Technical note: concentrations of soluble, insoluble, and total dietary fiber in feed ingredients determined using Method AOAC 991.43 are not different from values determined using Method AOAC 2011.43 with the AnkomTDF Dietary Fiber Analyzer

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ABSTRACT: The primary objective of this experiment was to test the hypothesis that concentrations of soluble (**SDF**), insoluble (**IDF**), and total dietary fiber (**TDF**) in feed ingredients used in diets for pigs and poultry analyzed using Method AOAC 2011.25 are greater than values determined using Method AOAC 991.43. A second objective was to determine the variation that may exist among 3 laboratories using the 2 methods with the Ankom[TDF] Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). The 3 laboratories were the Ministry of Agriculture Feed Industry Center (MAFIC) at China Agricultural University, Trouw Nutrition, and Hans H. Stein Monogastric Nutrition Laboratory at University of Illinois at Urbana-Champaign (UIUC). All laboratories analyzed SDF and IDF in feed ingredients in duplicate or triplicate using both methods AOAC 991.43 and 2011.25 with the Ankom[TDF] Dietary Fiber Analyzer. The 9 test ingredients were wheat, soybean meal, rapeseed meal, sugar beet pulp, peas, horse beans, native pea starch, and 2 samples of corn; 1 from Europe and 1 from China. All ingredient samples, with the exception of Chinese corn, were procured by Trouw Nutrition, ground to pass through a 0.5 mm screen, subsampled, and sent to MAFIC and UIUC. Data were analyzed using SDF, IDF, and TDF as response variables, replication as random effect, and method and location as fixed effects over all ingredients and within each ingredient. When averaged among 9 different ingredients, results indicated that SDF, IDF, and TDF values were not different with either method or at any laboratory. However, the concentration of IDF in corn, wheat, peas, and sugar beet pulp determined using Method AOAC 991.43 was greater ($P < 0.05$) compared with 2011.25. Soluble dietary fiber determined using Method AOAC 2011.25 was greater ($P < 0.05$) in corn, rapeseed meal, soybean meal, and sugar beet pulp compared with 991.43. There was no difference in TDF determined with either method, except for wheat having greater ($P < 0.05$) TDF when determined using Method AOAC 991.43. Interlaboratory variation for SDF, IDF, and TDF was 0.38, 0.87, 1.20, respectively, with Method AOAC 991.43 and 0.40, 0.93, and 1.27, respectively, with 2011.25. Therefore, values determined with the Ankom[TDF] Analyzer are repeatable among laboratories and can be used in feed formulation worldwide. In conclusion, it is recommended that Method AOAC 991.43 be used to determine SDF, IDF, and TDF in feed ingredients and diets for pigs and poultry.

Key words: feed analysis, insoluble dietary fiber, soluble dietary fiber, total dietary fiber

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INTRODUCTION

Total dietary fiber (**TDF**) in animal nutrition is best defined as carbohydrates and lignin resistant to enzymatic digestion by mammalian endogenous enzymes (Trowell, 1976). Resistant starch is not digested by endogenous enzymes of pigs and poultry and, therefore, should be classified as TDF if the previous definition is applied. It was demonstrated that resistant starch is a relevant component in swine and poultry feed ingredients and should be used in diet formulation and energy evaluation to best predict animal performance (Rojas and Stein, 2016; Karunaratne et al., 2018).

Total dietary fiber in animal nutrition is not often determined because it is laborious and its relationship with animal performance is not well documented. When TDF is reported, Method AOAC 991.43 (AOAC, 2007; Lee et al., 1992) is most often utilized to analyze feed ingredients and diets for insoluble dietary fiber (**IDF**) and TDF and the difference between TDF and IDF is reported as soluble dietary fiber (**SDF**). Recently, values for IDF, SDF, and TDF in feed ingredients and diets have been reported using Method AOAC 991.43 with the Ankom^{TDF} Dietary Fiber Analyzer (Jaworski and Stein, 2017). The instrument is used to reduce the laborious nature of the analysis and the only difference in Method AOAC 991.43 on the analyzer is that IDF and SDF are directly analyzed and TDF is calculated as the sum of IDF and SDF. It is known, however, that resistant starch is not analyzed in Method AOAC 991.43 and, as stated above, the definition of dietary fiber includes resistant starch. Therefore, Method AOAC 2011.25 (McCleary et al., 2012) was developed to include resistant starch in the analysis of TDF with the option to include the analysis of low-molecular weight sugars (i.e., nondigestible oligosaccharides) using HPLC. The option to determine IDF, SDF, and TDF using Method AOAC 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer is also available, but, to our knowledge, has not been used to analyze feed ingredients for pigs and poultry.

The aim of Method AOAC 2011.25 is to provide similar digestion conditions to those found in vivo and, therefore, TDF may be better quantified because resistant starch may be included in the analysis. However, the quantity of resistant starch present in feed ingredients for pigs and poultry is not well defined and is typically low (Cervantes-Pahm et al., 2014). Also, the sensitivity of Method AOAC 2011.25 may not be great enough to detect the low quantity of resistant starch present in feed ingredients. Therefore, an experiment was conducted to test the hypothesis that concentrations of SDF, IDF, and TDF in feed ingredients used in diets for pigs and poultry analyzed using Method AOAC 2011.25 are greater than values determined using Method AOAC 991.43. Also, the reproducibility of results determined using the Ankom^{TDF} Dietary Fiber Analyzer have not been published among animal nutrition laboratories and, therefore, a secondary objective of this study was to compare results obtained at different laboratories.

MATERIALS AND METHODS

The 3 laboratories that participated in the experiment were the Ministry of Agriculture Feed Industry Center (**MAFIC**) at the China Agricultural University (Beijing, China), Trouw Nutrition (Boxmeer, the Netherlands), and the Hans H. Stein Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign (**UIUC**). All laboratories analyzed SDF and IDF in feed ingredients using both methods AOAC 991.43 and 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY) and TDF was calculated as the sum of SDF and IDF. Low-molecular weight sugars (i.e., nondigestible oligosaccharides) were not determined in filtrate from Method AOAC 2011.25 and, therefore, SDF values do not include this fraction in either method.

The 9 test ingredients were wheat, soybean meal, rapeseed meal, sugar beet pulp, peas, horse beans, native pea starch, and 2 samples of corn; 1 from Europe and 1 from China. All ingredient samples, with the exception of the Chinese corn and soybean meal, which originated in Brazil, were produced in Europe and procured by Trouw Nutrition. All ingredients were ground to pass through a 0.5-mm screen, subsampled, and sent to MAFIC and UIUC. Corn, wheat, and soybean meal were selected because they are commonly used in swine and poultry diets worldwide. Peas, horse beans, and native pea starch were selected because they contain greater concentrations of resistant starch compared with corn, wheat, and soybean meal. Rapeseed meal was selected because it has a high concentration of dietary fiber, especially IDF, compared with the other ingredients and sugar beet pulp was selected because it has a high concentration of SDF.

Laboratory UIUC analyzed each ingredient 3 times in duplicate according to Method AOAC 991.43 and 2011.25. Trouw Nutrition analyzed each ingredient at least 6 times in duplicate according to both methods in order to establish internal laboratory standards. Also, the IDF residues from 3 replicates were removed from the Ankom IDF bags after analysis, the residues were pooled, and the concentration of total starch (ISO 15914:2004) was determined in the IDF residue. Laboratory MAFIC analyzed each ingredient 3 times in duplicate according to Method AOAC 991.43 and 2 times in duplicate according to Method AOAC 2011.25 due to the lack of sample to analyze 3 times. Resistant starch was determined in feed ingredients at Trouw Nutrition as any starch (International Organization for Standardization, 2004) not digested within a 6 h in vitro digestibility assay adapted from Boisen and Fernández (1997) and Englyst et al. (1996).

Homogeneity of the variance for each laboratory, method, and within each ingredient for determined SDF, IDF, and TDF values were confirmed using ESTIMATES option in the COVTEST statement of SAS (SAS Inst. Inc., Cary, NC) using the GLIMMIX procedure. Response variables (SDF, IDF, and TDF) were analyzed using the GLIMMIX procedure using replication as the random effect and method and laboratory as fixed effects over all ingredients and within each ingredient. Means were calculated using the LSMEANS statement and confidence intervals using the CI statement in SAS and differences were evaluated using the Tukey test. A P-value ≤ 0.05 was used to determine significance for all outcomes.

RESULTS

Effect of Method

Values determined for SDF, IDF, and TDF were not different ($P > 0.05$) between method AOAC 991.43 and 2011.25 or the 3 laboratories over all 9 ingredients analyzed (Table 1). The SEM was 0.40, 0.93, and 1.27 for SDF, IDF, and TDF, respectively, determined using Method AOAC 2011.25, while the SEM was slightly less using Method AOAC 991.43 and was 0.38, 0.87, and 1.20 for SDF, IDF, and TDF.

The concentration of SDF determined using Method AOAC 2011.25 was greater ($P < 0.05$) in corn, rapeseed meal, soybean meal, and sugar beet pulp compared with values determined using Method AOAC 991.43 (Table 2). Corn, wheat, peas, and sugar beet pulp all had greater ($P < 0.05$) concentrations of IDF determined using Method AOAC 991.43 compared with 2011.25. Total dietary fiber was greater ($P < 0.05$) in wheat using Method AOAC 991.43 compared with 2011.25. The variation in SDF analyzed using Method AOAC 991.43 was larger in peas and rapeseed meal compared with Method AOAC 2011.25 (Fig. 1), but variation in SDF analyzed using Method AOAC 2011.25 was larger in Chinese corn, native pea starch, soybean meal, and wheat. Variation in IDF values determined with Method AOAC 2011.25 was larger in soybean meal and sugar beet pulp, whereas variation in IDF values determined with Method AOAC 991.43 were larger for both corn samples, peas, and rapeseed meal (Fig. 2). Variation in TDF values for both corn samples were greater when determined with Method AOAC 991.43 compared with 2011.25, whereas variation in TDF values determined with Method AOAC 2011.25 was only larger for soybean meal compared with Method AOAC 991.43 (Fig. 3).

Table 1. Difference and confidence interval of the differences between methods and among laboratories of analyzed quantities of soluble dietary fiber, insoluble dietary fiber, and total dietary fiber (as-fed basis)

	Soluble dietary fiber	Insoluble dietary fiber	Total dietary fiber
Method[1]			
991.43 vs. 2011.25	−0.62 [−1.71, 0.47]	1.00 [−1.51, 3.51]	0.72 [−2.72, 4.16]
Laboratory[1]			
MAFIC vs. Trouw Nutrition	0.57 [−1.15, 2.24]	−1.28 [−5.20, 2.63]	−0.94 [−6.32, 4.44]
UIUC vs. Trouw Nutrition	0.50 [−1.09, 2.10]	0.30 [−3.39, 3.99]	0.49 [−4.54, 5.52]

[1]Results expressed as mean difference [95% confidence interval of the mean difference]. Soluble, insoluble, and total dietary fiber were not different ($P > 0.05$) between methods and laboratories over all 9 ingredients analyzed.

Table 2. Soluble, insoluble, and total dietary fiber analyzed in 9 feed ingredients using 2 different methods (as-fed basis)[1]

Ingredient	Method	*n*	SDF[2], %	IDF[2], %	TDF[2], %
Corn	991.43	14	0.39[b] [0.20, 0.57]	9.63[a] [8.93, 10.32]	9.87 [9.24, 10.50]
	2011.25	11	0.68[a] [0.47, 0.89]	8.49[b] [7.94, 9.05]	9.17 [8.46, 9.88]
Corn, China	991.43	10	0.37 [−0.01, 0.75]	10.85 [8.91, 12.79]	11.62 [9.74, 13.50]
	2011.25	11	0.67 [0.31, 1.03]	10.95 [9.14, 12.75]	11.62 [9.83, 13.41]
Wheat	991.43	15	1.02 [0.62, 1.42]	10.05[a] [9.60, 10.51]	11.08[a] [10.34, 11.82]
	2011.25	13	1.44 [1.02, 1.86]	8.68[b] [8.23, 9.14]	10.23[b] [9.49, 10.96]
Horse beans	991.43	15	2.06 [1.74, 2.38]	17.51 [16.64, 18.37]	19.57 [18.93, 20.21]
	2011.25	11	1.78 [1.32, 2.25]	17.17 [16.08, 18.27]	18.95 [18.08, 19.82]
Peas	991.43	18	0.51 [−0.02, 1.04]	16.08[a] [15.39, 16.77]	16.80 [16.25, 17.34]
	2011.25	11	0.79 [0.14, 1.45]	14.83[b] [14.09, 15.56]	16.03 [15.33, 16.72]
Native pea starch	991.43	10	−0.21 [−0.62, 0.20]	1.07 [0.66, 1.47]	0.90 [0.47, 1.33]
	2011.25	12	−0.02 [−0.37, 0.34]	0.43 [0.03, 0.84]	0.52 [0.13, 0.91]
Rapeseed meal	991.43	12	2.54[b] [2.06, 3.03]	29.63 [28.81, 30.46]	32.18 [31.55, 32.80]
	2011.25	11	3.64[a] [3.52, 3.75]	29.25 [28.55, 29.94]	32.88 [32.23, 33.53]
Soybean meal	991.43	10	1.42[b] [1.07, 1.77]	15.15 [14.65, 15.64]	16.70 [16.09, 17.31]
	2011.25	14	2.26[a] [1.95, 2.57]	15.00 [14.56, 15.43]	17.26 [16.74, 17.78]
Sugar beet pulp	991.43	12	12.83[b] [11.76, 13.91]	35.62[a] [34.34, 36.89]	48.45 [47.59, 49.31]
	2011.25	11	15.86[a] [14.81, 16.91]	31.72[b] [29.77, 33.67]	47.58 [46.69, 48.48]

[1]Results expressed as mean [95% confidence interval].

[2]SDF = soluble dietary fiber, IDF = insoluble dietary fiber, TDF = total dietary fiber.

[a,b]Different letters within ingredient and column indicate a significant difference between methods ($P < 0.05$).

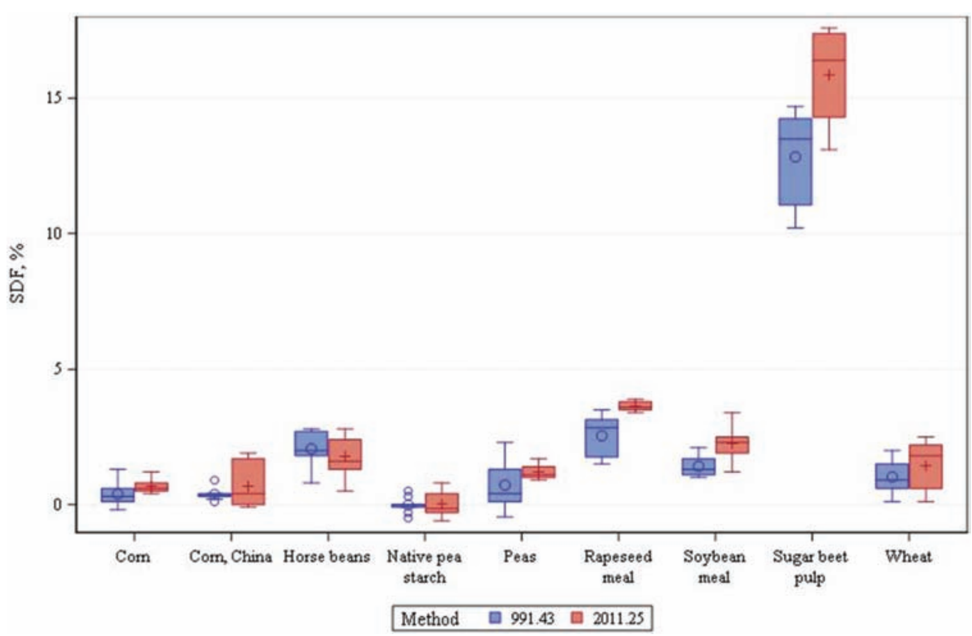

Figure 1. Soluble dietary fiber (SDF) analyzed in 9 feed ingredients using 2 methods

The analyzed concentration of total starch determined in the IDF residue was not different between methods and was lower compared to the quantity of resistant starch determined in the feed ingredients (Table 3).

Effect of Laboratory

The concentration of SDF determined with Method AOAC 991.43 in wheat at MAFIC was 1.80%, which was greater ($P < 0.05$) compared with 0.71% determined at Trouw Nutrition, while UIUC determined the SDF in wheat to be 1.17%, which was not different from either laboratory (Table 4; Fig. 4). Also, the SDF determined in wheat with Method AOAC 2011.25 was 2.35 and 1.66% at MAFIC and Trouw Nutrition, respectively, and these were greater ($P < 0.05$) compared with 0.17% SDF determined at UIUC. Soluble dietary fiber in horse beans determined with Method AOAC

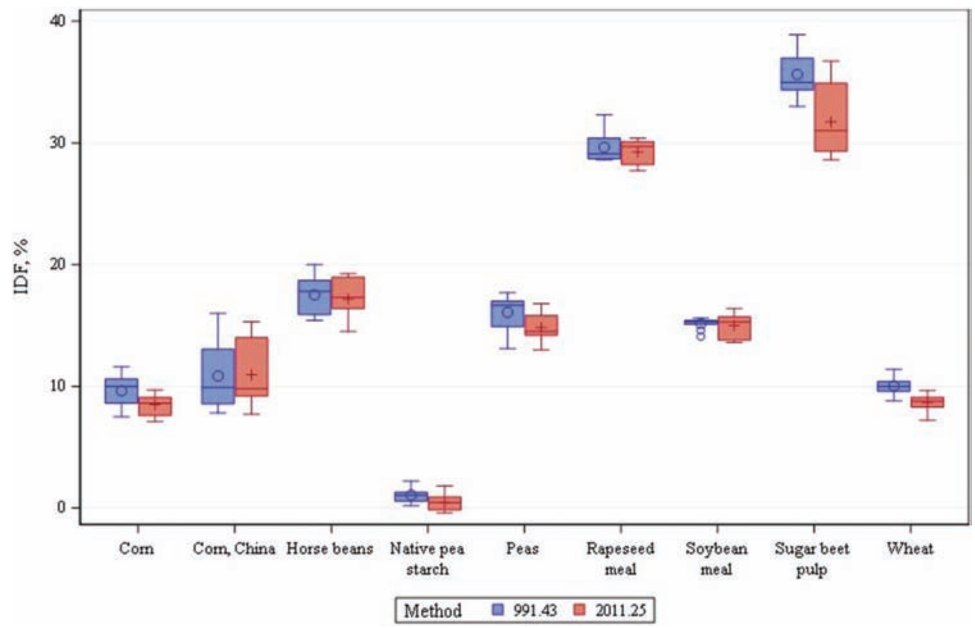

Figure 2. Insoluble dietary fiber (IDF) analyzed in 9 feed ingredients using 2 methods

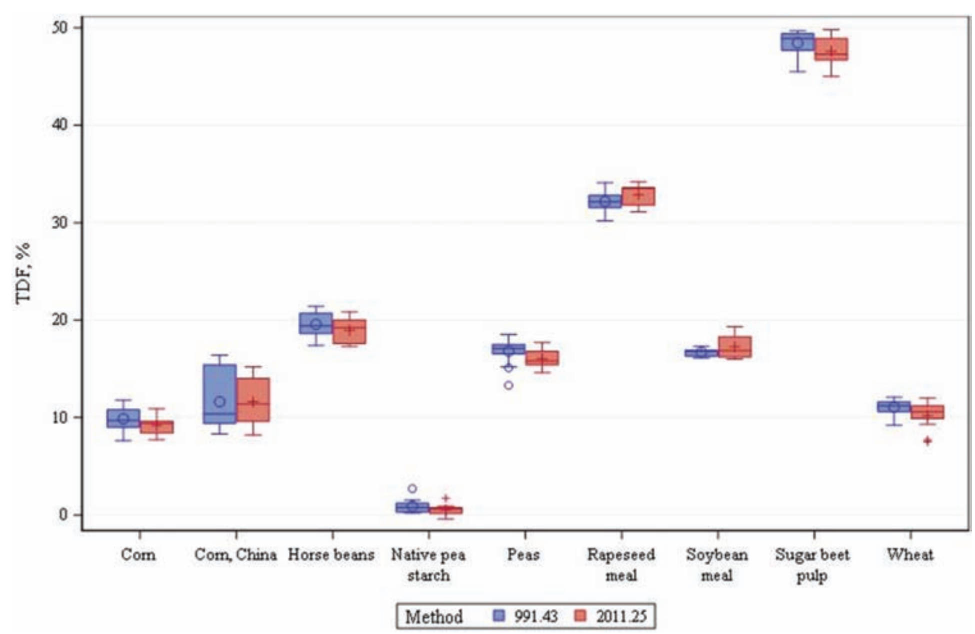

Figure 3. Total dietary fiber (TDF) analyzed in 9 feed ingredients using 2 methods

2011.25 was greater ($P < 0.05$) at MAFIC and UIUC compared with Trouw Nutrition. Peas had 1.65% SDF determined at MAFIC with Method AOAC 2011.25 which was greater ($P < 0.05$) compared with 1.10% SDF in peas determined at Trouw Nutrition and UIUC. The quantity of SDF determined in native pea starch using Method AOAC 2011.25 at UIUC was the greatest ($P < 0.05$) and at Trouw Nutrition the least, while the value determined at MAFIC was less than UIUC, but greater than Trouw Nutrition. The concentration of SDF in sugar beet pulp determined by Method 2011.25 at UIUC was 17.47%, which was greater ($P < 0.05$) compared with Trouw Nutrition (14.85%) and the value (16.50%) determined at MAFIC was not different from either lab.

Trouw Nutrition and UIUC determined greater ($P < 0.05$) concentrations of IDF in corn using both methods and in peas using Method AOAC 991.43 compared with MAFIC (Table 5; Fig. 5). The IDF in corn from China was greatest ($P < 0.05$) at UIUC, intermediate at Trouw Nutrition, and the least at MAFIC analyzed with either method. Trouw Nutrition analyzed greater ($P < 0.05$) IDF values for wheat compared with UIUC using either method and with MAFIC using

Table 3. Analyzed concentration of starch and resistant starch in ingredients and starch remaining in the insoluble dietary fiber (IDF) residue (as-fed basis)

	DM, %	Total starch, %	Resistant starch, %		Starch in IDF residue, %	
Ingredient, %				Method:	991.43	2011.25
Corn	86.50	66.01	5.35		3.90	3.20
Corn, China	87.70	63.40	6.40		NA	NA
Wheat	85.10	59.70	1.65		0.60	0.50
Horse beans	88.90	37.88	5.92		1.30	1.30
Peas	91.00	43.91	16.83		5.80	5.40
Native pea starch	90.60	89.19	42.73		NA[1]	NA
Rapeseed meal	88.40	NA	NA		NA	NA
Soybean meal	88.40	NA	NA		NA	NA
Sugar beet pulp	91.40	2.30	NA		NA	NA

[1]NA = not analyzed.

Table 4. Soluble dietary fiber analyzed in 9 feed ingredients at 3 laboratories (as-fed basis)[1]

		Soluble dietary fiber, %		
Ingredient	Method	MAFIC	Trouw Nutrition	UIUC
Corn	991.43	0.30 [−0.29, 0.89]	0.32 [−0.08, 0.72]	0.30 [−0.29, 0.89]
	2011.25	0.49 [0.10, 0.89]	0.73 [0.48, 0.98]	0.70 [0.39, 1.01]
Corn, China	991.43	0.35 [−0.07, 0.76]	0.42 [0.14, 0.70]	0.30 [−0.02, 0.62]
	2011.25	0.45 [−0.52, 1.42]	1.12 [0.56, 1.68]	−0.07 [−0.86, 0.73]
Wheat	991.43	1.80[a] [1.31, 2.29]	0.71[b] [0.43, 1.00]	1.17[ab] [0.67, 1.66]
	2011.25	2.35[a] [1.40, 3.30]	1.66[a] [1.21, 2.11]	0.17[b] [−0.61, 0.94]
Horse beans	991.43	2.33 [1.59, 3.08]	2.04 [1.61, 2.48]	1.83 [1.09, 2.58]
	2011.25	2.70[a] [2.13, 3.27]	1.27[b] [0.94, 1.60]	2.20[a] [1.74, 2.66]
Peas	991.43	0.83 [−0.12, 1.78]	0.49 [0.02, 0.97]	1.50 [0.55, 2.45]
	2011.25	1.65[a] [1.39, 1.91]	1.10[b] [0.95, 1.25]	1.10[b] [0.89, 1.31]
Native pea starch	991.43	0.00 [−0.41, 0.41]	0.05 [−0.31, 0.41]	−0.13 [−0.55, 0.28]
	2011.25	0.24[b] [0.02, 0.47]	−0.25[c] [−0.41, −0.11]	0.64[a] [0.45, 0.83]
Rapeseed meal	991.43	3.30 [2.44, 4.16]	2.40 [1.79, 3.01]	2.07 [1.21, 2.92]
	2011.25	3.65 [3.33, 3.97]	3.63 [3.27, 3.99]	3.63 [3.31, 3.96]
Soybean meal	991.43	1.10 [0.70, 1.50]	1.46 [1.15, 1.77]	1.67 [1.27, 2.07]
	2011.25	2.15 [1.02, 3.28]	2.22 [1.69, 2.76]	2.47 [1.54, 3.39]
Sugar beet pulp	991.43	12.70 [10.66, 14.74]	12.15 [10.71, 13.59]	14.33 [12.29, 16.37]
	2011.25	16.50[ab] [14.71, 18.29]	14.85[b] [13.81, 15.89]	17.47[a] [16.00, 18.93]

[1]Results expressed as mean [95% confidence interval].

[a,b]Different letters within a row indicate a significant difference between laboratories ($P < 0.05$).

Method AOAC 2011.25. Trouw Nutrition analyzed greater ($P < 0.05$) IDF values for horse beans and peas using Method AOAC 2011.25 and native pea starch using Method AOAC 991.43 compared with MAFIC, while UIUC values were not different from either laboratory. The IDF analyzed in rapeseed meal using Method AOAC 2011.25 was greatest ($P < 0.05$) at Trouw Nutrition, intermediate at UIUC, and the lowest at MAFIC. Trouw Nutrition analyzed greater ($P < 0.05$) IDF in soybean meal and sugar beet pulp using Method AOAC 2011.25 compared with MAFIC and UIUC.

Total dietary fiber determined in corn using either method was greater ($P < 0.05$) at Trouw Nutrition and UIUC compared with MAFIC (Table 6; Fig. 6). The TDF analyzed in Chinese corn and peas using Method AOAC 991.43 was greatest ($P < 0.05$) at UIUC, intermediate at Trouw Nutrition, and the lowest at MAFIC. Laboratory UIUC analyzed greater ($P < 0.05$) TDF in Chinese corn using Method AOAC 2011.25 compared with MAFIC and Trouw Nutrition. The TDF analyzed in wheat using Method AOAC 991.43 was greater ($P < 0.05$) at MAFIC compared with UIUC, but with Method AOAC 2011.25 the TDF analyzed in wheat was greater ($P < 0.05$) at MAFIC and Trouw Nutrition compared with UIUC. The TDF analyzed in rapeseed meal using Method AOAC 991.43

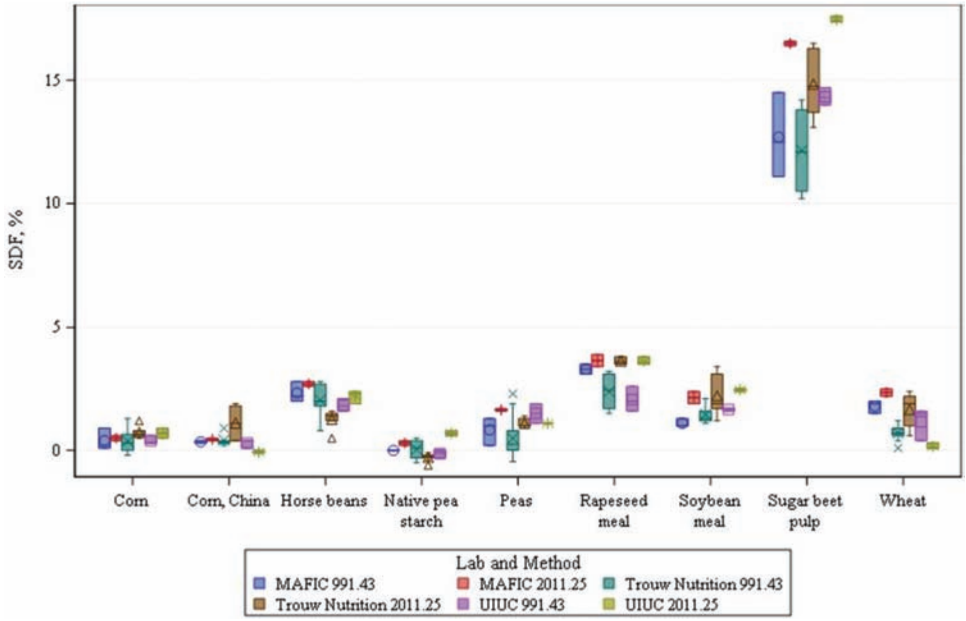

Figure 4. Soluble dietary fiber (SDF) analyzed in 9 feed ingredients at 3 laboratories using 2 methods

Table 5. Insoluble dietary fiber analyzed in 9 feed ingredients at 3 laboratories (as-fed basis)[1]

		Insoluble dietary fiber, %		
Ingredient	Method	MAFIC	Trouw Nutrition	UIUC
Corn	991.43	7.84[b] [6.70, 8.98]	9.85[a] [9.17, 10.52]	10.67[a] [9.53, 11.81]
	2011.25	7.20[b] [6.24, 8.17]	8.78[a] [8.23, 9.34]	8.77[a] [7.99, 9.55]
Corn, China	991.43	7.97[c] [7.17, 8.77]	9.90[b] [9.28, 10.52]	15.63[a] [14.83, 16.43]
	2011.25	7.80[c] [6.35, 9.25]	10.12[b] [9.28, 10.95]	14.70[a] [13.52, 15.88]
Wheat	991.43	9.93[ab] [9.38, 10.49]	10.39[a] [10.07, 10.71]	9.07[b] [8.51, 9.62]
	2011.25	8.27[b] [7.79, 8.75]	9.05[a] [8.65, 9.44]	7.59[b] [7.11, 8.07]
Horse beans	991.43	15.87 [14.09, 17.65]	17.96 [16.93, 18.98]	17.80 [16.02, 19.58]
	2011.25	14.60[b] [12.77, 16.43]	17.88[a] [16.82, 18.93]	17.47[ab] [15.97, 18.96]
Peas	991.43	13.70[b] [12.58, 14.82]	16.55[a] [15.99, 17.11]	16.57[a] [15.45, 17.68]
	2011.25	13.35[b] [12.03, 14.67]	15.43[a] [14.67, 16.20]	14.60[ab] [13.52, 15.68]
Native pea starch	991.43	0.27[b] [−0.31, 0.85]	1.48[a] [1.07, 1.89]	1.03[ab] [0.45, 1.61]
	2011.25	−0.21 [−1.10, 0.68]	0.88 [0.39, 1.36]	−0.18 [−0.87, 0.51]
Rapeseed meal	991.43	28.80 [27.38, 30.22]	30.45 [29.45, 31.45]	28.83 [27.42, 30.25]
	2011.25	27.66[c] [27.14, 28.18]	30.08[a] [29.58, 30.59]	28.57[b] [28.10, 29.03]
Soybean meal	991.43	15.30 [14.71, 15.89]	14.96 [14.51, 15.41]	15.30 [14.71, 15.89]
	2011.25	13.86[b] [13.12, 14.60]	15.56[a] [15.05, 16.07]	13.54[b] [12.90, 14.17]
Sugar beet pulp	991.43	34.30 [32.22, 36.38]	36.95 [35.48, 38.42]	34.27 [32.19, 36.35]
	2011.25	28.65[b] [25.91, 31.39]	33.87[a] [32.28, 35.45]	29.47[b] [27.23, 31.71]

[1]Results expressed as mean [95% confidence interval].

[a,b,c]Different letters within a row indicate a significant difference between laboratories ($P < 0.05$).

was greater ($P < 0.05$) at Trouw Nutrition compared with UIUC, but with Method AOAC 2011.25 the TDF was greatest ($P < 0.05$) at Trouw Nutrition, intermediate at UIUC, and least at MAFIC. Trouw Nutrition had greater ($P < 0.05$) TDF analyzed in sugar beet pulp using AOAC 991.43 compared with MAFIC and when Method AOAC 2011.25 was used Trouw Nutrition had greater ($P < 0.05$) TDF in sugar beet pulp compared with both MAFIC and UIUC.

DISCUSSION

In the present study, we found that concentrations of SDF, IDF, and TDF in 9 feed ingredients used in pig and poultry diets determined using Method AOAC 991.43 were not different than using Method AOAC 2011.25 with the Ankom[TDF] Dietary Fiber Analyzer. Also, the variation in the analysis of SDF, IDF, and TDF was determined to be low and acceptable among 3 animal nutrition

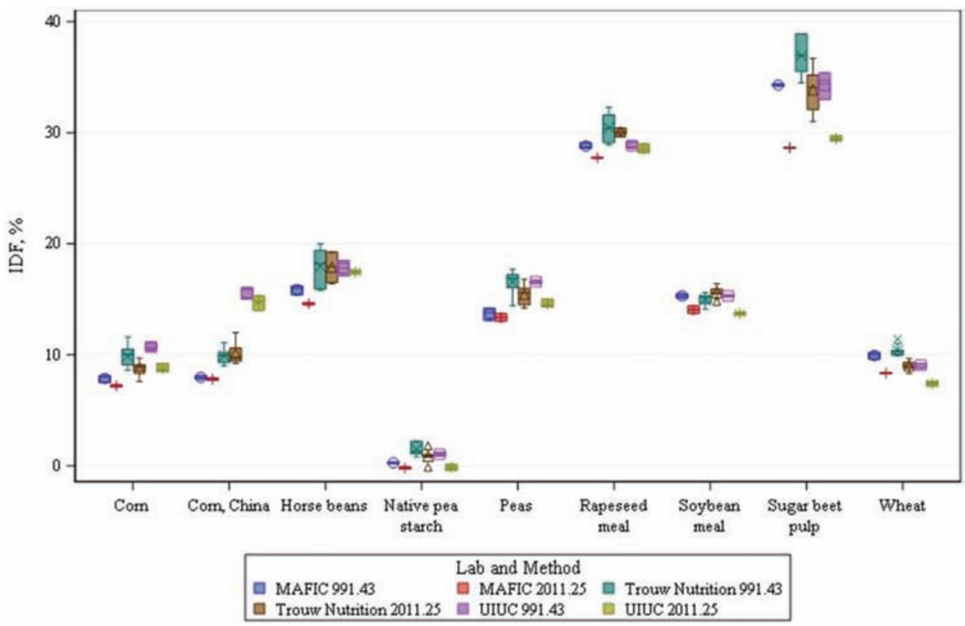

Figure 5. Insoluble dietary fiber (IDF) analyzed in 9 feed ingredients at 3 laboratories using 2 methods

Table 6. Total dietary fiber analyzed in 9 feed ingredients at 3 laboratories (as-fed basis)[1]

		Total dietary fiber, %		
Ingredient	Method	MAFIC	Trouw Nutrition	UIUC
Corn	991.43	8.27[b] [7.13, 9.40]	10.01[a] [9.32, 10.71]	11.10[a] [9.97, 12.23]
	2011.25	7.70[b] [6.63, 8.77]	9.52[a] [8.90, 10.13]	9.47[a] [8.59, 10.34]
Corn, China	991.43	8.63[c] [7.50, 9.76]	10.41[b] [9.17, 11.65]	15.93[a] [14.85, 17.01]
	2011.25	8.25[b] [6.05, 10.45]	11.23[b] [9.96, 12.50]	14.63[a] [12.84, 16.43]
Wheat	991.43	11.73[a] [10.93, 12.54]	11.10[ab] [10.64, 11.56]	10.23[b] [9.43, 11.04]
	2011.25	10.78[a] [9.19, 12.37]	10.73[a] [9.84, 11.61]	7.80[b] [6.21, 9.39]
Horse beans	991.43	18.20 [16.98, 19.42]	20.00 [19.29, 20.71]	19.63 [18.41, 20.86]
	2011.25	17.30 [15.51, 19.09]	19.15 [18.11, 20.18]	19.67 [18.21, 21.12]
Peas	991.43	14.53[c] [13.86, 15.21]	17.04[b] [16.71, 17.38]	18.07[a] [17.39, 18.74]
	2011.25	14.96 [13.60, 16.32]	16.53 [15.67, 17.39]	15.70 [14.68, 16.72]
Native pea starch	991.43	0.27 [−0.66, 1.19]	1.38 [0.57, 2.18]	0.90 [−0.03, 1.83]
	2011.25	0.02 [−0.94, 0.98]	0.67 [0.11, 1.22]	0.45 [−0.32, 1.21]
Rapeseed meal	991.43	32.10[ab] [31.25, 32.95]	32.85[a] [32.25, 33.45]	30.90[b] [30.05, 31.75]
	2011.25	31.29[c] [30.64, 31.94]	33.72[a] [33.03, 34.41]	32.20[b] [31.58, 32.82]
Soybean meal	991.43	16.40 [15.91, 16.89]	16.73 [16.30, 17.15]	16.97 [16.47, 17.46]
	2011.25	15.95 [14.27, 17.63]	17.75 [16.65, 18.85]	15.91 [14.52, 17.30]
Sugar beet pulp	991.43	47.00[b] [45.65, 48.35]	49.10[a] [47.93, 50.27]	48.60[ab] [47.25, 49.95]
	2011.25	45.15[b] [44.03, 46.27]	48.72[a] [48.07, 49.36]	46.93[b] [46.02, 47.85]

[1]Results expressed as mean [95% confidence interval].

[a,b,c]Different letters within a row indicate a significant difference between laboratories ($P < 0.05$).

laboratories using either Method AOAC 991.43 and 2011.25 with the Ankom[TDF] Dietary Fiber Analyzer over all ingredients, but variation amongst labs were larger for some ingredients using either method.

Concentrations of SDF, IDF, and TDF determined for soybean meal by Method AOAC 991.43 using the Ankom[TDF] Analyzer were in agreement (within the 95% CI for soybean meal values in this study) with previously determined values using the same method and analyzer (Jaworski and Stein, 2017). Corn and rapeseed meal, however, contained concentrations of SDF, IDF, and TDF that were different from values reported previously (Jaworski et al., 2017; Jaworski and Stein, 2017; Navarro et al., 2018), but SDF, IDF, and TDF in the corn from China was similar to those values previously reported. The concentration of SDF in wheat determined using Method AOAC 991.43 with the Ankom[TDF] Dietary Fiber Analyzer was similar to that reported by Abelilla (2018), but IDF and TDF

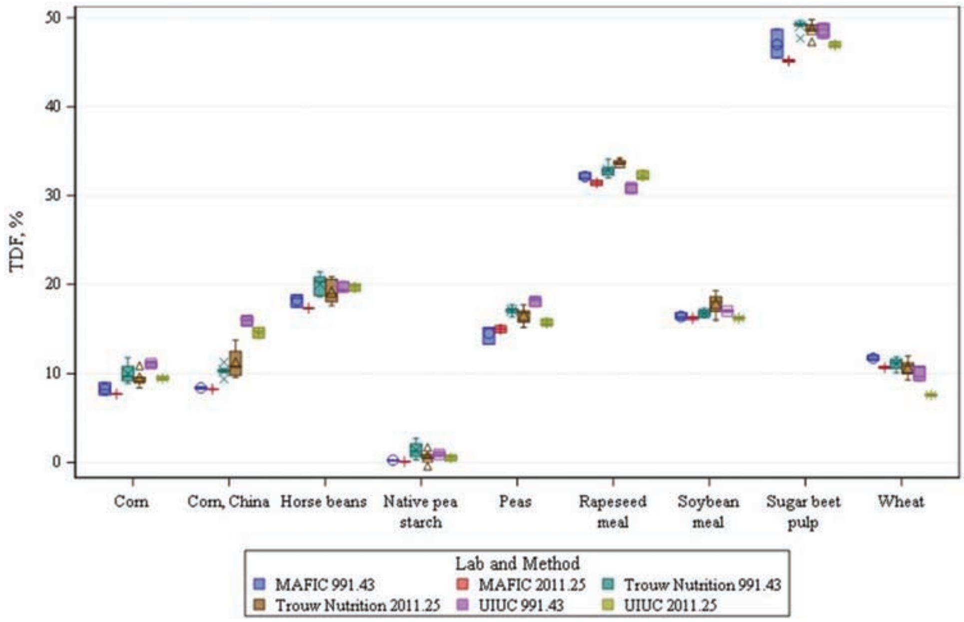

Figure 6. Total dietary fiber (TDF) analyzed in 9 feed ingredients at 3 laboratories using 2 methods

values reported in this study were less (Abelilla, 2018). The quantity of SDF in sugar beet pulp was greater and IDF lower, but TDF was similar compared with values determined by Navarro et al. (2018). This indicates that variation in SDF, IDF, and TDF in feed ingredients commonly used in diets for pigs and poultry exists worldwide and, subsequently, may be a reason for unexpected differences in animal performance and health. Therefore, routine analysis of SDF, IDF, and TDF should be performed.

The 2 main differences between the 2 methods used for analysis of SDF, IDF, and TDF with the Ankom^{TDF} Dietary Fiber Analyzer are: 1) Method AOAC 2011.25 has a less extreme, 16 h starch digestion phase at 37 °C compared with a 30 min starch digestion at 95 °C in Method AOAC 991.43 and 2) Method AOAC 2011.25 has a separate step in which low-molecular weight sugars are analyzed by HPLC. We did not determine the low-molecular weight sugars in the filtrate in Method AOAC 2011.25 because the objective of this study was to determine if resistant starch would be analyzed as TDF. It is, however, pertinent that low-molecular weight sugars be analyzed in feed ingredients used in diets for pigs and poultry to properly assess the quantity of TDF in feed ingredients for complete energy evaluation (Navarro et al., 2018).

Resistant starch is defined as starch that is not digested by mammalian endogenous enzymes (Berry, 1986; Englyst et al., 1996). Starch digestion in vivo is mostly dependent upon gelatinization, which is defined as the irreversible loss of native starch structure. The gelatinization process begins when the starch granule absorbs water and swells. Next, part of the amylose is exuded, making the granule susceptible to enzymatic degradation. Gelatinization requires energy (mechanical, thermal, chemical, or a combination), but the presence of free water is most critical.

Total starch analysis begins with complete starch granule gelatinization by boiling samples hydrated in water and this enables subsequent enzymatic digestion to free glucose, which passes freely through the Ankom filter bags and, therefore, is not analyzed as IDF or SDF. Method AOAC 991.43 uses thermal energy by heating samples in a buffer at 95 °C for 30 min to gelatinize starch granules making them susceptible to enzymatic digestion in vitro. Whereas Method AOAC 2011.25 simulates in vivo digestion by heating samples in a buffer at 37 °C for 16 h. Resistant starch types 1, 2, and 3 are insoluble and are analyzed in the IDF fraction, whereas type 4 resistant starches are chemically modified starches and are captured in the SDF fraction (Macagnan et al., 2016). However, no feed ingredients used in this study were expected to contain type 4 resistant starch and, therefore, values for IDF determined using Method AOAC 2011.25 were hypothesized to be greater compared with values determined for Method AOAC 991.43 if resistant starch was present in the feed ingredient. Peas, horse beans, and native pea starch were included because they contained greater concentrations of resistant starch compared with corn and wheat, whereas rapeseed meal, soybean meal, and sugar beet pulp contain very little total starch.

Antithetical to our hypothesis, the concentration of IDF in all ingredients analyzed using Method AOAC 2011.25 was not different and even less in corn, wheat, peas, and sugar beet pulp than IDF values analyzed using Method AOAC 991.43. Tobaruela et al. (2018) observed no difference in the quantity of IDF determined in 4 fruits using Method AOAC 991.43 compared with 2011.25. McCleary (2007) also found similar TDF values between the 2 methods in Novelose 240 and 330 (2 types of retrograded high amylose maize starch), and red kidney beans, but found much higher TDF values for many more food ingredients using Method AOAC 2011.25 compared with 991.43. Because of the discrepancy between our hypothesis and previous reports, the concentration of total starch that may have remained in the IDF residue, hence resistant starch, for a select number of samples was determined. Indeed, this confirmed our results because the IDF residue for corn, wheat, horse beans, and peas contained a similar or slightly higher concentration of total starch using Method AOAC 991.43 compared with 2011.25. It has been reported, however, that Method AOAC 991.43 may measure some, but not all resistant starch, and in particular, type 3 (i.e., retrograded starch; McCleary, 2007; Westenbrink et al., 2013). Our results also support this notion, but the quantity of starch remaining in the IDF residue was still less compared with the quantity of resistant starch determined using an in vitro digestion method. Recently, the 16 h incubation step in Method AOAC 2011.25 was criticized because that is much longer compared to in vivo upper gastrointestinal tract transit time. Therefore, McCleary et al. (2015) reduced the incubation time from 16 to 4 h in a modified Method AOAC 2011.25. We did not test this modified method in this study because the Ankom^{TDF} Dietary Fiber Analyzer was used and the objective of the study was to compare the 2 methods available on the analyzer. It is recommended that future research using Method AOAC 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer be conducted with an incubation time of 4 h because this is more similar to in vivo conditions and with the in vitro digestibility assay used to determine resistant starch in this study.

The concentration of SDF in feed ingredients determined in this study using Method AOAC 2011.25 were greater in corn, rapeseed meal, soybean meal, and sugar beet pulp compared with using Method AOAC 991.43. Tobaruela et al. (2018) also observed a greater concentration of SDF using Method AOAC 2011.25 compared with 991.43 in atemoya and coconut and reasoned that this may be explained by the many differences in enzymes and times and temperatures used for incubation in Method AOAC 2011.25 compared with 991.43. McCleary et al. (2015) indicated, however, that analyzing high starch products using Method AOAC 2011.25 may produce various maltodextrins, which are resistant to digestion in the assay and, therefore, may be analyzed as SDF, although pigs and poultry are capable of hydrolyzing maltodextrins by a mucosal α-glucosidase, thus, overestimating the quantity of TDF. This may be an explanation for the corn sample that was high in starch, but not for rapeseed meal, soybean meal, and sugar beet pulp. It is hypothesized that the longer incubation time used in Method AOAC 2011.25 provides longer time for sample hydration and, subsequently, an increased solubility of fiber. Furthermore, rapeseed meal, soybean meal, and sugar beet pulp are the only samples used in this study that contain significant quantities of pectin (Navarro et al., 2018), and it is hypothesized that pectins require a significant amount of hydration time for full solubility to occur.

Although differences in SDF and IDF were found between the 2 methods for some ingredients used in this study, the quantity of TDF determined for all samples with either method was the same except for wheat. This indicates that either method is appropriate if TDF is to be determined. However, caution is advised when interpreting SDF and IDF results from either method because both methods include a small quantity of resistant starch and this can lead to an overestimation of dietary fiber when the quantity of total starch or resistant starch is used together, which is often the case in feed ingredient evaluation for pigs and poultry.

A second objective of this study was to determine the between-laboratory variation for SDF, IDF, and TDF determined in the feed ingredients using either method with the Ankom^{TDF} Dietary Fiber Analyzer. In general, differences existed between-laboratory variation and the variation using Method 2011.25 was greater at all labs compared with 991.43. The interlaboratory variability for TDF in the samples observed using Method AOAC 991.43 and AOAC 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer were similar to those reported by McCleary et al. (2012) without the Ankom^{TDF} Dietary Fiber Analyzer. Also, the between-laboratory variation for SDF, IDF, and TDF using either method with the Ankom^{TDF}

Dietary Fiber Analyzer determined in this study was less compared with the variation reported for either method without using the Ankom^{TDF} Dietary Fiber Analyzer (Tobaruela et al., 2018).

In conclusion, when averaged among 9 different ingredients, the concentrations of SDF, IDF, and TDF determined using Method AOAC 991.43 were not different compared with Method AOAC 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer. Similarly, for individual ingredients, except for wheat, the concentration of TDF was not different when determined using either method. Insoluble dietary fiber in some feed ingredients, for example, corn, wheat, peas, and sugar beet pulp, analyzed using Method AOAC 2011.25 was even less than IDF values analyzed using Method AOAC 991.43, which was contrary to our hypothesis. The between- and within-laboratory variation in either method using the Ankom^{TDF} Dietary Fiber Analyzer was similar to previously reported variation produced without using the automated analyzer. However, the variation between- and within-laboratory was slightly greater when Method AOAC 2011.25 was used compared with 991.43. Method AOAC 2011.25, in the present study, did not show greater detection for IDF and, therefore, it is concluded that it did not capture more resistant starch in the feed ingredients tested compared with Method 991.43. It is recommended that SDF, IDF, and TDF in feed ingredients and diets for pigs and poultry be determined using Method AOAC 991.43, but future research should investigate the reduction in incubation time for Method AOAC 2011.25 with the Ankom^{TDF} Dietary Fiber Analyzer because this method may be a more physiologically relevant measurement of TDF. Finally, when the digestibility or fermentability of SDF, IDF, and TDF by pigs or poultry is to be determined, Method AOAC 991.43 is the only relevant method because it ensures a more complete removal of starch (95 °C for 30 min), rather than potential for incomplete starch removal (39 °C for 16 h) and animal digesta and feces should be analyzed using the former and not the latter in order to avoid underestimating dietary fiber digestibility or fermentability due to the inclusion of resistant starch.

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