1. GENERAL INTRODUCTION

Starch from cereals typically constitutes one of the most important sources of energy in the diets of human and animals. In particular, cereal grains and their co-products can be considered as the largest component in the diets specifically formulated for pigs (Back Knudsen et al., 2006).

In order to maximize starch utilization, a high starch digestion from grain-based feed would be desirable both for growing (Gidley et al., 2010) and for weaning pigs (Doucet et al., 2010).

The rate (kinetics) and extent of starch digestion in pigs are highly variable. Considerable differences in starch digestibility among cereal species and also among varieties within species have been observed (Svihus et al., 2005; Wiseman 2006; Sun et al., 2006). Principally, starch is digested in the small intestine by a combination of α-amylase and glucoamylase, but some can escape enzymatic digestion and can be fermented in the large intestine (Sun et al., 2006). Specifically, through works with humans and pigs, evidence arose (Englyst and Cumming, 1985; Regmi et al., 2010) that a specific fraction of starch cannot be digested in the small intestine: this fraction was termed resistant starch (Englyst et al., 1996). In addition, variations in the accessibility to digestion can influence the speed by which starch is degraded and the amount of enzyme resistant starch, thus affecting biological responses, feed intake and production performances of pigs (Hedemann and Bach Knudsen, 2007; Solà-Oriol et al., 2010; Regmi et al., 2010; Doucet et al., 2010).

The conventional approach for the evaluation of cereals used in pig diets has generally consisted of the determination of their chemical composition (dry matter, ash, crude protein, crude fat and starch) and of gelatinized starch (as a proportion of total starch). However, these measurements provide limited information on the properties of starch components in relation to its digestion potential and animal performance (van Kempen et al., 2007; Doucet et al., 2010).

Frequently overlooked factors associated with the starch source that might affect starch digestion include endosperm texture, nature of the starch, size of the granules, amylose-amylopectin ratio, association with other compounds such as lipid, fiber, protein fractions and minerals and processing method (Stevnebø et al., 2006). In particular, the protein fractions (especially prolamin proteins), gluing the protein

bodies into a matrix surrounding starch granules, can act as a barrier for enzyme access and thus degradation (Singh et al., 2010; Drew et al., 2011).

These limitations can be overcome through application of reliable quantitative laboratory tests in combination with animal trials. Gaining insight into biochemical and physicochemical factors that may influence starch digestion is an emerging issue in pig nutrition because of its possible implications for animal response and health (Menoyo et al., 2011; Drew et al., 2011). For instance, a better understanding of the potential effects of agronomic practices and of factors related to the composition of feeds on starch digestion (Doucet et al., 2010; Masoero et al., 2011), and of the intricacies of the latter on animal performances, would benefit the feed industry to improve the properties of commercial starch-rich products (van Kempen et al., 2007) by minimizing the variability of starch quality parameters (Masoero et al., 2011).

Laboratory techniques most commonly employed for the evaluation of the starch degradation and digestion potentials can be divided into two main groups:

- *in vivo* techniques;
- *in vitro* techniques.

Due to the complexity and cost of the *in vivo* starch evaluation, *in vitro* measurements are frequently utilized as alternative procedures (Englyst et al., 1992; Goñi et al., 1997; Capriles et al., 2008). *In vitro* enzymatic methods have been developed for estimation of starch digestion on mono-gastric species and the kinetics of *in vitro* digestion can be considered as an important characteristic both for food and feed.

The majority of these methods are based on a multi-enzyme digestion with α-amylase, pepsin, amyloglucosidase and pancreatin the most common enzymes employed which simulate digestive behavior in the alimentary tract and measures glucose release at different time (Stevnebø et al., 2006; Sun et al., 2006; van Kempen et al., 2010).

Although several workers have indicated a good relationship between the rate of *in vitro* starch digestion and *in vivo* animal response to food and feed (Goñi et al., 1997; van Kempen et al., 2010), more studies are currently needed to reach the goal of being able to predict cereal starch degradability potential in order to avoid or even eliminate the need of animals.

In addition, there is a need for efficient and reliable *in vitro* methods for determining the digestion potential in single feedstuffs for use in feed formulation.

There may be a potential in developing effective *in vitro* methods for the prediction of the susceptibility to enzymatic degradation of dietary starch, which would improve to feed evaluation systems.

Consequently, in an effort to elucidate details of the features of the digestion of starch, new analytical methodologies with advanced kinetics mathematical models, correlating structural and physical properties of starch to its digestion properties, are warranted.

In the present work, it has been attempted to examine the variability in starch hydrolysis and digestion from cereal grains and co-products and to study aspects related, in order to give a better understand of the science behind the differences in grains/feeds starch digestion potential. The general approach was to use a combination of *in vitro* and *in vivo* techniques.

In particular:

- A series of *in vitro* investigations (based on single-points measurements or on extended time-course evaluations) has provided data on the degradation potential of starch from cereal grains and co-products considering the influence of starch structure, cereal type, amylose-amylopectin ratio, heat processing, ensiling, stage of maturity and of agricultural practices.
- A laboratory-based functional evaluation of starch value of cereal grains based on a predicted glycemic index approach, combined with a mathematical first-order exponential model, has been proposed in order to allow an efficient screening of starchy-materials entering in the pig diet formulation.
- An *in vivo* experiment has been undertaken to investigate the postprandial plasma glucose response in pigs fed diets containing starch with a wide range in *in vitro* digestion patterns.

Lastly, taking into account the increasing attention of swine nutritionists on prolamin proteins (Drew et al., 2011), the most commonly used prolamin extraction procedures were compared and reinvestigated, in order to get useful information for routine lab analysis.

The current thesis is based on five *major papers* (full article manuscripts) and five *minor papers* (conference manuscripts), which will be referred to in the text by their roman numerals.