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Nutritional value and intake of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell.) in sows

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ABSTRACT

Aquatic ferns (AFs) such as *Azolla filiculoides* and *Salvinia molesta* are grown on swine lagoons in the tropics and used in diets for pigs. The present work is aimed at evaluating their potential as feed ingredients for sows. When presented with *ad libitum* AFs, gilts weighing 110 ± 14 kg (mean ± SD), were able to ingest 9.1–9.7 kg fresh AF per day (from 597 to 630 g dry matter (DM) per day) and from 1240 to 1428 g DM per day when presented in a dry, ground form. A digestibility study was conducted, using sows weighing 213 ± 9 kg (mean ± SD), which were fed diets containing maize, soybean meal and 0, 150 or 300 g AF kg^{-1} diet. The presence of AFs had a negative impact on the faecal digestibility of the crude protein, NDF and energy content of the whole diet ($P<0.001$) and on the ileal protein digestibility, especially with 300 g AFs kg^{-1} diet. The level of AFs in the diet had no effect on stomach weight ($P>0.05$) but increased the weight of the rest of the gastrointestinal tract ($P<0.001$). The rate of AF fibre fermentation in the pig large intestine was measured using an *in vitro* gas test. The rates were much lower than tropical tree foliage, which can also be used in pig diets in the tropics. This could partly explain the low apparent digestibility of AFs in pigs. In conclusion, the inclusion level of AFs in rations for sows should be limited to 150 g AFs kg^{-1} diet due to the low digestibility and energy density, as well as the negative impact on the digestibility of the whole diet.

There is a general need in tropical regions for increased livestock production to improve food security and alleviate poverty. In some countries, pork production is worth considering because pigs are very prolific, have a short generation interval and grow rapidly. Furthermore, pigs convert feed to meat relatively efficiently, production is not directly dependent on land – as compared with ruminants – and they are omnivorous animals (Holness, 1991; Leterme et al., 2007).

Sustainable systems in the tropics are based on perennial plants such as shrubs and trees that provide leaves with high protein and mineral content (Leterme et al., 2007). In this case, pig manure is used as a fertiliser, which ensures the sustainability of the system. Other systems involve aquatic ferns (AFs) such as *Azolla* sp. The latter are floating ferns with symbiotic N_2-fixing cyanobacteria that are used in Asia to fertilise rice fields (Arora and Singh, 2003). They are also used for pest and weed management (Kathiresan, 2007), as green manure (Bharati et al., 2000) or water purifiers (Oren Benaroya et

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al., 2004). They are usually integrated in more complex systems that include the production of fish and ducks (Kathiresan, 2007). More recently, there has been some interest to grow them on lagoons that collect pig slurry and to recycle them in swine or poultry nutrition due to their good protein quality (Becerra et al., 1990; Khatun et al., 1999) or mineral content (Chojnacka, 2006). However, despite the fact that millions of smallholders in the world feed their pigs with aquatic plants, very limited information is available on their actual nutritional value in pigs and on how to use them in swine nutrition, to improve productivity. In a previous study, we evaluated the nutritional value of two AFs, *Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell, in growing pigs and concluded that the ferns are a good source of minerals and essential amino acids but that their interest is attenuated by their poor digestibility and energy density (Leterme et al., 2009).

In backyard production systems, AFs are primarily offered to sows, due to their high capacity of ingestion and also because they better digest and ferment fibrous diets, due to their more developed gastrointestinal tract (Noblet and Shi, 1993; Jorgensen et al., 2007).

The present study was aimed at determining the nutritional value of *Azolla filiculoides* and *Salvinia molesta* in sows, at estimating their capacity of ingestion and at studying the rate of AF fibre fermentation in the sows' gastrointestinal tract, by means of an *in vitro* model. The rate of fibre fermentation was compared with several tree leaves, which are also used in sustainable pork production systems in the tropics.

1. Materials and methods

1.1. Production of aquatic plants and analysis of their chemical composition

The AFs were produced in lagoons used to collect slurry coming from the swine barn of the National University of Colombia in Palmira (Colombia), as well as from a commercial farm. The slurry was collected into three consecutive lagoons, connected with a pipe. The first lagoon was used to produce common duckweed (*Lemna minor* L.) and the other two for production of *Salvinia* and *Azolla*, respectively. The AFs were collected each week, sun-dried and ground with a hammer-mill (5 mm mesh screen). The whole process was described in detail elsewhere (Leterme et al., 2009). In total, approximately 5 t of each species were produced, over the course of several weeks, of which approximately 0.5 t was used in fresh form and the rest in dried and ground form.

The AF samples collected for analysis were freeze-dried and ground with a Pulverisette 14 (Fritch GmbH, Idar-Oberstein, Germany), through a 1-mm-mesh screen. The flour was analysed for its content in dry matter (DM; oven at 105 °C for 24 h), ash (furnace at 550 °C for 8 h), nitrogen (Kjeldahl method) and oil (ether extract by the Soxhlet system, using petroleum ether). NDFom, ADFom and ADL were determined by means of an ANKOM fibre analyser (Ankom Technology, Madecon, NY, USA) using nylon bags. Gross energy (GE) was determined using a Parr 1341 calorimeter (Parr Instruments, Moline, MA, USA).

1.2. Experiment 1: intake experiment

This experiment aimed at measuring the maximal level of AF intake in sows fed a diet composed of 0.6% of commercial diet and 0.4% of AFs in fresh or dry and ground form and distributed separately.

1.2.1. Animals

Eight gilts (PIC Andina, Santiago, Chile) weighing 110 ± 14 kg (mean ± SD) were used. They were housed for 3 weeks in large individual stalls (2 m × 1.5 m), with concrete floors and permanent access to fresh water. The day/night temperature was 30/18 °C during the whole study. The housing was well ventilated and protected from the sun by a roof at approximately 5 m from the ground. The experiment was in agreement with the guidelines of the National University of Colombia for care and use of laboratory animals (Mrad de Osorio and Cardozo de Martinez, 1999).

1.2.2. Diets

A basal diet (600 g maize kg^{-1} DM, 180 g soybean meal, 100 g sucrose, 60 g rice hulls, 56 g mineral/vitamin premix, 1.8 g lysine and 2.2 threonine; estimated content of 14.2 MJ digestible energy kg^{-1}) was formulated to meet the nutrient requirements of gestating sows (NRC, 1998). The fresh ferns were collected with nets and kept one night in Hessian bags, for elimination of the free water. The preparation of the dry ferns is explained above.

1.2.3. Methodology

The experimental design was a Latin square with two repetitions, that is, two gilts per treatment and four treatments tested during four consecutive periods. The gilts were fed 5 times per day, every 2 h, from 8 a.m. to 4 p.m., in identical conditions for each meal. They first received the concentrate, mixed with an equal amount of water. They were then offered either the dry AF or the fresh ferns. The dry meal was mixed with an equal amount of water. Initially, the gilts received 80 or 90 g DM kg^{-1} metabolic weight ($W^{0.75}$) day of fresh or dry AF-based meals, respectively, with 0.4 coming from the basal diet and 0.6 from the AF. The amount was adapted daily according to the appetite of each individual gilt. Feed intake was measured for 5 days. The diets were then permuted and the experiment repeated.

1.2.4. Statistical analyses

Basal diet and forage intakes were analysed using the animal as the experimental unit, with the MIXED procedure of the SAS 8.02 software (SAS Inc., Cary, NC, USA). The model included the effect of the forage source (*Azolla* or *Salvinia*), the form of the AF ferns (fresh or dry) as a class variable and the interaction between the forage and the form. Means were compared using the least square means method. Differences were considered significant at $P<0.05$.

1.3. Experiment 2: ileal and faecal digestibility in sows

1.3.1. Animals

Twenty gestating sows (PIC Andina, Santiago, Chile) between parities 2–4 and weighing 213 ± 9 kg (mean ± SD), were used for this experiment. They were housed for 3 weeks in farrowing crates adapted for total faecal collection. The floor was composed of several 30-cm-wide aluminium floor plates that were assembled and maintained together. The number of plates varied according to the length of the sow and the length of the cage was also adapted, so that the faeces would fall behind the floor on a metallic sheet with a 20° slope to allow for the separation between faeces and urine. Since the faeces were always dry, no problem of contamination or separation between faeces and urine was ever reported. The sows had permanent access to fresh water. The experiment was in agreement with the guidelines of the National University of Colombia for care and use of laboratory animals (Mrad de Osorio and Cardozo de Martinez, 1999).

1.3.2. Diets

A basal diet was formulated to meet the requirements of gestating sows in terms of digestible energy (14.2 MJ kg^{-1} DM), apparently digestible amino acids and minerals (NRC, 1998). Proportionally, 0.15–0.30 of the basal diet was then replaced by either *Azolla* or *Salvinia* to form four experimental diets (Table 1), offered in dried, ground form. The diets were mixed with water (1.5 l kg^{-1} diet). The inclusion rate of 300 g AF kg^{-1} diet was chosen as the maximum, based on the previous research (Leterme et al., 2009) where it was believed to constitute a compromise between accuracy (high level of AF in the diet) and a digestive process unaffected by too high a level of AF in the diet.

1.3.3. Methodology

The diets were randomly assigned to the sows (six sows/diet). After a 14-day adaptation to the diet, the amount provided to the animals was adapted according to feed intake and varied between 70 and 80 g DM kg^{-1} metabolic weight ($W^{0.75}$) per day. The sows were fed 3 times per day (7 a.m., 12 p.m. and 5 p.m.). Faeces were quantitatively collected once a day, for 10 days, homogenised and a sample, representing 0.1 of the total, was kept in a freezer. Feed refusals were collected 30 min after the meal and DM was immediately determined.

Table 1
Composition and analysis of the experimental diets in sows (g kg^{-1} DM).

Exp. 1, 2	Basal diet	Exp. 2 *Azolla*	Exp. 2	Exp. 1	Exp. 2 *Salvinia*	Exp. 2	Exp. 1
		150 g kg^{-1} DM	300 g kg^{-1} DM	400 g kg^{-1} DM	150 g kg^{-1} DM	300 g kg^{-1} DM	400 g kg^{-1} DM
Composition (g kg^{-1} DM)							
Maize	580	348	348	493	406	493	406
Soybean meal	200	120	120	170	140	170	140
Rice hulls	60	36	36	51	42	51	42
Dextrose	100	60	60	85	70	85	70
Min/vit premix	60	36	36	51	42	51	42
Azolla	–	400	–	150	300	–	–
Salvinia	–	–	400	–	–	150	300
Analysis (g kg^{-1} DM)							
Dry matter	895	908	901	889	886	893	890
Ash	82	86	90	93	97	102	105
Crude protein	176	184	191	196	170	163	159
NDFom	219	264	308	337	268	316	348
ADFom	55	97	140	168	105	156	189
ADL	14	32	49	61	32	50	63
GE (MJ kg^{-1} DM)	15.8	15.5	15.4	15.7	15.5	15.6	15.5
DE[a] (MJ kg^{-1} DM)	14.2						
AID[b] lysine	6.5						
AID threonine	4.3						
AID methionine-cysteine	4.3						
AID tryptophan	1.3						

Average composition of the AF alone: *Azolla*: 92.0% DM and per kg DM: 226 g crude protein, 110 g ash, 36 g ether extract, 515 g NDF, 337 g ADF, 132 g lignin and 15.3 MJ GE.
Salvinia: 92.2% DM and per g kg^{-1} DM: 132 g crude protein, 130 g ash, 42 g ether extract, 542 g NDF, 390 g ADF, 135 g lignin and 15.1 MJ GE.
[a] Digestible energy (DE was determined in a previous study; Leterme et al., 2009).
[b] Apparent ileal digestible content (NRC, 1998).

At the end of the experimental period, the animals received the same diet supplemented with the indigestible marker chromic oxide (2 g kg^{-1} diet), for 5 days. The distribution of the last meal started at 6 a.m. with the first sow, followed by the other sows at an interval of 30 min between each sow, so that they could be euthanised and their organs and organ contents collected exactly 4 h after their last meal. After the 4-h postprandial period, the sows were weighed, stunned by electric shock (250 V, 50 Hz), exsanguinated, placed on their back and their abdomen was opened by means of a scalpel. The total digestive tract (from pylorus to rectum) was extracted from the abdomen and the different components of the tract (stomach, duodenum and jejunum, ileum (last 1.5 m of the small intestine), caecum and colon) were isolated with small cords. Each section was weighed with its content, opened and the content was collected by gentle pressure on the organ and weighed. The content was electromagnetically stirred in a 150-ml-beaker and the pH immediately measured by means of a pH-meter (Metrohm 780; Polco s.a., Medellin, Colombia). The organs were then gently washed with distilled water (kept at 39 °C). Water in excess was removed by means of absorbent paper and the organs were weighed again.

1.3.4. Analyses

The diets were analysed for DM, ash, nitrogen (N), ether extract, NDFom, ADFom, ADL and GE, as described above. The faeces were frozen, freeze-dried and analysed for DM, N and GE. The ileum contents were analysed for DM, N, GE and chromium by colourimetry after nitro-perchloric hydrolysis, as described by Furukawa and Tsuhahara (1966).

1.3.5. Calculations and statistical analyses

The coefficients of total tract apparent digestibility (CTTAD) were calculated as follows:

$$\text{CTTAD} = \frac{X_i - f_X}{X_i}$$

where X_i is the amount of N, DM or energy ingested by the animal and f_X the amount of N, DM or energy excreted in the faeces.

The coefficients of ileal apparent digestibility (CIAD) were calculated as follows:

$$\text{CIAD} = 1 - \left[\left(\frac{M_{\text{diet}}}{M_{\text{ileum}}}\right)\left(\frac{N_{\text{ileum}}}{N_{\text{diet}}}\right)\right]$$

where M is the concentration of marker and N is the concentration of components in the diets or the ileal digesta.

Results were analysed using the MIXED procedure of the SAS8.02 software (SAS Inc., Cary, NC, USA) with the animal as the experimental unit. The model included the effects of the fern species as a class variable (*Azolla* and *Salvinia*), the concentration of fern as a continuous variable (150 and 300 g kg^{-1}) as well as the interaction. Linear and quadratic polynomial contrasts were used to determine the effects of increasing the dietary concentration of *Azolla* and *Salvinia*. Differences were considered significant at $P<0.05$.

1.4. Experiment 3: rate of fibre fermentation in the colon

The rate of colonic fermentation of the carbohydrate fraction of the AF, which was not digested in the small intestine, was evaluated by means of an *in vitro* technique. The ferns were evaluated together with the leaves of two tropical shrubs (*Trichanthera gigantea* Kunth. and *Morus alba* L.), which are used in many countries to feed pigs, thus acting as a reference for swine nutritionists. The method was divided in two distinct parts: (1) a treatment of the initial product with pepsin and pancreatin, to mimic the digestive processes in the stomach and the small intestine, followed by (2) the fermentation of the residue of enzymatic digestion in a buffer solution containing bacterial populations collected from faeces.

The methodology was described in detail by Bindelle et al. (2007). In brief, a sample was treated with pepsin (2000 FIP-U (Merck no. 7190) per g substrate) at 39 °C and pH 2 for 2 h. Then, the pH was adjusted to 6.8 and the sample was treated with pancreatin, a mixture of porcine pancreatin enzymes (Sigma no. P-1750; 0.2 g per sample) for 4 h. The residue was isolated by filtration (Nylon cloth, 42 μm), washed with ethanol (95%) and acetone (99.5%) and freeze-dried. An inoculum was then prepared with faeces from two sows, in a buffer solution supplemented with minerals. The sows were given a diet composed of 700 g of a commercial diet and 300 × *g* of tropical tree foliage (*Trichanthera gigantea* Kunth.). The residue (200 mg) was then incubated in glass syringes containing 30 ml of the inoculum solution for 144 h at 39 °C. The total gas production was measured after 0, 2, 5, 8, 12, 16, 20, 24, 48, 72, 96, 120 and 144 h of incubation. Twelve syringes were used for each substrate and three syringes, containing just inoculum, were used as blanks. All the syringes were put in the same water-bath and one inoculum was prepared for the whole study.

Gas-accumulation profiles were fitted to the model of France et al. (1993) as described by Leterme et al. (2006) and Bindelle et al. (2007). The method gives the maximum gas volume (in ml g^{--1} substrate), the lag time (h), which corresponds to the time before gas production actually starts, the half-time to asymptote (h) and the fractional rate of substrate degradation per hour.

Statistical analyses of the kinetics parameters were performed by analysis of variance (ANOVA) and a classification of means by the Student Newman–Keuls method using the GLM procedure of SAS 8.02 software (SAS Inc., Cary, NC, USA) with the forage species as sole class variable in the model.

2. Results

2.1. Experiment 1: intake experiment

The results of AF intake by the gilts are expressed per 100 kg body weight (BW) in order to take BW variation among gilts (94 kg) into account (Table 2). Despite the correction, the variability between gilts was still high, as shown by the high standard deviation. The gilts were able to ingest more than 9 kg of fresh AF per day, which represents a DM intake of approximately 0.6 kg DM per day. No difference was observed between AF species in fresh form (P>0.05) but less *Salvinia* was ingested when presented in dry form (P<0.001). When expressed per kilogram metabolic weight ($W^{0.75}$), the results followed the same pattern.

2.2. Experiment 2: ileal and faecal digestibility in sows

The CIAD and CTTAD of the AF-based diets are detailed in Table 3. Both the AF species and level in the diet affected the results and there was also an interaction between these two parameters (P<0.001). When the inclusion rate of *Azolla* in the diet was increased to 300 g AF kg^{-1}, the CIAD dropped (P<0.001). The opposite was observed for the *Salvinia*-based diets: the CIAD dropped dramatically, when 150 g *Salvinia* were added to the diet but the change in digestibility was limited between 150 and 300 g kg^{-1} diets. This difference in the response to AF level between both species was shown in the statistical analysis by the significant interactions between AF species as well as the level in the diet, despite significant quadratic effects for both species.

Similar trends were observed at the faecal level, except that no 'species × level' interaction was observed for the CTTAD of DM and NDF. The DE content of the diets followed a pattern similar to that of digestibility: the DE content of the diet containing 150 g *Azolla* kg^{-1} was similar to that of the basal diet, whereas that containing 150 g *Salvinia* was 25% lower. The DE contents of the diets with 300 g AF were 4.2 MJ lower per kg, that is, only two-thirds that of the basal diet.

The intake of AF had no effect on the length of visceral organs (Table 4; P>0.05), with the exception of a slight positive effect on the colon length (P=0.04). There was no difference between the AF for their effect on the relative organ weights (P>0.05). On the contrary, the intake of 300 g AF kg^{-1} diet slightly increased the weight of the empty small intestine, colon, caecum and that of the whole digestive tract (P<0.001). The effect of AF intake level on organ weight was linear for both AF species, with the exception of the stomach. The pH of the stomach content was affected by both AF species and AF level in the diet: the pH was higher with *Azolla* and when the diet contained 300 g AF kg^{-1} (P<0.001).

2.3. Experiment 3: rate of fibre fermentation in the colon

The parameters of kinetics of fibre fermentation of the AF in the pig large intestine and that of two tropical shrubs are detailed in Table 5. Compared with the rate of fibre fermentation of the tropical shrubs, the rate of the AF was both low and slow. The lag time, that is, the delay required before the fermentation process actually starts, was longer for the two AFs (P<0.001). The half-time of the asymptote, that is, the time necessary to reach half of the total gas production was also shorter for the tropical shrubs (P<0.001). The total gas production was 2 times higher for mulberry (*Morus*) than that for AF whereas the value obtained for *Trichantera* was intermediary (P<0.001).

3. Discussion

3.1. Experiment 1: intake experiment

Monogastric animals have difficulty meeting their energy requirements when consuming bulky diets, that is, diets with low energy concentration and which possibly swell in the hydrated form. In the present experiment, intakes of fresh AF were high (>9 kg per day) but this represents only 0.6 kg DM per day, that is, roughly 4.0 and 6.0 MJ per day for *Salvinia* and *Azolla*, respectively (Leterme et al., 2009). Drying and grinding doubled DM intake but not sufficiently to consider AF as a valuable energy source. The level of fresh AF that a sow can voluntarily ingest seems to be partially affected by DM intake. In a previous study with tree leaves containing 85% water, Leterme et al. (2006) measured intakes of fresh leaves of only 3.3 kg per day but DM intake was very similar to that observed here (approximately 0.5 kg per day for 100 kg pigs). The differences in water content between tree foliage and AF and in water intake have thus no major consequence on total DM intake. This contradicts the suggestion by Kyriazakis and Emmans (1995) that the ingestion of fibrous, bulky ingredients in pigs is proportional to the water-holding capacity of these ingredients.

3.2. Experiment 2: ileal and faecal digestibility in sows

In the present digestibility study, two inclusion rates were tested to evaluate the effect on digestibility. The decrease in digestibility following AF inclusions was not strictly linear (Table 3): digestibilities decreased slightly with the inclusion of 150 g *Azolla* kg^{-1} diet and more sharply with 300 g kg^{-1}, whereas the decrease was already significant with only 150 g *Salvinia* kg^{-1}. As a consequence, it was not possible to extrapolate for 100% AF and thus not possible to estimate the

Table 2
Experiment 1: voluntary intake of a basal diet and of forage provided *ad libitum*.

	Azolla diet		*Salvinia* diet		S.E.M.	Species	Significance	
	Fresh	Dry	Fresh	Dry			Form	Sp × F
Basal diet								
g fresh matter day^{-1}	1123 b(300)	1684 a(143)	1098 b(197)	1493 a(173)	57.0	0.160	<0.001	0.276
g DM day^{-1}	1018 c(233)	1479 a(128)	1001 c(168)	1316 b(152)	58.9	0.035	<0.001	0.057
g DM kg^{-1} BW$^{0.75}$ day^{-1}	33.5 b (7.5)	48.2 a (2.9)	32.8 b (5.7)	43.4 a (4.2)	1.49	0.160	<0.001	0.297
Aquatic ferns								
g fresh matter day^{-1}	9061 a(2558)	1428 b(83)	9677 a(1788)	1032 b(177)	778	0.844	<0.001	0.368
g DM day^{-1}	630 c(198)	1240 b(85)	597 c(120)	1428 a(83)	69.4	0.103	0.001	0.023
g DM kg^{-1} BW$^{0.75}$ day^{-1}	20.7 c (6.5)	40.5 a (4.2)	19.6 c (4.0)	28.7 b (4.5)	1.69	<0.001	<0.001	0.003

The results are expressed in g dry matter (DM) or fresh matter (FM) per 100 kg bodyweight (BW) or in g DM per kg metabolic weight (W$^{0.75}$).
a,b,c means in the same row with different letters differ significantly (P<0.05). Means are presented with their respective standard deviation (in brackets), in order to show the high variability.

Table 3
Experiment 2: CIAD, CTTAD and digestible energy content of aquatic fern-based diets and of the ferns alone in sows.

Diets	Control	*Azolla*		*Salvinia*		S.E.M.	P-values			Contrasts[a]			
	AF content	150 g kg^{-1} DM	300 g kg^{-1} DM	150 g kg^{-1} DM	300 g kg^{-1} DM		Species	Level	Species × level	*Azolla*		*Salvinia*	
										L	Q	L	Q
CIAD													
Dry matter	0.80	0.72	0.54	0.59	0.54	0.017	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Nitrogen	0.88	0.77	0.52	0.66	0.60	0.021	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NDF	0.63	0.63	0.43	0.52	0.49	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.047
Gross energy	0.82	0.72	0.45	0.50	0.50	0.024	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CTTAD													
Dry matter	0.84	0.79	0.71	0.68	0.56	0.017	<0.001	<0.001	0.376	<0.001	0.048	<0.001	<0.001
Nitrogen	0.85	0.76	0.50	0.68	0.53	0.023	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.512
NDF	0.67	0.63	0.46	0.45	0.35	0.023	0.001	<0.001	0.217	<0.001	0.148	<0.001	<0.001
Gross energy	0.80	0.79	0.55	0.61	0.54	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Digestible energy (MJ kg^{-1} DM)	12.6	12.4	8.46	9.56	8.35	0.323	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] P-values for the linear (L) and quadratic (Q) polynomial contrasts testing the influence of *Azolla* or *Salvinia* concentration in the diet.

Table 4
Experiment 2: length and weight of the digestive organs and pH of the organ contents of sows fed with diets supplemented with 150 or 300 g AF kg^{-1} diet.

	Control	*Azolla*		*Salvinia*		S.E.M.	P-values			Contrasts[a]			
		150 g kg^{-1} DM	300 g kg^{-1} DM	150 g kg^{-1} DM	300 g kg^{-1} DM		Species	Level	Species × level	*Azolla*		*Salvinia*	
										L	Q	L	Q
Length (m)													
Small intestine	20.2	20.9	20.2	20.4	20.8	0.156	0.251	0.701	0.131	0.966	0.164	0.215	0.799
Colon	6.5	7.6	7.3	7.6	7.4	0.136	0.044	0.342	0.847	0.099	0.056	0.064	0.077
Weight (g/100 kg bodyweight)													
Stomach	1369	1405	1404	1356	1408	16.0	0.693	0.519	0.498	0.534	0.712	0.469	0.493
Small intestine	3318	3454	3822	3497	3780	48.0	0.117	<0.001	0.351	<0.001	0.099	<0.001	0.168
Caecum	319	341	390	342	380	7.4	0.541	<0.001	0.611	<0.001	0.191	0.004	0.619
Colon	3487	3767	4189	3970	4135	64.8	0.108	<0.001	0.075	<0.001	0.420	<0.001	0.092
Gut	8493	8967	9805	9165	9703	118.0	0.274	<0.001	0.157	<0.001	0.096	<0.001	0.668
pH													
Stomach	3.5	3.7	4.2	3.9	3.3	0.078	<0.001	0.528	<0.001	0.027	<0.001	0.053	0.003
Caecum	6.5	6.7	6.8	6.9	6.8	0.043	0.043	1.000	0.191	0.027	0.642	0.031	0.006
Colon	6.5	6.9	6.9	6.9	6.9	0.054	0.097	0.899	0.899	0.021	0.117	0.047	0.149

Weight of the sows: 259, 222, 240, 228 and 225 kg, respectively, for the control diet and the diets containing 150 and 300 g *Azolla* kg^{-1}, 150 and 300 g *Salvinia* kg^{-1}.

[a] L: linear; Q: quadratic.

Table 5
Experiment 3: kinetic parameters, fitted according to the France model, of the gas-accumulation curves obtained after fermentation with faecal inoculum of tree leaf meals and aquatic ferns that were previously pre-digested with pepsin-pancreatin.

Substrate	Lag time to asymptote (h)	Half-time (h)	Fractional rate of degradation (per h)	Maximum gas volume (ml/g)
Morus	4.0 a	12.7 c	0.088 a	200.0 a
Trichanthera	3.8 a	22.0 b	0.039 b	134.5 b
Azolla	0.65 b	34.6 a	0.023 c	102.2 c
Salvinia	0.02 c	33.3 a	0.021 c	94.4 c
SEM	0.47	2.48	0.007	10.8
Significance	<0.001	<0.001	<0.001	<0.001

a,b,c means with different letters in a same column differ significantly ($P<0.05$). [a]Basal diet is not included in this analysis.

digestibility values and DE contents of the AF alone. An attempt was made to calculate the AF digestibilities by difference. The GE digestibility calculated from the results of the diets containing 150 and 300 g AF kg^{-1} were, for *Azolla*, respectively, 0.75 and −0.05 and, for *Salvinia*, −0.44 and −0.08, respectively. The results clearly indicate that the presence of 300 g *Azolla* or 150–300 g *Salvinia* kg^{-1} in the diet also negatively affects the digestion of the rest of the diet. Based on these results, it can be concluded that *Azolla* can represent up to 0.15 of a balanced diet for a pig but that *Salvinia* is of no interest for swine nutrition.

Similar trends were observed for the ileal protein digestibility of the AF. The inclusion of 150 g *Azolla* kg^{-1} resulted in a 12% decrease in digestibility, but when 300 g *Azolla* were added, the decrease was more pronounced (−32%). For *Salvinia*, the decrease was the most significant with 150 g added per kg. Similar to case with energy, the estimation of the protein digestibility of the AF alone was not possible.

3.3. Experiment 3: rate of fibre fermentation in the colon

Sows are generally able to make better use of fibrous feedstuffs, thanks to a better capacity to ferment the indigestible carbohydrates in the large intestine and transform them into short-chain fatty acids that will eventually be used as energy sources by the animals (Bindelle et al., 2009). The AF fibre fraction was not well fermented in the conditions of the study (Table 5), which means that their contribution to energy production was limited. The low rate of fermentation can possibly be ascribed to the high lignin content of the AF (104–168 g kg DM; Leterme et al., 2009), as opposed to the tree leaves studied here (29–80 g kg DM, respectively, for *Morus* and *Trichanthera*; Leterme et al., 2005). It is possible that the specific fibre fractions of the AF did not constitute a good substrate for bacteria either. The fibre of AF is composed of a complex matrix with pectic substances and galacturonans, among others (Golovchenko et al., 2002); however, little information is available on their susceptibility to fermentation. Based on the results of the present study, this susceptibility to fermentation is very limited. The fact that AF were not well digested either could be due to the resistance of the cell walls to disruption and fermentation, which would explain the low nutritional value of AF in swine.

4. Conclusions

A 100-kg sow has the capacity to ingest approximately 0.6 kg DM of AF when the latter are offered in the fresh form and roughly twice as much when presented in a dried and ground form. However, maximising intake is of no interest because: (1) high inclusion rates of AF in sow rations depress digestion and (2) the nutritional value of AF is low and would not contribute to meet the sow's requirements. Thus, AF should not represent more than 0.10–0.15 of the animal's DM intake. The low value is ascribable to the high dietary fibre content and to the fact that the latter is poorly fermented in the lower part of the gastrointestinal tract. *Azolla* can be considered for swine nutrition thanks to its relatively high crude protein content (>220 g kg^{-1} DM) and the fact that it does not prevent the digestive processes when incorporated in diets at a rate of 0.15, but *Salvinia* does not seem to present any nutritional advantage.

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