SHORT COMMUNICATION

Apparent digestibility of compound diets with increasing levels of perilla (*Perilla frutescens L.*) seeds in rabbit

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Abstract

The aim of this study was to determine the effects of three levels (0, 5 and 10%) of perilla (*Perilla frutescens* L.) seeds (PFS), included in isonitrogenous and isocaloric diets, on the apparent digestibility in rabbit aged of 73 days. The trial was carried out on 30 crossbred (Carmagnola Grey x New Zealand) rabbits randomly divided in three groups of ten animals each (five male and five female rabbits). Each of them was kept in individual cages. The faeces were collected during the last week of a growing trial that lasted 50 days. No obvious health problems were encountered during the experiment and no rabbits died during the trial. The measured parameters were digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fibre, acid detergent fibre and gross energy. The only parameter that was modified by the inclusion of PFS was the ether extract digestibility; it resulted higher in the 10% PFS diet (83.9%) than in the other two diets. Perilla seed may be used satisfactorily as a nutrient supplement for rabbits at levels of up to 10% in the diet with a better digestibility of ether extract than in the other two diets.

Introduction

Perilla (*Perilla frutescens*) is an annual herbaceous plant that belongs to the Labiatae family and it is native to Asia. It has recently been introduced in Europe, Russia and the USA as an oilseed crop (Nitta *et al.*, 2003). The seeds are used in particular in India (Sharma *et al.*, 1989) and in Korea where they are consumed as flavouring and nutritional sources in combination with cereals or vegetables after roasting (Shin and Kim, 1994). No ill effects have been reported due to the consumption of perilla seed or its oil in humans or in a short-term animal experiment (Longvah *et al.*, 2000). Longvah and Deosthale (1998) demonstrated that perilla seed is a potential source of food, rich in good quality fat (52%) and protein (17%), which could be used in both human and animal nutrition. The same authors demonstrated that the potential of perilla seed protein can be increased by dehulling the seed and then cooking it. Among the vegetable oils known as good sources of linoleic acid, perilla seed oil has the highest α-linolenic acid content (Longvah and Deosthale, 1991). Therefore, perilla seed could be used as an alternative protein source to soybean or as an alternative to others oilseeds (linseed, false flax and chia), aiming to increase the n-3 polyunsaturated fatty acid (n-3 PUFA) content (mainly α-linolenic acid) in rabbit meat. The production of PUFA enriched animal products based on perilla seed diets has been studied in pigs (Yamada *et al.*, 2001). Peiretti (2011) determined the fatty acid profile, chemical composition and gross energy content in perilla plants during the growth cycle. Nowadays, no study has been carried out about the digestibility and nutritive value of perilla seed on rabbits.

The current research aimed at evaluating the apparent digestibility of diets containing increasing levels of perilla seed in rabbits.

Materials and methods

Animals and diets

The study was carried out at the experimental rabbitry of the Department of Animal Sciences in Carmagnola (Torino, Italy).

Three isoproteic and isoenergetic diets were formulated with increasing levels of perilla (*Perilla frutescens* L.) seeds (PFS-0.5 and 10%), obtained from Manitoba Inc. (Winnipeg, Canada). All diets were pelleted and stored in darkness to avoid auto-oxidation of the lipid sources. The ingredients and chemical composition of the three diets are shown in Table 1. The diets were offered *ad libitum* to thirty weaned crossbred rabbits (Carmagnola Grey x New Zealand) aged of 30 days, randomly divided in three groups of 10 animals (five male and five female rabbits) with equal initial weight variability (1098±212 g, 1123±201 g and 1156±192 g, respectively).

The animals were single housed in three-floor cages at a temperature of 22°C±2°C and had free access to clean drinking water.

Digestibility trial and analytical methods

The apparent digestibilities of the three diets were determined in the last week of a growing trial, lasted 50 days. At the beginning of the digestibility trial, the mean live weights of three experimental groups were 2457±309 g, 2493±168 g and 2435±346 g for 0, 5 and 10% groups, respectively. The faeces were collected using a nylon net placed under each floor of cages, to avoid urine contamination. The faeces of each group (10 rabbits each) were collected and pooled daily over a period of five days, at approximately 09.00 h, before the next daily ration was provided. Each daily pooled fecal sample was placed in a two-layer plastic bag to prevent the loss of moisture and immediately frozen at -20°C. The frozen samples collected every day were individually mixed thoroughly and ground in a homogenizer (Tecator, Herndon, VA, USA). The representative samples were then weighed in an aluminium foil pan, dried in a draft oven at 80°C to constant weight and stored for chemical analysis.

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Key words: *Perilla frutescens*, *In vivo* digestibility, Rabbit.

Acknowledgments: the authors would like to thank G. Cerato for the preparation of the pelleted diets, V. Malfatto, L. Sterpone and C. Bianchi for their technical support and L. Sola and D. Sola for the rabbit care.

Received for publication: 8 July 2010.
Revision received: 16 September 2010.
Accepted for publication: 20 September 2010

Italian Journal of Animal Science 2010; 9:e81
doi:10.4081/ijas.2010.e81

All the analyses were carried out on duplicate samples. The diets and faeces were analyzed to determine total N content (AOAC, 1995), ash by ignition to 550°C, neutral detergent fibre (NDFom) without sodium sulfite and α-amylase and acid detergent fibre (ADFom), as described by Van Soest et al. (1991) expressed exclusive of residual ash. Lignin [lignin (sa)] was determined by solubilization of cellulose with sulphuric acid as described by Robertson and Van Soest (1981), gross energy (GE) using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany), and ether extract (EE) after acid-hydrolisis using the Soxlet method (AOAC, 1995). The acid insoluble ash content of the diets and faeces was determined according to the Van Keulen and Young' (1977) method.

Determination of digestibility coefficients and statistical analysis

The digestibility coefficients were calculated according to the indirect digestibility method (Furuichi and Takahashi, 1981) using acid insoluble ash (AIA) as an inert marker. The DM digestibility calculation procedure was as follows.

DM digestibility (%) = (1- A/B)*100, where A and B are the acid insoluble ash concentrations in the feed and faeces, respectively.

The digestibility of the other nutrients (X) was calculated as follows.

Digestibility (X in %) = (1-A/B*XB/XA)*100, where XA and XB are the concentrations of X in the feed and faeces, respectively.

Statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS, 2002). The analysis of variance was used to evaluate the effects of the diet on the *in vivo* digestibility. Significant differences among the treatments means were determined using Duncan's test.

Results and discussion

The protein content of the PFS found in our study was higher (23.9% DM) than reported by Longvah and Deosthale (1998), while the EE content was lower (43% DM) than the one found by the same authors in 1991. Other oilseeds used in rabbit digestibility trials, i.e. chia seed (Meineri and Peiretti, 2007) and golden flaxseed (Peiretti and Meineri, 2008) presented similar protein contents (both 23.5% DM), while the EE content of the PFS was higher than those of the other two oilseeds (31.1% and 36.9% DM for chia and golden flaxseed, respectively). The inclusion of PFS in the diets increased the EE and fibrous fractions contents with the exception of NDF (Table 1).

The apparent digestibility coefficients are reported in Table 2. The results show that the addition of PFS does not modify the digestibility of dry matter, organic matter, NDFom, ADFom, crude protein and gross energy.

On the light of experimental results and based on a literature review (De Blas *et al.*, 1992; De Blas and Mateos, 2010) these diets

Table 1. Ingredients and chemical composition of the experimental diets.

	Perilla seeds	Compound diets		
		0% perilla seeds	5% perilla seeds	10% perilla seeds
Ingredients				
Corn, %		14.4	18.0	17.0
Barley, %		20.0	18.0	16.7
Alfalfa meal, % (17% CP)		46.0	42.0	42.0
Soybean meal, % (45% CP)		12.0	11.0	9.0
Palm oil, %		3.60	2.00	1.30
Perilla seed°, %		0.00	5.00	10.0
Vitamin-mineral premix#, %		2.00	2.00	2.00
Lignosulphite, %		2.00	2.00	2.00
Chemical composition				
Dry matter, %	95.3	91.5	92.1	91.8
Organic matter, % DM	96.2	91.0	92.1	92.5
Crude ash, % DM	3.80	9.00	7.90	7.50
Acid insoluble ash, % DM	-	0.70	0.60	0.70
NDFom, % DM	29.7	27.6	28.8	28.3
ADFom, % DM	22.6	16.8	17.8	18.1
Lignin (sa), % DM	10.9	3.30	3.80	3.90
Ether extract, % DM	43.0	4.62	5.88	7.31
Crude protein, % DM	23.9	19.5	19.5	19.4
Gross energy, MJ/kg DM	28.0	18.4	18.8	19.4
Aminoacid calculated				
Lys, g/Kg DM	9.1	7.9	9.2	10.5
Met+Cys, g/Kg DM	10.0	5.6	7.5	9.3
Thr, g/Kg DM	9.8	6.4	8.1	9.7

°Essential amino acid composition of perilla seed (mg/g protein), Lys 38, Met+Cys 42, Thr 41 (as reported by Longvah and Deosthale, 1998); #per kg of diet: Vitamin A 200 U, α-tocopheryl acetate 16 mg, Niacine 72 mg, Vitamin B_6 16 mg, Choline 0.48 mg, DL-methionine 600 mg, Ca 500 mg, P 920 mg, K 500 mg, Na 1 g, Mg 60 mg, Mn 1.7 mg, Cu 0.6 mg; NDFom, neutral detergent fibre; ADFom, acid detergent fibre; Lignin (sulfuric acid), acid detergent lignin.

Table 2. Daily dry matter intake during the experimental period, *in vivo* apparent digestibility coefficients and nutritive value of the experimental diets.

	Perilla seed, % of diet			RSD	P
	0	5	10		
Number of animals	10	10	10	-	-
Daily dry matter intake, g DM/d	144	131	132	3.40	0.235
Apparent digestibility					
Number of observations	5	5	5	-	-
Dry matter, %	67.2	65.2	66.6	1.66	0.225
Organic matter, %	67.3	65.2	66.5	1.65	0.208
Neutral detergent fibre, %	27.7	23.2	25.2	3.51	0.205
Acid detergent fibre, %	21.8	18.6	20.9	2.74	0.446
Ether extract, %	73.8	77.5	83.9	4.64	0.000
Crude protein, %	70.2	68.8	69.2	1.95	0.641
Gross energy, %	66.4	64.2	66.5	1.83	0.109
Nutritive value					
Digestible energy, MJ/kg DM	12.2	12.1	12.9	0.45	0.009
Digestible protein, g/kg DM	136.8	133.9	134.5	3.84	0.587
DP/DE, g/MJ	11.2	11.1	10.4	0.37	0.001

resulted nutritionally balanced in relation to the requirements of growing and fattening rabbits.

As far as the fibre digestibility is concerned, our results did not show any significant differences, while the use of fat as a dietary energy source in other works induced a slight increase in fibre digestibility. Fernández *et al*. (1994) observed an increase in ADF digestibility (from 0.14 to 0.22) when fat was added to the diet, but most authors do not find significant differences in fibre digestibility (Xiccato *et al.*, 1995; Pérez *et al.*, 1996). In fact, in most of the works the differences seem to be attributed more to changes in dietary fibre content and nature than to the addition of fat itself.

The EE digestibility increased with increasing level of PFS with the high value recorded in rabbits fed 10% PFS diet (83.9%). The EE apparent digestibility coefficient is generally higher when the level of dietary fat is increased and its value usually depends on the type of added fat (Pascual *et al.*, 2002). Van Manen *et al.* (1989) described an increase in EE digestibility when fat was added to the diet and this could be due to the fact that with increasing fat intake the faecal excretion of endogenous fat had a diminishing effect on the calculated apparent digestibility.

EE digestibility found in our work has been affected by the decreasing level of palm oil and the increasing level of PFS. This result is associated to the use of pure fats-pure oils or high-fat raw materials. The PFS fat (mono-, di- and tri-glycerides) is more digestible compared to fats (terpens, waxes, etc.) contained in the other raw materials commonly used in rabbit feeding (Fernández *et al.,* 1994; Maertens, 1998; Fernández-Carmona *et al.,* 2000). There are also some differences of digestibility, depending on the unsaturation degree of fats used; in fact, the higher EE digestibility of unsaturated FAs diet seems to be related to its richness in polyunsaturated FAs, which are easier emulsified in the digestive tract than saturated FAs (Pascual *et al.*, 2002), and a negative relationship has been reported between the degree of saturation and fat digestibility in rabbits (Xiccato, 2010).

The crude protein digestibility obtained in this study resulted lower than what reported by Longvah and Deosthale (1998), who fed rats with dehulled perilla or wholeseed and with cooked dehulled perilla seed diets. It could be interesting to verify if the process of dehulling and/or cooking would improve the protein digestibility in rabbits as well. Oita *et al.* (2008) studied *in vitro* the digestibility of perilla seed protein and found that it could reasonably be expected to be used as food protein since most proteins are extractable with water and NaCl and are readily digestible.

As a result of the high digestibility of PFS fat, the digestible energy (DE) measured *in vivo* using the acid insoluble ash method was significantly higher in rabbits fed 10% PFS diet than the other groups, while the digestible protein (DP) did not differ among groups. The DP/DE ratio was significantly lower in rabbit fed 10% PFS diet compared with the other groups, due to the higher digestible energy of lipid fraction of PFS. These experimental data were compared with those calculated using some of the linear regression equations proposed by Villamide *et al.* (2009) and based on the chemical composition of the rabbit diets. As far as the digestibility of GE and digestible energy is concerned, our calculated data were similar in the prediction equation based on lignin(sa) or ADFom values, respectively, while resulted lower than the values calculated with the other proposed equations.

Conclusions

Perilla seed may be included in diets for growing rabbits up to 10% without consequences on diet utilization with a better digestibility of ether extract than in the other two diets that were examined. Due to the high content of α-linolenic acid in Perilla seeds, they could be used in PUFA enriched diets for growing rabbit in replacement of other seed rich in n-3 PUFA. Furthermore, due to the high nutritive value of the PFS they could also be used in substitution of other raw materials i.e. whole soybean meal.

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