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The importance of particle size on organic matter and crude protein *in vitro* digestibility of maize and soybean meal

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A B S T R A C T

Particle size plays an important role in the digestibility of nutrients by animals. Methodologies developed to simulate the digestive system to determine digestibility values require the grinding of samples to pass a specific sieve size (*e.g.* 1 mm) before *in vitro* digestion. The objective of this study was to evaluate if particle size affects *in vitro* (ileal) digestibility values of organic matter (OM) and crude protein (CP) in maize and soybean meal (SBM). Both ingredients were ground in a laboratory mill over four screens (1.50, 1.00, 0.75 and 0.50 mm) with trapezoidal holes in a sequential manner from large to fine. Particle size distribution, nutrient content and *in vitro* digestibility were determined of the various samples. With decreasing screen size, geometric mean diameter of particles significantly decreased from 351.4 to 203.2 μm for maize, and from 239.1 to 99.1 μm for SBM. Ash, CP and starch content were not affected whereas the neutral detergent fibre in maize and dry matter content in SBM differed after grinding with various sized screens ($P < 0.05$). The *in vitro* digestibility of OM and CP of maize and CP of SBM ground over the four different screens did not differ ($P > 0.185$). The OM *in vitro* digestibility differed ($P < 0.05$) with values for SBM ground over the 1.50 mm screen being lower ($P < 0.05$) than the smaller screen sizes: 0.814 (1.50 mm) *vs* 0.833 (1.00 mm), 0.829 (0.75 mm) and 0.834 (0.50 mm), respectively. Based on the current work and literature data, particles ≥ 595 μm of comminuted maize and SBM affect OM and CP *in vitro* digestibility. Analysis of three feeds and 10 feed ingredients ground over a 1.0 mm sieve showed that the fraction ≥ 595 μm can make up to 32.1% of the mass. It is recommended that before *in vitro* digestibility determination, particle size distribution is assessed, especially the mass fraction of particles ≥ 595 μm and further grinding is conducted to ensure that particles are < 595 μm for maize and SBM. The cut off size where particles size affects OM and CP *in vitro* (ileal) digestibility of other feeds or feed ingredients than maize and SBM should be determined.

1. Introduction

Particle size of diets and ingredients is important in animal nutrition. In pigs, it was found that reducing particle size can increase the surface area of particles for digestive enzymes to interact, resulting in a higher digestibility of nutrients as measured in both *in vivo*

Abbreviations: CP, crude protein; DM, dry matter; GMD, geometric mean diameter; GSD, geometric standard deviation; NDF, neutral detergent fibre; OM, organic matter; PSD, particle size distribution; SBM, soybean meal.

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and *in vitro* trials (Healy et al., 1994; Livesey et al., 1995; Wondra et al., 1995a; Blasel et al., 2006; Lyu et al., 2021a, b).

In vitro digestion methods have been widely used over many years to estimate *in vivo* nutrient digestibility, as they are relatively inexpensive and rapid. The most commonly used *in vitro* models simulate the physiological conditions in the gastrointestinal tract of animals and humans by adjusting the pH, use of buffers, addition of enzymes and bile salts (Butts et al., 2012) and mimicking absorption. The *in vitro* protocol developed by Boisen and Fernández (1995, 1997) in which it is prescribed that materials should be ground to pass a 1 mm sieve before digestion simulation has been, and still is, widely used in animal nutrition. Furuya et al. (1979) reported that grinding over a 1.0 mm screen of three out of four pig diets increased the dry matter (DM) and crude protein (CP) *in vitro* digestibility, with lower digestibility values showing larger effects of grinding material over a 1.0 mm before *in vitro* digestion. Although Löwgren et al. (1989) reported that an increased particle size requires a longer incubation time, these authors found no significant effect of incubations of ground (5.0, 2.0, 1.0 and 0.5 mm screens) barley with duodenal, ileal and faecal inocula on final digestibility values if incubations lasted > 45 h. Boisen and Fernández (1997) showed that the organic matter (OM) *in vitro* total tract digestibility of maize and soybean meal (SBM) decreased by only 1.4% and 0.4% units, respectively when ground over a 3 instead of 1 mm sieve. Recently, Lyu et al. (2021a, b), when studying fractionated hammer-milled maize and SBM, found that the grinding of fractions over a 1 mm sieve significantly increased the *in vitro* digestibility coefficient of OM up to 75% (from 0.16 to 0.91) for maize and for CP up to 7% in the case of SBM (from 0.88 to 0.95). The effect of grinding on *in vitro* digestibility was general seen for fractions collected on sieves $\geq$ 0.595 mm (Lyu et al., 2021a, b). There is a relative dearth of information in the literature whether particle size distribution (PSD) may explain the results of Lyu et al. (2021a, b). Further investigations are warranted to determine the influence of particle size on the values of OM and CP *in vitro* digestibility generated using the Boisen and Fernández (1995, 1997) assay.

The objective of this experiment was to evaluate the effect of mill screen size on the estimation of OM and CP *in vitro* digestibility of maize and SBM by the Boisen and Fernández (1995, 1997) assay.

2. Material and methods

2.1. Grinding

Two, 20 kg bags of French maize kernels and two, 20 kg bags of Brazilian SBM (both Research Diet Service B.V., Wijk Bij Duurstede, The Netherlands) both originating from one batch were used. For each ingredient, 4 kg/bag were randomly collected. Maize kernels were first ground using a cut mill with screen size of 4 mm (SM300, Retsch, Germany) to avoid excessive heat generation during subsequent milling at 1.5 mm. An ultra-centrifugal mill (ZM 200, Retsch, Germany) was used to grind the ground maize and SBM using screens with trapezoidal holes of 1.50, 1.00, 0.75 and 0.50 mm at a running speed of 12000 rpm. The 4 kg of ground maize and SBM were ground over the 1.50 mm screen before 1 kg was collected using a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands). The remaining material was ground again with the ultra-centrifugal mill using the 1.00 mm where after another 1 kg sample was collected using the multi-slot divider. This process was repeated with the 0.75 mm screen and the final material was ground over the 0.50 mm screen and collected. Great care was taken to ensure that the mill temperature remained similar to the initial starting temperature during grinding by slow feeding the material into the ultra-centrifugal mill and maintaining the running speed of 12000 rpm. After grinding and directly after collection, ground material was stored individually in airtight plastic bags and kept at 4 °C for further analysis.

2.2. Particle size analysis

The PSD of the various ground maize and SBM samples were determined (in duplicate) according to ASABE (2008) with sieve opening of 1.190 0.841, 0.595, 0.420, 0.297, 0.210, 0.149, 0.105, 0.074, 0.053 mm and a pan. In this method, the sieve shaker (AS 200 Control, Retsch, Haan, Germany) employed a 3-D throwing motion for 10 min with an amplitude of 2 mm and an interval shaking time of 6 s. Two rubber balls with a diameter of 20 mm were used as sieving aid on each sieve where the sieve opening was smaller than 300 μm while sieving SBM.

During the sieving procedure of maize, it was observed that very fine particles agglomerated on the sieves, which may be due to the molecular interactions like Van der Waals and electrostatic interactions. To reduce this agglomeration, four rubber balls were used for the sieves with an opening of $\leq$ 0.595 mm. Geometric mean diameter (GMD) and geometric standard deviation (GSD) were calculated based on the PSD according to ASABE (2008) and reported as the mean value of the duplicate samples.

2.3. Nutrient content and in vitro digestibility

The nutrient content of ground maize and SBM including DM, ash, neutral detergent fibre (NDF), CP and starch were analysed as previously described by Lyu et al. (2021a). The OM and CP *in vitro* digestibility coefficients of ground maize and SBM were determined as described by Lyu et al. (2021a, b) which is based on the method published by Boisen and Fernández (1995). Briefly, 10 g samples were first digested in a solution containing pepsin and pancreatin, where after the undigested residues were collected by filtration through nylon gaze with a pore size of 40 μm and porosity of 0.30 (PA 40/30, Nybolt, Switzerland) and then dried at 70 ℃ overnight. The DM, ash and CP content of residue was determined, and the digestibility coefficients calculated based on DM according to the difference in OM and CP content before and after digestion.

2.4. Statistical analysis

Data on nutrient content and *in vitro* digestibility of OM and CP for each ingredient were analysed by one-way analysis of variance using a general linear model in R 3.6.1 (R Core Team, 2019). The main factor was the screen size of the ultra-centrifugal mill containing four levels (1.50, 1.00, 0.75 and 0.50 mm). For each screen size, two bags of maize as well as SBM were used in duplicate, yielding 16 samples which each were analysed once. Multiple comparison of means was performed by Tukey's multiple comparisons using 'HSD. test' function in the 'agricolae' package (Mendiburu and Muhammad, 2020). The minimum significance threshold was set at 0.05.

3. Results

The recovery of maize and SBM ground over the four screen sizes (1.50, 1.00, 0.75 and 0.50 mm) during the determination of the PSD ranged from 98.86 ± 0.09–99.80 ± 0.20%. Screen size showed a significant effect (Table 1) on GMD and GSD of maize and SBM ($P < 0.001$). With a decreasing screen size, the GMD decreased from 351.4 to 203.2 µm, and from 239.1 to 99.1 µm for maize and SBM, respectively. The GSD decreased from 256.7 to 86.3 µm, and from 213.4 to 68.6 µm for maize and SBM, respectively.

As shown in Fig. 1, maize particles were mainly retained on the middle size sieves (from 74 µm to 420 µm). For the two smallest fractions (pan, and 53 µm sieve), a very small mass percentage was collected (<0.4%). The 841 and 1190 µm sieves collected less material if maize was ground over the 0.50, 0.75 and 1.0 mm screen (<0.3%), compared to milling over the 1.5 mm screen where these sieves contained 1.6% and 6.8% of the maize. On the 595 µm sieve, maize particles ground over the 1.5 mm screen collected the largest percentage (17.2%), followed by the maize ground over the 1.00, 0.75 and 0.50 mm screen (4.4%, 0.5% and 0.3%, respectively). For SBM, the mass collected on the pan and small sieves (53, 74, 105 and 149 µm) increased with a decreasing screen size during grinding, and a decreased mass was collected on the medium size sieves (297, 420, 595 µm) with increasing sieve size opening during grinding. Similar to the maize, little of the SBM material was found on the larger sized sieves (595, 841 and 1190 µm), especially for the SBM that was milled over the 1.00, 0.75 and 0.50 mm screens, which were less than 2.1%. The median particle size (D_{50}) was visually taken from cumulative PSD of maize and SBM (Fig. 1), and increased with increasing milling screen size: < 149, > 149, < 210 and < 297 µm (maize), and < 74, > 105, > 149 and > 210 µm (SBM) for screen size of 0.50, 0.75, 1.00 and 1.50 mm, respectively.

After grinding over the four screens, the nutrient content of comminuted maize and SBM was analysed (Table 2). There was no significant effect ($P > 0.05$) of mill screen size on the DM, ash, CP and starch content of maize, and no effect of mill screen size on CP, ash and NDF content of SBM. The NDF content of maize and DM content of SBM when ground over the different-sized screens were significantly different ($P < 0.05$). The maize that was ground over a 0.50 mm screen contained the lowest concentration of NDF (69.6 g/kg DM), and the maximum content of NDF (82.2 g/kg DM) was observed when maize was ground over a 1.00 mm screen. In SBM, the DM content decreased from 913 to 897.6 g/kg with increasing screen size.

The OM and CP *in vitro* digestibility of maize was not affected ($P > 0.05$) by screen size (Table 3). Similarly, the screen size of the mill did not show a significant effect on CP *in vitro* digestibility of comminuted SBM. However, the OM *in vitro* digestibility of comminuted SBM was significantly different ($P = 0.028$) although the differences were small (0.02 between lowest and highest value). The lowest value (0.814) was recorded for screen size 1.50 mm which was not significantly different to the value (0.829) for screen size 0.75 mm.

4. Discussion

Sequential grinding was used to obtain the 1.00, 0.75 and 0.50 mm comminuted maize and SBM. In addition, the maize kernels were first ground using a cut mill with screen size of 4 mm before grinding over the 1.50 mm screen. This scheme, the slow and careful

Table 1
Geometric mean diameter and geometric standard deviation of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size (mm)	Geometric mean diameter (µm)	Geometric standard deviation (µm)
Maize[a]	0.50	203.2[b]	86.3[b]
	0.75	219.0[c]	111.4[c]
	1.00	260.3[b]	148.2[b]
	1.50	351.4[a]	256.7[a]
SEM		21.77	24.61
P-value		< 0.001	< 0.001
Soybean meal	0.50	99.1[a]	68.6[a]
	0.75	133.3[b]	104.8[b]
	1.00	186.2[c]	163.6[c]
	1.50	239.1[d]	213.4[d]
SEM		20.09	20.92
P-value		< 0.001	< 0.001

SEM, standard error of the mean.
[a] Ground first using cut mill with 4 mm screen size.
[b] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).

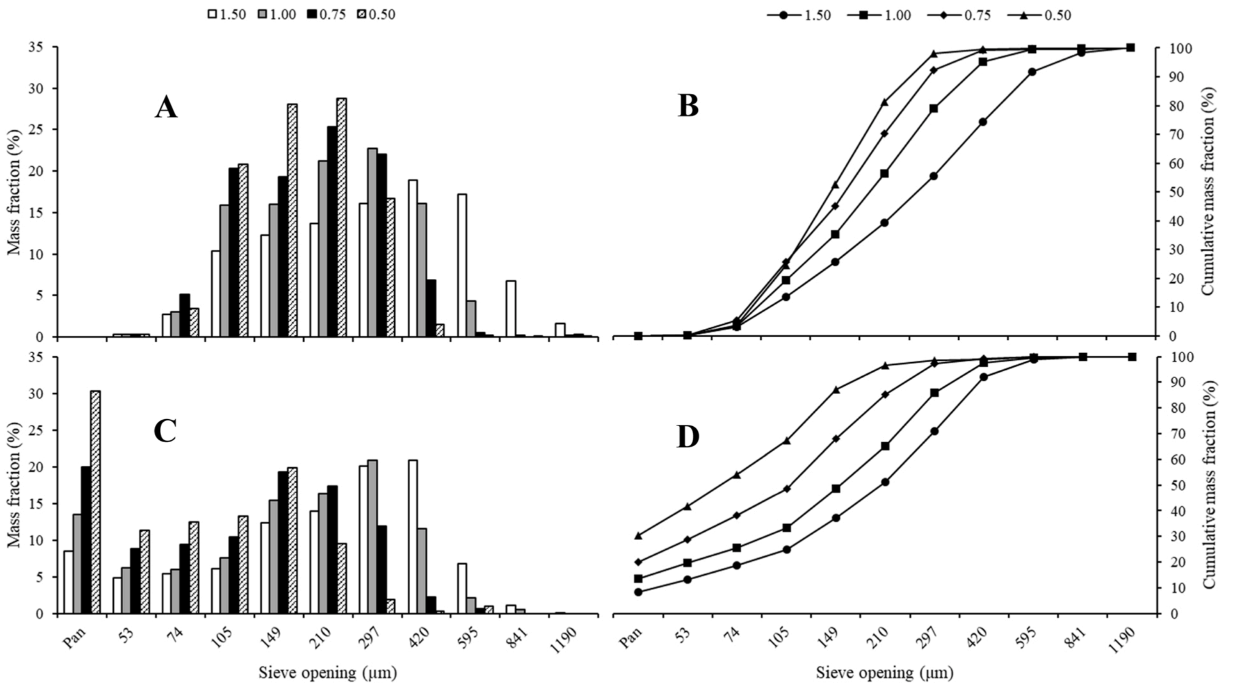

Fig. 1. Mass and cumulative mass fraction distribution of comminuted maize (A, B, resp.) and soybean meal (C, D, resp.) using mill screen sizes of 1.50, 1.00, 0.75 and 0.50 mm. Maize was ground first using a cut mill with 4 mm screen size.

Table 2
Nutrient content of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size (mm)	Dry matter (g/kg)	Ash (g/kg dry matter)	Crude protein	Starch	Neutral detergent fibre	Remaining fraction[a]
Maize[b]	0.50	902.6	14.5	90.4	643.9	69.6[c]	181.6
	0.75	899.9	14.2	90.2	699.2	75.4[c]	121.1
	1.00	897.1	14.6	89.6	707.4	82.2[a]	106.3
	1.50	897.3	14.4	89.3	687.3	78.4[b]	130.6
SEM		0.95	0.06	0.28	11.00	1.74	12.2
P-value		0.068	0.058	0.565	0.144	<0.001	0.089
Soybean meal	0.50	913.0[a]	73.5	551.1	–	146.8	228.6
	0.75	906.1[a]	72.7	549.6	–	89.7	288.0
	1.00	897.8[b]	72.9	549.6	–	93.2	284.2
	1.50	897.6[b]	73.1	548.3	–	97.7	280.9
SEM		2.63	0.16	0.48	–	10.68	10.9
P-value		0.041	0.473	0.237	–	0.174	0.146

[a] 1000-ash-crude protein-starch-neutral detergent fibre.
[b] Ground first using a cut mill with 4 mm screen size.
[c] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).

grinding, the quantitative collection of all ground material, proper mixing and division of material were employed in order to not only avoid unwanted heat generation but also to obtain representative samples differing only in particle size as the aim was the determination of the influence of particle size on *in vitro* digestibility.

Grinding over a smaller-sized screen decreased the GMD and GSD in maize and SBM. Similar results of a decreased GMD and GSD with reducing diameter of screen holes (6/64″, 10/64″ and 16/64″) while grinding maize in a hammer mill were also reported by Saensukjaroenphon et al. (2017). Wondra et al. (1995b) reported that hammer milling maize over a screen opening of 9.53 (3/8″) and 1.59 (1/16″) mm reduced the GMD of particles from 826 to 419 μm and the GSD from 2.5 to 1.7 μm, respectively.

The non-significant effect of milling screen size on CP and starch in the present study, is as expected. Grinding mainly changes the physical properties such as the particle size and/or particle shape instead of chemical composition. Although there was no clear direction of the effect, the NDF content between the four comminuted maize samples were significantly ($P < 0.001$) different. It is highly likely that this is the result of the analytical method by which NDF is determined. Unlike CP and starch, in the NDF analysis a filtration step is used where finer particles already present or generated due to treatment with laurate and amylase, are not retained. Although differences were small (0.4 g/kg between smallest and largest value), a trend in ash content was observed for maize. Possibly this may

Table 3
In vitro digestible organic matter and crude protein (g/g dry matter) of comminuted maize and soybean meal as affected by mill screen size.

Ingredient	Mill screen size (mm)	*In vitro* organic matter digestibility (g/g dry matter)	*In vitro* crude protein digestibility (g/g dry matter)
Maize[a]	0.50	0.888	0.898
	0.75	0.897	0.903
	1.00	0.884	0.881
	1.50	0.871	0.867
SEM		0.0045	0.0067
P-value		0.257	0.194
Soybean meal	0.50	0.834[b]	0.958
	0.75	0.829[a,b]	0.957
	1.00	0.833[b]	0.958
	1.50	0.814[b]	0.953
SEM		0.0032	0.0009
P-value		0.028	0.185

[a] Ground first using a cut mill with 4 mm screen size.
[b] Values with different superscripts within a column per ingredient are significantly different ($P < 0.05$).

be related to the NDF effect. The NDF content of SBM ground over different sized screens did not differ due to the large standard error of the mean (10.7 g/kg DM). The DM content increased with increasing fineness of grind with significant differences observed in SBM samples and a trend for maize. This loss of moisture can likely be attributed to the additional grinding of the 1.00, 0.75 and 0.50 mm samples and may indicated that, although great care was taken, some additional heat was generated that increased moisture loss. Probst et al. (2013) reported that a significant amount of heat was generated in the grinding chamber due to particle-particle and particle-hammer friction when hammer milling maize over a 1.6 mm screen. In addition, the increase in surface area of the broken particles can further facilitate moisture loss. Alternatively, the additional processing time required for material with a finer screen size (exposure to air) may have affected the DM content.

The present study was designed to determine the influence of mill screen size on the estimation of OM and CP *in vitro* digestibility of maize and SBM by the Boisen and Fernández (1995) assay as previously Lyu et al. (2021a, b) found a major effect of grinding of fractions on *in vitro* analysis. The effect of particle size of the material on the digestibility of nutrients is also observed in *in vivo* trials (Kim et al., 2005; Lahaye et al., 2008; Ball et al., 2015; Rojas and Stein, 2015) with smaller particles resulting in higher nutrient digestibility values in pigs. In the present study, particle size did not significantly affect OM and CP *in vitro* digestibility of maize and CP digestibility of SBM. These results are in line with data of Boisen and Fernández (1997) who showed that grinding of maize and SBM over a 3 instead of 1 mm sieve had a minor effect (−1.4 and −0.4% units, respectively) on OM *in vitro* total tract digestibility. Effects on OM *in vitro* digestibility may, however, be larger compared to total tract digestibility as the additional step of using a mixed multi-enzyme complex containing a wide range of microbial carboanhydrases including arabinase, cellulase, B-glucanase, hemicellulase, xylanase and pectinase, is omitted. Lyu et al. (2021b) found that the grinding of hammer-milled and size fractionated maize and SBM over a 1 mm screen (as per specification of the assay) significantly increased the OM and CP *in vitro* digestibility coefficients: grinding maize particles > 3360 μm increased the OM *in vitro* digestibility value from 0.161 to 0.907 and grinding SBM particles > 1190 μm increased CP *in vitro* digestibility from 0.783 to 0.830. The authors also reported a significant interaction effect between grinding before *in vitro* digestion and sieve size indicating different effects depending on sieve size. In general, *in vitro* digestibility of both OM and CP were significantly lower for fractions collected on sieves ≥ 0.595 mm with smaller particles showing no significantly lower values (Lyu et al., 2021a, b). The non-significant results in the present study can be explained by the fact that most of the maize and SBM particles were < 595 μm (Fig. 1). The mass of particles ≥ 595 μm made up less than 5% of the material milled over the 0.50, 0.75 and 1.00 mm screens with maize ground over the 1 mm screen having a value of 2.6% and SBM 4.6%. As such, the data of Lyu

Table 4
Mass fraction of particles larger than 595 μm of three compound feeds and 10 commonly used feed ingredients milled over a 1 mm sized screen.

Material[a]	Mass fraction (%)
Weaned piglets compound feed	13.4
Lacto sow compound feed	10.1
Poultry compound feed	11.9
Maize	12.8
Soybean meal	8.0
Barley	19.4
Rye	32.1
Peas	28.9
Oats	28.9
Wheat	12.3
Wheat bran	25.6
Rapeseed meal	14.8
Sunflower seed meal	17.3

[a] One feed or ingredient was analyzed.

et al. (2021a, b) and those reported here appear to be in agreement. As the fraction of material collected on sieves ≥ 595 μm was small, the *in vitro* digestibility values were not significantly affected. The major effect observed in the studies of Lyu et al. (2021a, b) were due to the particles ≥ 595 μm making up approximately 88% for maize and 70% for SBM. Especially the grinding over a 1.0 mm screen of these larger fractions (≥595 μm) significantly increased *in vitro* digestibility.

From the above it is clear that it is important that before OM and CP *in vitro* digestibility determination according to the Boisen and Fernández (1995) method and likely also other methods, the PSD is determined, especially the mass fraction of particles ≥ 595 μm for at least maize and SBM. In order to provide first estimates, we determined this mass fraction (including use of additional rubber balls during sieving) for three compound feeds and 10 commonly used feed ingredients after direct grinding (not sequential) over a 1 mm screen with trapezoidal holes as would be practiced in the assay (Table 4). Soybean meal had the lowest percentage (8.0%) of mass fraction ≥ 595 μm. The three compound feeds, together with maize wheat and rapeseed meal showed values between 10% and 15% followed by sunflower seed meal and barley with 17.3% and 19.4%. For rye, oats and wheat bran, more than 25% of the mass was collected on sieves with a size ≥ 595 μm with rye having the largest mass fraction of 32.1%. As expected, the percentages for maize (12.8 *vs* 2.6%) and SBM (8.0 *vs* 4.6%) were greater than the values obtained when the sequential grinding was used. It should be noted that the values in Table 4 were determined on one sample for each feed/ingredient and that variation within feed/ingredient may affect this percentage. Whether the cut off value of 595 μm also applies to these feeds/ingredients as is the case for maize and SBM is unknown. As feed/ingredient breaking characteristics, mill design, mill operation, screen design and other factors can affect PSD, the variation in PSD within and between feeds and ingredients and its effect on OM and CP *in vitro* digestibility should be further investigated. Particle size distribution next to composition of materials may be a significant contributor to the variation in OM and CP *in vitro* digestibility values.

5. Conclusions

Grinding maize and soybean meal over a ≤ 1 mm sieve did not affect the determination of organic matter and crude protein *in vitro* (ileal) digestibility. Particles of ground maize and soybean meal should not exceed 595 μm as this appears to be the cut off for the influence of particle size on *in vitro* digestibility of organic matter and crude protein for these two feed ingredients. The grinding of feeds and feed ingredients over a 1 mm sieve shows a significant mass fraction (8.0–32.1%) exceeding 595 μm that may affect digestibility values. Breaking behavior of feeds and feed ingredients should be investigated in more detail as well as the cut off where particle size affects *in vitro* (ileal and total tract) digestibility values of other ingredients than maize and soybean meal, and of feeds. Furthermore, future *in vitro* assay development should incorporate additional *in vivo* digestion processes and variables (*e.g.* chewing, passage rate, (anti)peristalsis, absorption, microbiota).

CRediT authorship contribution statement

F. Lyu: Conceptualization, Methodology, Investigation, Formal analysis, Project administration, Writing – original draft. **M. Thomas:** Resources, Writing – review & editing, Supervision. **A.F.B. van der Poel:** Writing – review & editing, Funding acquisition, Supervision. **W. H. Hendriks:** Conceptualization, Methodology, Writing – review & editing, Methodology, Resources, Funding acquisition, Supervision.

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Conflict of interests

None of the authors have a conflict of interest regarding this manuscript.

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