.

In a field study of 32 high-producing commercial dairy herds in the Upper Midwest, Powel-Smith et al. (2015) used lignin and uNDF (240 h) as indigestible markers to determine in vivo TTSD and total tract NDF digestibility (**TTNDFD**) for diets. Measurements of ruminal in vitro starch digestibility (**ivSD**; 7 h) were unrelated (R^2 = 0.00) to TTSD. For TTNDFD, measurements of ruminal in vitro NDF digestibility (**ivNDFD**; 24 h) and uNDF were poorly (R^2 = 0.13 and 0.21, respectively) related.

Lopes et al. (2015), using in vivo TTNDF data from 21 treatment diets in 7 lactating dairy cow feeding trials conducted at the University of Wisconsin, evaluated uNDF (240 h) and the Combs rumen in vitro estimate of total tract NDF digestibility (**ivttNDFD**). Diet uNDF (240 h) was negatively related (R^2 = 0.40) to TTNDFD; each 1%-unit increase in uNDF (240 h) was associated with a 0.96%-unit decrease in TTNDFD. Mean values, however, were 15%-units greater for uNDF-predicted total total tract NDF digestibility compared to the observed TTNDFD. The ivttNDFD calculations included diet uNDF (240 h), potentially-digestible NDF and NDF k_d determined using the in vitro procedure of Goeser and Combs (2009), assumed k_p, and assumed hindgut NDF digestion. The R^2 for the relationship between ivttNDFD and TTNDFD was 0.68 and mean values differed by only 1%-unit, showing promise for this approach.

The remainder of this paper will focus primarily on review and discussion of the effects of starch by NDF interactions and DMI on in vivo starch and NDF digestibilities.

Corn Silage

Substantially (10-15%-units) greater ivNDFD for brown midrib 3 mutation (**bm_3**) whole-plant corn silage (**WPCS**) hybrids associated with reduced lignin content compared to conventional hybrids is well established (Jung and Lauer, 2011; Jung et al., 2011). However, greater ivNDFD for bm_3 hybrids has sometimes, but not always, translated into greater in vivo NDF digestibility (Oba and Allen, 1999; Tine et al., 2001; Jung et al., 2011; Ferraretto and Shaver, 2015). Variable TTNDFD response to feeding bm_3 WPCS is influenced by the DMI response to the greater ivNDFD (Oba and Allen, 1999; Tine et al., 2001), while WPCS type (bm_3 versus near-isogenic or conventional WPCS hybrids) by dietary forage-NDF (Oba and Allen, 2000; Qiu et al., 2003), starch (Oba and Allen, 2000) and CP (Weiss and Wyatt, 2006) concentration or supplemental corn grain endosperm type (Taylor and Allen, 2005) interactions were undetected.

With approximately 10%-units greater ivNDFD for bm_3 compared to near-isogenic or conventional WPCS hybrids, DMI and TTNDFD responses were, respectively, 2.1 kg/d per cow and 1.8%-units (Oba and Allen, 1999), 0.8-1.4 kg/d per cow and non-significant (Oba and Allen, 2000), and 0.9 kg/d per cow and 2.5%-units (meta-analysis by Ferraretto and Shaver, 2015). Furthermore, Oba and Allen (1999) observed a negative linear relationship between DMI and TTNDFD responses for bm_3 WPCS, which was likely related to a faster passage rate through the rumen associated with greater DMI (NRC, 2001), with the regression indicating a zero TTNDFD response at a 3 kg/d per cow DMI response.

Tine et al. (2001) fed bm_3 WPCS TMR ad libitum or restricted to the DMI of the TMR containing near-isogenic WPCS to lactating dairy cows, while dry cows were fed bm_3 and near-isogenic WPCS TMR at maintenance intake levels. For dry cows, TTNDFD was 10%-units

greater for the bm_3 diet, while for the lactating cows TTNDFD was 9%-units or 7%-units greater, respectively, for restricted-fed or ad libitum-fed cows compared to near-isogenic WPCS control diets. Averaged across treatments, TTNDFD was 67% in dry cows and 54% in lactating cows. Results from this study show a negative relationship between DMI and TTNDFD and TTNDFD response to bm_3 WPCS. While diet net energy for lactation (NE_L) concentrations were unaffected by treatment ($P > 0.10$), numerically diet NE_L content was 9% greater in dry cows, but only 2% greater in lactating cows, for bm3 compared to near-isogenic WPCS diets. In Tine et al. (2001), DMI and milk yield were 2.4 and 3.1 kg/d per cow, respectively, greater for cows fed bm_3 WPCS compared to cows fed near-isogenic WPCS.

It is evident that the milk yield response to greater ivNDFD in bm_3 WPCS derives primarily through increases in DMI. Based on this research, the MILK2006 update of the MILK2000 WPCS hybrid evaluation model included discounts for estimating the NE_L content of WPCS from predicted increases in DMI in response to greater ivNDFD, so that increases in estimated milk per ton in relationship to greater ivNDFD derive primarily through increases in DMI (Shaver, 2006; Shaver and Lauer, 2006). Prediction of DMI by NRC (2001), however, is not influenced by diet composition or forage ivNDFD.

From a meta-analysis, Ferraretto and Shaver (2015) reported 7%-unit and 2%-unit reductions in vivo for ruminal (**RSD**) and total tract (**TTSD**) starch digestibility, respectively, in bm_3 compared to near-isogenic or conventional WPCS hybrids. Compared to leafy hybrids, TTSD was 5%-units lower for bm_3 WPCS hybrids. Reduced starch digestibility for bm_3 WPCS hybrids could be due to greater kernel vitreousness (Fish, 2010; Glenn, 2013) and/or faster passage rate through the digestive tract associated with increased DMI (NRC, 2001; Ferraretto et al., 2013). Ferraretto et al. (2015a) reported 5%-units greater TTSD for lactating dairy cows fed

an experimental floury-leafy WPCS hybrid compared to cows fed a bm_3 WPCS hybrid that appeared related to reduced kernel vitreousness and greater WPCS ruminal ivSD (7 h) and in situ (12 h) starch digestibility for the floury-leafy hybrid. However, ivNDFD (30 h), DMI and milk yield were 11%-units, 1.7 kg/d per cow and 2.2 kg/d per cow, respectively, greater for the bm_3 WPCS treatment. In agreement with previously discussed trials, TTNDFD was similar for the 2 diets despite the large ivNDFD difference between the WPCS treatments. Greater ivNDFD, DMI and milk yield for a bm_3 WPCS hybrid compared to an experimental floury-leafy WPCS hybrid has also been reported by Morrison et al. (2014).

These results underscore the importance of ivNDFD for WPCS hybrid selection from the standpoint of DMI and milk yield responses, and when attempting to incorporate parameters associated with greater starch digestibility into new WPCS hybrids. For example, improving starch digestibility of bm_3 hybrids through genetics appears to be a logical WPCS hybrid development strategy.

Ferraretto and Shaver (2012a), from a meta-analysis of WPCS trials with lactating dairy cows, reported the following: processing (1 to 3 mm roll gap) increased diet TTSD compared to 4 to 8 mm processed and unprocessed WPCS; processing increased TTSD for diets containing WPCS with 32% - 40% DM; processing increased diet TTSD when length of chop was set for 0.93 - 2.86 cm. Ferraretto and Shaver (2012b) and Vanderwerff et al. (2015) reported greater TTSD in lactating dairy cows fed Shredlage™ compared to conventional-processed WPCS. Clearly, physical form of WPCS affects starch digestibility. Grinding incubation samples for in vitro or in situ analysis through a common screen (e.g. 4 or 6 mm) may mask differences in particle size among WPCS that impact starch digestibility. Furthermore, incorporating measures

of starch digestibility into WPCS hybrid selection is difficult because starch digestibility increases over time in storage (Ferraretto et al., 2015b).

Dietary Starch and Forage NDF

Presented in Figure 1 (meta-analysis by Ferraretto et al., 2013) is the effect of dietary starch concentration on fiber digestibility. Increased dietary starch concentration reduced ruminal NDFD in vivo ($P = 0.01$) and TTNDFD ($P = 0.001$). The digestibility of dietary NDF decreased 0.61%-units ruminally and 0.48%-units total-tract per %-unit increase in dietary starch content. Decreased fiber digestibility may be partially explained by a decrease in rumen pH as a consequence of greater amounts of starch (kg/d) being digested in the rumen as starch intake increases. Low rumen pH is known to affect microbial growth and bacterial adherence and thereby fiber digestion. Also, the inherently high fiber digestibility of non-forage fibrous by-products used to partially replace corn grain in reduced-starch diets may be partly responsible.

Weiss (2014; unpublished from 28th ADSADiscover Conf. in Starch for Ruminants) used the slope of the Ferraretto et al. (2013) in Figure 1, or 0.5%-unit change in TTNDF for each 1%-unit change in dietary starch content, to calculate effects on dietary energy values. In the Weiss (2014) example, a 5%-unit increase in dietary starch content (e.g. 30% vs. 25%) reduced TTNDF 2.5%-units (46.5% to 44.0%) which resulted in a 5.3% increase in diet NE_L content compared to a 6.5% increase had TTNDFD not been adversely affected by increased dietary starch content. Greater TTSD (>90%) than TTNDFD (<50%) tempers the negative impact on diet NE_L content of reduced TTNDFD with greater dietary starch concentrations.

Effects of dietary forage-NDF concentration on nutrient digestibilities were reported in the meta-analysis of Ferraretto et al. (2013). Fiber digestibility was unaffected by FNDF concentration in the diet either ruminally or total-tract. Similar results were reported by Zebeli et

al. (2006). Furthermore, starch digestibility decreased only 0.17%-units per %-unit increase in dietary FNDF total-tract ($P = 0.05$), but not ruminally (Ferraretto et al., 2013). Thus, if dietary starch and total NDF concentrations are held constant, the primary effect of dietary FNDF was on DMI ($P = 0.04$) with a 0.17 kg/d per cow decrease in DMI per 1%-unit increase in dietary FNDF (Ferraretto et al., 2013). For example, a 3%-unit increase in dietary FNDF (25% vs. 22%, DM basis) would result in a 0.51 kg/d per cow decrease in DMI.

Site of Starch Digestion

Relationships between ruminal, post-ruminal and total-tract starch digestibilities from the meta-analysis by Ferraretto et al. (2013) are presented in Figures 2 and 3. The RSD and TTSD were related positively ($P = 0.04$; Figure 2), with an increase of 0.19%-units total-tract per %-unit increase ruminally. Post-ruminal starch digestibility measured as percentage of flow to the duodenum was positively related to TTSD ($P = 0.001$; Figure 3). In feedstuffs with a high proportion of rumen-digested starch, e.g. corn silage or high-moisture corn, in vitro or in situ measurement of starch digestibility may be a useful predictor of TTSD if particle size differences among test feeds were not masked by grinding of the incubation samples to a similar particle size.

Conclusions

Generally, lab analyses related to starch and NDF digestibilities should be viewed as relative index values for comparison among feeds/diets or over time within feeds/diets, rather than as predictors of in vivo digestibility.

The milk yield response to greater ivNDFD in bm_3 WPCS derives primarily through greater DMI rather than diet TTNDFD or NE_L content. Reduced RSD and TTSD in bm_3

compared to near-isogenic or conventional WPCS hybrids suggests potential for genetic improvement of bm_3 hybrids with a more floury-type endosperm.

Grinding incubation samples for in vitro or in situ analysis may mask differences in particle size among WPCS that impact starch digestibility, and incorporating measures of starch digestibility into WPCS hybrid selection is difficult because of ensiling effects on starch digestibility.

Increased concentrations of dietary starch decrease fiber digestibility. The negative effect, however, on calculated diet NE_L content is not large, and thus still favors higher starch diets. Comparisons among sites of starch digestion indicate that greater ruminal starch digestibility increases starch digestibility in the total-tract. However, the proportion of starch digested post-ruminally can be high for some feedstuffs and diets which would go undetected by rumen in vitro or in situ starch digestibility measurements.

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Table 1. Survey of websites and sample reports of 4 major dairy feed testing labs in USA for analyses related to starch and NDF digestibilities.

NDF; NDF_{OM}; Lignin; uNDF (Lignin × 2.4)
Starch; Prolamin; Ammonia; Particle Size; UW Feed Grain Evaluation; Processing Score
TMR-D; Rumen in vitro total tract NDFD (Combs-ivttNDFD)
Traditional (Goering – Van Soest) NDFD; Standardized (Combs – Goeser) NDFD
NDF k_d calculated from 24, 30, 48, 120-h NDFD (Combs – Goeser)
NDF k_d Mertens; NDF k_d Van Amburgh
24-h NDFD; calculated B_2/B_3 kd
30, 120, 240-h NDFD – forages; 12, 72, 120-h NDFD – byproducts
4, 8, 12, 24, 48, 72, 120, 240-h NDFD lag, pools & rates
120-h uNDF; 240-h uNDF
3-h, 7-h Rumen in vitro or in situ starch digestibility (ivRSD); k_d
Fecal Starch; Dietary Total Tract Starch Digestibility (TTSD)
Fermentrics™ (gas production system)
Calibrate™

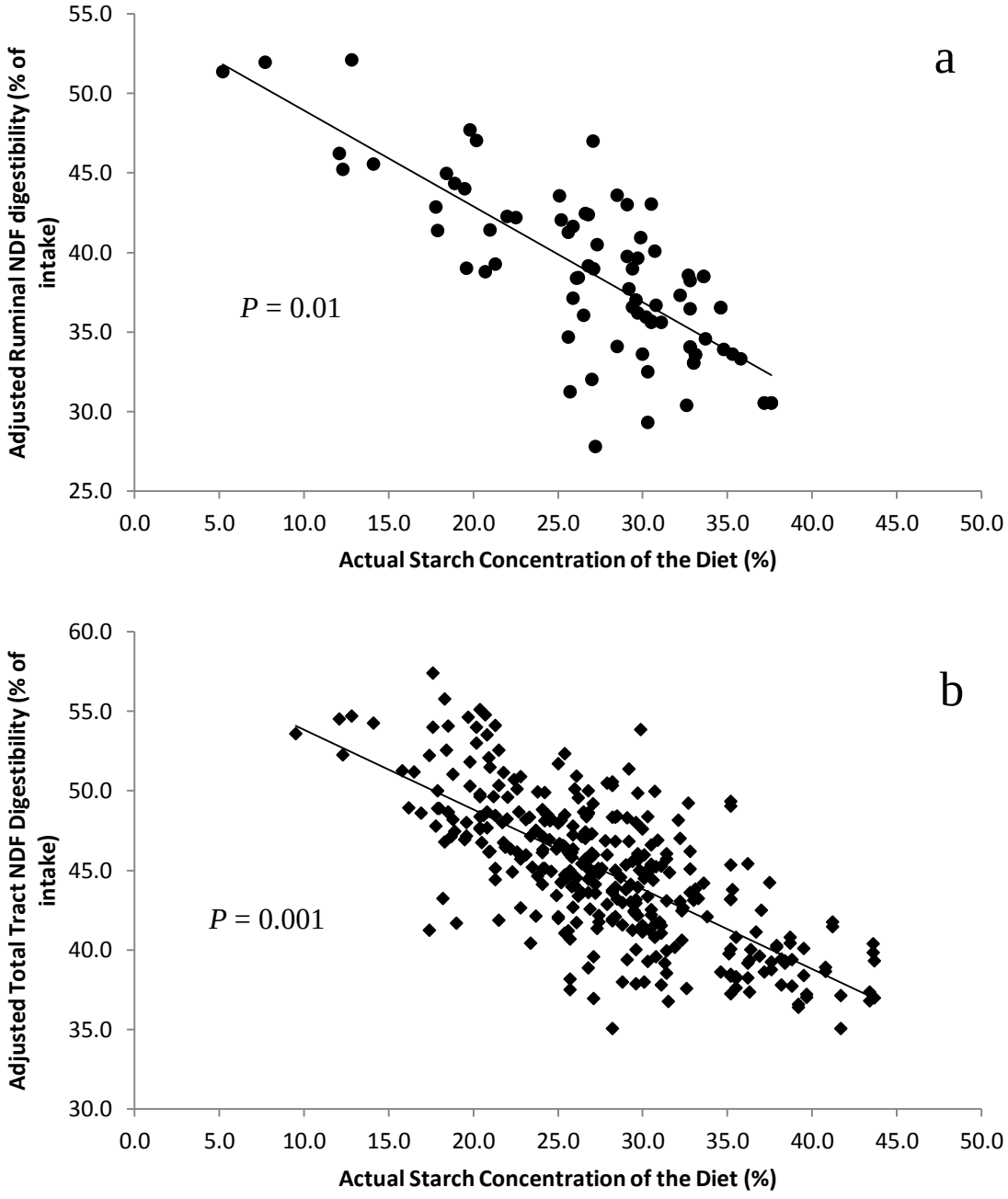

Figure 1. Effect of starch concentration of the diet on ruminal and total-tract digestibility of diet NDF adjusted for the random effect of trial. Ruminal digestibility data (Panel a) predicted from equation: y = 54.9746 + (-0.605*starch concentration) + (0.063 $\pm$ 3.524); n = 70, RMSE = 3.55. Total-tract digestibility diet (Panel b) predicted from equation: y = 58.2843 + (-0.4817*starch concentration) + (0.059 $\pm$ 3.191); n = 320, RMSE = 3.20. Ferraretto et al., 2013.

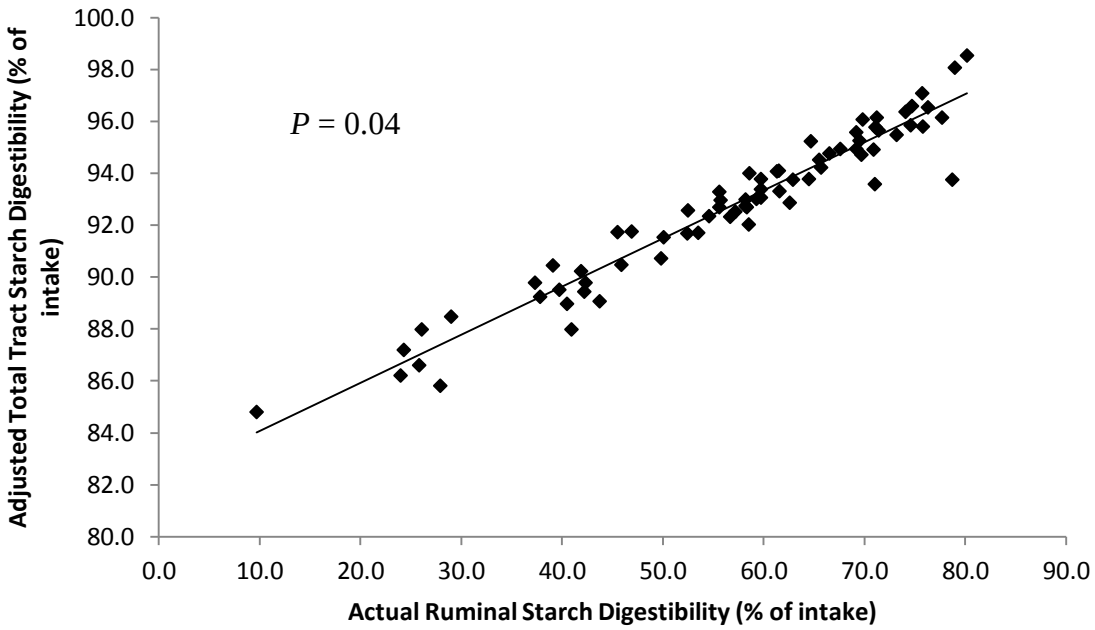

Figure 2. Relationship between ruminal and total-tract starch digestibility adjusted for the random effect of trial. Prediction equation: y = 82.224 + (0.185*ruminal) + (-0.002 $\pm$ 0.772); n = 72, RMSE = 0.78. Ferraretto et al., 2013.

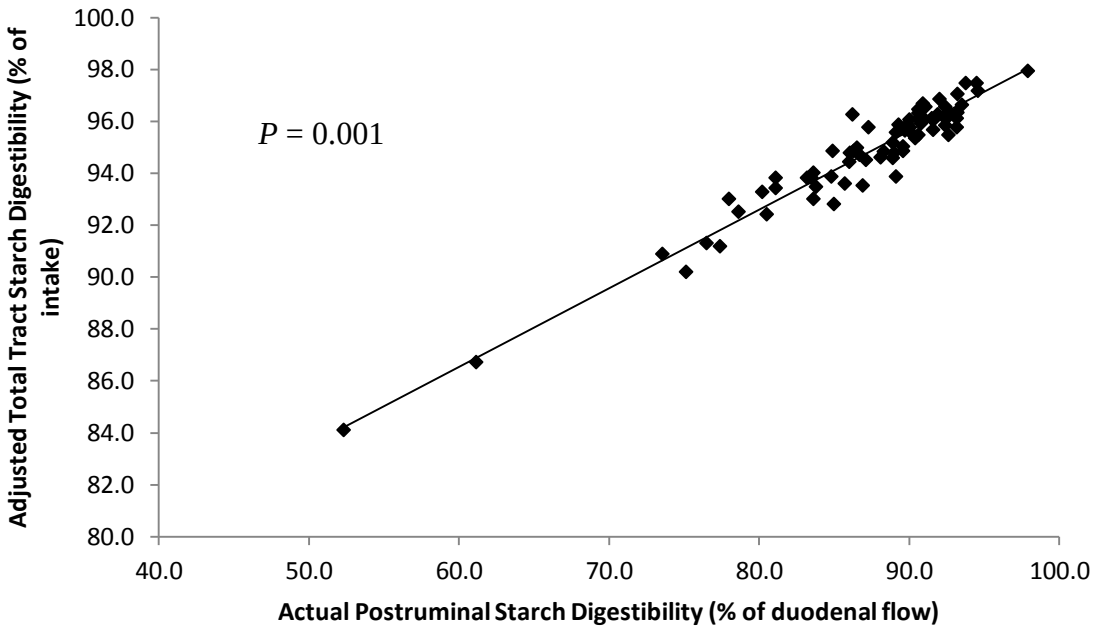

Figure 3. Relationship between postruminal starch digestibility as a percentage of duodenal flow and total-tract starch digestibility adjusted for the random effect of trial. Prediction equation: y = 68.287 + (0.304*postruminal % of flow) + (0.013 $\pm$ 0.574); n = 72, RMSE = 0.58. Ferraretto et al., 2013.