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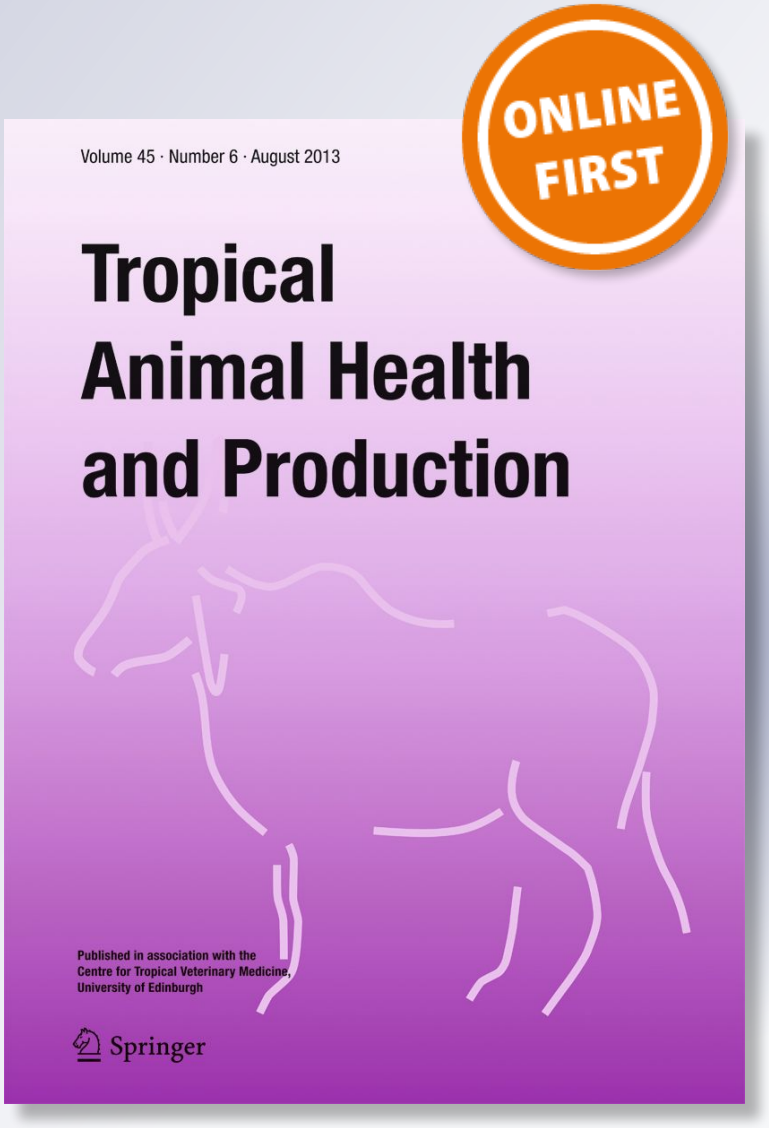

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REGULAR ARTICLES

Evaluation of the feeding value of *Dichrostachys cinerea* pods for fattening pigs in Cuba

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Abstract *Dichrostachys cinerea* (L.) Wight & Arn. is a tropical leguminous shrub widely regarded as an invasive species in Cuba, after having invaded a significant proportion of its arable land during the past decades. Concurrently, smallholder pig producers are highly constrained by the scarcity of protein feeds. This study aimed to assess the feeding value of *D. cinerea* pod meal (DCPM) as an alternative protein supplement for pigs in Cuban smallholder production systems. An on-farm feeding trial was carried out with three groups (N = 10) of growing-fattening pigs over 60 days, where DCPM replaced 0, 15, and 30% in DM of a dietary commercial concentrate. Then, in an in vivo digestibility trial with eight growing pigs, apparent digestibilities of DCPM were determined for dry matter (DM), organic matter (OM) and crude protein (CP). Finally, in vitro digestibilities for OM (fecal and ileal) and CP (ileal) were determined. In the feeding trial, pig body weight gains were not affected by increased dietary substitution levels of concentrate for DCPM. Blood parameters, with a few exceptions, did not show significant differences among groups. Values for in vivo OM and CP digestibilities were 40.81 and 50.26%, and substantially higher than in vitro values. In conclusion, our results showed that at least 30% of DM in commercial concentrate could be substituted by DCPM without affecting pig growth performances under Cuban smallholder conditions. The low digestibility of DCPM is, however, not acceptable for intensive pig production systems. In vitro enzyme digestibility methods developed for commercial pig feeds are not suitable for DCPM without further calibration.

Keywords *Dichrostachys cinerea* · In vivo digestibility · In vitro digestibility · Growth trial · Pig · Cuba

Abbreviations

AD	Apparent digestibility
ALAT	Alanine amino transferase
BW	Body weight
CP	Crude protein
DM	Dry matter
NDF	Neutral detergent fiber
OM	Organic matter

Introduction

Dichrostachys cinerea (L.) Wight & Arn. (commonly known as Marabú) is a tropical leguminous shrub originating from tropical Africa and spread out to tropical Asia, Australia and America (Southern and Central) (Heuzé et al. 2016). In Cuba, Marabú is regarded as a major threat to agricultural production and food security: according to the earliest available data, around 18% of all the arable land is infested by this species (Cordero 2012). Furthermore, its control by the use of traditional methods has resulted inefficient, a situation contributing to the problem remaining unsolved (Nielsen et al. 2013). Concurrently, the Cuban pig farming sector—especially the small-scale private sector—is experiencing low productivities and a high dependency on expensive imported feeds. Current research efforts in Cuba are therefore exploring alternative

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local feed sources with the aim to potentially substitute part of these imports (Almaguel et al. 2011; Ortiz et al. 2011; Cabrera et al. 2012). *D. cinerea* pods, containing between 11 and 18% of CP in DM, have been reported as a useful feed for livestock (Heuzé et al. 2016). Digestibility values for pigs might even fall within acceptable ranges (53% digestible energy) for Cuban smallholder conditions (Nielsen et al. 2013; INRA et al. 2016), despite their high values in antinutritional compounds (18 and 47% in DM for total tannins and NDF, respectively) (Mlambo et al. 2004). Adding value to the biomass of Marabú could furthermore increase incentives for farmers to engage in the harvesting of pods, thus preventing the spread of the plant and integrating it into more sustainable land use systems.

We hypothesized that *D. cinerea* pods can constitute a useful feed supplement in pig diets with a low basal concentration of protein, as found in conditions prevailing in the Cuban small-scale pig production sector.

The objective of this study was, therefore, to assess the value of *D. cinerea* pods as a potential alternative feed source, with a specific focus on Cuban smallholder conditions. The specific objectives were as follows: (1) to evaluate the effects of an increased inclusion of *D. cinerea* pod meal (DCPM) in pig fattening diets on growth and blood parameters, in a smallholder production setting, and (2) to assess in vivo and in vitro digestibility values of DCPM for pigs.

Materials and methods

The test feed of this study was *D. cinerea* pod meal (DCPM). Two months prior to the start of the study, pods were harvested from trees in a site with predominance of cambisol soils. The pods were in an advanced stage of development (brown and partially dry), with fully developed seeds. After harvest, the pods were air dried in storage conditions for 10 days and then finely ground in order to avoid risk of seed germination after animal excretion. Prior to the start of the experiments, the chemical composition of DCPM was determined according to A.O.A.C. (1965), containing 92.43% DM, 15.14% (DM) CP, 47.45% (DM) NDF, and 5.13% (DM) ash.

Feeding trial

This part of the study was carried out at the "La Gran Señora" farm, member of the Cooperative of Credits and Services (CCS) "El Vaquerito", in Santa Clara (Cuba).

Pigs, design, and diets

An initial population of 30 110-day old growing-fattening pigs (Yorkshire × Landrace; 15 castrated males + 15 females) was used, with an initial average weight of 63.9 ± 8.5 kg. Prior to entering into the experiment, the animals had been fed with the same ration and raised under the same management conditions. The pigs were randomly allocated to three pens with equal number of animals ($N = 10$ in each group). Due to practical inconveniences of re-grouping, full homogeneity in the initial weights was not obtained (a statistical correction for this is later described).

Three different experimental diets were fed to each pen. Diets were formulated following the NRC (2012) nutrient requirements for swine. Each diet contained the same basal diet, consisting of locally produced feeds (hydrated yam and plantain, hydrated cassava, and organic canteen waste), corn meal, and a protein-mineral supplement. This basal diet was supplemented with a commercial fattening concentrate (19.5% CP), which was substituted by DCPM at three substitution rates: 0, 15, and 30% of DM. Daily provision and relative administration of the basal diet and supplement were further adjusted according to the growth stage of the pigs, as summarized in Table 1.

The government collected 30 of the animals after the initial 30 days, and group size was therefore halved from day 30 to the end of the experiment. This was accounted in the statistical model described later.

Measurements and analytical methods

The feeding trial was carried out for a period of 60 days. Feed was offered twice a day (at 06 and 18 h) in equally sized meals. Daily DM provision increased from 2.5 kg (days 0 to 30) to 3 kg (days 30 to 60) per pig and day (Table 1), and water was available ad libitum. Individual live weights were indirectly estimated through girth circumference measurements, using the transformation tables provided by IIP (2014) at days 0, 10, 20, 30, 40, 50, and 60 of the experiment. Total feed intake (as per pen) was not measured, as there were no or negligible amounts of leftovers during the whole experiment.

At days 8 and 60, blood samples were obtained from four randomly selected pigs from each pen for further assessment of blood parameters (hemoglobin, hematocrit, glucose, total proteins, albumin, globulin, and alanine amino transferase (ALAT)). Two samples of 5 ml were taken from each animal by orbital sinus puncturing and stored in two sterilized sampling tubes: one with 5 mg of EDTA (for hematocrit and hemoglobin determination) and another without anticoagulant (for the remaining parameters). The hemoglobin was determined by the cyanmethemoglobin method (Drabkin and Austin 1932), and the hematocrit by the microhematocrit method with capillary tubes (ICSH 1982). Leucocytes were determined in an IDEXX VetAutoread™ equipment (IDEXX LABORATORIES VetLab®, USA), according to the manufacturer's procedures. For the biochemical analyses, the blood samples were centrifuged at $3500 \times g_{av}$ during 15 min and blood serum was then frozen at −10 °C until analyzed. Total serum protein was determined by the Biuret method (Gornall

Table 1 Composition of the three experimental diets fed during the first and last 30 days of the 60-day experimental period

	Diet (% of DCPM substitution rate)					
	A (0%)		B (15%)		C (30%)	
	0–30 days	30–60 days	0–30 days	30–60 days	0–30 days	30–60 days
Ingredients	% DM	% DM	% DM	% DM	% DM	% DM
Hydrated yam + plantain	4.7	0	4.7	0	4.7	0
Hydrated cassava	7.7	12.3	7.7	12.3	7.7	12.3
Fattening concentrate	47.4	44.3	40.3	37.6	33.2	31
Nuprovim (protein/mineral supplement)	2.4	0	2.4	0	2.4	0
Canteen food residues	33.4	23.9	33.4	23.9	33.4	23.9
Maize flour	4.3	19.5	4.3	19.5	4.3	19.5
DCPM	0	0	7.1	6.6	14.2	13.3
Daily DM offer per animal (kg)	2.55	2.97	2.55	2.97	2.55	2.97
CP (% DM)	16.7	14.6	16.5	14.3	16.2	14.0
Crude fiber content in diet (% of DM)	4.2	4	5.3	5.1	6.4	6.1
Digestible energy (MJ/kg MS)	14.45	14.81	14.15	14.53	13.84	14.24

et al. 1949) and the albumin level was quantified by serum electrophoresis. Globulins were calculated by the total protein-albumin difference. The ALAT was analyzed by the IFCC optimized UV kinetic method (Bergmeyer et al. 1986) in an AIRONE 200 (Crony Instruments) equipment, using the ALT-test reactive (Empresa de Producción de Biológicos "Carlos J. Finlay", Cuba). Serum glucose concentration was determined by ultra-violet visible spectrophotometry (GENESYS™ 6, U.S.A.), according to the manufacturer's guidelines and using the RapiGluco-Test reactive (Empresa de Producción de Biológicos "Carlos J. Finlay", Cuba).

Finally, a cost-benefit assessment was carried out, based on a comparison between the 0 and 30% substitution regimes. This was done by calculating the difference of costs between both scenarios as if each was applied to all the animals throughout the study period. From direct observations and interviewed sources, sufficient data was collected in order to estimate DCPM production costs (harvesting and milling), as well as the real costs—as of October 2015—of the commercial fattening concentrate substituted in this study.

In vivo digestibility trial

The in vivo digestibility trial was carried out at the Experimental Agricultural Station of the Central University "Marta Abreu" of Las Villas, in Santa Clara, Cuba.

Pigs, design, and diets

Eight male pigs with an initial average weight of 80.0 ± 0.47 kg were randomly assigned to eight metabolic cages. All the animals were litter mates, with an age of 222 days at the start of the trial. The breed used was a hybrid between a Yorkshire × Landrace sow and a L31 hybrid (Pietrain × L63) boar.

The experimental feed mixture was composed of 50% DCPM and 50% final sugarcane molasses (80.0% DM; 90.2% OM; 3.0% CP) (Figueroa 1989; Reinoso-Pérez et al. 2005), on a fresh matter basis. Throughout the study period, the diet was offered in the form of a wet mash (7% addition of water), while water was available ad libitum. The pigs were offered 2.3 kg of DM per day, distributed equally in morning and afternoon feedings (7 and 17 h).

Measurements and analytical methods

The trial started with a 4-day adaptation period to the experimental feed, followed by a 4-day adaptation period to both the cages and the feed. The sampling period was then carried out during 5 days. The total amounts of feed orts and excreta (feces and urine) were determined from each animal at 24-h intervals, before each morning feeding. On days 1, 3, and 5; 20 g of feces and 100 ml of urine were randomly sampled from each animal and pooled for later chemical characterization. All samples were stored at −20 °C until analyzed.

Fecal samples were analyzed for DM, OM, and N, whereas urine samples were analyzed for total N, according to A.O.A.C. (1965). Apparent fecal digestibilities (AD) of DM, OM, and CP (N × 6.25) of *D. cinerea* pods were calculated by following the same principles as in the difference method (Fan and Sauer 1995). In this case, an indirect calculation was made, based on known digestibility values for the basal feed (final molasses) and assuming

that there was no interference in digestibility between DCPM and final molasses.

$$AD\% = 100 \times [I(DCPM) - [F(total) - I(molasses) \times (1 - AD(final\ molasses))]] / I(DCPM)$$

(where I = intake; F = fecal output).

For this calculation, the assumed AD values of the accompanying feed (final molasses) were the same as those previously reported by Xandé et al. (2010): DM = 85.2%; OM = 84.4%; and CP = 68.1%.

Finally, N retention (metabolizable N) was assessed by subtracting total N output (feces + urine) from N intake (g/day). This balance predominantly represented the N retention from *D. cinerea*, as the N content in the final molasses is considered almost negligible.

In vitro digestibility trials

Contents of enzyme degradable nutrients in ground DCPM were determined by three in vitro analysis methods: (1) fecal enzyme digestible OM (total digestive tract); (2) enzyme digestible OM by the end of the ileum and (3) enzyme digestible nitrogen by the end of the ileum. The analyses were conducted at the commercial laboratory Eurofins Steins Laboratorium A/S (Vejen, Denmark) and performed according to the procedures and calculations described by Boisen and Fernández (1995) and Boisen and Fernández (1997).

Statistical analyses

For the growth trial, daily individual weights and weight gain results were analyzed with the package lme4 (Bates et al. 2015) for linear mixed models and using the R statistical software (R Core Team 2013). The initial body weights for the control group were significantly less than those of the other two groups, and therefore initial body weight was used as a covariate for weight and gain models. Both time and animal were investigated as random effect variables and the final model was chosen based on the best AIC value. The final model for gains included only diet as a fixed variable and animal as a random effect variable. The final model for absolute weight included time and diet as fixed variables and animal as a random effect variable. This statistical approach accounted for the reduction of animals at day 30 and for differences in initial average body weights between pens at the onset of the experiment. For all the blood parameters, the differences among groups at both sampling times were assessed by a Duncan test (Duncan 1955). Differences with $P < 0.05$ were considered to be statistically significant.

For all the digestibility trials (in vivo and in vitro), descriptive statistics (mean and standard deviation) were applied to all the obtained parameters.

Results

Feeding trial

The average weights increased significantly over time for all groups (Fig. 1). The average daily gain (ADG) did not differ significantly between the groups at any given time, either before or after the untimely removal of half of the experimental animals. Likewise, the ADG for the first 30 days was similar to the ADG in the remaining pigs after half of them had been removed. The 60-day ADG was described by the following linear equations:

1. Control group; $Y(\text{kg}) = 492\times + 59.63$ ($R^2 = 0.9909$)
2. 15% DCPM; $Y\ (\text{kg}) = 561\times + 64.549$, ($R^2 = 0.9916$)
3. 30% DCPM; $Y\ (\text{kg}) = 467\times + 67.654$ ($R^2 = 0.9917$)

where Y is the weight in kg and x is ADG (grams).

The pigs consumed the entire daily ration throughout the study period, with only occasional leftovers related to changes in the basal diet, but without differences in DCPM acceptability among groups. No significant detrimental effect ($P < 0.05$) was observed on the growth rate of pigs when DCPM replaced commercial fattening concentrate at 15 and 30% rates.

Values for the blood parameters are shown in Table 2. All values were within the normal ranges reported for pigs (Kaneko et al. 2008), except for lower glucose values in all the groups at both samplings, as well as for ALAT at the second sampling. No animals showed any clinical signs of illnesses at any time during the study. The only significant differences related to feeding treatment were observed for albumin, where plasma levels were higher at the second sampling in the 15 and 30% substitution groups as compared to the control group.

The estimated cost reduction of substituting commercial fattening concentrate for DCPM was up to 1.0 Cuban pesos (CUP) or 0.04 USD per kg of substituted feed. This value was calculated based on an estimated cost of 397 CUP or 14.7 USD per ton of DCPM produced (harvesting + milling costs), and a cost of 1397 CUP or 52.7 USD per ton of fattening concentrate (as of October 2015). When comparing the costs of the 0 and 30% treatments in the current study (for 30 pigs and during 60 days), the total estimated savings would have been up to 726 CUP or 27.4 USD for the 30% scenario, representing a 21.1% cost reduction. Translated into costs per kg of live weight produced, such savings would amount to 0.09 pesos per kg.

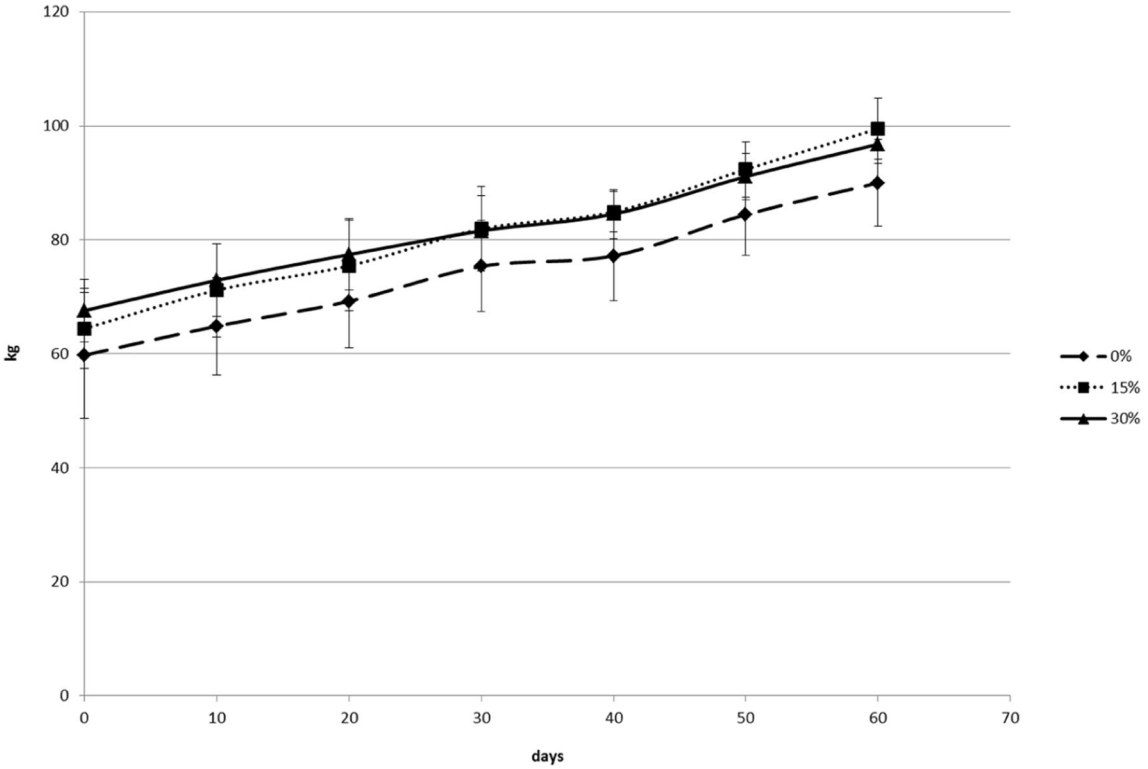

Fig. 1 Weights of Cuban pigs fed 0, 15, or 30% Marabu in ration DM (sd shown by *vertical lines*)

Table 2 Average values of biochemical blood parameters among groups, at two samplings (days 8, 60)

				Diet (% of DCPM substitution rate)			
	Unit	Normal values	Sampling	A (0%)	B (15%)	C (30%)	SE ±
Hematocrit	L/L	35–46	1st	35.2[a]	34.8[a]	35.8[a]	0.01
			2nd	44.0[ab]	39.3[a]	46.7[b]	0.01
Hemoglobin	g/L	85–140	1st	114.8[a]	115.6[a]	116.4[a]	1.71
			2nd	136.0[a]	124.6[b]	140.3[a]	1.70
Leucocytes	10^9/L	6–18	1st	12.18[a]	10.62[b]	11.66[ab]	0.32
			2nd	11.56[a]	12.1[a]	11.93[a]	0.28
Glucose	mmol/L	4.0–6.38	1st	2.46[a]	2.44[a]	2.38[a]	0.05
			2nd	2.7[a]	3.06[a]	2.8[a]	0.21
Total proteins	g/L	60–85	1st	68.12[a]	67.94[a]	67.94[a]	0.19
			2nd	64.7[a]	69.6[a]	62.7[a]	1.62
Albumins (A)	g/L	16–38	1st	36.0[a]	35.98[a]	36.18[a]	0.19
			2nd	33.53[a]	35.33[b]	36.0[b]	0.50
Globulins (G)	g/L	–	1st	32.12[a]	31.96[a]	31.76[a]	0.13
			2nd	30.36[a]	29.06[a]	33.6[a]	1.32
A/G	g/g	0.5–2.2	1st	1.1[a]	1.1[a]	1.1[a]	–
			2nd	1.1[a]	1.2[a]	1.1[a]	–
ALAT	IU/L	21–46	1st	30.56[a]	31.0[a]	31.6[a]	0.27
			2nd	17.76[a]	16.83[a]	12.8[a]	1.32

Different superscripts (a, b) between groups within same blood variable indicate significant differences with $P < 0.05$ (Duncan 1955)

SE standard error

Digestibility trials

As shown in Table 3, in vivo digestibilities of DM, OM, and CP were 38.8, 40.8, and 50.3%, respectively. The average N retention from the total diet was 8.7 g/day per animal, representing a net protein utilization of 35.4%.

In vitro digestibilities for OM were 32.2% at the total digestive tract level and 21.5% at the ileal level, respectively. In vitro ileal digestibility for N was 25.0%.

Discussion

The current study is the first of its kind where *D. cinerea* pods have been tested in a swine feeding trial. Most of the research done so far with woody leguminous species as alternative feed sources for pigs in Cuba has focused on the use of leaves. Examples include *Cajanus cajan* (19.3% CP; 24.1% CF) and *Gliricidia sepium* (22.3% CP; 19.7% CF) (Heuzé and Tran 2015; INRA et al. 2016), having proven a certain potential for use in pig diets at moderate rates of inclusion (up to 16 and 20% of fresh matter, respectively) without significantly affecting productive parameters (Vásquez and Rosso 1997; Trómpiz et al. 2001). The results of this study showed that a 14.2% dietary inclusion rate in DM (30% DM substitution rate) of DCPM (Table 1) had no negative effect on growth rates in growing-fattening pigs within a smallholder contract farming scenario. This suggests that these pods could be used as an alternative dietary source of energy and protein, at least at moderate inclusion rates under conditions where alternative protein sources are scarce or unavailable. The latter would especially be the case for smallholder pig farmers who depend entirely on locally available feed resources (i.e., farmers without state contracts ensuring supply of concentrates).

The results provided in this study further suggested that there was a potential saving of 21% of the feeding costs under the smallholder contract farming scenario. This underlines the potential of DCPM as an alternative feed source for the private smallholder sector, and this is backed by the fact that the tested health parameters (pig blood profiles) did not show any anomalies. In one of the blood samplings, albumin levels were even significantly higher in pigs fed with diets containing DCPM; which might—if anything—suggest an improvement in protein supply (Jahoor et al. 1996) as compared to the diet without DCPM.

The in vitro digestibility values were much lower than those determined in vivo. The reasons for this can probably be attributed to the fact that the laboratory in vitro digestibility methods applied were developed for use on easily digestible feeds from the Danish industrialized pig production sector (Boisen and Fernández 1997) and may thus not be applicable to a feed like DCPM without further calibration.

No published data was found on digestibility of pods from *D. cinerea* or any other tropical woody leguminous species in pigs. The in vivo DM digestibilities found in this study were, however, within the same ranges reported for leaves from other locally available woody legume species, such as *G. sepium* and *Calliandra surinamensis* (46.9 and 46.4%, respectively) (Espinosa-Sifontes and Pedraza-Olivera 2013). The in vivo CP digestibility obtained in this study (50.3%) was markedly higher than values reported for other previously tested feeds derived from legume species, such as forages from *L. leucocephala* (38.2%) (Santos-Ricalde and Abreu-Sierra 1995; Ly 1996), *Sesbania rostrata* (41.4%; Ly 1996) and *Medicago sativa* (43.9%; Dierick 1991). DCPM may therefore be a relevant alternative protein source to consider under smallholder conditions where other and better protein sources are not available.

A high content of fibers and biologically active compounds is the most likely reason for the rather low digestibility of DCPM compared with other conventional protein sources used in pig feeding, such as soybean meal. The total tannin content in the pods has been reported to be as high as 18% in DM (Mlambo et al. 2004), while our study and other reports (Heuzé et al. 2016) have shown a large content of NDF in dry pods of around 47% of DM. In this study, the high fiber values obtained can be attributed to the fact that the pods were harvested at a late stage of development. Even in ripe pods, the seeds constitute a very small fraction, and are surrounded by a very hard shell.

Given the relatively high protein content of *D. cinerea* pods and the high prevalence and risk of spread of the plant in Cuba, further possibilities for the use of these pods as feed for pigs in the Cuban smallholder sector are definitely worth exploring. In order to further prove the potential of DCPM as an alternative source of energy and protein in pig diets, future studies must establish if NDF content could be reduced by

Table 3 In vivo and in vitro digestibility values for *Dichrostachys cinerea* pod meal

	Content in feed	Digestibility		
		In vivo	In vitro (fecal level)	In vitro (ileal level)
DM	92.4 ± 0.28%	38.83 ± 7.8%	–	–
OM	94.9 ± 0.17% (DM)	40.81 ± 7.6%	32.2% (n = 1)	21.6% (n = 1)
CP	15.1 ± 0.70% (DM)	50.26 ± 5.8%	–	25.0% (n = 1)

harvesting pods at an earlier stage of development, as well as explore ways to reduce the content or activity of antinutritional compounds.

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Compliance with ethical standards

Statement of animal rights All the experimental procedures involving the use of animals (growth trial and digestibility trial) were carried out in compliance with both the animal welfare regulations of the Cuban Institute of Veterinary Medicine and with the EU Directive for animal experiments (2010/63/EU) as an essential requirement for performing animal experiments under representation of the University of Copenhagen. Field work supervision on the compliance of such procedures has been carried out by the Coordinator of the Animal Production Group of the Central University "Marta Abreu" of Las Villas (Cuba).

Conflict of interest The authors declare that they have no conflict of interest.

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