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Acerola seed meal (*Malpighia emarginata*) as a source of dietary fibre in starter piglet diets

Farelo de semente de acerola (*Malpighia emarginata*) como fonte de fibra dietética em dietas de leitões iniciantes

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Highlights

The acerola seed meal (ASM) can be an alternative ingredient used for piglets.
The ASM affected the animals' daily body weight gain and average daily feed intake.
The inclusion level of ASM that promoted the best weight gain for piglets was 3.05%.

Abstract

Three experiments were carried out to determine the nutritional value and the effect of acerola seed meal (ASM) for starter piglet diets on feed digestibility, nitrogen balance (NB), growth performance and blood metabolites. Twenty-four crossbred entire male piglets (21.07 ± 3.07 kg) were assigned to a digestibility assay (Exp. I) and distributed in a randomised block design (RBD) with two treatments composed of reference diet (RD) or test diet (20% replacement with ASM), 12 replications and with one pig per experimental unit (EU). An NB study (Exp. II) was conducted with four levels of ASM (0, 4, 8 and 12%) and 24 crossbred entire male piglets (20.78 ± 1.84 kg) allocated in an RBD of six replications and one pig per EU. The growth performance study (Exp. III) involved 120 crossbred piglets: 60 entire males and 60 females (13.85 ± 1.49 kg). Treatments consisted of a gender combination and five levels of ASM (0, 3, 6, 9 and 12%), distributed in an RBD with

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six replications and four animals per EU. The physicochemical composition results indicated 86.12% of dry matter (DM), 8.03% of crude protein (CP) and 4,553 kcal kg-1 of gross energy (GE) as well as apparent digestibility coefficients of 39.04 and 28.21% for CP and GE, respectively. The levels of ASM that provided the maximum estimate for final body weight, daily body weight gain and average daily feed intake were 2.93% (p = 0.037), 3.05% (p = 0.040) and 4.27% (p = 0.043), respectively. ASM can be indicated as a dietary fibre source for starter piglet diets because it contains relevant nutritional and energy values, without affecting the nitrogen balance and blood urea concentration.
Key words: By-product. Digestibility. Fibrous ingredient. Growth performance. Nitrogen balance. Plasma urea.

Resumo

Três experimentos foram realizados para determinar o valor nutricional e o efeito do uso do farelo da semente de acerola (FSA) na dieta de leitões em fase inicial sobre a digestibilidade de ração, balanço de nitrogênio (BN), desempenho zootécnico e metabólito sanguíneo. Vinte e quatro leitões mestiços, machos inteiros (21,07 ± 3,07 kg) foram atribuídos a um ensaio de digestibilidade (Exp. I.) e distribuídos em um delineamento experimental de bloco ao acaso (DBC), com dois tratamentos, compostos de uma dieta referência ou teste (substituição em 20% por FSA na dieta referência), 12 repetições e com um suíno por unidade experimental (UE). O ensaio de BN (Exp. II) foi conduzido com quatro níveis crescentes de inclusão de FSA (0, 4, 8 e 12%) e 24 leitões mestiços machos inteiros (20,78 ± 1,84 kg), alocados em um DBC de seis repetições e um suíno por UE. O estudo de desempenho zootécnico (Exp. III) envolveu 120 leitões mestiços, 60 machos inteiros e 60 fêmeas (13,85 ± 1,49 kg). Os tratamentos consistiram da combinação de dois gêneros e cinco níveis crescentes de inclusão de FSA (0, 3, 6, 9 e 12%), distribuídos em um DBC com seis repetições e quatro animais por UE. Os resultados de composição físico-química do FSA indicaram 86,12% de matéria seca (MS), 8,03% de proteína bruta (PB) e 4.553 kcal kg -1 de energia bruta (EB), e coeficientes de digestibilidade aparente de 39,04 e 28,21% para PB e EB, respectivamente. Os níveis de FSA que proporcionaram a máxima estimativa de peso corporal final, ganho de peso corporal diário (GPCDM) e consumo de ração diário médio foram 2,93% (p = 0,0372), 3,05% (p = 0,0407) e 4,27% (p = 0,0432), respectivamente. O FSA pode ser indicado como uma fonte de fibra dietética na alimentação de suínos em fase inicial por conter valores nutricionais e de energia relevantes sem afetar o balanço de nitrogênio e concentração de ureia no sangue.
Palavras-chave: Balanço de nitrogênio. Co-produto. Desempenho zootécnico. Digestibilidade. Ingrediente fibroso. Ureia plasmática.

Introduction

Corn is an ingredient commonly used in pig feed, and it is responsible for half of the production costs (Caldarelli & Bacchi, 2012). Moreover, there is competition with human food and variation in the price of this ingredient, which are important factors in the search for alternative strategies and/or alternative ingredients that can make production systems economically viable. On the other hand, Brazil is an important producer of fruits destined for pulp extraction because of its climate and adequate territory. After fruit processing, part of the total produced becomes residue and when improperly discarded can negatively

affect the environment (Almeida et al., 2014). To reduce this environmental impact and feed costs, this residue material from the fruit industry can be processed and used as an ingredient in pig feed.

The production of acerola is highlighted in Brazil because its main characteristic is a high content of vitamin C as well as carotenoids and antioxidant compounds (Ritzinger & Ritzinger, 2011; Pereira et al., 2013; Araújo et al., 2014). Residues from its industrialisation are largely formed by seeds and peel, and after their proper treatment, their perishability is reduced and can be used for alternative purposes (Pereira et al., 2013). The evaluation of the physicochemical composition of the acerola residue indicates that the by-product has the potential to be used in animal feeding because it contains considerable values of CP (13.39%), GE (4,757 kcal kg^{-1}) and dietary fibre (76.47%) (Castelini, 2015).

Dietary fibre can be described as polymers and oligomers of carbohydrates, which are undigested in the small intestine and partially or completely fermented in the large intestine (Jones, 2014). The inclusion of these compounds in pig diets favours the production of short-chain fatty acids by intestinal bacteria and intestinal morphology (Shang, Liu, Liu, He, & Piao, 2019) and increases the activity of digestive enzymes (Pluschke, Williams, Zhang, & Gidley, 2018).

The most common classification used to differentiate fibre types is as soluble and insoluble, with the capacity of dispersion in aqueous medium as for hemicellulose and pectin and lower dispersion capacity as for cellulose and lignin (Williams, Mikkelsen, Flanagan, & Gidley, 2019). Soluble fibres are fermentable by intestinal microbiota and increase the viscosity of the digesta, while insoluble fibres are less fermentable and increase the volume of the digesta (Rebello, O'Neil, & Greenway, 2016) and intestinal flow (Zhuang et al., 2019).

Physicochemical analyses of ASM determined by different authors (Diógenes et al., 2014; Castelini, 2015) showed that its nutritional content is rich in fibre in the form of cellulose and lignin, and it is characterised as a source of insoluble fibre. However, few experiments have tested ASM as a source of dietary fibre in animal nutrition or whether the use of the residue could affect animal production and excretion of nitrogen. In this context, this study aimed to evaluate the use of ASM in piglet feeding during the starter phase to determine the nutritional composition and to verify its effects on feed digestibility, N balance, growth performance and blood metabolites. We hypothesised that the inclusion of ASM in diets would promote relevant digestibility results with positive effects on growth performance and that it could be used as an alternative energy ingredient for starter piglet diets.

Material and Methods

The experiment was conducted in the Swine Sector of the Experimental Station of the State University of Western Paraná (UNIOESTE, Marechal Cândido Rondon, Paraná, Brazil). All procedures using animals were approved by the Ethics Committee on the Use of Animals (CEUA-UNIOESTE).

Procedure for obtaining and analysing acerola seed meal

The ASM (*Malpighia emarginata*) was obtained from the fruit pulp company Fruteza Sucos Naturais Ltda. (Dracena, São Paulo, Brazil) and processed by the company Nutra-Animal Nutrition (Dracena, São Paulo, Brazil). Initially, the fruits were selected for impurities removal (pieces of plant branches and leaves as well as spoiled or unripe fruits). After the selection, the fruits were pre-washed with abundant drinking water, sanitised by immersion, and then the pulping process was performed. The peel and seed residues obtained were frozen at −22°C for further drying. For the drying process, the residues were thawed at room temperature and placed in drying trays. The dehydrated residues were ground and stored in packaging and sealed for later use. Samples of the by-product were sent for analyses of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), ash and gross energy (GE) according to the methodology described by Silva and Queiroz (2002). The ASM aminogram was performed by the company EVONIK® (São Paulo, SP, Brazil).

Animals, experimental design, housing and experimental treatments

In Experiment I (digestibility assay), a total of 24 entire male piglets crossbred for high deposition of lean meat with average initial body weights (AIBWs) of 21.07 ± 3.07 kg were distributed in a randomised block design (RBD). There were two treatments composed of a reference diet (RD) or a test diet (20% replacement by the ASM in the RD), 12 replications and one pig per experimental unit (EU). The RD was formulated based on corn and soybean meal to meet the nutritional requirements of the pigs in the starter phase according to the recommendations proposed by Rostagno et al. (2011) (Table 1) and a test diet in which the ASM represented 20% and RD 80%.

Table 1
Centesimal and chemical composition of reference diet used in the digestibility trial in Exp. I (as-fed basis)

Ingredients	Composition (kg per 100 kg)
Ground corn 7.88% CP	65.95
Soybean meal 45.22% CP	28.78
Monocalcium phosphate	1.370
Calcitic limestone	1.064
Common salt	0.400
Vitamin and mineral premix[1]	0.500
Soybean oil	1.348
Lysine sulfate 50.7%	0.420
DL-methionine 98%	0.088
L-threonine 99%	0.075
Tiamulin	0.005
Calculated composition	
Crude protein, %	18.32
Metabolisable energy, kcal kg^{-1}	3,230
Total calcium, %	0.768
Available phosphorus, %	0.380
SID lysine, %	1.093
SID methionine + cyst., %	0.612
SID threonine %	0.270
Neutral detergent fibre %	13.01
Acid detergent fibre %	4.31

[1]Contained per kilogram of product (5 g of premix per kg of feed): folic acid (103.12 mg); pantothenic acid (2,249.99 mg); biotin (16.88 mg); chloro hydroxyquinoline (15.00 g); copper sulfate (22.07 g); ethoxyquin (206.00 mg); iron sulfate (6,733.40 mg); iodine (37.51 mg); lysine (123.76 g); manganese (1,866.71 mg); methionine (110.25 g); niacin (4,687.50 mg); sodium selenite (43.75 mg); threonine (46.64 g); vit. A (14,375 IU); vit. B_1 (224.96 mg); vit. B_{12} (2,537.50 mg); vit. B_2 (537.50 mg); vit. B_6 (437.50 mg); vit. D_3 (262,500 IU); vit. E (4,250 IU); vit. K_3 (375.00 mg); zinc oxide (1,000 mg). SID: standardized ileal digestibility.

The piglets were housed individually in metal metabolism cages, with manual adjustment for the size of the animal, a plastic screen attached to a rectangular tray to collect feed leftovers and avoid faecal sample losses, a urine collecting hopper at the bottom of the cage and a faeces collection box at the back, equipped with a feeder-drinking, similar to those described by Pekas (1968).

The provision of feed and collection of faeces and urine were performed according to the methods previously described by Sakomura and Rostagno (2016). The amount of feed supplied was calculated based on the metabolic body weight (BW0.75) of each pig, and the average feed intake was recorded during the acclimation period. The diets were provided at 08h00 and 14h00 and moistened

with water in an amount corresponding to 20% of the total amount of feed supplied to avoid waste, reduce powdery properties and improve palatability. After each meal, water was supplied through a feeder-drinking in the proportion of 3 mL g-1 feed consumed to equalise daily water intake between animals. The experimental period lasted 12 days, with seven days of acclimation of the animals to cages and diets, and five days of collection of faeces and urine.

To mark the beginning and end of the faeces collection period, 1.5% of ferric oxide (Fe3O2) was added to the feed as a faecal marker. Faeces were collected twice a day, weighed and placed in labelled plastic bags and stored in a freezer (-18°C). Subsequently, the material was homogenised, weighed on a digital scale and a composite sample of 100 g was removed and dried in a forced ventilation oven (55°C) for 72 hours, and ground in a knife mill (1 mm sieve). Urine was collected daily in plastic buckets containing 20 mL of 1:1 HCl to avoid nitrogen volatilisation and bacterial proliferation. The total volume of urine was measured and an aliquot (10% by the total volume of urine) was conditioned daily in polyethene terephthalate bottles and frozen at -18°C. Samples of feed, faeces and urine were sent to the laboratory for determination of DM, OM, CP, NDF, ADF and GE.

In Exp. II (NB study), a total of 24 entire male piglets crossbred for high deposition of lean meat, with an AIBW of 20.78 ± 1.84 kg were distributed in an RBD into four treatments, six replications and one animal per EU. The treatments consisted of four diets with increasing levels of ASM (0, 4, 8 and 12%) (Table 2). The diets were isoenergetic and isoproteic as proposed by Rostagno et al. (2011). The procedure for handling and collection of faeces and urine were as described in Exp. I. The analyses of feed, faeces and urine were performed following the procedures described in Exp. I. The N content of diets, faeces and urine samples was analysed using the Kjeldahl method.

The growth performance test (Exp. III) involved 120 crossbred piglets (60 entire males and 60 females) of a commercial lineage bred for the high deposition of lean meat, with AIBWs of 13.85 ± 1.49 kg at 41 days of age and final weight of 25.08 ± 1.06 kg at 63 days of age that were allocated in an RBD, which was composed of five treatments, six replications and four animals per EU. The AIBWs of the animals were used as a blocking factor, in which each EU consisted of four animals.

The animals were housed in a masonry shed, with suspended nursery stalls (1.54 m²) and polyethene plastic flooring, equipped with nipple-type drinkers and gutter-type feeders, arranged in two rows and divided by a central corridor. The treatments consisted of two genders (males and females) and increasing levels of ASM inclusion (0, 3, 6, 9 and 12%). The experimental diets were formulated to meet the nutritional requirements for starter piglets (Table 3) recommended by Rostagno et al. (2011). The animals received feed and water ad libitum throughout the experimental period.

Table 2
Centesimal and chemical composition of diets with different levels of acerola seed meal inclusion for piglets in Exp. II (as-fed basis)

Ingredients	Levels of inclusion, %			
	0	4	8	12
Ground corn 7.88% CP	69.37	63.08	56.79	50.49
Soybean meal 45.22% CP	26.72	27.27	27.82	28.38
ASM	0.00	4.00	8.00	12.00
Monocalcium phosphate	1.298	1.311	1.323	1.335
Calcitic limestone	1.019	1.014	1.009	1.004
Soybean oil	0.603	2.321	4.038	5.755
Common salt	0.400	0.400	0.400	0.400
L-lysine HCl	0.301	0.297	0.294	0.290
Trace mineral premix[1]	0.100	0.100	0.100	0.100
DL-methionine	0.070	0.082	0.095	0.107
L-threonine	0.061	0.069	0.078	0.086
Vitamin premix[1]	0.050	0.050	0.050	0.050
Calculated composition				
Crude protein (%)	17.898	17.663	17.427	17.191
Total calcium (%)	0.733	0.733	0.733	0.733
Metabolisable energy (kcal kg^{-1})	3,230	3,230	3,230	3,230
Available phosphorus (%)	0.363	0.363	0.363	0.363
SID lysine (%)	1.037	1.037	1.037	1.037
SID met. + cyst. (%)	0.581	0.581	0.581	0.581
SID threonine (%)	0.653	0.653	0.653	0.653
SID tryptophan (%)	0.187	0.187	0.187	0.187
Neutral detergent fibre (%)	13.20	14.62	16.03	17.44
Acid detergent fibre (%)	4.26	5.89	7.52	9.14

[1]Contained per kilogram of product (5 g of product per kg of feed): folic acid (103.12 mg); pantothenic acid (2,249.99 mg); biotin (16.88 mg); chloro hydroxyquinoline (15.00 g); copper sulfate (22.07 g); ethoxyquin (206.00 mg); iron sulfate (6,733.40 mg); iodine (37.51 mg); lysine (123.76 g); manganese (1,866.71 mg); methionine (110.25 g); niacin (4,687.50 mg); sodium selenite (43.75 mg); threonine (46.64 g); vit. A (14,375 IU); vit. B_1 (224.96 mg); vit. B_{12} (2,537.50 mg); vit. B_2 (537.50 mg); vit. B_6 (437.50 mg); vit. D_3 (262,500 IU); vit. E (4,250 IU); vit. K_3 (375.00 mg); zinc oxide (1,000 mg). SID: standardized ileal digestibility.

Table 3
Centesimal and chemical composition of diets with different levels of acerola seed meal inclusion for piglets in Exp. III (as-fed basis)

Ingredients	Levels of inclusion, %				
	0	3	6	9	12
Ground corn 7.88 CP	64.07	59.64	55.21	50.77	46.34
Soybean meal 45.22% CP	28.64	28.85	29.06	29.27	29.48
ASM	0.00	3.00	6.00	9.00	12.00
Fish meal 53% CP	2.500	2.500	2.500	2.500	2.500
Monodicalcium phosphate	1.195	1.188	1.181	1.174	1.167
Soybean oil	1.015	2.259	3.502	4.745	5.989
Common salt	0.364	0.366	0.369	0.372	0.375
Vitamin and mineral premix[1]	0.500	0.500	0.500	0.500	0.500
Lysine sulfate 50.7%	0.557	0.562	0.567	0.572	0.577
DL-methionine 85%	0.124	0.135	0.145	0.156	0.166
L-threonine 98%	0.116	0.125	0.134	0.143	0.151
L-tryptophan 99%	0.011	0.012	0.013	0.014	0.015
Calculated composition					
Crude protein, %	18.32	18.32	18.32	18.32	18.32
Metabolisable energy, kcal kg^{-1}	3,230	3,230	3,230	3,230	3,230
Total calcium, %	0.768	0.768	0.768	0.768	0.768
Available phosphorus, %	0.380	0.380	0.380	0.380	0.380
SID lysine, %	1.093	1.093	1.093	1.093	1.093
SID methionine + cyst., %	0.612	0.612	0.612	0.612	0.612
SID threonine, %	0.418	0.450	0.483	0.515	0.544
SID tryptophan, %	0.036	0.039	0.043	0.046	0.049
Neutral detergent fibre, %	9.110	10.490	11.870	13.250	14.630
Acid detergent fibre, %	4.580	5.770	6.950	8.140	9.320

[1]Contained per kilogram of product: folic acid (103.12 mg); pantothenic acid (2,249.99 mg); biotin (16.88 mg); chloro hydroxyquinoline (15.00 g); copper sulfate (22.07 g); ethoxyquin (206.00 mg); iron sulfate (6,733.40 mg); iodine (37.51 mg); lysine (123.76 g); manganese (1,866.71 mg); methionine (110.25 g); niacin (4,687.50 mg); sodium selenite (43.75 mg); threonine (46.64 g); vit. A (14,375 IU); vit. B_1 (224.96 mg); vit. B_{12} (2,537.50 mg); vit. B_2 (537.50 mg); vit. B_6 (437.50 mg); vit. D_3 (262,500 IU); vit. E (4,250 IU); vit. K_3 (375.00 mg); zinc oxide (1,000 mg). SID: standardized ileal digestibility.

The animals were weighed (stainless steel digital scale, model UL50i) at the beginning and the end of the experimental period. Leftover feed was collected, weighed and discounted from the feed supplied. The variables evaluated during the experimental period (41 to 63 days of age) were the final body weight (FBW, kg), average daily body weight gain (ADBWG, kg day^{-1}), average daily feed intake (ADFI, kg day^{-1}) and feed conversion ratio (FCR, kg kg^{-1}).

Blood sampling and analysis procedures

On the last day of the Exp. II and at the beginning (first day baseline) and the end (last day) of the Exp. III, the animals were subjected to an eight-hour fast. The collection was performed by puncture of the anterior cranial vena cava, using 1.20 × 25 mm gauge needles. The blood samples obtained were transferred to glass tubes with and without urea anticoagulant (ethylenediaminetetraacetic acid EDTA) and centrifuged (Excelsa® II Centrifuge, Model 206-R) at 3,000 rpm for 15 minutes to obtain the blood serum. Then, the serum samples were transferred to 1.5 mL Eppendorf polyethene tubes in duplicates and stored in a freezer at -5°C for further analysis. Blood urea nitrogen and plasma urea were determined with an automated biochemical analyser (model Flexor EL 200, Vitória, Espirito Santo, Brazil), using specific ELI Tech kits (Clinical Systems).

Calculations and statistical analysis

The total carbohydrate contents (TCC) were calculated according to the equations indicated by Sniffen, O'Connor, Van Soest, Fox and Russell (1992), where TCC = 100 - (% CP + % EE + ash) and the non-fibre carbohydrate content (NFC) by the formula NFC = TCC - NDF. The apparent digestibility coefficients of DM (ADCDM), GE (ADCGE), OM (ADCOM), CP (ADCCP), NDF (ADCNDF), ADF (ADCADF), and the apparent metabolisability coefficients of GE (AMCGE) were calculated according to the methodology described by Matterson, Potter, Stutz and Singsen (1965). The values of N intake (NI) and N excretion (faeces and urine) were obtained by multiplying the nitrogen contents by the amounts of consumed feed, faeces and urine excreted, respectively. From these values, the N absorbed (NA), N retained (NR), NR/NI and the NR/NA were calculated according to Adeola (2001).

The normality of experimental errors and the homogeneity of variances between treatments for the several variables were previously assessed using the Shapiro-Wilk and Levene's tests, respectively. The statistical model used for the variables of growth performance and blood metabolite was $Y_{ijk} = m + T_i + b_j + \beta (X_{ijk} - \bar{X}...) + \varepsilon_{ijk}$. The effects of factors included in the model are described by Y_{ijk}, which is the average observation of the dependent variable in each plot, measured in the *i*-th level of ASM, in the *j*-th block and the *k*-th replication; m is the effect of the overall average; T_i is the effect of ASM levels, for i = (1, 2, 3, 4...); bj is the effect of blocks, for j = (1 and 2); β is the regression coefficient of Y over X; X_{ijk} is the average observation of the covariate (initial body weight or initial urea) in each plot, measured in the *i*-th level of ASM, in the *j*-th block and the *k*-th replication; $\bar{X}...$ is the overall average for the covariate; Y_{ijk} is the random error of the plot associated with level *i*, block *j* and replication *k*, independent, homoscedastic and with a normal distribution. For the N balance, the statistical model used

was mentioned, without the inclusion of the covariate effect.

The effects of ASM levels were verified using analysis of variance (ANOVA). When significant in ANOVA, the effects of ASM levels were estimated by regression models. Five regression models were adjusted based on the ASM inclusion levels, according to the significance of ANOVA. The initial body weight (IBW) was used to adjust the observed averages of FBW, ADBWG and ADFI. Initial blood urea (IU) data were used to adjust the final blood urea (FU) data. In both situations, the use of models with covariate inclusion was intended to increase experimental accuracy, when compared to the respective models that did not use covariables. The linear regression coefficients of FBW, ADBWG and ADFI on IBW and FU on IU were estimated by analysis of covariance (ANCOVA). The homogeneity between the regression coefficients, considering each treatment, was assessed using the ANOVA F-test, with model adjustment including the interaction between treatment and covariate (IBW or IU) to test the hypothesis of nullity between the betas (H0: $\beta T_1 = \beta T_2 = \beta T_3 = \beta T_4 = \beta T_5$).

For the FCR and N balance, the statistical model was the one previously mentioned, without the covariate inclusion. The effects of ASM levels on FBW, ADBWG, ADFI and FU were verified using ANCOVA and on effects on FCR were tested using ANOVA. When significant, the effect of ASM levels on dependent variables was estimated using linear regression models. Five linear regression models were adjusted to the averages by treatment of the variables from ASM values (0, 3, 6, 9 and 12%) to select the predictive model that best fits the average values to estimate the level of ASM that would promote the maximum pig performance.

The ordinary least squares method was used to estimate the parameters of the regression models. The significance of each parameter was assessed using the partial t-test, where the nullity hypothesis tested was H0:*i* = 0. The adherence of the models to the adjusted averages of FBW, ADBWG, ADFI and FU and to the observed averages of FCR was evaluated by observing the adjusted coefficient of determination (R^2 adj.). For the data selected by treatment, confidence intervals were estimated for the population averages adjusted for FBW, ADBWG, ADFI and FU and not adjusted for FCR at a 95% confidence index.

The contrasts of adjusted averages for FBW, ADBWG, ADFI and FU and observed averages of FCR and the N balance between each level of ASM relative to the reference treatment (0%) were assessed using Dunnett's test. The significance level of 5% was adopted in all hypothesis tests. The analyses were performed using the R software (Core Development Team, 2013).

Results and Discussion

Feed digestibility trial and determination of the nutritional value of ASM

The results of the chemical composition of the ASM (Table 4) show that the values found for lysine (0.296%), arginine (0.522%) and isoleucine (0.304%) were higher than for corn grain (lysine 0.23%; arginine 0.37%; isoleucine 0.26%) described by Rostagno et al. (2017). These amino acids are among those considered essential for the adequate growth of young pigs, including arginine, which is not produced by piglets in sufficient quantity to meet the metabolic demand (Rezaei et al., 2013).

Table 4
Chemical composition and energy value of acerola seed meal based on natural matter

Analysed composition	
Gross energy, kcal kg^{-1}	4,553
Dry matter, %	86.12
Organic matter, %	96.75
Crude protein, %	8.03
Total carbohydrates, %	85.33
Non-fibre carbohydrates, %	30.24
Neutral detergent fibre, %	55.09
Acid detergent fibre, %	44.60
Lignin, %	22.26
Cellulose, %	32.90
Ash, %	2.80
Ether extract, %	3.84
Total phosphorus, %	0.110
Total calcium, %	0.300
Total lysine, %	0.296
Total methionine, %	0.109
Total cysteine, %	0.109
Total threonine, %	0.273
Total arginine, %	0.522
Total isoleucine, %	0.304
Total leucine, %	0.506
Total valine, %	0.358

Based on the results, ASM can be characterised as a fibrous ingredient, composed of greater amounts of insoluble fibre, with contents of 32.90% cellulose and 22.26% lignin, 4,553 kcal kg-1 GE, 8.03% CP and high levels of NDF and ADF. In a study conducted by Castelini (2015), who also analysed ASM, the values obtained for DM = 89.22%, ash = 3.14%, OM = 96.86%, GE = 4,757 kcal kg^{-1}, NDF = 76.47, ADF = 63.78%, cellulose = 45.90%, hemicellulose = 12.69% and CP = 13.39% were higher than those that were found in our study. In addition, different composition values were found by Vieira et al. (2018), who analysed acerola bagasse (AB) and reported OM = 97.18%, ash = 2.82, NDF = 52.15%, ADF = 47.13%, EE = 3.59% and CP = 8.14%. These differences in the chemical compositions found for the acerola residues occur mainly because they are by products of the juice pulp industries, still without a defined standard and may vary according to the place of cultivation, variety, percentage of peel, seed and fruit pulp (Castelini, 2015).

The ADCDM (19.96%) and DDM (17.19%) values found in the present study (Table 5) were low, which can relate with Figueiredo et al. (2012), who included 30%

of cassava branch hay in piglet feed and also obtained low ADCDM (36.54%). The GE and CP values found for the ASM were higher than those of the corn grains were (GE = 3,901 kcal kg^{-1} and CP = 7.86%) described by Rostagno et al. (2017). However, there was less use of these ASM nutrients by pigs when compared to the same corn grain, which has digestible values for CP of 82.70% and GE of 88.23%. This lower availability of nutrients can be attributed to the fibrous content present in the ASM, since fibres can affect the digestibility of nutrients by increasing the viscosity of the digesta or even by reducing its length of stay in the GIT, preventing the proper action of digestive enzymes (Zhang et al., 2013; Brambillasca, Zunino, & Cajarville, 2015).

Table 5
Apparent digestibility coefficient (ADC), apparent metabolisability coefficient (AMC), digestible nutrients and energy values of acerola seed meal for starter pigs in Exp. I

Items	%
ADC of dry matter	19.96
ADC of organic matter	29.88
ADC of crude protein	39.04
ADC of gross energy	28.21
AMC of gross energy	27.84
ADC of neutral detergent fibre	16.54
ADC of acid detergent fibre	10.27
Digestible nutrients and energy	Natural matter[1]
Digestible dry matter, %	17.19
Digestible organic matter, %	24.90
Digestible protein, %	3.14
Digestible energy, kcal kg^{-1}	1,253
Metabolisable energy, kcal kg^{-1}	1,236
Digestible NDF, %	9.11
Digestible ADF, %	4.58
ME:DE	0.987

Fachinello et al. (2015) analysed the digestibility of passion fruit seed meal (DM = 92.2%, CP = 11.3%, GE = 5,565 kcal kg^{-1}, NDF = 50.2%, ADF = 43.7%) and obtained an ADCDM of 67.6%, ADCGE of 71.4%, ADCCP of 70.5%, ADCNDF of 49.9% and ADCADF of 44.8%. The nutrients present in the passion fruit seed meal (PSM) had higher digestibility coefficients when compared to those of the ASM, which was associated with the lower lignin content present in the PSM (5.77%) relative to the ASM (22.26%). The lignin molecule is linked by strong chemical bonds to cellulose and hemicellulose, preventing its digestion (Noblet & Le Goff, 2001) and when present in greater concentration in an animal diet also reduces digestibility of other nutrients (Zhao, Windisch, Roth, Eder, & Ettle, 2007; Schedle et al., 2008).

Nitrogen balance and blood urea nitrogen

There was no effect (p > 0.05) of the experimental treatments on the N balance and concentration of blood urea nitrogen (mg dL-1) (Table 6). This is contrary to the study by Castelini (2015), who observed a quadratic effect on faecal N in growing and finishing pigs fed with increasing levels of acerola meal (0, 9, 18 and 27%). However, the diets in the present experiment were isoenergetic and isoproteic, different from those used by the aforementioned author, which may have influenced the feed intake, and consequently, the N ingested and excreted.

The fact that piglets fed with different levels of ASM did not show differences in blood urea nitrogen concentrations shows that the addition of this fibrous ingredient did not impair protein utilisation, given that this parameter is related to the process of deamination of amino acids by the body, either by an imbalance or to compensate for energy production, with the release of urea into the bloodstream for further excretion (Bertechini, 2012). Shang, Ma, Liu, Liu and Piao (2020) evaluated the effects of diets with corn or corn replaced by 6% of wheat bran as a source of insoluble fibre (37.36% NDF) on the serum parameters of weaned piglets and found no difference between treatments for BUN.

Table 6
Average values of nitrogen balance for piglets according to the levels of acerola seed meal inclusion in the experimental diets in Exp. II

Items[1]	Levels of ASM inclusion, %				SEM[2]	*p-value*[3]
	0	4	8	12		
N intake (g day^{-1})	22.68	25.69	24.55	24.70	0.874	0.703
N excreted in faeces (g day^{-1})	4.32	4.15	4.81	5.25	0.303	0.575
N absorbed (g day^{-1})	18.36	21.53	19.73	19.45	0.638	0.400
N excreted in urine (g day^{-1})	6.67	6.9	6.58	5.61	0.214	0.176
N retained (g day^{-1})	11.69	14.63	13.15	13.84	0.553	0.342
N retained/intake (%)	51.39	56.32	53.84	56.04	0.778	0.147
N retained/absorbed (%)	63.64	66.94	67.37	71.04	1.059	0.158
Blood urea nitrogen (mg dL^{-1})	12.16	14.28	13.36	11.60	0.458	0.189

[1] CP = 6.25 * N.
[2] SEM: standard error of the mean.
[3] Significance level.

Growth performance testing and plasma urea

No gender effect (p > 0.05) was found on growth performance parameters and plasma urea concentration, which was removed from the statistical models to promote greater experimental accuracy. There was an effect of covariates IBW on the FBW (p < 0.0001), ADBWG (p = 0.0001) and ADFI (p = 0.0003) and IU on the FU (p = 0.043), indicating the need for adjustment of the observed averages of these characteristics, considering the averages of IBW and IU in experiments with starter pigs. There was no effect (p = 0.702) of IBW on FCR; therefore, the observed averages of FCR were not adjusted by the average values of IBW.

The unique estimates found indicated that the increase in one unit of IBW corresponded to the average increase of 1.44 kg in the FBW, 24.2 g in the ADBWG and 42.6 g in the ADFI. Similarly, the one-unit increase (mg dL^{-1}) in IU promoted an estimated average increase of 0.3902 mg dL^{-1} in FU.

There was an effect of ASM addition on FBW (p = 0.037), ADBWG (p = 0.040) and ADFI (p = 0.043) which increased compared to the reference diet. However, there was no effect of ASM levels on FCR (p = 0.199) and FU (p = 0.271) (Table 7).

Table 7
Adjusted average values for the growth performance and plasma urea of starter piglets fed with increasing levels of acerola seed meal in Exp. III[1]

Items[2]	Levels of ASM inclusion, %					SEM[3]	*p-value*[4]
	0	3	6	9	12		
Initial body weight (kg)	14.01	14.66	13.63	13.45	13.58	-	-
Final body weight (kg)**	24.67	25.76*	25.34	25.16	24.46	0.132	0.0372
Average daily body weight gain (kg day^{-1})**	0.581	0.638*	0.619	0.614	0.572	0.007	0.0407
Average daily feed intake (kg day^{-1})**	0.896	1.035*	1.005	0.992	0.968	0.014	0.0432
Feed conversion ratio (kg:kg)	1.546	1.628	1.624	1.616	1.691	0.018	0.1996
Plasma urea (mg dL^{-1})**	76.44	77.27	68.62	54.96	61.33	3.64	0.2713

[1] Means followed by an * within the line differ from the reference treatment (0%) by the Dunnett's test at 5% of probability level.
[2] **Covariance analysis with homogeneous betas.
[3] SEM: standard error of the mean.
[4] Significance level.

Fachinello et al. (2015) evaluated the growth performance of starter piglets fed with PSM and did not observe differences with the addition of up to 16%. Pascoal et al. (2012) tested the use of different insoluble fibre sources in the feeding of weaned piglets and found no differences in growth performance up to 63 days of age, differing from that observed in the present study. The results with fibre in piglet diets differ due to the chemical and physical characteristics of each source used and its degree of lignification, in addition to the different levels of inclusion in the feed (Budiño, Prezzi, Rodrigues, Monferdini, & Otsuk, 2015).

The regression models that best fitted the adjusted averages of FBW, ADBWG and ADFI were: $y = 24.55 - 0.392x + 1.342x^{0.5}$ (FBW), $y = 0.574 - 0.020x + 0.071x^{0.5}$ (ADBWG) and $y = 0.885 - 0.032x + 0.134x^{0.5}$ (ADFI). The adjusted determination coefficient values of the models were $R^2_{adj.}$ = 92.89% (FBW), $R^2_{adj.}$ = 88.98% (ADBWG) and $R^2_{adj.}$ = 91.07% (ADFI) (Figures 1 and 2). The results suggested that the use of up to 12% of ASM in diets for starter piglets does not change the average values of FCR and FU. The estimated ASM levels that provided the maximum estimate of FBW, ADBWG and ADFI were 2.93%, 3.05% and 4.27%, which resulted in values of 25.70 kg, 0.636 kg and 1.15 kg, respectively.

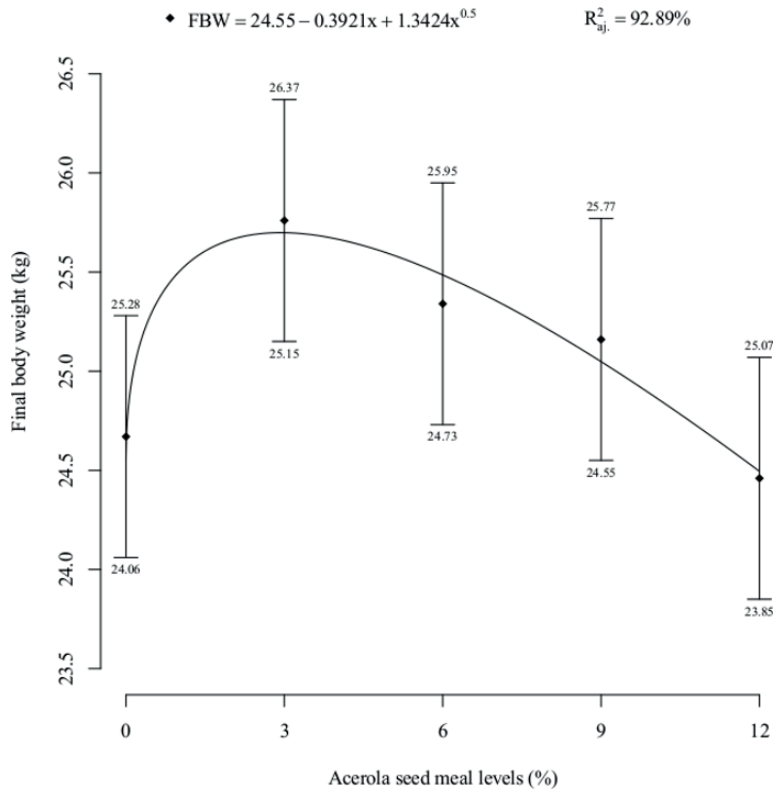

Figure 1. Linear regression models of final adjusted body weights of pigs in initial phase according to acerola seed meal levels (%)

The presence of fibre affects the energy digestibility of the diet (Zhang et al., 2013), and pigs can regulate intake according to the content present in the feed, increasing consumption if there is a decrease in digestible energy content to maintain their normal metabolism. Higher fibre intake affects the digestion volume and GIT distension, besides altering the intestinal flow, but only up to a certain limit, from which it is not possible to compensate for the energy decline, generating a reduction in feed intake by the animal (Ratanpaul, Williams, Black, & Gidley, 2019), directly affecting growth performance. Carvalho et al. (2014), who investigated the growth performance of piglets in the nursery phase fed with different inclusion levels of coconut meal (0, 7, 14 and 21%) reported that levels above 7% resulted in a negative linear effect on daily weight gain of the animals, from 21 to 42 days of age.

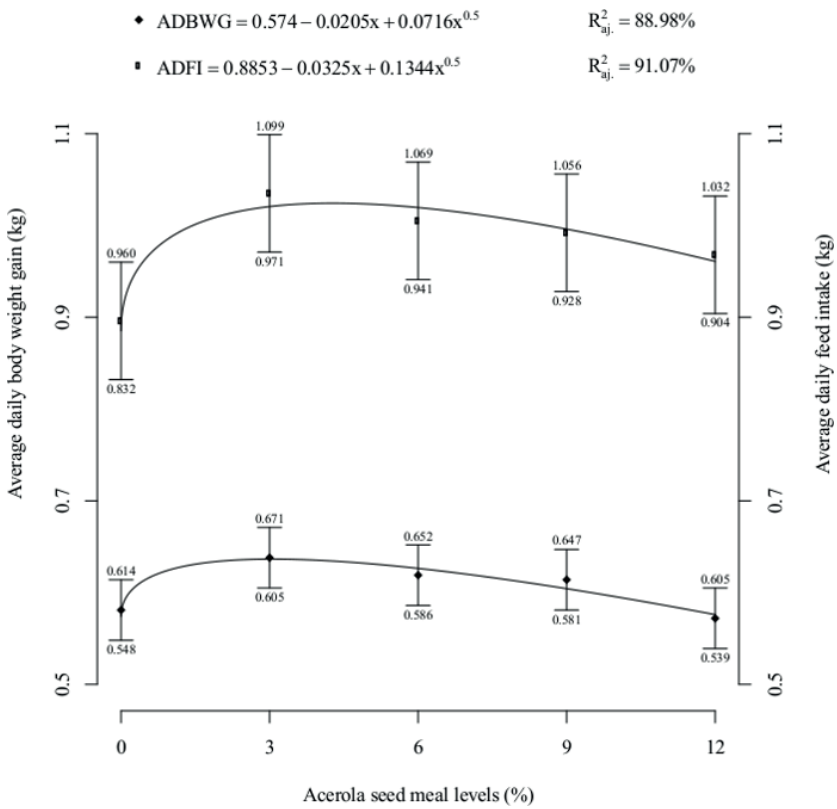

Figure 2. Linear regression models of average daily body weight gain and average daily feed intake adjusted of pigs in initial phase according to acerola seed meal levels (%)

Another important point to be considered when adding fibre to pig diets is the GIT development stage. In a study conducted with finishing pigs, Taranu et al. (2018) found that the addition of 5% grape seed cake (66.22% NDF) to the animal's feed did not affect growth performance. The capacity of pigs to use diets containing dietary fibre increases with the development of the animal, i.e. younger animals have less utilisation of fibre due to the smaller size of the GIT because there is less surface area available for the action of the microbial population in the large intestine and greater rate of digesta passage, which limits the fermentation of these components (Noblet & Le Goff, 2001; Pascoal et al., 2012). Thus, it may be justified that piglets did not present better growth performance with higher levels of ASM inclusion.

The results of the blood parameters coincide with the findings of Fachinello et al. (2015), who verified that the urea concentrations in the plasma of pigs did not differ with the inclusion of different levels of agroindustry by-products. The protein sources used in the diets of this study were of high quality and feed digestibility was not influenced by the addition of ASM; therefore, it did not interfere with plasma urea parameters. Urea is the final component of amino acid catabolism, and its levels may vary according to the protein and amino acid composition of the diet or in

cases of fasting, where cell proteins are used as an energy source for metabolism (Nelson & Cox, 2014).

Conclusion

Based on the findings, ASM can be indicated as a dietary fibre source in the feeding of starter pigs because it contains relevant nutritional and energy values. The inclusion of ASM in piglet diets did not affect the nitrogen balance and urea nitrogen in serum. In addition, the optimal level of inclusion of ASM in starter pig diets was 2.93% for FBW, 3.05% for ADBWG and 4.27% for ADFI. The best level of inclusion of ASM was 3.05%, which promotes greater ADBWG without affecting plasma urea concentration.

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