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Combined effects of supplementation of diets with hops and of a substitution of starch with soluble fiber on feed efficiency and prevention of digestive disorders in rabbits

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ABSTRACT

The aim of this work was to determine the effects of hops supplementation and of substitution of starch with soluble fiber in diets for growing rabbits. These factors were factorially combined in four experimental diets. Twenty four individually caged rabbits (New Zealand × Californian) were allocated at random to the experimental diets to measure growing performance and fattening mortality. In addition, mortality was also measured in 374 collectively caged rabbits per treatment in four independent trials at two experimental facilities. Apparent fecal digestibility of dry matter, organic matter, energy, protein and fiber was determined in 20 growing rabbits 56 days old. Caecotrophy trials were also conducted on a total of 228 animals to determine soft feces excretion and composition and *Clostridium perfringens* enumeration. Substitution of starch with soluble fiber increased fecal digestibility of all the constituents of the fibrous fraction (P<0.001). Moreover, digestibility of soluble fiber was high (0.746 in the high soluble fiber diet), so that a decrease of 51 g/kg of the dietary starch concentration was compensated with an increase in 71 g/kg in the total digestible fiber content and no differences in feed efficiency in individually caged animals between treatments were observed. Inclusion of soluble fiber also led to a decrease of pH (from 5.90 to 5.75, P=0.005) and *Cl. perfringens* counts in soft feces (by 17.4%, P=0.080). Fattening mortality was also reduced by increasing soluble fiber in each of the trials conducted (from 26.3 to 16.7%, P<0.001 as overall of the five trials). Hop supplementation had no influence on soft feces production and composition, but decreased (P<0.05) nutrient digestibility especially that of hemicelluloses (by 14.6%, P<0.001). An interaction between treatments was observed, as this effect was higher in the low soluble fiber diet (by 25.5%, P=0.013). Addition of hop flowers increased soft feces pH (from 5.77 to 5.88, P=0.029), and ammonia *N* concentration only in the case of the high soluble fiber diets (P=0.04). It also improved slightly (by 3.5%, P=0.050) feed efficiency and decreased the overall fattening mortality, but only in the case of the low soluble fiber diet (from 29.2 to 23.4%, P=0.063). In conclusion, the results obtained show promising results both of soluble fiber and hops supplementation of fattening rabbit diets, and deserve further research in order to improve performance and gut health.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; AOAC, Association of Official Analytical Chemists; DM, dry matter; CP, crude protein; DE, digestible energy; ERE, epizootic rabbit enteropathy; HSLSF, high starch low soluble fiber; LSHSF, low starch high soluble fiber; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; SD, standard deviation; SEM, standard error of means; TDF, total dietary fiber.

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1. Introduction

Nutritional digestive disturbances constitute a major concern in commercial rabbit rearing. Feed formulation and the use of phytogenic products are among the main nutritional tools at present in the EU to mitigate this problem (De Blas et al., 2012). A minimal concentration of insoluble fiber is widely considered as the main dietary factor to prevent digestive disorders in fattening rabbits (De Blas et al., 1986; Pérez et al., 1996; Gidenne et al., 2010). This effect might be related to (i) an increase of rate of passage of digesta (García et al., 2002) that implies a shorter fermentation time (Gidenne et al., 2010) and might lead to a lower total caecal bacteria enumeration (Michelland et al., 2011) and microbial nitrogen recycled through caecotrophy (García et al., 2000), and (ii) by a decrease of fermentable substrates available for the fibrolytic flora, which might alter the equilibrium among the microbial species. Moreover, several studies have shown that the additional inclusion of soluble fiber (defined by the sum of fructans, galactans, β-glucans, pectins and resistant starch; Gidenne et al., 2010) might contribute to a further decrease of fattening mortality (Jehl and Gidenne, 1996; Gidenne et al., 2004; Gómez-Conde et al., 2009). This effect was parallel to a decrease in the frequency of detection of several potential harmful bacteria as *Clostridium perfringens* and *Campylobacter* spp at the ileal and caecal contents (Gómez-Conde et al., 2007). According to García et al., 2002, soluble fiber reduced transit time compared to starch but, because of its high fermentatibility, induces significant changes in caecal environment (acidity, volatile fatty acids and ammonia concentrations that might modify caecal flora composition (García et al., 2002). Furthermore, the partial replacement of insoluble with soluble fiber minimizes the deterioration of intestinal villi (Alvarez et al., 2007; Gómez-Conde et al., 2007), which might explain the improvements observed in starch digestibility and immune response (Gómez-Conde et al., 2007). The combined changes of the carbohydrate proportions in rabbit diets have then major consequences on health and performance. However, there is a great variability in the responses observed in previous studies depending on the type of nutrients substituted and the microbism of the experimental farm. Most of the previous research has been done by substituting dietary starch with insoluble fiber or with a mixture of insoluble and soluble fiber.

Hop (*Humulus Lupulus* L.) flowers and extracts are used to impart the bitter taste and aroma by the brewing industry, but they were originally added as a preservative to inhibit the growth of lactic acid bacteria that spoil the beer. The main antimicrobial components in bitter hops (150–250 g/kg dry matter depending on cultivars) are humulone, lupulone (typically called α- and β-acids, respectively) and their isomers, which inhibit most Gram-positive bacteria (Simpson and Smith, 1992; Moir, 2000). Recent works (Siragusa et al., 2008; Tillman et al., 2011) have showed that hop β-acids extracts added to drink water decreased *Cl. perfringens* counts in caecal contents of challenged chickens, without significant changes in the overall microbiota for the caecum or midgut. Moreover, it has been previously reported (Cornelison et al., 2006) that hop flowers milled into feed pellets and supplemented at a rate of 227 mg/kg improved feed utilization and growth rate of broilers chickens compared to birds on feed without antibiotics. Antimicrobial activity of hop acids is pH dependent (Simpson and Smith, 1992), with β-acids having greater activity at higher pH values than α-acids because of its higher hydrophobicity and pKa (6.1 and 5.4 for β- and α-acids, respectively; Blanco et al., 2006). Previous works on the effects of hop flower supplementation in feeds (Cornelison et al., 2006; Wang et al., 2010; Narvaez et al., 2011) have used a non-brewing hop cultivar (*var.* Teamaker) rich in β- and low in α-acids (84–93 and 6–11 g/kg DM, respectively), which is not currently available at the market. The antimicrobial activity of the bitter hop commercial genotypes used at present by the brewing industry (between 70 and 150 g/kg DM of α-acids), and its interaction with the different gut pH induced by increasing levels of soluble fiber in rabbit diets might therefore modulate the response to hop flower supplementation.

The aim of the current study was to assess in farms affected by epizootic rabbit enteropathy (ERE) the effects of hop flowers (c.v. Nugget) supplementation and to evaluate the influence of a substitution of starch with soluble fiber in diets containing similar and above recommendations levels of insoluble fiber on digestion efficiency, fattening performance, mortality or proliferation of *Cl. perfringens* in the hindgut.

2. Material and methods

Animals (New Zealand White × Californian rabbits originating from strains genetically improved at the Universidad Politécnica of Valencia, Spain) were kept in closed buildings located at the experimental facilities of Polytechnic University of Madrid and of a commercial company (COREN S.C.G.), with partial environmental control (room temperature between 16 and 24 °C; 12 h of light per day). Rabbits were handled according to the principles for the care of animals in experimentation published by Boletín Oficial del Estado (Boletín Oficial del Estado (BOE), 2005).

2.1. Diets

Two diets were formulated to substitute starch with soluble fiber while maintaining at similar levels the concentration of insoluble fiber. Essential nutrients were included according to the recommendations of De Blas and Mateos (Fundación Española Desarrollo Nutrición Animal, 2010) for fattening rabbits. The ingredient and chemical composition are shown in Tables 1 and 2. Each of these diets was supplemented with 500 mg/kg of whole hops to obtain the four experimental feeds. Hops (*H. Lupulus*, c.v. Nugget) were supplied as pellets and according to the supplier (Sociedad Anónima Española para el Fomento del Lúpulo, Villanueva de Carrizo, León, Spain) contained 124 g of α-acids, 37 g of ß-acids