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Particle size distribution, energy consumption, nutrient composition and *in vitro* ileal digestion characteristics of hammer milled maize and soybean meal affected by moisture content

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A B S T R A C T

Grinding is an important feed processing technology, determining physical and nutritional characteristics of ground materials, which affects nutrient digestion in animals and their growth performance. This study aimed to clarify if differences in moisture content (MC) lead to differences in nutrient composition over various particle size fractions after grinding that have potential to affect feed manufacturing characteristics or animal performance. Maize and soybean meal (SBM) with targeted MC of 120, 140 and 160 g/kg (adding no (0), 30 and 60 g/kg of tap water, respectively) were hammer-milled and the physical and chemical characteristics as well as *in vitro* apparent ileal digestibility (AID) of particle size fractions were determined. The mill was fitted with a 6-mm (maize) or 2-mm (SBM) sized screen, with milled material subsequently separated by dry sieving (size ranging from $<$ 0.075 to $>$ 3.36 mm) and each fraction was analyzed for its nutrient composition, morphology characteristics and *in vitro* AID of organic matter (OM) and crude protein (CP). For the latter assay, specific particle size fractions were additionally ground using a laboratory mill (1 mm screen). Geometric mean particle size diameter and energy consumption increased with increasing MC ($P < 0.05$). Chemical composition, physical characteristics and *in vitro* AID of particle size fractions were significantly different ($P < 0.001$). Moisture addition had no significant effect on nutritional and physical parameters except for solidity in maize, ash content, projected area, circularity, and solidity in SBM. Physical characteristics of particles, especially particle size affected *in vitro* AID of OM and CP most ($P < 0.05$). Additional grinding of samples before determination of *in vitro* AID increased the OM digestibility up to 0.684 in maize ($P < 0.001$). Additional grinding of particles larger than 0.595 mm increased *in vitro* AID of OM and CP ($P < 0.05$). In summary, increasing MC has limited effect on the breakage behavior of maize and SBM, but increased energy consumption during hammer-milling. *In vitro* AID measurement of fractionated particles appears to require material should be ground to pass a 0.595 mm sieve rather than the prescribed 1 mm.

Abbreviations: AID, apparent ileal digestibility; SBM, soybean meal; CP, crude protein; DM, dry matter; GMD, geometric mean diameter; GSD, geometric standard deviation; MC, moisture content; NDF, neutral detergent fiber; OM, organic matter; PSD, particle size distribution.

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1. Introduction

Grinding performance is important in feed processing as it not only significantly contributes to the feed processing costs (energy consumption), but more importantly determines the physical and nutritional characteristics of the final ground material (Jagtap et al., 2008), including gastro-intestinal digestibility and kinetics of nutrient absorption. There are many factors affecting grinding performance including those intrinsic to the material to be ground such as initial particle size, moisture content (MC), material properties and those related to the equipment used in grinding such as sieve opening, (tip) speed of hammers, feed rate settings, geometry of the grinding chamber with respect to position and number of breaker plates (Chen et al., 1999; Mani et al., 2004; El Shal et al., 2010; Yancey et al., 2013; Gil and Arauzo, 2014; Guo et al., 2016; Mugabi et al., 2017). Moisture content plays an important role and can affect various intrinsic properties of ingredients such as strength, stiffness, elasticity and plasticity (Jung et al., 2018) which, in turn, influences breaking behavior and energy consumption (Jagtap et al., 2008) during grinding of feed raw materials.

Many studies have showed that MC affected the energy consumption, particle size distribution (PSD) of ground material (Velu et al., 2006; Doblado-Maldonado et al., 2013; Lee et al., 2013; Moon and Yoon, 2018). In addition, many studies reported that after grinding and classifying, the nutrient content, physical characteristics and *in vitro* apparent ileal digestibility (AID) of nutrients are distributed differently (Sundberg et al., 1995a; b; Maaroufi et al., 2000, 2009; Al-Rabadi, 2013; Acosta et al., 2019; Lyu et. al, 2021). However, few studies have been done in estimating the effect of MC on the physical, nutritional and *in vitro* digestion characteristics with respect to PSD after grinding. We hypothesized that MC influences the grinding behavior of ingredients and results in different PSD, nutrient content, and physical characteristics. Such studies are important, as the results have potential for developing grinding strategies (*e.g.*, use moisture of ingredients as a control measure) to produce higher-digestibility feeds and improve animal growth performance utilizing tailor-made selection of fractions. The main objective of this study was to determine the effect of MC on breaking behavior of maize and SBM *via* the physical and nutritional characteristics of size fractionated particles.

The digestibility of ground ingredients and feeds is one of the primary criteria in animal nutrition to obtain an efficient and sustainable animal production. High digestibility values mean less waste and a higher nutrient utilization from ingredients/feed. *In vitro* digestion simulation assays are a cost and time saving technology and widely used to provide an indication of *in vivo* digestibility of nutrients of ingredients/feeds for a variety of animal species (Jobling and Sumpter, 1993; Theodorou et al., 1994; Boisen and Fernández, 1995; 1997). Recently, Lyu et al. (2021) unexpectedly observed that the routinely used Boisen and Fernández (1995) assay to determine *in vitro* AID of organic matter (OM) and CP was affected by the size of maize and SBM particles although the developers of the assay reported minor differences (−1.4 to −0.4% units) between ingredients ground over a 1- and 3-mm sieve. We hypothesize that the current used *in vitro* digestion measurement is negatively affected by the large particle size fraction in the sample and that additionally grinding particles > 1 mm will increase the *in vitro* AID of these large particles. Therefore, another purpose of this research was to study the effect of additionally grinding fractionated material before *in vitro* digestion analysis on the measurement of *in vitro* AID of OM and CP.

2. Material and methods

Moisture-treated and -untreated maize and SBM were hammer-milled. Then the energy consumption of grinding was record, and PSD, geometric mean diameter (GMD) and geometric standard deviation (GSD) of hammer-milled material were measured. After sieving, the nutrient composition, and physical characteristics of particles from different size fractions were determined. In addition, in order to corroborate or contradict the preliminary observation by Lyu et al. (2021) that the *in vitro* OM and CP digestibility assay of Boisen and Fernández (1995, 1997) is affected by PSD, the effect of additional grinding of size-fractionated particles on *in vitro* digestibility of OM and CP was investigated.

2.1. Sample preparation

2.1.1. Material moisture adjustment

Whole maize kernels (France) and Brazilian SBM with initial GMD of 0.80 mm (Research Diet Service B.V., Wijk bij Duurstede, The Netherlands) originating from a single batch were divided over six, 20 kg plastic lined paper bags per ingredient. The MC of four bags (2 bags of each MC treatment) per ingredient was increased by gradually adding either 0.6 (+30 g/kg, M3) or 1.2 kg (+60 g/kg, M6) tap water while the ingredient was stirred in a paddle mixer (Forberg, type F60, Larvik-Norway) for 120 s. The other two bags per ingredient were also individually stirred in the mixer for 120 s but no water was added (M0). All six bags (M0, M3 and M6, duplicates) per ingredient were individually sealed in air-tight plastic bags and kept at 4 °C for at least 48 h to ensure uniform water distribution throughout the material. At least 12 h before hammer-milling, each sealed bag was acclimatized to room temperature.

2.1.2. Hammer-milling

Twenty kg of maize or SBM (from the same original batch) was first hammer-milled (Engl hammer mill, Dongen, The Netherlands, type 30, 7.5 kW motor) to warm up the machine and to ensure all batches were ground at the same technological conditions for each ingredient. After the warming-up run, the order of milling was M0, M3 and M6 with duplicate batches following each other and thorough cleaning of the hammer mill with a vacuum cleaner between runs. For each of the three MC levels (M0, M3 and M6), the two 20 kg bags of maize and SBM were first poured into a feeding hopper of which the adjustable inlet was opened to the 80% position and fixed when the hammer mill running speed reached 1500 rpm. All batches were hammer-milled at a fixed running speed of 157.1 rad/s, with a 6- and 2-mm screen-sized plate sieve used for maize and SBM, respectively. The choice for the sieve openings was based on the

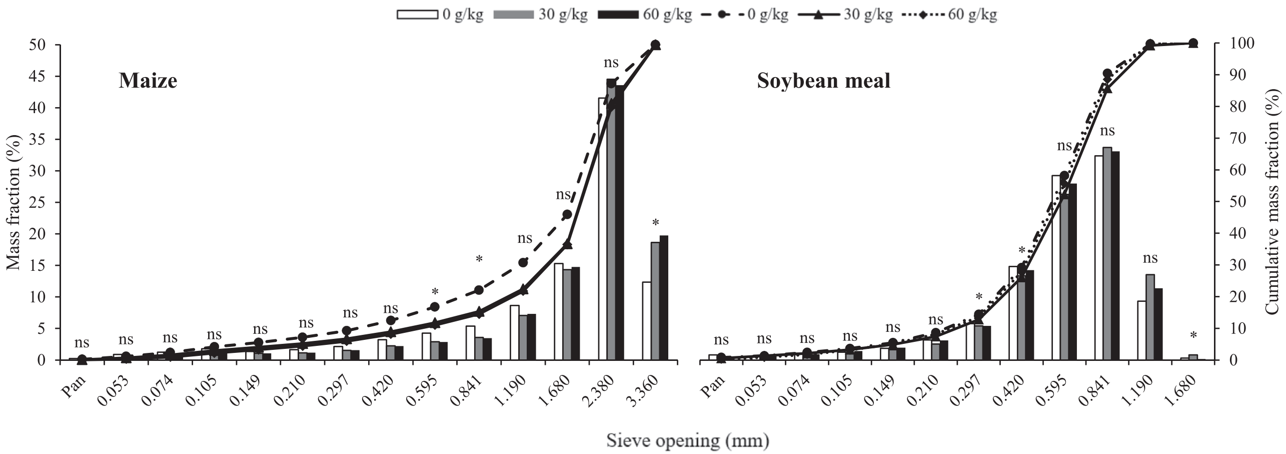


Fig. 1. Mass (bar) and cumulative mass (line) distribution of hammer-milled maize and soybean meal as affected by no (0 g/kg), 30 and 60 g/kg moisture addition to the ingredient. *Significant ($P < 0.05$) different mass fractions within sieve opening, ns = not significant ($P > 0.05$).

study of Lyu et al. (2021) which ensured balanced mass fractions across size classes. A data-logger (Hiflex, OPT-2–2USB485-OBUS, The Netherlands) connected to the controller was used to record various parameters (*e.g.* motor current, motor voltage, grinding time) every second during grinding from which effective energy consumption was calculated by subtracting idle load from the total load (kJ/kg).

2.1.3. Sampling and sieving

After hammer-milling, approximately 5 kg of a representative subsample was collected from each 20 kg run using the quartering and coning method (Campos-M and Campos-C, 2017) from which ~1.25 kg of subsample was collected using a multi-slot divider (Mooij-Argo, Hegelsom, the Netherlands) to determine PSD (in duplicate) using the 15-sieve method (ASABE, 2008). 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. Geometric mean diameter and GSD were calculated based on the PSD according to ASABE (2008) and reported as the mean value of the duplicate samples. The remainder of the hammer-milled material (~3.75 kg) was used to obtain six particle size fractions for each of the batches by additional sieving. For this purpose, five sieves were selected from the PSD determination data to yield fractions with sufficient mass for subsequent chemical and physical analysis. For maize these sieves included 3.360, 2.380, 1.680, 0.841 and 0.420 mm (+pan), and for SBM 1.190, 0.841, 0.595, 0.420 and 0.210 mm (+pan). Multiple sieving (at least 10 times) was performed to obtain sufficient material for each fraction (> 70 g) for physical/chemical analyses and *in vitro* digestion. The material on each sieve layer (+pan) from the multiple sieving was collected, pooled per treatment, thoroughly mixed and kept at − 20 °C until further analysis.

2.1.4. Additional grinding of samples

To determine the effect of particle size on the *in vitro* OM and CP AID, material retained on the five sieves and collected in the pan were additionally ground at 1256.4 rad/sin a centrifugal mill (ZM200, Retsch GmbH, Hann, Germany) fitted with a 1.0 mm screen, as prescribed for the *in vitro* digestion analysis protocol of Boisen and Fernández (1995).

2.2. Physical characteristics and chemical composition analysis

Physical characteristics of hammer-milled particles retained on the five sizes and collected in the pan were measured using the image analysis methods as described in Lyu et al. (2021). Briefly, multiple images were taken by a laboratory microscope combined with a digital camera (Bresser, microcam 3.0, megapixel, software version 7.2.1.7), and analysed with ImageJ (software version 1.51f) software. For the finest particles, < 420 µm (maize) or < 210 µm (SBM) microscopical resolution was insufficient to obtain clear images and for these fractions additional image analyses were conducted using a Morphologi 4 (Malvern Panalytical Ltd, Almelo, The Netherlands). The analyzed physical characteristics included projected area, projected perimeter, circularity, aspect ratio, roundness and solidity. The calculations were based on the illustration as provided in Fig. 1 of Lyu et al. (2021).

For chemical analyses, the various samples were dried in an air circulation oven at 103 °C for 4 h before determination of residual dry matter (DM) content (ISO, 6496, 1999) and calculation of DM. Ash content was determined after dried samples were combusted at 550 °C for 3 h in a muffle furnace (ISO, 5984, 2002). Neutral detergent fiber (NDF) was determined with heat-stable amylase (thermamyl) and alcalase, using the standard procedure of Van Soest et al. (1991). Nitrogen (N) content was determined by the DUMAS technique (ISO, 16634–1, 2008), and CP calculated by multiplying the N content by 6.25. Starch content of maize samples was determined using an enzymic method (ISO 4, 1591, 2004).

2.3. In vitro apparent ileal digestibility

The *in vitro* AID of both hammer-milled and additionally ground samples was determined as described by Lyu et al. (2021) which is based on the method published by Boisen and Fernández (1995). Briefly, 10 g of sample was mixed with 250 m phosphate buffer (0.1 M, pH 6.0) and 20 ml HCl solution (1 M) in a 600 ml beaker before being incubated with freshly prepared pepsin solution (10 ml, 10 g/l) at pH 3.5 and 39 °C for 90 min under constant magnetic stirring. To mimic small intestine digestion, 100 ml phosphate buffer (0.2 M, pH 6.8) and 30 ml NaOH (1 M) were added to the mixture, followed by incubation with freshly prepared pancreatin solution (10 ml, 100 g/l) and bile solution (10 ml, 150 g/l) at pH 6.8 and 39 °C for 210 min under constant magnetic stirring. The undigested residues were then collected by filtration through nylon gaze with a pore size of 40 µm and porosity of 0.30 (PA 40/30, Nybolt, Switzerland) using a vacuum pump. After sequential washing of all material with 70% ethanol and acetone, residues were dried overnight in an oven at 70 °C before determination of DM, ash and CP. Digestibility was calculated according to the difference in nutrient content before and after digestion.

2.4. Plastic deformation

The plastic deformation of moisture-treated and ground maize and SBM were determined by compressing using a plunger in a barrel (Ø 60 mm × 65 mm high) with an Instron 3366 series. A constant force of 4.5 kN with the total percentage displacement relative to the initial height recorded after 6.5 h.

2.5. Statistical analysis

The single hammer mill runs on moisture treated maize and SBM were used as experimental units. Data on physical characteristics and nutrient content of fractionated particles for the different MC treatments were analyzed by two-way analysis of variance using the general linear model in R 3.6.1 (R Core Team, 2019). The statistical model used was:

$$\gamma_{ijk} = \mu_0 + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$$

where γ_{ijk} = response variable ($k = 1$ or 2, the number of measurements), μ_0 = overall mean, α_i = effect of moisture content i ($i = 1.3$), β_j= effect of fraction j ($j = 1.6$), $(\alpha \times \beta)_{ij}$ = interaction of moisture content i and fraction j and ε_{ijk} = residual error with a mean of 0 and variance σ^2. α_i and $\beta_j$were fixed effects and the minimum significance threshold was set at 0.05.

3. Results

3.1. Grinding performance

The recovery of the hammer-milled maize and SBM during the determination of the PSD (14 sieves + pan) across treatments (M0, M3 and M6) ranged from 98.8 ± 0.35–99.9 ± 0.05%. After the addition of 30 and 60 g/kg water, the MC of maize increased from 122.7 to 144.2 and 161.8 g/kg, respectively ($P < 0.05$), with the MC of SBM increasing from 117.1 to 142.7 and 164.4 g/kg (Table 1), respectively ($P < 0.05$). Moisture content showed a significant effect on the GMD of maize ($P = 0.043$) with the M3 and M6 treatments (2.01 and 2.07 mm, respectively) having higher values than the M0 treatment (1.71 mm). There was no effect of MC treatment on GSD ($P = 0.253$). Fig. 1 shows the mass and cumulative mass distribution of the hammer-milled maize and SBM as affected by MC. The PSD of ground maize and SBM with different MC showed different patterns ($P < 0.05$), and the differences were mainly observed in the middle (0.297–0.420 mm in maize and 0.595–0.841 mm in SBM) and largest (1.680 mm in SBM and 3.360 mm in maize) particle size fractions. The cumulative mass fraction reached 50% at an approximate sieve opening of 1.68 mm for maize and 0.595 mm for SBM.

Fig. 2 reports the net energy consumption required for the hammer-milling of the different MC maize and SBM. Maize showed a linear increase in net energy consumption with all three treatments being significantly different from each other ($P < 0.001$) while for SBM, the M6 treatment requiring significantly more energy for milling the same mass material ($P = 0.014$). No significant difference was observed between M0 and M3 treatments for SBM ($P > 0.05$).

3.2. Physical characteristics of particles

Physical characteristics of particles from the different size fractions for the three MC treatments of maize and SBM are presented in Fig. 3 and Fig. 4, respectively. Projected area and perimeter increased from 1647 to 508,982 μm^2, and 123–3236 μm, respectively in maize and from 1629 to 69,348 μm^2 and from 120 to 1121 μm in SBM ($P < 0.05$). The circularity of particles in the various fractions was approximately 0.60 – 0.70 except for the particles in the pan fraction, which were in the range of 0.85 – 0.90. In general, the solidity of maize particles increased with particles > 0.841 mm ($P < 0.05$). The range in solidity values of SBM particles among the different MC treatments was rather small with a maximum value of 0.942 and minimum of 0.956 (Fig. 4). In both maize and SBM, the effect of fraction was significant ($P < 0.001$) for all measured physical parameters (projected area, projected perimeter, circularity, aspect ratio, roundness and solidity). In maize, interaction effects between MC and fraction were observed for circularity ($P = 0.045$) and solidity ($P < 0.001$). In SBM, projected area ($P = 0.018$), circularity ($P = 0.005$) and solidity ($P = 0.009$) were affected by MC, and the interactions between size fractions and MC were also observed in projected area and solidity.

Table 1
Geometric mean diameter (GMD) and standard deviation (GSD) of hammer-milled[1] maize and soybean with different moisture contents.

Ingredient	Moisture addition (g/kg)	Moisture content (g/kg)	Feed rate (kg/s)	GMD (mm)	GSD (mm)
Maize	0	122.7	0.348	1.71^{b}	1.79
	30	144.2 (+21.5)[2]	0.322	2.01^{ab}	1.85
	60	161.8 (+39.1)	0.320	2.07^{a}	1.77
SEM			0.007	0.077	0.019
P-value			0.260	0.043	0.253
Soybean meal	0	117.1	0.231	0.67^{b}	0.44
	30	142.7 (+25.6)	0.212	0.72^{a}	0.46
	60	164.4 (+47.3)	0.135	0.70^{ab}	0.43
SEM			0.021	0.007	0.005
P-value			0.082	0.020	0.066

^{a,b} Values with different superscripts within column per ingredient are different ($P < 0.05$). SEM: Standard error of the mean.
[1] Screen size used for maize 6 mm and soybean meal 2 mm.
[2] Values between brackets are the increase in moisture content compared to 0 moisture addition per ingredient.

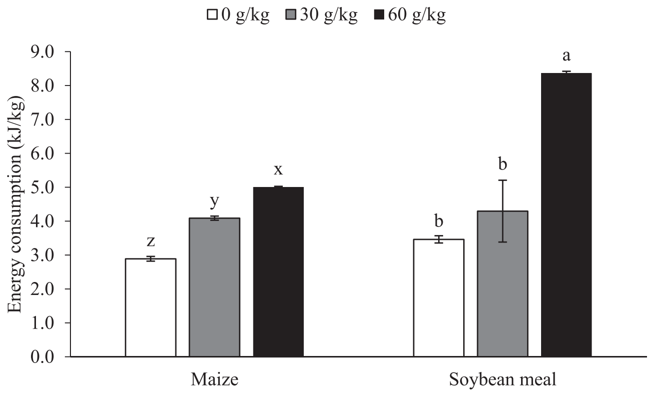
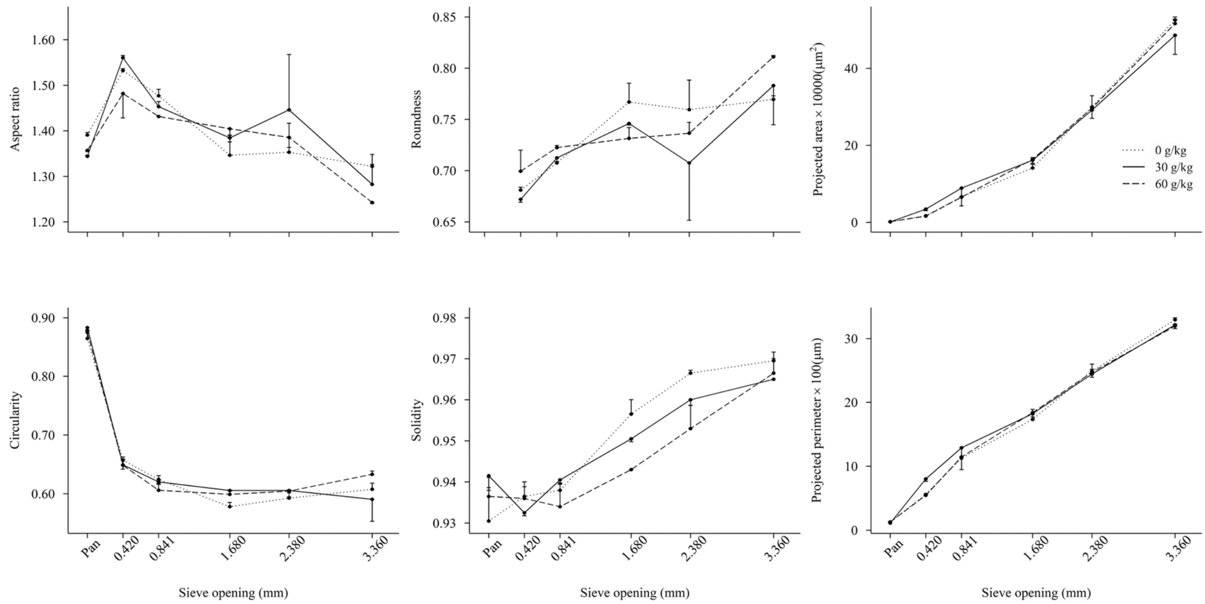


Fig. 2. Net energy consumption of hammer-milled maize and soybean meal as affected by no (0 g/kg), 30 and 60 g/kg moisture addition to the ingredient. Error bars represent standard deviations. Values with different superscripts within ingredient are significantly different (P < 0.05).

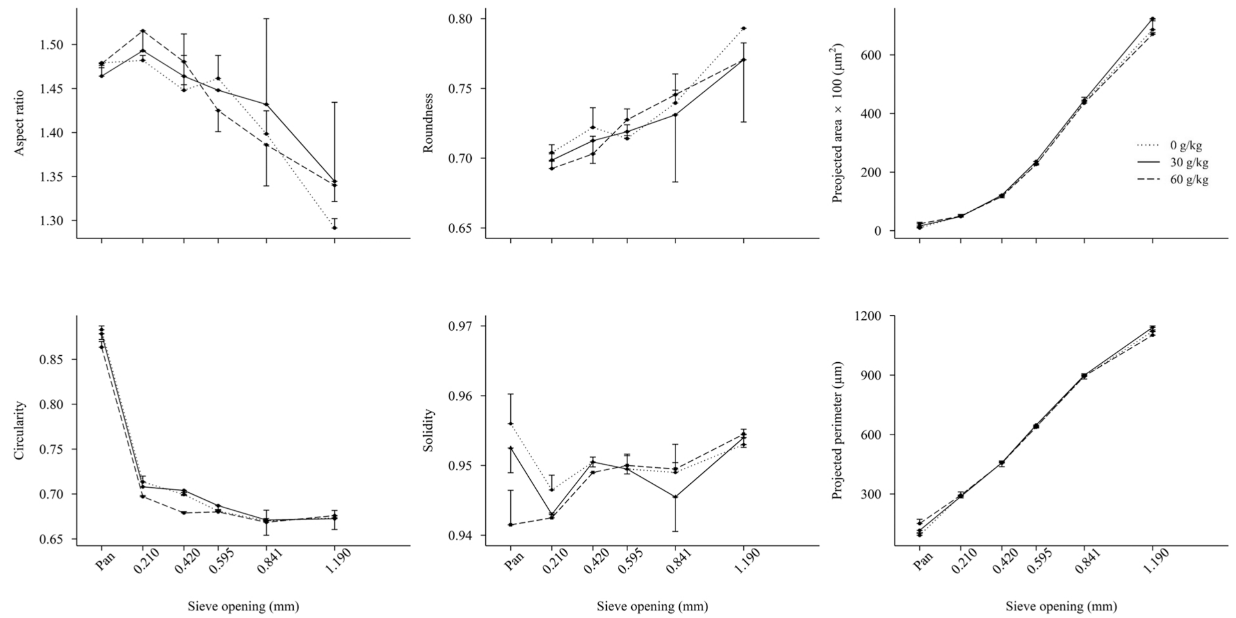


Variable	Projected area	Projected perimeter	Circularity	Aspect ratio	Roundness	Solidity
Moisture	0.963	0.228	0.341	0.250	0.225	0.016
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	0.513	0.290	0.045	0.224	0.219	0.019

Fig. 3. Physical characteristics of particles retained on different sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling of maize. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.

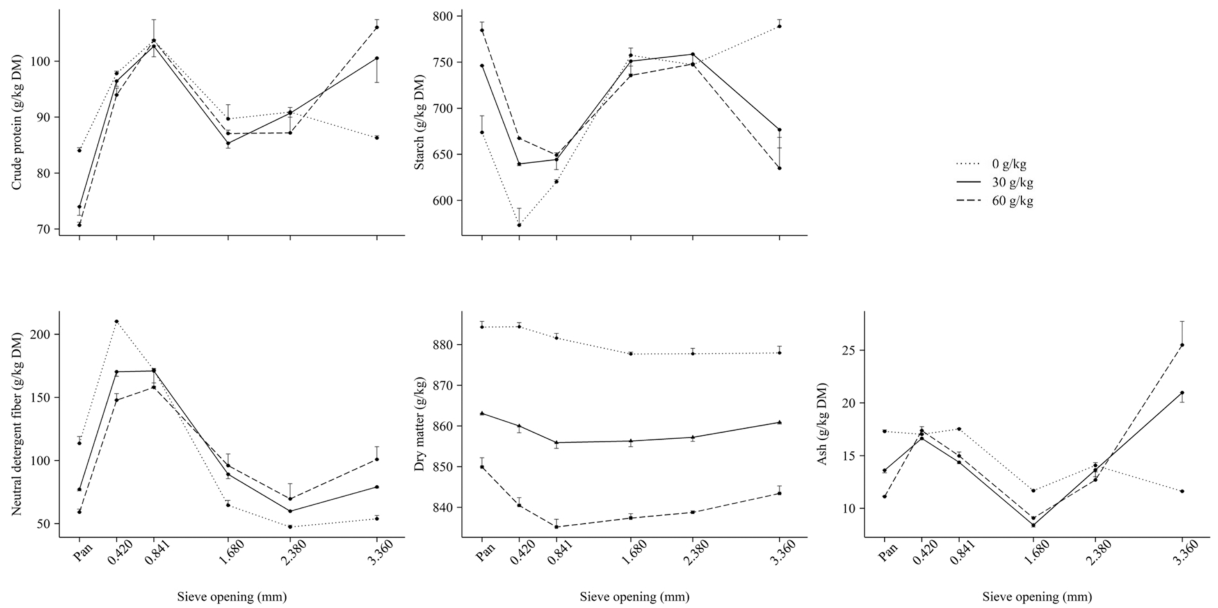
3.3. Nutrient content

Fractionation showed marked differences in the nutrient content among particle size fractions in both maize and SBM (Fig. 5 and Fig. 6, respectively). Moisture content did not significantly influence the nutrient content between particle fractions (P > 0.05), except for the DM content in both ingredients and ash content in SBM (P < 0.001). For SBM, as Fig. 6 showed, the ash content in fractions were relatively similar and close to 72 g/kg, except for the pan fraction in which contained 84 g/kg ash. The larger the particle size, the higher the CP content (~500–570 g/kg) in the case of SBM (P < 0.001). The CP content of maize fractions was low in the pan fraction and increased in the fractions collected on the 0.420- and 0.841-mm sieves (P < 0.001), then decreased on the 1.680 mm sieve fraction (P < 0.001). With increasing particle size, the DM content first decreased and then increased for fractions collected on the 0.841 (M0 and M3) and 1.680 mm (M6) sieve in maize (P < 0.001). A similar pattern was observed for SBM, where the minimum DM content was observed in the fraction collected from the 1.680 mm (M0) and 0.841 mm (M3 and M6) sieves (P < 0.001). Neutral detergent fiber in maize showed a similar pattern to CP, first increasing and then decreasing when particle size increased, with particles > 0.841 mm



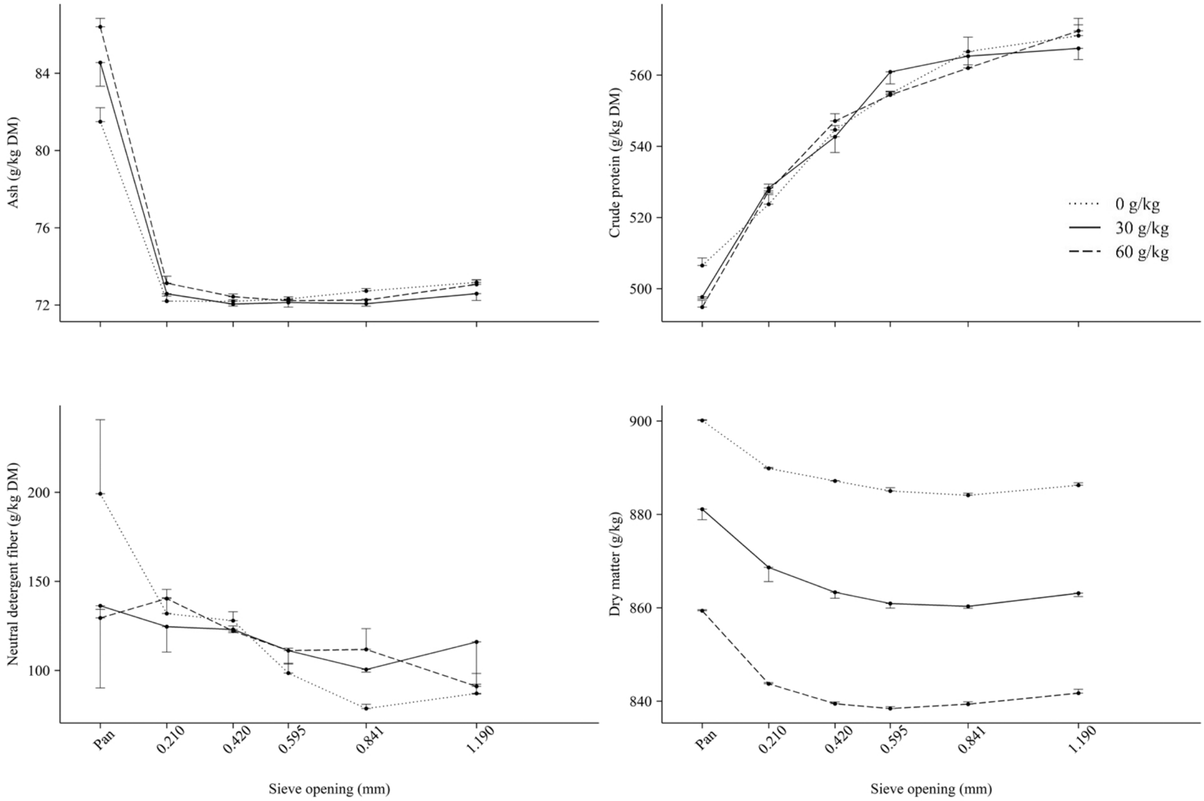
Variable	Projected area	Projected perimeter	Circularity	Aspect ratio	Roundness	Solidity
Moisture	0.018	0.270	0.005	0.658	0.617	0.033
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	0.024	0.055	0.192	0.838	0.911	0.009

Fig. 4. Physical characteristics of particles retained on different sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer-milling of soybean meal. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.



Variable	Ash	Crude protein	Starch	Neutral detergent fiber	Dry matter
Moisture	0.170	0.715	0.145	0.136	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	<0.001	<0.001	<0.001	<0.001	<0.001

Fig. 5. Nutrient content of particles retained on different sized sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer milling of maize. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.



Variable	Ash	Crude protein	Neutral detergent fiber	Dry matter
Moisture	<0.001	0.390	0.919	<0.001
Fraction	<0.001	<0.001	<0.001	<0.001
Moisture × Fraction	<0.001	0.005	0.028	0.008

Fig. 6. Nutrient content of particles retained on different sized sieves (+pan) as affected by no (0 g/kg), 30 and 60 g/kg moisture addition before hammer milling of soybean meal. Error bars represent the standard deviation of duplicate measurements. Probability values of effects are provided in the table.

containing more NDF ($P < 0.001$). In maize, the ash content of particles on the 1.680 mm sieve at each moisture level was the lowest among all fractions ($P < 0.001$).

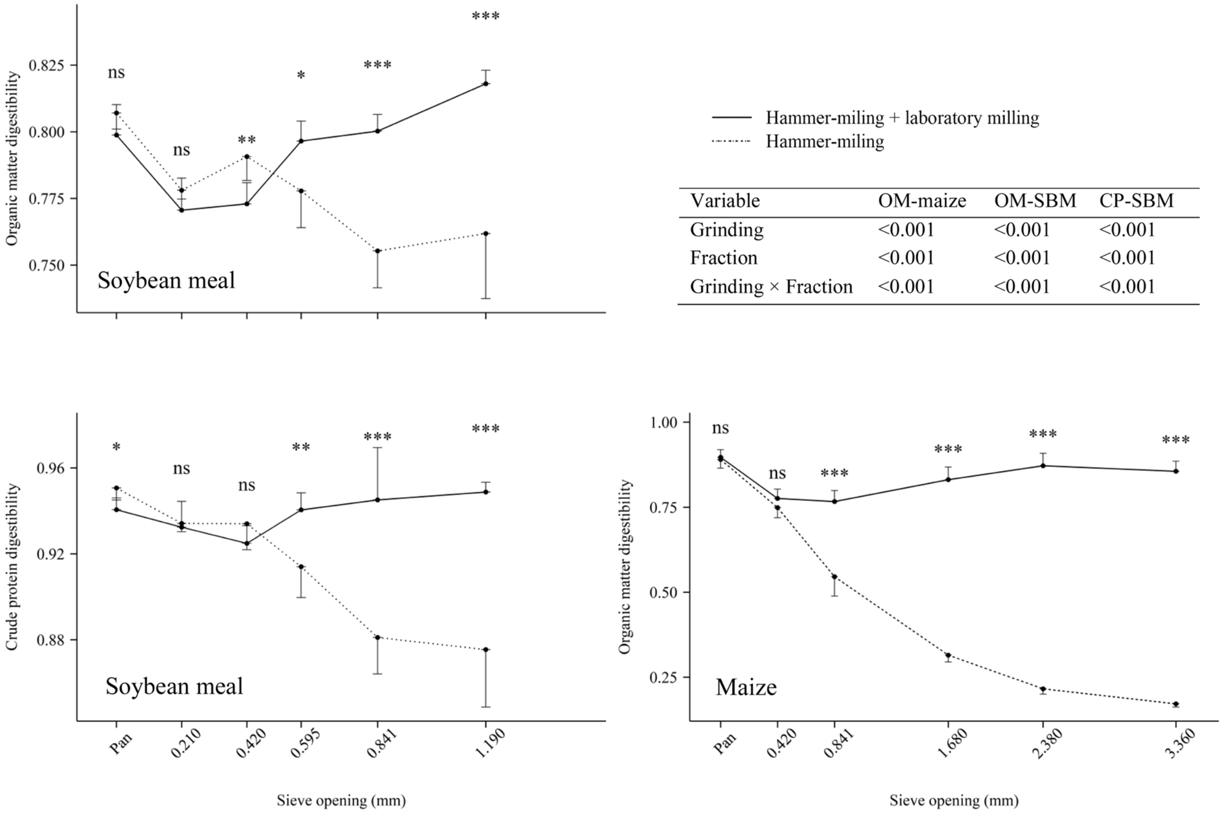
3.4. *In vitro* apparent ileal digestibility

The moisture adjustment of maize and SBM did not affect ($P > 0.05$) the *in vitro* AID of OM and CP among the fractions and the data were, therefore, combined per MC.

treatment. Fig. 7 shows the *in vitro* AID of OM (maize and SBM) and CP (SBM) of particle size fractions originating from the hammer-milled as well as the additional grinding (1 mm). A significant effect of fraction and additional grinding as well as an interaction between the two was observed on *in vitro* OM and CP AID for both ingredients ($P < 0.001$). For the hammer-milled maize, the *in vitro* OM AID increased with decreasing particle size from 0.172 in the 3.360 mm sieve to 0.890 in the material collected in the pan ($P < 0.001$). Unlike the hammer-milled fractions only, the *in vitro* OM AID values of the additionally ground particles were relatively similar, ranging from 0.767 to 0.897. Additional grinding increased the *in vitro* OM AID for maize fractions ≥ 0.841 mm compared to the hammer-milling only ($P < 0.001$). In SBM, the additional grinding of particles ≥ 0.595 mm increased the *in vitro* OM and CP AID values with a higher value ($P < 0.01$) reported for the material collected on the 0.420 mm sieve. For CP, a higher *in vitro* AID value was observed for the material collected in the pan ($P < 0.05$) that was only hammer-milled.

3.5. Plastic deformation

The measurement of plastic deformation of both maize and SBM increased with an increase in MC, with a percentage of total displacement of M0, M3 and M6 of 22.0%, 26.5% and 31.0% for maize and 54.9%, 65.3% and 75.6% for SBM, respectively.



Variable	OM-maize	OM-SBM	CP-SBM
Grinding	<0.001	<0.001	<0.001
Fraction	<0.001	<0.001	<0.001
Grinding × Fraction	<0.001	<0.001	<0.001

Fig. 7. *In vitro* digestibility coefficient of organic matter (OM) and crude protein (CP) of hammer-milled maize and soybean meal (SBM) fractionated by sieving (⋯) and the same fractions additionally ground over a 1.0 mm sieve (–) as per assay requirements. Error bars represent the standard deviations. Probability values of effects are provided in the table. Significance levels within sieve opening: *** , P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

4. Discussion

The present study encompasses three main parts: (1) grinding performance by the hammer mill of maize and SBM with different MC; (2) physical and chemical characteristics of fractionated particles of moisture-treated maize and SBM; (3) effect of additional grinding of fractionated particles of hammer-milled maize and SBM on *in vitro* OM and CP AID. Although, 30 and 60 g/kg water was added to the two ingredients during mixing, the water was not completely absorbed by the ingredients with some moisture lost during mixing and storage. The SBM was already ground and compared to the intact maize kernels, the water could have been more easily distributed and absorbed. Moisture loss during hammer-milling as a result of heat generation is unlikely. Under the assumption that all energy in grinding was converted to heat and using a heat capacity of 1.8 kJ/kg/K, the maximum temperature rise is 4.7 K for the SBM M6 treatment. For the other treatments, the temperature rise would be lower. This is only a slight temperature increase, insufficient to cause moisture loss due to grinding. Probst et al. (2013) reported that there was significant loss of moisture after hammer-milling maize containing 162 and 196 g/kg moisture but not for lower MC (104 g/kg) maize, supporting the observations in the present study.

4.1. Grinding performance

Increasing the MC before hammer-milling of whole maize kernels resulted in more energy consumption, higher yield of coarse particles and a larger GMD, this is in line with results of previous studies (Velu et al., 2006; Dziki, 2008; Doblado-Maldonado et al., 2013; Lee et al., 2014; Braun et al., 2019). Higher MC increases the plasticity of material, which makes it more difficult to grind and therefore more energy was consumed (Mabille et al., 2001; Dziki et al., 2012; Deng and Manthey, 2017; Hassoon and Dziki, 2018). In addition, increased plastic deformation in the material leads to increased grinding energy absorption, and transformed into heat. Therefore, less energy is being transferred to create new surfaces, so that more coarse particles produced (Dziki and Laskowski, 2005; Probst et al., 2013; Hassoon and Dziki, 2018). The increased amount of coarse particles with increased MC in maize could also be attributed to the decrease in secant modulus of elasticity. The destruction of the kernel most likely took place with the appearance and spread of large cracks resulting in large fragments (Haddad et al., 1999; Babic et al., 2013). The non-significant difference in GMD of the M3 and M6 maize may be attributed to the role of the seed coat. High MC makes seed coats less affected by rupture force (Jung et al., 2018), and the M3 and M6 maize may have had similar conditioned seed coats, resulting in similar GMD. It may also be due to the relatively small range of MC. Dziki (2008) reported similar results in grinding wheat at MC of 120, 140 and 160 g/kg. In SBM, the GMD of the M6 was not different from the M3 treatment. However, the net energy consumption was more than double in the M6 treatment. Material with higher MC has a lower flowability (Seifi and Alimardani, 2010) which results in a lower feed rate at a fixed adjustable inlet: it took a longer time to grind the same amount of material. This results in a difference in degree of fill or active volume in the mill which may account for the observed differences or, as indicated, differences in plasticity of the material as indicated by the creep test may account for the large effect on net energy consumption in the M6 SBM treatment.

4.2. Physical and chemical characteristics

Fractionated material of maize and SBM showed marked differences in physical and chemical characteristics, which are in good agreement with results obtained by Lyu et al. (2021). Such results were also observed in the study of grinding other ingredients: barley (Sundberg et al., 1995a, b), sorghum (Al-Rabadi et al., 2009, 2012), rice grains (De La Hera et al., 2013), peas (Maaroufi et al., 2000), and maize (Acosta et al., 2019). However, information on the effects of adding moisture before hammer-milling and possible changes in chemical and physical characteristics of fractionated materials are scarce.

Studies investigating nutrient distribution over fractionated materials can potentially be used to remove specific components from raw materials in food industries (Maaroufi et al., 2000). On the other hand, the differences in nutrient levels could also be considered as indicators for breakage behavior of materials after grinding (Lyu et al., 2021), because such differences can be due to a separation of different botanical constituents of the seed when impacted upon in the hammer mill chamber. For example, NDF content was mainly present in maize particles collected on the 0.420- and 0.841-mm sieves. Since the maize kernel hull contains 51% of the total grain fiber (Watson, 1987), it can be inferred that in the grinding of maize kernels, the hull is probably ground to particles that can pass a 1.680 but not a 0.420 mm sieve, which could also be observed from the images (not provided) taken of fractionated maize.

Moisture content is considered to influence the hardness of materials (Lee et al., 2013) and the strength of the seed coat (Jung et al., 2018), which is also likely, therefore, to influence the grinding behavior of materials. Moisture content in maize only showed a significant effect on solidity which is a measure of the overall concavity of a particle, a lower solidity is an indication of a more fragmented particle and can show an increase in brittleness (Olson, 2011). According to Barbosa-Cánovas et al. (2005) and Jung et al. (2018), a low MC material is more likely to be broken by shattering and tends to produce more irregular-shaped particles which increases solidity values. Soybean meal as a by-product obtained after the oil extraction of soybeans, was already in a ground form before hammer milling in the current study. As such, the physical factors related to grinding might be different from those of whole grain kernels. In SBM, MC showed a significant impact on projected area and solidity, as well as ash content. Interactions between MC and fraction were also observed.

Generally, the nutrient composition and morphology characteristics of maize and SBM fractions is influenced to a minor extent by MC before hammer-milling. The Instron measurements showed changes in plastic deformation for both maize and SBM, but this influence appears to have limited impact on the final physical and chemical characteristics of fractionated material after hammer-milling, although the MC levels studied in the current study are relevant to the feed industry.

4.3. In vitro apparent ileal digestibility

The *in vitro* AID of OM and CP were different among various size fractions for both maize and SBM, which agrees with the data of Lyu et al. (2021). *In vitro* AID of CP increased with particle size decreasing, while the degree of improvements is smaller when particle size is smaller than 0.595 mm. Also, additional ground SBM < 0.595 mm did not improve the *in vitro* AID of CP. Similar results were also observed by Fastinger and Mahan (2003) in grower-finisher swine trials, who reported that the largest improvement *in vivo* AID of amino acid was occurred when particle size was reduced from 949 to 600 μm. However, limited increase in *in vitro* AID was observed when the particle size of SBM was further reduced from 600 μm to 300, and 150 μm.

The *in vitro* digestion was performed based on the method described by Boisen and Fernández (1995), in which the material is prescribed to be ground over a 1-mm sieve. Lyu et al. (2021) reported preliminary results that the additional grinding of hammer-milled maize and SBM fractions with a GMD larger than 1 mm, significantly affected the *in vitro* OM AID (CP was not measured). For hammer-milled maize particles with a GMD of 3.999 mm, additional grinding increased *in vitro* OM AID from 0.161 to 0.907 (Lyu et al., 2021). In order to corroborate the results of the latter authors and extend the measurement to also include CP, all fractions collected in the present study were additionally ground in a standard laboratory mill to pass a 1 mm sieve size, as the assay specifications of Boisen and Fernández (1995) before determination of *in vitro* OM and CP AID. The additional grinding was observed to have a major effect on *in vitro* AID values especially for OM in maize. This indicates that the *in vitro* AID values were highly dependent on the particle size in contrast to observations of Boisen and Fernández (1997) who showed that the *in vitro* total tract OM digestibility of maize and SBM was only reduced by 1.4% and 0.4% units, respectively when ingredients were ground at 3 instead of 1 mm. An explanation could be that Boisen and Fernández (1997) investigated the whole ingredient while the present study focused on fractionated materials, which contain different levels of nutrients. In the study of Boisen and Fernández (1997), results for samples with a particle size larger than 1 mm were shown to be less reproducible. According to the results in the present study, particles collected on sieves smaller than 0.595 mm, the effect of additional grinding was largely non-significant. Further studies should be conducted to ascertain the importance of particle size on the *in vitro* AID assay as published by Boisen and Fernández (1995, 1997).

5. Conclusion

Increasing the moisture content of maize and soybean meal can increase the plasticity of the material which results in increased energy consumption during and an increased geometric mean diameter of particles after hammer-milling. Size fractionated particles differ in terms of nutrient content and morphological characteristics after the hammer-milling of maize and soybean meal which may be indicators for breaking behavior. The effects of moisture addition on the breakage behavior of maize and soybean meal during hammer-milling, however, may be limited. The *in vitro* apparent ileal digestibility of OM and CP appears to be highly dependent on particle size when it comes to fractionated ingredients with particles $\geq$ 0.595 mm.

CRediT authorship contribution statement

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

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