



PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021

ISSN 2308-1910

Session **NUTRITION & FEEDING**

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How to cite this paper

Ribeiro D.M., Martins C., Pinho M., Freire J.P., Falcão-e-Cunha L., 2021. Effect of using carob pulp in growing rabbit diets on performance, digestibility, intestinal morphology and caecal parameters. Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication N-09, 4 pp. + presentation

EFFECT OF USING CAROB PULP IN GROWING RABBIT DIETS ON PERFORMANCE, DIGESTIBILITY, INTESTINAL MORPHOLOGY AND CAECAL PARAMETERS

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ABSTRACT

The objective of this study is to evaluate the effect of including carob pulp in rabbit diets and the consequent growth performance, feed digestibility, intestinal mucosa morphology and caecal fermentative activity. Early weaned (22 days old) rabbits (N=39) were divided among three diets with 0%, 12.5% and 25% carob pulp, all with the similar CP and NDF content. During the 5th week of trial, faeces were collected to determine feed digestibility. At the end, rabbits were slaughtered. Caecal content was harvested and a section from the middle of the small intestine was taken to analyse intestinal morphology. Carob pulp inclusion did not affect growth performance with the exception of feed conversion which deteriorated 12% at 25% of inclusion. The digestibility of all fractions lowered significantly with 25% inclusion of carob pulp with the exception of ether extract which was unaffected. Dry matter digestibility lowered from 67.0% to 62.2%; organic matter and energy digestibility decreased between 7.5 and 8.9%, whereas crude protein digestibility decreased 21.1% (83.3% vs 65.6%). NDF digestibility was around 24% lower at higher level of inclusion. Neither intestinal morphology nor parameters of caecal fermentation, caecal pH and molar proportions of volatile fatty acids were affected by the inclusion of either 12.5% or 25% carob pulp in the diets of fattening rabbits.

Keywords: carob pulp, dig

estibility, mucosa morphology, caecal fermentative parameters

INTRODUCTION

The carob tree (*Ceratonia siliqua L.*) grows in the Mediterranean area. Its fruit is traditionally used as animal fodder after seed extraction. Carob pulp, which represents 90% of the fruit, has a high content of soluble sugars (45-52%), 2-7% of crude protein (CP) and a low content of fat, under 1% (Nasar-Abaas *et al.*, 2016). In addition, it has a high content of condensed tannins, which may interfere with the digestive process.

The objective of this study was to feed early-weaned rabbits with carob pulp and study its effect on growth performance, feed digestibility, intestinal morphology and caecal fermentation.

METHODS

Animals and experimental design

Three diets were formulated with similar CP and neutral detergent fibre (NDF) contents and with 0, 12.5% and 25% carob pulp, partially or fully replacing oat (Table 1). These diets were provided *ad libitum* for 7 weeks for each group containing 13 rabbits balanced for live weight, weaned at 22 days old. Live weights were registered each week and feed intake was measured 3 times per week. During the fifth week, faeces were collected to analyse apparent digestibility. At the end of the experimental period, rabbits were slaughtered and caecal content samples were taken. The pH and dry matter of the caecal contents were determined. Approximately 5-10 g of fresh caecal contents were

stored in a bottle with 4 mL of a 0.03 M H₃PO₄ solution for later determination of volatile fatty acids (VFA). One section was taken from the middle of the small intestine for histological analysis of the mucosa. Tissue samples were fixed in 10% neutral buffered formalin and processed for paraffin embedding for microscopical examination of intestinal villi and crypts.

Table 1: Ingredients and chemical composition (% as fed basis) of the experimental diets

	Carob pulp' level				Carob pulp level		
	0%	12.5%	25%		0%	12.5%	25%
Ingredients (%):				Chemical composition (%)			
Wheat	16	14.5	13	Dry matter	91.3	91.0	90.3
Carob pulp ¹	0	12.5	25	Organic matter	84.8	84.5	83.6
Oat	30	15	0	Crude protein	16.1	15.2	15.0
Soybean meal,	11.5	12.5	13.5	Ether extract	7.6	7.0	6.3
Sunflower meal 28%	10	11	12	NDF	32.3	33.0	33.4
Dehydrated alfalfa meal	20	19	18	ADF	17.1	20.7	22.7
Wheat straw	6	9	12	ADL	3.4	5.7	7.3
Lard	5	5	5	Hemicellulose	15.2	12.3	10.7
L-lysine	0.02	0.06	0.11	Cellulose	13.8	15.0	15.3
DL-methionine	0.18	0.18	0.20	Gross energy (kcal/kg)	4265	4213	4134
ClNa, CaHPO ₄ , CaCO ₃ , premix ²	1.30	1.26	1.19				

¹ carob pulp: 4.8% CP; 25.5% NDF as feed basis

²Premix provided per kg of complete diet: vitamin A, 1000 UI; vitamin D3, 1500 UI; vitamin E, 15 mg; vitamin K3, 1.5 mg; vitamin B1, 1 mg; vitamin B2, 2 mg; vitamin B6, 1.5 mg; vitamin B12, 0.01 mg; pantothenic acid, 8 mg; nicotinic acid, 25 mg; biotin, 0.02 mg; betain, 136.5 mg; robenidine, 50 mg; Co, 0.7 mg; Cu, 5 mg; Fe, 30 mg; I, 1 mg; Mn, 15 mg; Se, 0.2 mg; Zn, 30 mg; ethoxyquin 12.5 mg; butylated hydroxytoluene 12.5 mg.

Chemical Analyses

Dry matter (DM), organic matter (OM), CP, ether extract (EE), NDF, acid detergent fibre (ADF), acid detergent lignin (ADL) and gross energy of feed and faeces were determined according to the procedures proposed by EGRAN (2001). The pH values of caecal contents were measured with a glass electrode pH meter. VFA levels in caecal contents were measured by gas chromatography.

Histological Analysis

Microscopic examination and measurement of villi and crypt were performed in 7 µm thick tissue sections, stained with haematoxylin-eosin from 10 rabbits from each treatment. An Olympus BX 511 microscope equipped with 4× and 10× lenses was used. Images were digitally captured with an Olympus DP 11 camera and the Olympus DP Soft software was used to measure the height and width of the villi and the depth of the crypts. Ten intact and correctly oriented villi and crypts were selected per animal.

Statistical Analysis

Data concerning growing performance, apparent faecal digestibility, intestinal morphology and fermentative activity parameters were subject to analysis of variance of one factor (the diet) by using the SAS System software (SAS, 1991).

RESULTS AND DISCUSSION

Albeit this study was not a zootechnical trial, growth performance was analyzed. Dietary inclusion of carob pulp did not significantly affect daily feed intake or average daily gain. Only feed conversion was significantly higher (worse) in the diets with 25% carob pulp inclusion (Table 2). This resulted from the negative effect of carob pulp over the digestibility of nearly all analytic fractions considered, with the exception of EE digestibility which remained relatively constant around 88% in all diets.

Regarding DM, OM and energy digestibility, these decreased from 7.2 to 8.9% at a 25% inclusion level of carob (Table 3). Reduced NDF digestibility higher than 20% with carob inclusion occurs due to the increased content of ADL and the total and partial replacement of oat with carob pulp, which

has different contents of hemicellulose. Contrarily, cellulose has similar digestibility across all diets. Crude protein digestibility decreased more than 20% at 25% carob inclusion level compared to control. The presence of condensed tannins could be responsible for this difference, since they can bind to endogenous proteins, mainly digestive proteases, as well to dietary protein during the digestive process (Velickovic and Stanic-Vucinic, 2018).

Table 2: Effect of inclusion of carob pulp in diet on performances of growing rabbits

	Carob pulp' level			RMSE ²	P value
	0%	12.5%	25%		
Rabbits, no.	13	13	13		
Live weight 22 d (g)	368	363	366	35.00	0.949
Live weight 70 d (g)	2568	2615	2514	280.1	0.657
Feed intake (g/d)	111.1	116.2	120.0	15.86	0.385
Average daily gain (g/d)	46.3	46.9	44.8	5.46	0.600
Feed conversion	2.39 ^a	2.47 ^a	2.68 ^b	0.12	<0.001

¹Means with different letters on the same row differ significantly at P<0.05; ²RMSE: root mean square error.

Hafsa *et al.* (2017) have reported that an inclusion of 10% carob pulp in rabbit diets significantly lowered all animal performance indicators and lowered the digestibility of DM and CP at around 7%. However, with 5% inclusion the results were improved by comparison with 10% inclusion. Decreased digestibility in parallel with increasing carob pulp inclusion has also been reported in other species, such as pigs (Kotrotsios *et al.*, 2010).

Nonetheless, phenolic compounds in carob had no effect over non-nitrogenous cellular content (NNCC= OM-CP-NDF) since their digestive availability, albeit statistically significant, is total in carob diets.

Table 3: Effect of inclusion of carob kibbles in diet on total tract apparent digestibility

Coefficients of total tract apparent digestibility ¹	Carob pulp' level			RMSE ³	P value
	0%	12.5%	25%		
Dry matter	67.0 ^a	65.1 ^a	62.2 ^b	2.50	<0.001
Organic matter	67.6 ^a	65.4 ^b	62.5 ^c	2.46	<0.001
Crude protein	83.3 ^a	75.7 ^b	65.7 ^c	2.66	<0.001
Ether extract	87.5	89.5	88.3	2.53	0.158
NDF	29.9 ^a	26.5 ^a	22.6 ^b	5.18	0.004
ADF	22.4	22.9	19.6	5.47	0.250
ADL	12.7 ^a	9.9 ^a	-8.3 ^b	9.14	0.001
Hemicellulose (NDF-ADF)	38.3 ^a	32.5 ^b	29.1 ^b	5.59	<0.001
Cellulose (ADF-ADL)	24.8	28.0	29.4	5.47	0.110
NNCC ²	96.2 ^a	98.2 ^b	101.0 ^c	0.90	<0.001
Energy	68.2 ^a	65.8 ^b	62.1 ^c	2.51	<0.001

¹Means with different letters on the same row differ significantly at P<0.05;

²NNCC= non-nitrogenous cellular content;

³RMSE: root mean square error

No statistically significant differences were obtained regarding intestinal morphology (Table 4), which indicates no negative effect of carob over digestion and absorption of these diets. Similar results have been obtained in other studies, although the nature and concentration of the dietary tannins might contribute to the low statistical significance of the observed effects. This is the case for hydrolysable tannins in finishing pig diets (Bilić-Šobota *et al.*, 2016) or tannin supplements in sheep (Zhao *et al.*, 2019).

Likewise, the fermentative activity measured by pH and proportion of VFA in the caecal content of rabbits was also unaffected by the dietary inclusion of carob pulp (Table 5). Neither phenolic compounds nor carob fibre seem to influence microbial action during digestion.

Table 4: Effect of inclusion of carob pulp in diet on intestinal morphology

	Carob pulp' level			RSME ¹	P value
	0%	12.5%	25%		
Rabbits, no.	10	10	10		
Villus height (µm)	797	807	875	134.91	0.3143
Villus width (µm)	77.5	81.3	79.7	11.58	0.7577
Crypt depth (µm)	98.6	108.5	117.3	29.23	0.3101
Villus height/crypt depth	8.67	7.99	8.38	2.72	0.8485

¹RMSE: root mean square error

Table 5: Effect of inclusion of carob pulp in diet on caecal pH and on molar proportions of VFA

	Carob pulp' level			RMSE ¹	P value
	0%	12.5%	25%		
pH	6.07	6.03	6.05	0.23	0.902
C2 (mol/100 mol)	78.5	78.7	79.7	2.10	0.401
C3 (mol/100 mol)	7.1	6.8	6.7	1.25	0.718
C4 (mol/100 mol)	14.3	14.4	13.8	2.43	0.791

¹RMSE: root mean square error

CONCLUSIONS

In our experimental conditions, it was possible to include carob pulp in rabbit feed up to 12.5% without negatively affecting growth performance, albeit there is a decrease of protein digestibility. Dietary carob pulp had no effect over intestinal morphology or caecal fermentation parameters.

ACKNOWLEDGEMENTS

Authors acknowledge funding from FCT - Fundação para a Ciência e a Tecnologia (Lisbon, Portugal) in the form of Research Center Programs LEAF (Linking Landscape, Environment, Agriculture and Food: authors JF, DR, CM and LFC), CIISA (Interdisciplinary Animal Health Research Center: author MP) and the PhD grant SFRH/BD/143992/2019 awarded to DR.

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EFFECT OF USING CAROB KIBBLES IN GROWING RABBIT DIETS ON PERFORMANCE, DIGESTIBILITY, INTESTINAL MORPHOLOGY AND CAECAL PARAMETERS



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BACKGROUND

- The carob tree (*Ceratonia siliqua* L.), widely distributed in the Mediterranean region, is cultivated for its **edible pods**.
- Carob kibbles¹ represent 90% of the pod, with high content of **soluble sugars** and **fibre**. They have **condensed tannins** that have an anti-nutritional effect for monogastric animals.
- The objective** of this study is to evaluate the dietary incorporation of carob kibbles in weaned rabbit diets and its effect on performance, feed digestibility, intestinal morphology and caecal fermentation.

¹ Chemical composition	Proportion (%)
Total sugars	45 to 52
Crude fiber	Up to 40
Protein	2 to 7
Ash	2 to 3
Fat	0.5 to 1
Total polyphenols	1.4 to 2.0

MATERIALS AND METHODS

A total of 39 rabbits were divided into three experimental groups (n=13)

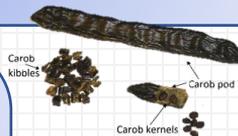
0, 12.5% and 25% carob kibble, partially or fully replacing oat²

Carob kibbles: 4.8% CP; 25.5 NDF % as feed basis

Rabbits were fed ad libitum for 7 weeks, faecal collection on the 5th week

Rabbits were slaughtered and samples were taken from intestinal tissue and caecum contents

Feed and faecal chemical characterization: Dry matter (DM), organic matter (OM), CP, ether extract (EE), NDF, acid detergent fibre (ADF), acid detergent lignin (ADL) and gross energy. Caecum pH and volatile fatty acid content were determined. Small intestinal histological measurements were taken



Source: doi.org/10.1016/j.jfloodeng.2015.06.011

Source: doi.org/10.1111/1541-4337.12177

Carob inclusion ²	0%	12.5%	25%
Chemical composition (%)			
Dry matter	91.3	91.0	90.3
Organic matter	84.8	84.5	83.6
Crude protein	16.1	15.2	15.0
Ether extract	7.6	7.0	6.3
NDF	32.3	33.0	33.4
ADF	17.1	20.7	22.7
ADL	3.4	5.7	7.3
Hemicellulose	15.2	12.3	10.7
Cellulose	13.8	15.0	15.3
Gross energy (kcal/kg)	4265	4213	4134

RESULTS AND DISCUSSION

GROWTH PERFORMANCE

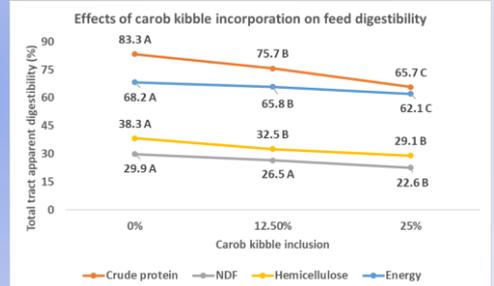
- Rabbit **growth was not affected** by carob kibble inclusion. Feed conversion ratio was higher (p<0.05) in the group fed with 25% inclusion.

FEED DIGESTIBILITY:

- DM, OM, CP, NDF, ADL, hemicellulose and energy** digestibility significantly decreased with dietary carob kibble.
- Replacement of oat with **carob decreased NDF digestibility** due to different hemicellulose levels of these feedstuffs.
- Dietary **tannins** inhibited digestive enzyme access to CP, **reducing its digestibility**.

INTESTINAL MORPHOLOGY

- No effects on intestinal morphology or caecum content properties.



CONCLUSIONS

Carob inclusion up to 12.5% has no detrimental effect on the performance of rabbits, albeit lowering TTAD of some feed fractions. Tannin content needs to be considered when using carob kibbles in rabbit diets.