In vitro fermentation of diets incorporating carob pulp using inoculum from rabbit caecum

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Abstract

The aim of this work was to evaluate the nutritive value of carob pulp for rabbits using the *in vitro* digestibility and gas production techniques with inocula from caecal content of rabbits. Experimental diets contained 0% (D1), 10% (D2), 20% (D3) and 100% (D4) carob pulp on a dry matter (DM) basis and were incubated in glass syringes for 72 h at 39 ºC. Carob pulp contained 313 g neutral detergent fibre/kg DM with a high acid detergent fibre (263 g/kg DM) content, resulting in a low hemicellulose content of 50 g/kg DM. Potential gas production ranged from 123 (D1) to 179 (D4) mL/g DM and was similar for the D1 (123 mL/g DM), D2 (126 mL/g DM) and D3 (130 mL/g DM) treatments. The lowest pH value of 6.47 and the highest organic matter degradation (OMD, 64.3%) were observed in the 100% carob pulp (D4) treatment, while its inclusion at 10% and 20% tended to improve the OMD of the diets. These results show that carob pulp is well fermented by the caecal micro-organisms of rabbits. Although its inclusion at 20% did not improve *in vitro* fermentation and degradation of the commercial concentrate, it was concluded that carob pulp has potential as an unconventional feed resource for rabbits. Its utilization could have a positive effect on intestinal microbiota owing to its high content of soluble fibre.

Keywords: *Ceratonia siliqua*, by-product, chemical composition, gas production, organic matter degradation
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Introduction

Conventional by-products and unconventional materials from the food processing industry have frequently been included in livestock diets. Their utilization can have nutritional, economic and ecological advantages. The carob pod is the fruit of the carob tree (*Ceratonia siliqua* L.), and is mostly used in the food industry for carob bean gum and locust bean gum, which are polysaccharides in the seeds (Battle & Tous, 1997). Nevertheless, carob pod consists mainly of pulp (90%), which is especially rich in sugars (48% - 56%) and in gross energy (Petit & Pinilla, 1995), making it a high-energy feed for animal nutrition. Owing to its high content in sugars, carob pulp is very palatable. Its inclusion increases palatability (Cheeke, 1987) and reduces feeding costs in lamb fattening diets (Guessous *et al*., 1989; Lanza *et al*., 2001; Obeidat *et al*., 2011), but animal response is variable. In a previous experiment, average daily gain of fattening lambs was improved when carob pulp was included up to a dietary level of 30% as a substitute for citrus pulp (Guessous *et al*., 1988). Performance of growing calves was also improved by replacing barley with carob pulp at a dietary level of 30% (Louca & Papas (1973). In contrast, deleterious effects of a diet containing carob pulp were recorded on lamb growth and meat quality (Priolo *et al*., 2000). Bugarski *et al*. (1971) suggested that the incorporation of carob pulp should not exceed 10% of the diet, while Ouchkif (1988) recorded the best performance in growing lambs at an inclusion of 20%. One of the main problems with the use of carob pulp in animal feeding is the presence of condensed tannins (CT). To reduce the impact of CT, Priolo *et al*. (2000)

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supplemented a diet based on carob pulp (56% as fed) with 40 g of polyethylene glycol (PEG) per kg of feed. The authors found significant improvements of lamb growth and carcass and meat quality, compared with the animals fed the same diet without the addition of PEG.

Carob pulp could also be used as a supplement for rabbits. However, there are no specific studies on the optimal level of its inclusion in a practical diet. In rabbits, the caecum and proximal colon are important sites of digestion. Caecal microorganisms ferment nutrients to short-chain fatty acids (SCFA), ammonia and gases (Gidenne, 1992). *In vitro* gas production is used as an indicator for assessing the potential nutritive value of feedstuff for ruminants (Menke & Steingass, 1988). This technique could also be used to characterize feed fermentation in the large intestine of non-ruminant animals (Bindelle *et al*., 2007).

The aim of this investigation was to study *in vitro* fermentation of diets containing different levels of carob pulp, using the caecal content of rabbits to evaluate its possible incorporation as an energy supplement in rabbit feed formulation.

Materials and Methods

Four diets were formulated to contain an increasing proportion of carob pulp: 0%, 10%, 20% and 100% DM for diets, D1, D2, D3 and D4, respectively, by replacing a decreasing proportion (100%, 20%, 10% and 0%) of a typical commercial rabbit concentrate. Weighed feed ingredients were mixed with water to achieve a 25% - 30% moisture content, pelleted with an electric meat mincer, and oven dried at 40 ºC for two days.

In vitro gas production was determined according to the Menke & Steingass (1988) technique. Two hundred mg of sample were anaerobically incubated for 72 hours in triplicate in 100 mL glass syringes containing 30 mL of inoculum. The inoculum was prepared according to the modified method of Calabro *et al.* (1999). Fresh samples of caecal content were collected from eight 78-day-old New Zealand White rabbits randomly chosen prior to slaughtering. The rabbits were fed a commercial compound feed *ad libitum* from weaning at 35 days of age. The feed was withdrawn 12 hours before sampling, but water was still available *ad libitum*. After slaughtering, the caecum was isolated by tying off the two extremities with nylon string to prevent movement of digesta. Caecum content was diluted (1:50 w/v) with the buffer solution prepared according to Menke & Steingass (1988), and then strained through four layers of cheesecloth and held under CO_2 in a water-bath at 39 ºC. Syringes were pre-warmed (40 ºC) before the injection of 30 mL of caecal liquid per buffer mixture into each syringe, followed by incubation in a ventilated oven (39 ± 1 ºC). Thirty min after the start of incubation the syringes were shaken gently and then every hour for the first 10 h of incubation. Three control syringes containing only caecal liquid and medium (buffer) were used as blanks, so that the gas produced by caecal contents without substrate could be subtracted from the total gas production. The production of gas resulting from microbial fermentation was measured manually at the following intervals: 0, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours.

At the end of fermentation, the pH of the fermented solution in each syringe was recorded using a pH meter, and syringes were removed from the oven and put in a refrigerator at 4 ºC. The solid and liquid matter in each syringe were separated by filtration using a vacuum pump. The dry matter (DM) degraded, was measured after drying at 100 ºC until a constant weight was obtained. The organic matter (OM) degraded, was measured after ashing (6 h/500 ºC). Incubations were repeated three times.

Samples were analysed for DM, OM, nitrogen (N) and crude fibre (CF) according to AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the procedure of Van Soest *et al.* (1991). Hemicellulose (HC) and neutral detergent soluble (NDS) were calculated according to the equations:

HC% = NDF% - ADF% and NDS% = 100 % - NDF%; respectively.

Results were analysed (ANOVA) using the generalized linear model procedure (SAS, 1988) with the diets as the treatment factor based on the following statistical model:

$Yij = \mu + Ti + eij$,

where *Yij* is a dependent variable (gas production, pH and OMD), μ is the population mean, Ti is a diet and eij is the residual error. The means were compared by Scott-Knott test at a probability level of 5%.

Results and Discussion

Table 1 shows the chemical composition of the experimental diets, which were previously used for an *in vivo* trial (Gasmi-Boubaker *et al.*, 2007). The low CP content (64 g/kg DM) of carob pulp is comparable with values reported in the literature (Albanell *et al*., 1991; Petit & Pinilla, 1995; Williams *et al*., 2005). The ADF content of carob pulp (263 g/kg DM) was higher at than the 170 g/kg and lower than the 290 g/kg, the range recommended by Gidenne (2003) to prevent digestive troubles in the growing rabbit. This author observed that the sanitary risk (SR = mortality + morbidity) increased from 18% to 28% when the dietary ADF content decreased from 19% to 15%.

An examination of carob pulp showed that NDF and ADF values are very close, resulting in a low HC content (50 g/kg DM), when the HC fraction is calculated as the difference between NDF and ADF values. In this study, the analytical method to measure ADF concentration might have caused some kind of polymerization of sugars with the fibre fraction, resulting in an artificial increase in apparent ADF content, and thus underestimating the HC value. Although this method is not biochemically valid for estimating hemicellulose, this is the only available approach for a routine laboratory analysis of feed ingredients. This problem is demonstrated in a study by Milad *et al*. (2010) on kibbled carob pods. They, surprisingly, reported that the content of ADF was greater than that of NDF, and that the HC content was negative.

There has been recent interest in the role of soluble fibre in rabbit feeding owing to alleged positive effects on digestive health of rabbits. As can be seen in Table 1, the NDS proportion was higher in carob pulp (687 g/kg DM) than in treatments D1 (548 g/kg DM), D2 (563 g/kg DM) and D3 (578 g/kg DM). The utilization of this by-product as a nutrient source for rabbit could therefore have a positive effect on intestinal microbiota. It was reported that the most important properties of soluble fibre are its higher fermentability and its capacity to increase viscosity in the digestive tract (Rodriguez-Romero *et al*., 2011).

Table 1 Diet formulation and chemical composition of diets incorporating 0%, (D1), 10% (D2), 20% (D3) and 100 % (D4) carob pulp

	Diets			
	D1	D2	D3	D4
Ingredients (%)				
Commercial concentrate	100	90	80	0
Carob pulp	0	10	20	100
Total	100	100	100	100
Chemical composition (g/kg DM)				
Dry matter	882	882	891	917
Organic matter	907	909	912	957
Crude protein (N × 6.25)	169	164	159	64
Crude fibre	210	202	198	135
Neutral-detergent fibre	451	437	424	313
Acid-detergent fibre	218	223	227	263
Hemicellulose	233	214	197	50
Neutral detergent soluble	548	563	578	687

Table 2 shows means of pH, total gas production and OM degraded for the experimental diets after 72 h of fermentation *in vitro*. Overall, gas production ranged from 123 to 179 mL/g DM and did not differ (P >0.05) between D1, D2 and D3. In accordance with published results, it could be suggested that the highest amount and the majority of gas produced from carob pulp (D4) derived from sugars (glucose and saccharose), which are important components of carob pulp. Thomson (1971) investigated 40 cultivars of carob pods and observed high levels of sugars, ranging from 37% to 62%. These soluble carbohydrates are readily degraded by gut microorganisms. In rabbits, caecal micro-organisms ferment available nutrients,

mainly polysaccharides, to short-chain fatty acids, ammonia and gases. The amount of gas produced in *in vitro* fermentation is directly related to the amount of SCFA production (Getachew *et al.*, 1998). Consequently, results presented in Table 2 suggest that large amounts of SCFA will be produced with consequent reduction of caecal pH when carob pulp is fermented.

Table 2 Mean (± SE) pH, gas production (mL/g DM) and organic matter (OM) degraded (expressed as %) of diets incorporating different levels of carob pulp after 72 h of fermentation *in vitro*

Diets	pH	Gas (mL/g DM)	OM degraded (%)
D1 (0% carob pulp)	6.65^{a}± 0.08	123^{b}± 22	58.58^{b} ± 3.70
D2 (10% carob pulp)	6.66^{a} ± 0.03	126^{b}± 12	60.39^{b}± 4.43
D3 (20% carob pulp)	6.64^{a} ± 0.04	130^{b}± 26	62.01^{b}± 4.17
D4 (100% carob pulp)	6.47^{b} ± 0.07	179^{a} ± 24	64.27^{a} ±3.46
Probability.	<0.001	0.007	0.043

[a,b,c] Column means with different superscripts differ significantly at P <0.05.

The variation in *in vitro* fermentation may be related to differences in chemical composition of the diets. Abreu & Bruno-Soares (1998) found a positive correlation between gas production and the OMD (r = 0.69; P <0.05). Of all diets, carob pulp (D4) had a higher (P <0.05) *in vitro* OM matter degradation (64.3%) than the D1 (58.6%) treatment, while that in D2 (60.3%) and D3 (62%) were similar (P >0.05) to D1. The OMD of carob pulp is in accordance with the *in sacco* degradation of DM (65.4%) (Albanell *et al.*, 1991). An *in vivo* trial (Gasmi-Boubaker *et al.*, 2007) showed that the addition of carob pulp to the diet consumed by rabbits tended to improve DM and OM digestibility with increasing the level of carob pulp in the diet.

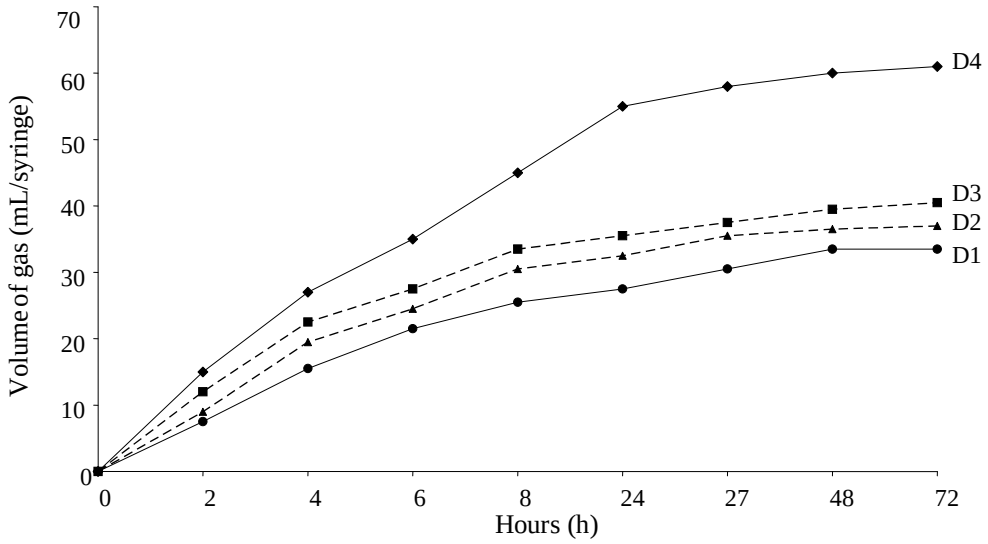

Figure 1 Gas production profile of diets incorporating 0% (D1), 10% (D2), 20% (D3) and 100% (D4) carob pulp and incubated with inoculum from rabbit caecum.

On the basis of both gas production and OMD, the feed classification is D1< D2<D3<D4. As stated by Bindelle *et al.* (2007), the ranking of *in vitro* fermentative characteristics of substrates would probably

remain the same as in *in vivo*, even if the diversity of various conditions cannot be fully reproduced by *in vitro* methods. However, under practical conditions, higher microbial fermentation of carob pulp might imply high energy losses. Moreover, and owing to its low CP content, the administration of carob pulp as a single ingredient would not be recommended, because additional protein supplementation is required.

In vitro gas production can reflect the extent to which substrates are fermented by gut microorganisms (Schofield, 2000). The trend of gas production is well described by Figure 1. It demonstrates the difference in cumulative gas production between D1, D2, D3 and D4. The plateau of the curves was reached at 72 h and the initial phase of fermentation was short and does not differ between the four diets. It is speculated that before fermentation started, the time needed for hydration was short and the microbial colonization was very quick. After 2 hours and during 72 h of incubation, carob pulp (D4) produced the highest volume of gas compared with D1, D2 and D3. It is reasonable to expect such a trend when carob pulp is incubated alone, because this by-product is rich in sugars and NDS. Sugars are positively correlated with the maximum volume of gas produced at t (Piquer *et al*., 2009), and ferment more quickly than starch and much quicker than cellulose and hemicellulose (Sniffen *et al.*, 1992). Diets incorporating of 0%, 10% and 20% carob pulp would have a much lower content of sugars and NDS. Therefore, total potential gas productions would also be lower.

Conclusion

Carob pulp is highly fermented by the caecal micro-organisms of rabbits. Although, its inclusion at 20% did not significantly improve *in vitro* fermentation and degradation of the commercial concentrate, it can be concluded that carob pulp has potential as an unconventional feed resource for rabbits. Its utilization could have a positive effect on intestinal microbiota owing to its high content in soluble fibre.

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