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 H_3PO_4 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
[2]Premix 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

[1]Means with different letters on the same row differ significantly at $P<0.05$; [2]RMSE: 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

[1]Means with different letters on the same row differ significantly at $P<0.05$;
[2]NNCC= non-nitrogenous celular content;
[3]RMSE: 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 statistically 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

[1]RMSE: 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

[1]RMSE: 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.

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REFERENCES

Bilić-Šobota D., Valentina Kubaleb V., Škrlepc M., Čandek-Potokara M., Prevolnik Povše M., Fazarincb G., Škorjanca D., 2016. Effect of hydrolysable tannins on intestinal morphology, proliferation and apoptosis in entire male pigs. *Archives of Animal Nutrition, 70, 5, 378–388.*

EGRAN, 2001. Technical note: attempts to harmonize chemical analyses of feeds and faeces, for rabbit feed evaluation *World Rabbit Science, 9, 2, 57-64.*

Hafsa S.H.A, Ibrahim S.A., Hassan A.A. 2017. Carob pods (Ceratonia siliqua L.) improve growth performance, antioxidant status and caecal characteristics in growing rabbits. *Journal of Animal Physiology and Animal Nutrition, 101, 1307–1315.*

Kotrotsios N, Christaki I.E., Bonos E., Florou-Paneri P. 2010. Dietary carob pods on growth performance and meat quality of fattening pigs. *Asian-Australasian Journal of Animal Sciences, 25, 6, 880–885.*

Nasar-Abbas S.M., Zill-e-Huma, Vu T. H., Khan M.K., Esbenshade H., Jayasena V. 2016. Carob Kibble: A Bioactive-Rich Food Ingredient. *Comprehensive Reviews in Food Science and Food Safety, 15, 63-72.*

SAS. 1991. SAS System for Linear Models 3rd ed. *SAS institute Inc., Cary, NC.*

Velickovic T.D.C, Stanic-Vucinic J. 2018 The role of dietary phenolic compounds in protein digestion and processing technologies to improve their antinutritive properties *Comprehensive Reviews in Food Science and Food Safety, 17, 82-103.*

Zhao M.D., Di L.F., Tang Z.Y., Jiang W., Li C Y. 2019. Effect of tannins and cellulase on growth performance, nutrients digestibility, blood profiles, intestinal morphology and carcass characteristics in Hu sheep *Asian-Australasian Journal of Animal Sciences, 32(10), 1540-1547.*