



## The use of chestnuts (*Castanea sativa* Mill.) as a source of resistant starch in the diet of the weaned piglet



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### ABSTRACT

Twenty-four piglets, with a mean weight of 6.11 kg, were allocated to three experimental diets (8 piglets per diet). The starch content of the experimental diets was 39% consisting either of 100% wheat starch (CD), 50% wheat starch and 50% chestnut starch (CN50) or 100% chestnut starch (CN100). The piglets received these diets for 35 days. Total tract apparent digestibility (TTAD) was measured when piglets were aged 32–39 days and 46–53 days. Afterwards, the animals were sacrificed, their gastrointestinal compartments weighed, and the contents of their caecum and colon collected. Diet did not influence the feed intake or growth rate of the piglets, but had a significant effect on their feed conversion ratio which was 1.27, 1.30 and 1.37 for diets CD, CN50 and CN100, respectively ( $P=0.042$ ). Piglets receiving the CN50 diet exhibited lower faecal scores compared to CD and CN100 groups ( $\chi^2 = 39.31$ ,  $P<0.001$ ). There was a trend ( $P=0.067$ ) towards an increase in the TTAD of dry matter (DM) in the two chestnut-containing diets, but only when piglets were younger (32–39 days). The TTAD of crude protein (CP) was decreased ( $P<0.001$ ) in CN100 group relative to CD and CN50 groups, independently of age. Relative to the CD group, the coefficient of TTAD of NDF was increased by 0.18 units in the CN50 group and 0.25 units in the CN100 group, when the piglets were aged 32–39 days. These differences were reduced to 0.09 and 0.17 units, respectively, when the piglets were aged 46–53 days. There were tendencies ( $P<0.10$ ) towards increases in the full and empty weights of the large intestine in CN100 group. Moreover, diet had no significant effect on villus height and width. In the jejunum, crypt depth was reduced in CN100 group ( $P=0.006$ ), while the villus height: crypt depth ratio was increased with the CN50 and CN100 diets ( $P=0.018$ ). Diet had no significant effect on DM and pH of the caecal or colonic contents. In the caecum, the concentration ( $\text{mmol l}^{-1}$ ) of propionic acid was 5.15, 9.43 and 14.7 ( $P=0.042$ ) for diets CD, CN50 and CN100, respectively. The concentration ( $\text{mmol l}^{-1}$ ) of butyric acid was 3.50, 4.90 and 9.84 ( $P=0.025$ ). Conversely, diet had no significant effect on the concentration of short chain fatty acids in the colon. In conclusion, chestnuts could be used as an alternative source of RS in the diet of weaned piglets, although their feed conversion ratio (FCR) may be worsened.

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**Abbreviations:** ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; CD, control diet; CN50, diet containing 50% wheat starch and 50% chestnut starch; CN100, diet containing 100% chestnut starch; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; FCR, feed conversion ratio; LR, Landrace; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; RDS, rapidly digestible starch; RS, resistant starch; SCFAs, short chain fatty acids; SD, standard deviation; SDS, slowly digestible starch; TDF, total dietary fibre; TTAD, total tract apparent digestibility.

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## 1. Introduction

The chestnut (*Castanea sativa* Mill.) is a traditional nut from European Mediterranean countries, and one of the most popular in the world. In Europe, Portugal is one of the biggest producers; in 2010, it produced approximately 22,000 tonnes, representing 3% of world production (INE, 2011).

Starch accounts for 50–80% of the dry matter content of European chestnuts cultivars, while protein represents between 6.0 and 8.6% (Míguez et al., 2004). According to Pizzoferrato et al. (1999), 21.5% of raw chestnut starch takes the form of rapidly digestible starch (RDS), 20.9% is slowly digestible starch (SDS), and 57.6% can be termed resistant starch (RS).

Resistant starch provides a fermentable substrate for caecal and colonic microflora. Thus, including RS in the diet will influence microbial metabolism in the large intestine; increasing the size of the bifidobacteria and lactobacilli populations (Bird et al., 2007). Recently, it was shown that including 7% of raw potato starch (as a source of RS) in the diet of weaned piglets improved their faecal scores, and modified the Bacteroidetes, Firmicutes and Proteobacteria populations in the colon but not in the ileum (Bhandari et al., 2009). These prebiotic actions are comparable to those of established prebiotic oligosaccharides, notably the insulin-type fructans (Robertfroid, 2001).

In the large intestine of piglets, the concentration of short chain fatty acids (SCFAs) – in particular butyrate – increases with the inclusion of RS in the diet (Hedemann and Bach Knudsen, 2007). Moreover, dietary RS has been shown to prevent pathogen infections and diarrhoea in monogastric animals (Williams et al., 2001), and to improve colonic mucosal morphology and integrity (Hedemann and Bach Knudsen, 2007; Nofrarias et al., 2007).

In pigs, dietary RS increases faecal nitrogen excretion and decreases the total tract apparent digestibility (TTAD) of crude protein (CP) (Rideout et al., 2008). Seemingly, the increase in faecal nitrogen after RS consumption is not due to any effect on the secretion of mucin or endogenous nitrogen in the small intestine (Morel et al., 2005). Instead, it can be attributed to an increase in bacterial nitrogen through the stimulation of bacterial growth in the hindgut (Heijnen and Beynen, 1997).

In the present study, chestnuts were used as an alternative source of RS in the diet of weaned piglets. Control animals were fed a standard diet containing 100% wheat starch, while experimental animals received one of two alternative diets. One diet contained 50% wheat starch and 50% chestnut starch, while the other contained 100% chestnut starch. The objective was to determine the effect of these diets on growth performance, total tract apparent digestibility, small intestine mucosal morphology and hindgut microbial activity.

## 2. Materials and methods

### 2.1. Animals and housing

Twenty-four male piglets (Duroc × LR), weaned at 3 weeks of age, with a mean weight of 6.11 kg (SD=0.49 kg) were used. Piglets were assigned to 3 groups (8 piglets per group), according to live weight. Each group received one of three experimental diets. Animals were individually housed in metabolic cages (110 cm × 80 cm) equipped with stainless steel plates for separate collection of faeces and urine. Room temperature was initially set at 28 °C, and was decreased by 2 °C each week until it reached a final temperature of 24 °C.

After an acclimatization period of three days, the piglets completed 5 weeks (35 days) of experimental observation. Piglets were weighed at the beginning of each week, and faeces were collected during two periods of 7 days (second and fourth experimental weeks) to calculate TTAD. The consistency of the faecal matter was recorded daily, according to the scale proposed by Marquardt et al. (1999): 0 = normal, 1 = soft faeces, 2 = moderate diarrhoea and 3 = liquid diarrhoea.

At the end of the experiment, the piglets were sacrificed in the morning (08:00) by electrocution following 12 h of food deprivation. The weight of the pancreas, the liver, and the compartments of the gastrointestinal tract were then recorded. The length of the small and large intestine was also measured. The contents of the caecum and colon were immediately collected for the analysis of pH, DM content, microbial enzymatic activity and levels of SCFAs. Tissue samples from three segments of the small intestine were also collected: the duodenum (10 cm from the pylorus), jejunum (5.5 m from the pylorus) and ileum (60 cm before the ileo-cecal valve). The tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and then sectioned and mounted for microscopic examination of the intestinal villi and crypts.

### 2.2. Chestnuts preparation and diets formulation

Fresh chestnuts were obtained and mechanically peeled to remove the tegument and pericarp. The chestnuts were then dried at 40 °C for 48 h. This reduced their moisture content from around 50% to 10–12%. The dried chestnuts were milled through a 2 mm screen before they were added to the diets. Their chemical composition is reported in Table 1. Three experimental diets were prepared (Table 2). The starch content of the experimental diets was 39%; consisting of either 100% wheat starch (CD), 50% wheat starch and 50% chestnut starch (CN50), or 100% chestnut starch (CN100). The diets were formulated according to the NRC (1998) recommendations for 5–20 kg piglets. They were pelleted (3 mm) and given to the piglets on a single meal per day basis. Animals had access to water *ad libitum*.

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