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PII: S0377-8401(18)30411-5
DOI: https://doi.org/10.1016/j.anifeedsci.2019.03.010
Reference: ANIFEE 14158

To appear in: *Animal Feed Science and Technology*

Received date: 23 March 2018
Revised date: 3 December 2018
Accepted date: 25 March 2019

Please cite this article as: Sakkas P, Royer E, Smith S, Oikeh I, Kyriazakis I, Combining alternative processing methods for European soybeans to be used in broiler diets, *Animal Feed Science and Technology* (2019), https://doi.org/10.1016/j.anifeedsci.2019.03.010

Combining alternative processing methods for European soybeans to be used in broiler diets

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[1]The Feed-A-Gene Project has received funding from the European Union's H2020 Programme under grant agreement n° 6336531.

Highlights

- European soy beans were processed to produce 4 SBM products by grinding, dehulling (NH) or not (H) and subjected to either extrusion (E) or flaking-pressing-cooking (F).
- Diets were offered to starter and grower broilers in a 2x2 design: processing method (E) vs (F) x hulling methods (NH) vs (H).
- Treatments equally promoted performance and there were no differences in measured variables other than a higher GIT development and lower carcass yield in H in comparison to NH products.

Abstract

Locally produced, expeller soybean meal (SBM) may be an important constituent of European broiler diets in the future. In the present trial 4 SBMs were produced from European grown soybeans, using different processing methods; this is the first time that the combination of these methods has been applied. Starter (d0-14) and grower (d15-28) diets were offered to 288 Ross 308 male broilers in a 2x2 design: 2 processing methods ((Extrusion-pressing (E) vs Flaking-pressing-cooking (F)) x 2 hulling methods ((with hulls (H) vs no hulls (NH)). Variables measured consisted of average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio, apparent ileal dry matter (DM) and crude protein (CP) digestibility, jejunal histomorphometry at d14 and d28 and ileal digesta viscosity at d28 of age. In addition, carcass and carcass part yield, organ weight, and empty gastrointestinal tract weight and length per small intestinal segment were assessed at d28. Processing method did not affect any of the variables tested. On the other hand, hull presence increased ($P < 0.05$) ADFI over the starter period, but not over the grower period. Presence of hulls increased proventriculus, gizzard and jejunum weight, and reduced carcass yield at d28 of age, likely due to the higher fibre content. Method of processing and hulling significantly interacted ($P < 0.05$) for ADFI and ADG at the end of the starter period, being highest for the E/H treatment, but overall broiler performance was similar between dietary treatments. Similarly, small intestinal architecture and DM and CP digestibility were not affected by dietary treatments at either d14 or d28 post hatch. Although there was some variation in soybean protein solubility and trypsin

inhibitors amongst SBM products these factors did not appear to affect any of the measured variables. In conclusion, all 4 methods of production resulted in comparable results in relation to performance variables. Hull removal did not confer a significant advantage, aside from increased carcass yield, possibly due to the adaptive growth of the gizzard and proventriculus.

Keywords: extrusion, flaking, hulling, European grown soybean meal, broiler, digestibility

1. Introduction

Soybean meal (SBM) represents 61% of the protein sources used to feed livestock, whilst the European Union (EU) has a self-sufficiency of 3% for its soybeans and SBM needs, which is considered unsustainable for a variety of reasons (Leinonen et all, 2013; Boerema, et al., 2016; Zander, et al., 2016). The increased demand for imported soybean, especially by the non-ruminant feed industry, is attributed to its high crude protein (CP) content and amino acid (AA) profile, which is complementary to that of cereals, and its high AA digestibility (de Visser 2014; Ravindran, et al., 2014). Increasing EU production of domestic soybean and processing at medium plants from local, non-genetically modified crops may contribute to the reduction of dependency on imported SBM (Martin, 2015). Following years of decline, EU soybean production has increased over the last years, which is attributed to the change in policy support as well as premium prices for non-genetically modified soybeans (Eurostat, 2016; European Commission, 2017). However, because of the opening up of the European market to imports of biodiesel from third countries and current discussions around the Renewable Energy Directive (European Commission, 2009), oil and meal markets may be volatile in the next years. Nonetheless, before any investment phase by oil operators, the feasibility of small or medium-sized SBM production for use as a local feedstuff must be assessed.

The classical processing of soybeans involves dehulling, defatting with hexane, treatment with equate ethanol under vacuum to eliminate soluble carbohydrates, and drying with ethanol. However, hexane is extremely flammable and non-renewable, and is regulated as a hazardous air pollutant (O'Quinn, et al., 1997). In addition, solvent extraction requires a considerable capital investment, a consistent supply of soybeans to operate continuously throughout and imposes high energy consumption (Pacheco, et al., 2013). Although expeller extraction of soybeans is an alternative to the use of hexane, performance responses to expeller extracted SBM are rather inconsistent and suggest variations in nutritional value depending on the seed source and the process used (Karr-Lilienthal et al., 2006; Opapeju et al, 2006).

In the present study alternative processes for the production of expeller SBM from European grown soybeans were compared. Following grounding, soybeans were dehulled or not and then either extruded or flaked and cooked and finally pressed to extract the oil, resulting in four SBM products. This is, to our knowledge, the first time the combination of these processing and hulling methods has been applied. SBM products were then incorporated into broiler starter and grower diets to compare their effect on performance, digestibility as well as organ and gastrointestinal tract (GIT) development. Based on the analysed soybean potassium hydroxide, KOH solubility index and trypsin inhibitor units (TIU; mg/kg) we hypothesised that diets incorporating the dehulled, flaked and cooked SBM product would result in superior performance and digestibility. Furthermore, we hypothesized that hull presence would penalise performance variables, due to the potential effects of the increased fibrous content deriving from soy hulls on GIT development and nutrient digestibility.

2. Material and methods

2.1 Animals and husbandry

All procedures were approved by the Animal Welfare and Ethical Review Body (AWERB) of Newcastle University. Two-hundred and eighty-eight male Ross 308 day old chicks were

obtained from a commercial hatchery and were housed in a windowless, thermostatically controlled building in 24 pens of 1.7 m^2. Each pen was equipped with a tube feeder and a bell-drinker, and wood shavings were used as litter to a depth of 5cm. Birds had *ad libitum* access to feed and water throughout the trial. Temperature at pen level was monitored daily and maintained to meet Aviagen recommendations (Aviagen, 2014), starting at 34 °C at chick placement and gradually reduced to 20 °C by 25 days of age. Light intensity at pen level ranged from 80 to 100 lux, whilst a lighting schedule of 23L:1D was applied for the first 7 days of age and switched to 18L:6D for the remainder of the trial.

2.2 Soybean meal products and diets

The soybeans (var. Ecudor, Euralis Semences, Lescar, France) were grown near Toulouse in the South of France and yielded around 3000 kg/ha; they were harvested in September 2015. A small quantity of 4200 kg of soybeans was processed at the OLEAD technology platform (Pessac, France), following the methods detailed by Quinsac et al. (2012) to produce flaked-cooked-pressed SBM with hulls (831 kg), or without hulls (634 kg), and extruded-pressed SBM with hulls (708 kg) or without hulls (718 kg). Processing parameters of the soybeans for the production of the experimental SBM products are outlined in Table 1. Briefly, soybeans were ground using a cylindrical grinder with corrugating rolls (Damman-Croes, Roeselare, Belgium), and were then dehulled or not with a cleaner separator (D50, Ets Denis, Brou, France). The two products were either extruded using single-screw extrusion (FEX1, France extrusion) at 140°C at around 100 kg/h, or cooked (horizontal cooker, Olexa, Arras, France) at 150°C for 3600 seconds after flaking with contra-rotating smooth cylinders. All beans were then pressed (MBU 75, Olexa, arras, France) to extract the oil and the quality of the SBMs was determined (Table 2). Following processing of the soybeans, SBM products were transported to UK for their incorporation to the starter and grower broiler diets.

Raw soybeans and SBM products were analysed at a COFRAC accredited commercial laboratory of Terres Inovia (Ardon, France) for dry matter (DM) (ISO 665 for beans and 771 for meals, respectively), crude protein (CP) (ISO 5983-2), crude fat (NF V03-908 for beans and ISO 22630 for meals), crude fibre (NF V03-040), KOH protein solubility (ISO 14244). Trypsin inhibitor contents [1 TIU/mg = 1.9 trypsin inhibitor activity (TIA) mg/g] (TIU/mg) were analysed at a COFRAC accredited commercial laboratory (InVivo Labs, France) using the AOCS Ba 12-75 SN method (Table 2). Total, soluble and insoluble non starch polysaccharides (NSP) and their constituent sugars were determined as alditol acetates by gas-liquid chromatography (GLC) for neutral sugars and by colorimetric method for uronic acids using the modified Uppsala method (Knudsen, 1997).

Thereafter, starter (d0-14) and grower (d15-28) mash diets were formulated deriving from the 4 SBM products (2 processing methods ((Extrusion-pressing (E) vs Flaking-pressing-cooking (F)) x 2 hulling methods ((with hulls (H) vs no hulls (NH)). Synthetic AAs were added to cover limiting EAA requirements at the same level on a digestible AA basis for all starter or grower diets (Table 3). Differences in AME of the 4 dietary treatments were accounted for by supplementation of soybean oil. All diets contained titanium dioxide (0.5%) as an indigestible marker. All diets were analysed at a UKAS accredited commercial laboratory to the internationally recognised standard for competence ISO/IEC 17025:2005 (Table 3; Sciantec Analytical Services, Cawood, UK). Briefly, DM was measured by determining the loss in weight of the feed sample after heating at 103 - 105°C for 3 hours. Crude fat was determined by extraction with petroleum ether and total fat by acid hydrolysis followed by petroleum ether extraction. CP was determined with the Dumas method on a LECO FP-528 Nitrogen Analyser (Leco Instruments UK, Cheshire), Crude fibre and acid detergent fibre (ADF) using an Ankom 220 Analyser (Ankom, Technology, Fairport, NY, USA). Ash was determined gravimetrically after incinerating the sample at 510 °C for 4 hours. TiO2 analysis in feed was according to the method described by Vogel (1961). Neutral detergent fibre (NDF) was estimated by enzymatic gravimetry.

2.3 Experimental design and sampling

Upon arrival at the facilities birds were randomly allocated to one of four diets, each containing one of the four SBM products (E/H, E/NH, F/H and F/NH). Starter diets were offered from d0-14 and grower diets from d15-28. Each treatment was replicated in 6 pens with 12 birds per pen. Pen body weight (BW) was measured at arrival and pen BW and average daily feed intake (ADFI) at the end of the starter and grower period.

Three randomly selected chickens with a BW close to the pen average were individually weighed at d14 and d28 of age and removed from their home pens. Following euthanasia with a lethal injection of sodium pentobarbitone (Euthatal®, Merial, Harlow, United Kingdom), the ileum was excised, cut in 3 smaller segments and intestinal contents were collected from the lower 2/3 of the ileum up to 2cm from the ileocecal junction with gentle finger stripping and pooled for each pen. Afterwards 5 cm of intestinal tissue were excised from one of the three birds from the region immediate to Meckel's diverticulum, and were fixed in 10% buffered formalin for morphometric assays under light microscopy. From the birds killed at d28 of age, a subsample of pooled ileal digesta from the upper 1/3 of the ileum were also collected and immediately stored at -80 °C pending determination of viscosity. An additional bird per pen was selected at d28 of age and its gastrointestinal tract and organs were removed to obtain the empty gizzard and proventriculus weight, full ceca weight and liver and pancreas weight. In addition, the length of duodenum, jejunum and ileum were determined along with the weight of each segment following finger stripping. Subsequently, the carcass was de-feathered, its shanks, legs and head removed and dissected to its parts and the weight of the breast muscle, thighs and wings were determined.

2.4 Histology

Excised, formalin-fixed intestinal sections from the duodenum and jejunum were dehydrated through a series of graded ethanol baths followed by xylene in a Shandon™ Excelsior™ ES Tissue

Processor (Thermo Fisher Scientific Inc., Waltham, Massachusetts), before being embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin/eosin. Histological sections were examined under a Zeiss Primostar light microscope and images were captured using ZEN imagine software (Zeiss Germany, Oberkochen, Germany). Images were viewed to measure morphometric features of the intestinal structure at 10× magnification. From sections, the villus height (VH) and the crypt depth (CD) were determined using ImageJ (NIH) software (Schneider, et al., 2012). The VH was estimated by measuring the vertical distance from the villus tip to the villus-crypt junction for 10 villi/section, and the CD by the vertical distance from the villus-crypt junction to the lower limit of the crypt, for 10 corresponding crypts/section. The ratio between VH:CD was calculated (VCR).

2.5. Laboratory analyses

Frozen ileal digesta samples originating from the upper 1/3 of the ileum were thawed in a water bath at 40 °C. After defrosting the samples were centrifuged (with 12 000 × g for 10 min), 0.5 ml of supernatant was then loaded to a Brookfield Digital Viscometer (Model LDVI+CP, Brookfield, Engineering Laboratories, Stoughton, MA) with a plate-plate geometry and a gap of 2 mm appropriate for small volumes to carry out digesta viscosity measurements. The temperature of the sample was maintained at 40°C during the measurements. The results are presented as solution viscosity in centipoise (CPs). The ileal digesta samples collected from the lower 2/3 of the ileum were freeze dried overnight and along with samples of the diets, and were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at −20°C pending chemical analyses. All samples were analysed for CP with the Dumas combustion method using Leco's CNS 2000 analyser (Leco Instruments UK, Cheshire), and titanium according to the methodology of Short et al., (1996).

2.6 Calculations and statistics

All statistical analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). For all statistical assessments pen was considered the experimental unit and data were analysed with PROC GLM with processing method and hulling as fixed factors and their interaction. Weight of carcass parts, organs and GIT segments as well as length of small GIT segments, obtained at d28 of age were expressed as a ratio to the sampled empty carcass weight of the slaughtered bird. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P < 0.05$. All values are expressed as model-predicted least square means with the SEM.

3. Results

3.1 Performance

There were two mortalities over the production period, classified as starve -outs, on d2 and d4 of age for EH and FNH, respectively. Effects of processing method, hulling method and their interaction on performance variables are presented in Table 4. There was no effect of processing method on any of the performance variables. Hulling method affected ADFI ($P = 0.032$) during the starter period, which was significantly higher for birds when hulls were present in the diet rather than not. However, hulling method did not affect performance neither during the grower period nor over the whole growing period. Processing method and hulling method interacted for average daily gain (ADG) during the starter period ($P = 0.004$), being significantly lower ($P < 0.05$) for E/NH and F/H, compared to E/H treatments. Furthermore, processing method and hulling method interacted for ADFI during the starter period, which was significantly lower for the E/NH than the E/H treatment.

3.2 Ileal digestibility and digesta viscosity

Effects of processing method, hulling method and their interaction on ileal apparent CP and DM digestibility and ileal digesta viscosity are presented in Table 5. There was no significant effect of processing method, hulling method or their interaction, on any parameter.

3.3 Carcass and carcass part yields

Effects of processing method, hulling method and their interaction on carcass and carcass part yields are presented in Table 6. There was no significant effect of processing method, hulling method or their interaction on carcass part yield at d28. However, hull presence significantly reduced carcass yield ($P < 0.05$).

3.4 Histology

Effects of processing method, hulling method and their interaction on VH, CD and VCR are presented in Table 7. There was no effect of processing method, hulling method or their interaction on VH, CD and VCR.

3.5 Gastrointestinal tract development

Effects of processing method, hulling method and their interaction on GIT and organ weight and on small intestinal segment length are presented in Table 8. Processing method did not affect GIT and organ measurements; this was also the case for the interaction between processing method and hulling method. However, hull presence significantly increased gizzard, proventriculus and jejunal weight ($P < 0.05$).

4. Discussion

The motivation for this study was the reliance of European broiler systems on imported SBM and the possibility of introducing novel SBM products from locally grown soybeans (Watson et al 2017). Sustainability is an important aspect of the evaluation of alternative sources of protein

for non-ruminant farm animals. Feed provision represents the poultry industry's biggest environmental issue (Leinonen et al., 2012; Tallentire et al., 2017), exacerbated by the inclusion of imported soybeans from South America where they are grown in vast monocultures on land obtained via deforestation (de Visser et al., 2014; Kebreab et al., 2016). The growing concern about the production systems of soy and impact that they have on deforestation and soil decline has led to initiatives for sustainable soy, like the RTRS soy (The Round Table on Responsible Soy) and the Danube Soy initiative (Donau Soja Association). Partially defatted SBM could have an additional economic advantage against imported SBM related to its non GMO character and its local origin (Le Cadre et al, 2015).

Extrusion, or cooking processes were used in combination with dehulling and pressing to produce four partially defatted SBMs from the same batch of beans. The crushing costs and net margins of both E and F methods can be estimated as rather similar, but a Flaking cooking pressing unit may also be used for sunflower and rapeseed meal oil extraction (Quinsac et al, 2012). The chemical characteristics of these SBMs are in good agreement with the meal quality of the F/NH meals produced with soybeans of the 2010 French harvest in the study reported by Labalette et al. (2013). As expected, the E process was more effective than F for de-oiling the meals. All combinations of preparation and processing methods resulted in acceptable levels for KOH protein solubility index and residual trypsin inhibitor activity, whereas E was shown to be less effective than F for deactivation of trypsin inhibitors in the previous work of Quinsac of al. (2012). However, the present processing of SBMs resulted in variable KOH protein solubility index, TIU and fibre content of the products. Although the dehulling step resulted in an increase of almost 3 g/100 g protein (58.8 and 58.4 g/100 g for E/NH and F/NH products, respectively, and 56.0 and 55.8 g/100 g for whole E/H and F/H meals, respectively, on a fat free DM basis), diets within a growing stage were designed to have the same CP content. On the other hand, dietary levels of NDF and ADF were increased in diets which included products with hulls, as expected due to the higher

concentration in soluble and insoluble NSP, along with overall lower levels of KOH solubility index values and lower TIU values.

In assessing the quality of the SBM products, it is important that urease or trypsin inhibitor activity is analysed in conjunction with protein solubility to obtain an accurate assessment of processing adequacy. As far as the TIU values in this experiment were concerned, soybean products had 2.6, 3.5, 3.6, and 7.6 TIU/mg for E/H, E/NH, FN/NH and F/NH meals, respectively. TI reduce the proteolytic action of the pancreatic enzyme trypsin and chymotrypsin (Liener, 1994), affecting protein digestibility and growth rates despite potential increases in feed intake (Perilla, et al., 1997). Although heat processing reduces levels of heat labile trypsin inhibitors, it may also adversely affect protein digestibility by decreasing the availability of heat-sensitive AA due to the formation of Maillard reactions. This is reflected in the KOH protein solubility index which decreases as the degree of heat treatment associated with soybean processing increases. It has been suggested that a KOH index higher than 85% indicates under processing, whereas a protein solubility index less than 74% infers overheating (Dozier, et al., 2011). Only the F/NH product had TIU levels above the commercial recommendation of 4 TIU/mg which may be attributed to the lower dryer outlet temperature measured for the dehulled beans compared to whole beans (90 vs. 97°C). It has been previously suggested that even lower levels are required for optimum performance of grower chicks (Clarke, et al., 2005). However, the higher TIU levels measured in the F/NH dietary treatment did not penalise performance, or digestibility, which may be attributed to its KOH protein solubility index value which was the highest among SBM products. Regardless of TIU levels, dietary treatments did not differ in terms of small intestinal architecture or pancreas weight; a high level of protease inhibitors is known to lead to reduced villi height whilst causing hypertrophy and hyperplasia of the pancreas (Leeson and Summers, 2001, Rocha, et al., 2014).

Contrary to expectations, CP and DM digestibility was not affected by hull presence. In terms of their total dietary fibre composition, hulls contain 83.3% total dietary fibre with an insoluble to soluble fibre ratio of 5.0 (Dust, et al., 2004). Based on the analysed NSP contents, SBM

products contained a similar content of soluble NSP, apart from the lower content of the F/NH treatment (≃2 % vs 4 % DM). One would expect that the increased soluble fibre content deriving from soy hulls would have resulted in lower digestibility values for CP (Choct, et al., 2010). However, this was not observed in the present study, which may be attributed to the fact that diets were wheat- rather than corn-based to reflect European feeding practices. Wheat has a higher soluble NSP fraction than corn (22 % vs 12 %), mainly in the form of soluble arabinoxylan (Knudsen, 2014), which likely negated in part the contribution of soy hull inclusion to the total soluble fibre content of the hulled treatments and associated effects on resulting digesta viscosity and nutrient digestibility. To that end, the ability of the products to promote performance and nutrient digestibility should be further investigated in wheat based diets with xylanase inclusion. On the other hand, hulled treatments led to higher proventriculous and gizzard development which could be primarily ascribed to their increased total NSP content which predominantly derived by an increase in the insoluble NSP content. It has been previously shown that addition of soy hulls at 3% in low fibre diets results in increased gizzard development (Jiménez-Moreno, et al., 2009), which may contribute to increased grinding of the diets and as a result facilitate DM and CP digestibility. A well-developed gizzard has been associated with increased grinding activity, enhanced reverse peristalsis, and greater absorption of nutrients (Amerah, et al., 2007). Although effects on proventriculus and gizzard development were only assessed at d28 of age, they may have been present earlier, thus accounting for the absence of adverse effects of soy hull inclusion on DM and CP digestibility at the end of the starter period. Previous studies have indicated that increasing dietary fibre levels may induce changes in GIT morphometric features, which nonetheless vary according to the type of the fibre used, its level of incorporation, the type of cereal grain used, the feed form and particle size of the diet offered (Mateos et al., 2012). In the present study there was no difference on villi height, crypt depth or their ratio which are indicative of GIT absorptive capacity. However, an increase in relative jejunum empty weight was observed as well as a reduced carcass yield which reflects a higher GIT development in birds offered hulled treatments.

As far as performance is concerned, the EH dietary treatment group showed ~ 6 % higher ADFI in comparison to the E/NH treatment, as well as ≃ 5.5 % higher ADG in comparison to both F/H and E/NH treatment groups over the starter period. However, these differences were absent by the end of the grower period and over the growing period as a whole. One would expect that hull presence would have increased maintenance requirements considering the lower carcass yield observed at d28 of age and the higher GIT development, therefore resulting to overall higher FCR. In the absence of effects on DM and CP digestibility it is possible that AME digestibility was improved although it was not measured in the present trial.

In this study we concentrated on the performance consequences of the four SBM products. Due to the small scale and experimental nature of the study, it was not deemed appropriate to conduct an environmental and economic life cycle assessment of their consequences. In conclusion, both processing methods of locally produced soybeans resulted in products which equally promoted broiler performance over the grower and the overall growing period. Although there were some differences in KOH solubility and TIU levels, these were poor predictors of DM and ileal CP digestibility. Hulled products resulted in similar performance, which could be ascribed to their effect on proximal GIT development. This nonetheless was achieved at an additional cost; slightly higher proportions of the hulled products were used to achieve similar CP content.

Declarations of interest: none

Competing interests

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The Feed-A-Gene Project has received funding from the European Union's H2020 Programme under grant agreement n° 6336531.

Acknowledgements

Appreciation is expressed to James Taylor for assisting in the performance of the experiment and carrying laboratory procedures. We would like to thank to Professor Knud Erik Bach Knudsen for providing us with the analysis of the NSP fraction of the SBM products, and Patrick Carré and Alain Quinsac for producing the SBM products.

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Tables and figures

Table 1. Processing parameters of soybeans for the production of experimental SBM batches

Extrusion-Pressing								
	Extrusion step[1]			Pressing step[3]				
	Throughput rate	Last barrel temperature	Motor power	Oil flow	Meal flow	Rotation speed	Motor power	Cage temperature
Product	kg/h	°C	kW	kg/h	kg/h	rpm	kW	°C
E/NH	115	141	25.0	19	98	15	4.8	98
E/H	100	138	19.5	14	76	15	5.9	113
Flaking-cooking-pressing								
	Cooking step[2]			Pressing step[3]				
	Cooker exit temperature	Steam injection	Dryer exit temperature	Oil flow	Meal flow	Rotation speed	Motor power	Cage temperature
Product	°C	kg/h	°C	kg/h	kg/h	rpm	kW	°C
F/NH	86	9.7	90.3	26	184	15	7.3	0
F/H	91	9.5	97.1	19	150	14	5.3	104

[1] Extrusion was obtained with a single-screw extruder (FEX1, France extrusion) of 50-300 kg/h capacity powered by a 30 kW motor with adjustable rotating speed. The screw has a diameter of 82 mm and a length 720 mm. The extruder was used with a soybean configuration at 9 rpm. Restriction disks were placed between the last screw elements and the die was mounted on a threaded insert allowing adjustment of the gap between the tapered tip of the screw and the die.

[2] At first, a flaking was conducted with contra-rotating smooth cylinders (Croix, France). Distance between rolls was adjusted to obtain flakes without almonds residues. The horizontal cooker (Olexa, Arras, France) was composed of two superposed horizontal cylinders of 900 mm in diameter and 2000 mm in length. Each stage was heated by 4 resistances of 4 kW, the heat being transported by a thermal fluid circulation in cooker jacket. Inside the cooker, flakes were mixed by a helical ribbon. The flakes were dried in the bottom part of the cooker which was connected to a mist extracting fan. Cooker and dryer set temperatures were 145 and 155 °C respectively and the average length of stay was around 3600 seconds.

[3] Final pressing of materials was carried with a screw press (MBU75, La Mecanique Moderne, Arras, France). The screw length was 1800 mm and the diameter was 180 mm diameter in the feed area and 150 mm in high pressure area. The semi-compressing arrangement of the press was used for the tests (non-compressing arrangement with addition of the two rings at the last part of the screw).

Table 2. Chemical analysis of raw soybeans and of the four soybean products produced through different processing (Extrusion pressing vs Flaking-pressing-cooking) and hulling methods (No hulls vs With hulls).

Proximate analysis (%)	Raw soybeans	Extrusion-pressing		Flaking-pressing-cooking	
		No hulls	With hulls	No hulls	With hulls
DM [1]	86.6	93.85	94.2	92.3	91.3
Crude fat	17.8	4.8	4.6	5.9	7.8
CP [1]	38.36	52.3	50.1	50.5	46.6
Soluble proteins (% CP)	36.5	39.7	35.2	44.9	38.2
Protein solubility KOH (%)	95	75.9	70.2	88.8	82
Crude fibre	4.8	2.9	5.53	3.19	5.06
Trypsin inhibitors (TIU/mg)	25	3.5	2.6	7.6	3.6
Soluble NSP [1] (% DM)	-	3.8	3.9	2.2	4.4
Insoluble NSP [1] (% DM)	-	12.6	17.2	15.3	16.9
Total NSP [1] (% DM)	-	16.4	21.1	17.5	21.3

[1] (DM) dry matter, (CP) crude protein, (NSP) non starch polysaccharides.

Table 3. Composition of the 4 diets produced through the combination of different processing and hulling methods, offered to broilers over the starter (0-14) and grower periods (15-28).

	Starter (d1-14)				Grower (d15-28)			
Processing Method [1]	E		F		E		F	
Hulling Method [2]	NH	H	NH	H	NH	H	NH	H
Ingredient (%)								
Maize	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat	52.9	51.7	53.2	50.7	56.9	55.3	56.1	53.4
E/NH	29.0				25.0			
E/H		30.0				26.5		
F/NH			29.0				26.0	
F/H				32.0				29.0
Soybean oil	3.00	3.20	2.70	2.20	3.50	3.65	3.35	3.00
Limestone	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Monocalcium phosphate	1.25	1.25	1.50	1.50	1.25	1.25	1.25	1.25
Vitamin and mineral premix [3]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine HCL	0.40	0.41	0.44	0.43	0.30	0.30	0.30	0.30
DL-Methionine	0.35	0.35	0.41	0.41	0.35	0.35	0.35	0.35
L-Threonine	0.15	0.16	0.17	0.17	0.15	0.15	0.15	0.15
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Coccidiostat	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Calculated composition (%)								
AME_n (MJ/kg) [4]	12.79	12.80	12.80	12.80	13.02	13.01	13.04	13.05
Calcium	0.97	0.98	0.98	0.98	0.92	0.92	0.92	0.93
Total phosphorous	0.72	0.72	0.72	0.73	0.64	0.65	0.65	0.66
Available phosphorous	0.48	0.48	0.48	0.49	0.42	0.42	0.42	0.42
Digestible lys	1.31	1.31	1.32	1.32	1.13	1.13	1.13	1.14
Digestible met + cys	1.00	1.00	1.00	1.00	0.91	0.91	0.91	0.91
Digestible thr	0.83	0.83	0.83	0.83	0.76	0.77	0.76	0.77
Digestible try	0.24	0.23	0.23	0.23	0.21	0.21	0.22	0.22
Analysed composition (%)								
Dry matter	90	90	89.4	89.4	89.1	89.3	89.1	88.9
Crude protein	23.1	23	22.9	22.8	21.6	21.1	21.8	21.6
Crude fibre	2.3	2.8	2.2	2.5	2.2	2.7	2.3	2.6
Ash	7.6	7.2	6.8	6.6	6.2	6.4	7	6.4
NDF [4]	7.8	9.3	7.8	8.8	7.7	8.8	7.8	8.5
ADF [4]	3.1	3.7	3.1	3.5	2.7	3.6	3.1	3.5
Crude fat	6.5	6.2	6.0	5.5	5.9	6.2	7.2	7.1
Total oil	7.7	7.4	6.9	6.8	7.0	7.5	8.1	8.0
Titanium dioxide	0.610	0.631	0.549	0.551	0.530	0.500	0.581	0.531

[1] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[2] (H) with hulls, (NH) no hulls.

[3] The premix supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.30 mg; cholecalciferol, 5000 IU/kg µg; folic acid, 2.2 mg; menadione, 3.2 mg; niacin, 60 mg; pyridoxine, 5.4 mg; trans-retinol, 13.000IU/kg; riboflavin, 8.6 mg; thiamine, 3.2 mg; dl-α-tocopheryl acetate 80IU/kg; choline chloride, 1700 mg;; Cu, 16 mg; Fe, 20 mg; I, 1.25 mg; Mn, 120 mg; Mo, 0.5 mg; Se, 300 µg; Zn, 110 mg.

[4] (AME_n) apparent metabolisable energy, (ADF) acid detergent fibre, (NDF) neutral detergent fibre.

Table 4. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods, on performance variables of broilers over the starter (d1-14) and grower periods (d15-28).

Measured responses [1]		BW	ADFI			ADG			FCR		
Days of age		0	1-14	15-28	1-28	1-14	15-28	1-28	1-14	15-28	1-28
Processing Method [2]											
E		37.1	37.8	110.9	74.3	28.3	66.2	47.3	1.33	1.63	1.57
F		36.7	37.6	113.1	75.4	28.2	68.0	48.1	1.34	1.63	1.57
Hulling Method [3]											
NH		37.1	37.2	111.3	74.2	28.2	66.4	47.3	1.32	1.65	1.57
H		36.6	38.3	112.7	75.5	28.4	67.9	48.1	1.35	1.62	1.57
SEM		0.32	0.34	0.98	0.54	0.30	0.77	0.41	0.013	0.018	0.015
Processing × Hulling Method											
E	NH	37.2	36.7[a]	110.3	73.5	27.5[a]	65.5	46.5	1.33	1.65	1.58
	H	37.0	38.9[b]	111.4	75.1	29.1[b]	66.9	48.0	1.33	1.61	1.57
F	NH	37.0	37.6[ab]	112.2	74.9	28.8[ab]	67.2	48.0	1.30	1.64	1.56
	H	36.3	37.7[ab]	114.1	75.9	27.6[a]	68.9	48.2	1.37	1.62	1.57
SEM		0.45	0.48	1.39	0.76	0.43	1.09	0.59	0.019	0.025	0.021
Source						*Probabilities*					
Processing Method		0.341	0.705	0.116	0.184	0.832	0.114	0.157	0.783	0.965	0.804
Hulling Method		0.298	**0.032**	0.295	0.104	0.660	0.186	0.164	0.094	0.279	0.975
Processing × Hulling Method		0.571	**0.043**	0.758	0.698	**0.004**	0.913	0.293	0.090	0.709	0.513

a,b Means within a row with different letters differ significantly ($P < 0.05$).

[1] (BW) body weight, (ADFI) average daily feed intake, (ADG) average daily gain, (FCR) feed conversion ratio.

[2] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[3] (H) with hulls, (NH) no hulls.

Table 5. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods, on the coefficient of apparent ileal dry matter (DM) and crude protein (CP) digestibility at the end of the starter (d14) and grower period (d28) and on ileal viscosity at the end of the grower period.

		DM		CP		Viscosity (Cps)
Days of age		14	28	14	28	28
Processing Method [1]						
E		0.709	0.727	0.842	0.858	5.93
F		0.711	0.723	0.824	0.837	5.48
Hulling Method [2]						
NH		0.720	0.724	0.834	0.842	5.68
H		0.700	0.726	0.832	0.852	5.73
SEM		0.0107	0.116	0.064	0.091	0.24
Processing × Hulling Method						
E	NH	0.723	0.728	0.843	0.855	5.76
	H	0.695	0.727	0.840	0.860	6.10
F	NH	0.717	0.720	0.825	0.830	5.60
	H	0.705	0.726	0.823	0.843	5.37
SEM		0.0151	0.0164	0.0090	0.0130	0.33
Source				*Probabilities*		
Processing Method		0.906	0.785	0.069	0.118	0.203
Hulling Method		0.201	0.877	0.778	0.486	0.881
Processing × Hulling Method		0.615	0.861	0.958	0.751	0.414

[1] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[2] (H) with hulls, (NH) no hulls.

Table 6. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods on carcass yield and carcass part yield (% of carcass weight) at d28 of age.

		Carcass yield (%)	Breast meat (%)	Wing (%)	Thigh (%)
Processing Method [1]					
E		67.1	33.6	11.7	28.2
F		67.8	34.1	11.7	27.5
Hulling Method [2]					
NH		68.9	33.8	11.5	27.4
H		66.0	33.9	11.9	28.2
SEM		0.84	0.64	0.27	0.48
Processing × Hulling Method					
E	NH	68.9	34.0	11.5	27.5
	H	65.3	33.2	11.9	28.8
F	NH	68.9	33.6	11.5	27.3
	H	66.7	34.6	11.8	27.6
SEM		1.12	0.90	0.38	0.68
Source			*Probabilities*		
Processing Method		0.616	0.540	0.936	0.294
Hulling Method		**0.023**	0.909	0.342	0.260
Processing × Hulling Method		0.550	0.342	0.963	0.483

[1] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[2] (H) with hulls, (NH) no hulls.

Table 7. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods on jejunal histomorphometry at the end of the starter (d14) and grower period (d28).

Days of age		14			28		
Measured responses [1]		VH (μm)	CD (μm)	VCR	VH (μm)	CD (μm)	VCR
Processing Method [2]							
E		494	75.5	6.80	748	99.8	7.85
F		502	78.6	6.65	773	101.9	7.89
Hulling Method [3]							
NH		509	77.3	6.85	776	104.9	7.78
H		487	76.8	6.60	745	96.8	7.95
SEM		21.2	2.52	0.237	29.6	4.64	0.198
Processing × Hulling Method							
E	NH	491	77.4	6.67	775	102.1	8.03
	H	497	73.7	6.92	722	97.5	7.67
F	NH	528	77.3	7.03	778	107.6	7.53
	H	477	79.9	6.28	768	96.1	8.24
SEM		30.0	3.56	0.335	41.8	6.6	0.280
Source		*Probabilities*					
Processing Method		0.786	0.404	0.676	0.569	0.757	0.896
Hulling Method		0.476	0.886	0.462	0.457	0.235	0.546
Processing × Hulling Method		0.354	0.389	0.153	0.608	0.609	0.068

[1] (VH) villi height, (CD) crypt depth, villi height: crypt depth ratio (VCR).

[2] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[3] (H) with hulls, (NH) no hulls.

Table 8. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods on intestinal segment length and weight relative to eviscerated carcass weight at the end of the grower period (d28) (cm/kg and g/kg of carcass weight, respectively).

Measured responses [1]		Duo L	Jej L	Ile L	Duo W	Jej W	Ile W	Pro W	Giz W	Liv W	Pan W	Cec W
Processing Method [2]												
E		27.9	70.3	72.8	11.2	20.4	16.7	6.90	28.1	35.1	3.54	16.4
F		30.6	73.6	77.2	10.6	19.8	16.5	7.27	31.0	31.8	3.50	16.1
Hulling Method [3]												
NH		28.4	70.6	74.4	10.4	18.8	16.7	6.55	27.9	32.8	3.58	15.9
H		30.1	73.3	75.7	11.4	21.5	16.5	7.61	31.6	34.1	3.46	16.5
SEM		1.47	3.03	3.01	0.47	0.68	0.69	0.302	1.20	1.47	0.20	0.74
Processing × Hulling Method												
E	NH	27.5	69.0	72.8	10.1	18.6	15.8	6.47	27.2	33.0	3.33	15.2
	H	28.4	71.6	72.9	12.3	22.2	17.5	7.32	29.8	36.2	3.75	17.6
F	NH	29.4	72.2	76.0	10.6	19.0	17.5	6.62	28.6	32.6	3.83	16.6
	H	31.7	75.0	78.5	10.6	20.7	15.4	7.91	33.4	31.0	3.17	15.5
SEM		2.09	4.29	5.15	0.67	0.96	0.99	0.427	1.70	2.09	0.287	1.04
Source						*Probabilities*						
Processing Method		0.225	0.447	0.315	0.387	0.564	0.852	0.394	0.156	0.127	0.670	0.809
Hulling Method		0.449	0.532	0.762	0.123	**0.012**	0.858	**0.021**	**0.041**	0.536	0.479	0.591
Processing × Hulling Method		0.732	0.985	0.784	0.127	0.346	0.067	0.606	0.509	0.179	0.129	0.103

[1] (Duo) duodenum, (Jej) jejunum, (Ile) ileum, (L) length, (W) weight, (Pro) proventriculus, (Giz) gizzard, (Liv) liver, (Pan) pancreas, (Cec) ceca.

[2] (E) extruded using single-screw extrusion process at 140°C at around 100 kg/h, (F) flaked and cooked at 150°C for 3600 seconds.

[3] (H) with hulls, (NH) no hulls.