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Effect of carob seed (*Ceratonia siliqua* L.) in broiler chick diets on nutrient digestibility and intestinal viscosity

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Summary

Inclusion of carob seed at levels of 60 (CS-60 diet) and 90 g/kg (CS-90 diet) in a reference diet caused a marked (P<0.01) depression in the utilization of nutrients. The reduction in the apparent digestibility of crude protein and crude fat was 12.7 and 20.9% for CS-60 diet, and 29.5 and 23.8% for CS-90 diet, respectively. The apparent metabolizable energy value followed a similar depressing trend than that observed for crude fat digestibility. Viscosity of the jejunal digesta was drastically increased by the presence of carob seed in the diet, and it might explain most of the results obtained in this study.

Keywords: carob seed, digestibility, intestinal viscosity, chicks

Resumen

La inclusión de garrofín a niveles de 60 (ración CS-60) y 90 g/kg (ración CS-90) en una ración referencia produjo una marcada (P<0.01) disminución de la utilización de los nutrientes. La reducción en la digestibilidad aparente de la proteína bruta y de la grasa bruta fue del 12.7 y 20.9% en la ración CS-60, y del 29.5 y 23.8% en la ración CS-90, respectivamente. La energía metabolizable aparente disminuyó siguiendo una tendencia similar a la observada en la digestibilidad de la grasa bruta. La viscosidad de la digesta en el yeyuno se incrementó acusadamente por la presencia de garrofín en la ración, lo que podría explicar la mayoría de los resultados obtenidos en este estudio.

Palabras clave: garrofín, digestibilidad, viscosidad intestinal, pollos

Introduction

Carob bean is the fruit of *Ceratonia siliqua* L., a perennial legume tree native to the Mediterranean basin and southwestern Asia. The seeds contain a very high level of gum (42-46% in weight; Saura-Calixto, 1987), which is basically constituted of a neutral galactomannan polymer.

Numerous studies have shown that soluble polysaccharides, such as β-glucans and arabinoxylans, can depress the digestion and absorption of nutrients in monogastric animals (Campbell & Bedford, 1992; Choct *et al.*, 1996). On the other hand, it is known that certain

types of soluble fibre can have beneficial effects on human and animal health, such as to reduce the risk of developing coronary heart disease by lowering blood pressure and serum cholesterol level (Williams *et al.*, 1995; Beagger et al., 1996).

The aim of the current study was to evaluate the effect of dietary inclusion of carob seed on the apparent digestibility of protein and fat, apparent metabolizable energy (AME) content and intestinal viscosity in broiler chicks.

Material and methods

The carob seeds used in this study were obtained from a commercial supplier in Spain (Valencia). The seeds were ground and analyzed by the methods described below.

Five-day-old male broiler chicks (Cobb) were randomly distributed into three experimental groups of 10 chicks each. They were housed in wire metal cages, two birds per cage, and fed during 16 d (5 to 21 d of age) on a reference diet (RD) or one of two test diets containing carob seed at 60 (CS-60) and 90 (CS-90) g/kg (Table 1). Titanium dioxide was added to the diets as an indigestible marker. All diets were formulated to meet at least the National Research Council (1994) requirements for broiler chicks.

Excreta samples were collected from each cage During the last three days of the experiment and used to estimate crude fat digestibility. On day 22, all birds were slaughtered and the jejunum and ileum from each bird bisected out, the contents being flushed into plastic containers. Jejunal contents were used for viscosity measurement, and ileal contents for pH measurement and dry matter, nitrogen and titanium analyses to estimate crude protein digestibility. Analyses were performed on pooled samples from the two birds in the same cage.

Table 1. Composition of experimental diets (g/kg, as fed basis).

	Diets		
	RD	CS-60	CS-90
Ingredients			
Maize	479	397	356
Soyabean meal	410	404	400
Sunflower oil	66	94	109
Carob seed	0	60	90
Methionine	2	2	2
Titanium dioxide	5	5	5
Basal mixture[1]	38	38	38

[1]Basal mixture provided the following as g/kg: sodium chloride, 3.0; calcium carbonate, 10.0; dicalcium phosphate, 19.0; BHT, 1.0; vitamin and mineral premix, 5.0. The composition of the vitamin and mineral premix has been described (Alzueta *et al.*, 2003).

Dry matter, crude protein, soluble and insoluble dietary fibre and ash were analyzed by standard procedures (AOAC, 1995). Crude fat was determined using petroleum ether following acidification of the samples with 4N HCl (Wiseman *et al.*, 1992). Neutral and acid detergent fibre and lignin analyses were performed as described by Robertson & Van Soest (1981). Gross energy was measured with an adiabatic bomb calorimeter. Titanium was determined colorimetrically according to Short *et al.*, (1996). Total phenolic and condensed tannin contents were determined by the Folin-Denis (Burns, 1963) and vanillin-hydrochloric acid (Broadhurst & Jones, 1978) procedures, respectively. Viscosity of jejunal digesta and pH of ileal digesta were measured with a digital viscometer and micro pH-electrode, respectively.

Results were subjected to analysis of variance using the Statgraphics software package (version 5.0, Statistical Graphics Corp., Rockville, MD, USA).

Results and discussion

The carob seed composition is shown in Table 2. The seeds contained 681 g/kg DM of dietary fibre, approximately the half of which being soluble fibre.

Table 2. Analyzed composition of carob seed (g/kg DM).

Crude protein	183.0	Neutral detergent fibre	300.1
Crude fat	18.8	Acid detergent fibre	123.3
Ash	45.1	Lignin	9.8
Total phenolics[1]	32.1	Soluble fibre	346.8
Condensed tannins[2]	8.0	Insoluble fibre	334.4

[1]Expressed as tannic acid equivalents (g/kg DM). [2]Expressed as catechin equivalents (g/kg DM).

The results of this experiment (Table 3) show that the inclusion of carob seed in the diet had a depressing effect ($P<0.01$) on the digestibion of nutrients, this effect being more marked as the inclusion rate increased from 60 to 90 g/kg of the ration.

Table 3. Effect of dietary inclusion of carob seed on the apparent digestibility of crude protein and crude fat, AME content (MJ/kg DM), jejunal digesta viscosity (cP) and pH of ileal digesta measured in 22-d-old broiler chicks.

Inclusion (g/kg)	0	60	90	Pooled SEM	ANOVA *P*-value
Crude protein	0.800[a]	0.698[b]	0.564[c]	0.0259	<0.01
Crude fat	0.870[a]	0.688[b]	0.663[b]	0.0113	<0.01
AME	14.22[a]	11.49[b]	11.41[b]	0.111	<0.01
Viscosity	1.2[a]	115.2[b]	nm	7.00	<0.01
pH	6.8[a]	6.4[ab]	5.7[b]	0.27	<0.05

nm = not measurable
[a,b,c] Mean values within a row with no common superscript differ significantly.

The apparent digestibility coefficients for crude protein of CS-60 and CS-90 diets were reduced by 12.7 and 29.5%, respectively, in comparison to the reference diet. For crude fat digestibility, the corresponding digestibility coefficients for these diets decreased by 20.9 and 23.8%, respectively. Carob seed in the diet also had a negative effect on the AME content, the values showing a similar decreasing trend to that observed for crude fat digestibility. These findings agree with the work reported by Vohra & Kratzer (1964), who found that the growth of chickens was inhibited about 26% by the dietary inclusion of carob gum at the level of 20 g/kg. Harmuth-Hoene & Schwerdtfeger (1979) also observed a marked reduction of protein digestibility when rats were fed on a diet with 100 g/kg carob gum.

Feeding the carob seed containing diets to chicks increased drastically digesta viscosity and caused sticky droppings. In chicks fed on CS-90 diet, the jejunal content was gel-like and viscosity could not be measured because no supernant was obtained after centrifugation. Viscosity is considered to be the mechanism by which soluble fibre components can depress nutrient digestion by limiting the diffusion of digestive enzymes and nutrients and by

stimulating the microbial activity in the small intestine resulting in a greater competition with the host animal for nutrients (Bedford, 1995; Choct *et al*., 1996). Taking into account this hypothesis, it is conceivable that the lower ileal pH found in the carob seed-fed chicks than in the chicks consuming the reference diet might indicate a microbial degradation of carob gum, yielding lactic and short chain fatty acids, since this neutral galactomannan polymer is highly susceptible to fermentative breakdown (Bravo, 1999). In addition to the effect of intestinal viscosity, the presence of tannins in seed carob was probably another factor that contributed to the depression of nutrient digestion, particularly that of protein. Regarding this, it is well known that tannins, specially condensed tannins, can inhibit the activity of some digestive enzymes and form tannin-protein complexes resistant to enzymic hydrolysis (Marquardt, 1989). This might be the reason for what the decreasing values found for protein digestibility were not in line with those observed for fat digestibility and AME content.

In conclusion, results from this study showed that inclusion of carob seed impaired the nutritive value of the diet and it was associated with a drastic increase in the intestinal viscosity. The reduced ileal pH found might be a reflection of microbial activity in the small intestine, an aspect that would require further research employing microbial methods.

References

1. Alzueta, C., Rodríguez, M.L., Cutuli, M.T., Rebolé, A., Ortiz, L.T., Centeno, C. & Treviño, J., 2003. Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilisation and intestinal microflora. *British Poultry Science*, 44: 67-74.
2. Association of Official Analytical Chemists International, 1995. *Official Methods of Analysis*, (Arlington), VA, AOAC.
3. Beagger, M., Andersen, O., Nielsen, J.D., Rytting, K.L., 1996. Dietary fibres reduce blood pressure, serum total cholesterol and platelet aggregation in rats. *British Journal of Nutrition*, 75: 483-493.
4. Bedford, M.R., 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology*, 53: 145-155.
5. Bravo, L., 1999. Propiedades y aplicaciones de la fibra de la algarroba (*Prosopis pallida* L.). *Alimentaria*, marzo: 67-73.
6. Broadhurst, R.B. & Jones, W.T., 1978. Analysis of condensed tannins using acidified vainillin. *Journal of the Science of Food and Agriculture*, 29: 788-794.
7. Burns, R.E., 1963. Methods of tannin analysis for forage crop evaluation. Georgia Agricultural Experiment Station. Technical Bulletin no. 32 (Athens, University of Georgia College of Agriculture).
8. Campbell, G.L.& Bedford, M.R., 1992. Enzymes applications for monogastric feeds: a review. *Canadian Journal of Animal Science*, 72: 449-456.
9. Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J.& Annison, G., 1996. Increased small intestinal fermentation is partly responsible for the antinutritive activity of non-starch polysaccharides in chickens. *British Poultry Science*, 37: 609-621.
10. Harmuth-Hoene, A. & Schwerdtfeger, E., 1979. Effect of indigestible polysaccharides on protein digestible and nitrogen retention in growing rats. *Nutrition and Metabolism*, 23: 399-407.
11. Marquardt, R.R., 1989. Dietary effects of tannins, vicine and convicine. In: Recent Advances of Research in Antinutritional Factors in Legume Seeds (J. Wiseman, T.F.B. van der Poel and I.E. Liener, editors). PUDOC Wageningen, The Netherlands, pp. 141-155.

12. National Research Council, 1994. Nutrient Requirements of Poultry, 9th ed. National Academic Press, Washington, DC.
13. Robertson, J.B. & Van Soest, P.J., 1981. The detergent system of analysis and its application to human food, in: James, W.P.T. & Theander, O. (Eds). *The Analysis of Dietary Fibre in Foods*, pp. 123-158 (New York, Marcel Dekker).
14. Saura-Calixto, F., 1987. Determinación de la composición química de algarroba (*Ceratonia siliqua*). Azúcares, taninos, pectinas y aminoácidos. *Anales de Bromatología*., XXXIX: 81-93.
15. Short, F.J., Gorton, P., Wiseman, J. & Boorman, K.N., 1996. Determination of titanium dioxide added as inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, 59: 215-221.
16. Vohra, P. & Kratzer, F.M., 1964. Growth inhibitory effect of certain polysaccharides for chickens. *Poultry Science*, 43: 1164-1169.
17. Williams, C.L., Bollella, M., Spack, A.& Puder, D., 1995. Soluble fiber enhances the hypocholesterolemic effect and the step I diet in chilhood. *Journal of the American College of Nutrition*, 14: 251-257.
18. Wiseman, J., Edmunds, B.K & Shepperson, N., 1992. The apparent metabolizable energy of sunflower oil and sunflower acid oil for broiler chickens. *Animal Feed Science and Tecnology*, 36, 41-51.