Review:

Chestnut and quebracho tannins in pig nutrition: a review of the effects on performance and intestinal health

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Abstract

Natural extracts are frequently adopted as a valuable alternative to antibiotics in intensive animal farming. Their diverse bioactive constituents such as phytosterols glucosinolates, carotenoids and polyphenols have shown antioxidant, anti-inflammatory and antibacterial effects. Tannins are the largest class of polyphenol compounds of plant extracts, which can be classified into two hydrolysable or condensed subgroups. Poultry and swine nutrition are the most important sectors in which tannins have been used, firstly adopting tannin-rich feedstuffs and more recently, using tannin extracts from different plants. Several commercial products are available containing tannins extracted from the European chestnut tree (Castanea sativa Mill.) and the American quebracho (Schinopsis spp.). Tannins extracted from these plants have been applied on intensive swine farms due to their ability to improve animal performance and health. These positive and prominent effects are frequently associated with the antinutritional effects in reducing feed palatability, digestibility and protein utilization of feed. Some criticisms and contrasting results regarding pig performance and intestinal health have been reported. This paper provides an overview of the effects of chestnut and quebracho tannins on growth performance and intestinal health of pigs in order to clarify the appropriate dosage and response in the various physiological stages.

Keywords Polyphenol, natural extract; livestock production; feed supplements; swine

Implications

Natural extracts, such as tannins, has been widely studied for their promising antioxidant, anti-inflammatory and antibacterial properties. This review explores the effects of chestnut and quebracho tannins on the growth performance and intestinal health of pigs during different physiological stages. Data from different *in vivo* trials could be exploited to establish new recommendations on the nutritional and functional activities of tannins for the post-weaning, growing and finishing phases. The information described in this review could be useful for future research into alternatives to antibiotics in intensive animal farming.

Introduction

The increase in antibiotic resistance has led to development of protective actions (e.g. prudent use of antimicrobials) based on the “One Health” approach (EFSA, 2017; WHO, 2017). The future actions and the World Health Organization action plan against antimicrobial resistance are based on best practices in implementing and monitoring the “one health” plans; supporting novel solutions to prevent and treat infections, thereby increasing the efforts in terms of combating antimicrobial resistance and related risks worldwide.

In pig farming the development of antibiotic resistance can lead to multifactorial infections (Rossi *et al.,* 2013; Rossi *et al.,* 2014a). Antimicrobials and/or additives based on pharmacological levels of zinc and copper oxide (2 000-3 000 ppm) are commonly used during the post-weaning phase (Hejna *et al.,* 2019; Hejna *et al.,* 2020). However, many international organisations have recommended decreasing the use of copper and zinc oxide due to the suspected resistance to certain bacteria and risks associated with the impact of these heavy metals on the environment (EFSA, 2017; WHO, 2017).

Alternatives to in-feed antibiotics and zinc oxide are thus required in order to prevent antimicrobial resistance and natural extracts could usefully replace antibiotics in swine industry (Onelli *et al.,* 2017; Dell'Anno *et al*., 2020; Sotira *et al*., 2020). Natural extracts have several biologically active constituents, which have different bio-activities on animal metabolism (Perricone *et al*., 2020; Huang *et al.,* 2018).

Polyphenolic compounds are ubiquitous in all plant organs and commonly distributed in legumes, cereals, and fruits (Huang *et al.,* 2018). Tannins are an important class of polyphenol compounds, and are mainly classified into two hydrolysable (HTs) or condensed (CTs) subgroups. They are present in a wide variety of animal feed ingredients such as forage, legumes, fruits, cereals and grains (Jansman, 1993).

Poultry and swine are the most important sectors in which tannins have been tested, firstly adopting tannin-rich feedstuffs (such as sorghum) and more recently using tannin extracts from different plants (Jansman, 1993). In poultry, early studies were conducted on the effects of tannin-rich feedstuffs on growth performance, feed efficiency, and antibacterial capacity (Huang *et al.,* 2018). These promising effects led researchers to also test tannins in swine industry (Huang *et al.,* 2018). However, in the early 1980s most studies performed in swine were based on the effects of sorghum, barley, maize, faba bean tannins on pigs' digestibility, protein use, and growth performance (Mitaru *et al.,* 1984). Since then, various sources and tannin extracts have been adopted in pigs, such as tannins from grape seeds, grape pomace, tannic acid, acorns (Huang *et al.,* 2018).

Other relevant sources of tannins are the European chestnut tree (*Castanea sativa Mill.)* and the American quebracho (*Schinopsis spp*.). Traditionally chestnut and quebracho extracts has been adopted in leather tanning industry. Although, in the last decade these extracts have been applied in animal nutrition, due to the high concentration of HTs and CTs contained in chestnut and quebracho extracts. Hence, raised attention has been made on chestnut and quebracho tannins application as feed supplements. In addition, several additives containing chestnut and quebracho tannins are available in the market, confirming the great interest of animal feed sector. The increasing application of these extracts is also related to the continued availability of such products over the year, compared with other sources (e.g grape pomace).

In monogastric animals, supplementation with tannins at different concentrations has positive effects because of their antioxidant, anti-inflammatory and antibacterial activities (Biagi *et al.,* 2010; Huang *et al.,* 2018). Tannins extracted from chestnut and quebracho have been exploited on intensive swine farms and adopted in the different physiological stages of swine (post-weaning and growing/finishing phases) to enhance growth performance, modulate intestinal microbiota and decrease the incidence of diarrhoea in particular during the post-weaning period (Girard *et al.,* 2019; Girard and Bee, 2020). Moreover, contrasting results on the effective supplementation of chestnut and quebracho on animal performance and intestinal health have been observed and, therefore, reported and discussed in the present review. This review analyses the effects of chestnut and quebracho tannins on the growth performance and intestinal health of pigs in order to clarify the appropriate dosage and response in the various physiological stages of pigs.

Classification and composition of tannins

Tannins are polyphenolic secondary metabolites generally defined as "water-soluble phenolic metabolites" or "macromolecular phenolic substances" (Khanbabaee and van Ree, 2001). They can be classified by: i) their molar mass between 300 and 3 000 Da; ii) their molecular structures, divided into two main groups (hydrolysable and condensed tannins); and iii) their structural properties (gallotannins, ellagitannins, complex tannins, and condensed tannins) (Khanbabaee and van Ree 2001). Due to the wide differences in the classification of tannins, for practical reasons we adopt the "hydrolysable and condensed tannins" classification.

Hydrolysable tannins can be fractionated hydrolytically into their components (Khanbabaee and van Ree, 2001) by treatment with hot water or with the enzyme tannase, which catalyses the hydrolysis of tannins. HTs are composed of esters of gallic (gallotannins) or ellagic (ellagitannins) acid and glucose (Smeriglio *et al.,* 2017). Therefore, the term 'hydrolysable tannins' includes both gallotannins and ellagitannins. In fact, ellagitannins, are not hydrolysable, but for historical reasons are still classified as hydrolysable tannins (Khanbabaee and van Ree, 2001).

Condensed tannins are non-hydrolysable oligomeric and polymeric proanthocyanidins, consisting of coupled catechin units. The coupling pattern of the catechin units in condensed tannins can vary extensively. Thus, different tannins present diverse structures and degrees of polymerization. The tannin synthesis occurs in the plants as protection against insects, diseases or grazing animals. Plants accumulate a considerable amount of tannins in the bark, roots, wood, leaves and fruits. CTs can be found in ferns, fern allies, gymnosperms, and many dicotyledonous and monocotyledonous flowering plants (Constabel *et al.,* 2014). However, HTs are limited to dicotyledonous plants (Constabel *et al.,* 2014). Tannins can also be found in several other plants conventionally used in animal nutrition, such as forage, shrubs, cereals and medicinal herbs (Huang *et al.,* 2018). Several tannin-rich by-products such as grape pomace, olive, peanut, green tea, fruit and vegetable co-products have been extensively studied in animal nutrition (Brenes *et al.,* 2016). In addition, tannins can be directly extracted from different plants such as mimosa, oak, chestnut, quebracho trees or from by-products such as grape seeds, and acorns (Krisper *et al.,* 1992; Huang *et al.,* 2018). Several studies have reported the use of tannins extracts from the chestnut and quebracho. Compared to other tannin-rich commercial products, chestnut and quebracho tannin extracts are widely studied in animal nutrition worldwide. The increasingly utilisation of these compounds and the growing of plant extracts market have led to develop specific regulations. In light of this, tannins are recognised as safe ingredient by U.S food and drug (FDA) (listed in 21 CFR 184.1097; 21 CFR 173.310). Chestnut and quebracho extracts are classified as “Natural flavouring substances and natural substances used in conjunction with flavours” in the Food Additives Status List (FDA, 21 CFR 172.510), whereas in the European Union Register of Additives (annex I, 2019) chestnut (*Castanea sativa Mill.*) and quebracho (*Schinopsis spp.*) tannin extracts are under the subclassification “Natural products – botanically defined”. Despite the large application of various tannin extracts in animal nutrition, chestnut and quebracho extracts are the only ones listed in the European Union Register of additives. Although, limited authorizations were enquired to the European Food Safety Authority for the authorization of commercial products (EFSA, 2005; EFSA, 2016).

Chestnut (Castanea sativa Mill.) and quebracho (Schinopsis spp.) trees as a source of tannins

Chestnut tannins

Chestnut trees are a source of hydrolysable tannins, in which ellagitannins and gallotannins are the main representative classes. Ellagitannins are chemically characterized by at least two galloyl units (C—C coupling) and do not contain a glycosidically-linked catechin unit (Landete, 2011). The hydroxydiphenoyl residue undergoes lactonization to produce ellagic acid, which is not easily hydrolysed because of the further C–C coupling of the polyphenolic residue with the polyol unit. The main ellagitannins are vescalagin, vescalin, vascalignin, castalagin, castalin and castaligin, while the most representative complex tannins are acutissimin A (Khanbabaee and van Ree, 2001).Gallotannins are composed of galloyl units or their meta-depsidic derivatives bound to different polyol-, catechin-, or triterpenoid units (Landete, 2011). The hydrolysis of gallotannins produces glucose and gallic acid.

Chestnut tree (Castanea sativa Mill.)

Chestnut, *Castanea sativa Mill.*, (Fagaceae) is the predominant sweet chestnut tree in Europe (Živković *et al.,* 2009). Sweet chestnut has an important economic value in timber production, besides being a valuable food and feed source. Several chestnut-based products are on the food market (traditional dried chestnuts, chestnut flour, marrons glacés, frozen chestnut products, chestnut flakes, beer or liquors) and feed market (chestnut flour, chestnut extracts).

The high content of tannins also increases the value of sweet chestnut trees. The tannin concentration largely depends on the season, and the age and part of the tree. Živković *et al. (*2009) showed a higher content of total phenolic compounds and total condensed tannins in catkin (3.28%), chestnut bark (3%), the red internal seed shell (2.82%), and brown seed shell (1.19%). The bark contains a high tannin concentration, from 5 to 10 %, due to its protective function against vermin and UV light. Krisper *et al.* (1992) reported that the tannin concentration of wood increases linearly with the age of the tree.

Extraction methods

The most common method used for tannin extraction is aqueous extraction at 100-120°C or the solvent extraction method with ethanol, methanol, acetone, methylethylketone, toluene: ethanol in different concentrations are applied to bark, wood chestnut flowers, leaves and shells (Živković *et al.,* 2009). The extract obtained is clarified, evaporated under vacuum and spray-dried. Normally, the tannin concentration of spray-dried chestnut extract is 75%, together with 17.6% non-tannin, 0.4% insoluble substances, 7% water, 3.5 pH (Krisper *et al.,* 1992). Despite the type of extraction adopted, about 89% of the tannin components are hydrolysable tannins such as castalagin (53%), vescalagin (35%), castalin (3%), and vescalin (8%) (Krisper *et al.,* 1992). Campo *et al.* (2016) showed that the liquid fraction contains 43% castaligin and vascalignin, and 6.1% castalin and vescalin of the total tannins, while the spray-dried fraction contains 37.8% castaligin and vascalignin, and 7.8 % castalin and vescalin.

Quebracho tannins

Quebracho trees are a source of condensed tannins, of which polymeric proanthocyanidins are the most representative type. Condensed tannin or proanthocyanidins are oligomeric and polymeric proanthocyanidins containing flavan-3-ols the catechins units (Khanbabaee and van Ree, 2001). Oligomeric or polymeric proanthocyanidins (procyanidin B2, proanthocyanidin A1, proanthocyanidin A2, and proanthocyanidin C1), and related flavanol residues, typically produce anthocyanidins (e.g. cyanidin and pelargonidin) on acid degradation (Khanbabaee and van Ree 2001). Condensed tannins present polymers such as procyanidin, and typical CTs with unsubstituted catechin units include: (Khanbabaee and van Ree, 2001).

Quebracho tree (Schinopsis spp.)

Quebracho trees (*Schinopsis spp*., family *Anacardiaceae*) grow in South America, mostly in northern Argentina and eastern Paraguay. The most representative quebracho trees belong to *Schinopsis lorentzii* and *Schinopsis balansae* species, characterized by red hardwood. A third tree species is the white quebracho (*Aspidosperma quebracho-blanco,* family Apocynaceae). However, the “true quebracho” is referred to as *Schinopsis lorentzii* and *Schinopsis balansae* from the *Anacardiaceae* family (Venter *et al.,* 2012).

These trees are converted into different products such as railroad ties, boards, beams, poles, piles, fence posts, and cross-arms. Quebracho trees are not generally used for construction proposes due to their heavy weight and short length.

Quebracho trees are principally used for their hardwood content of tannins, which are used in the tanning of high-grade leathers and adhesive manufacturing (Pizzi, 2019). Quebracho extract obtained from *Schinopsis lorentzii* contains 15% to 21% of pure tannin, whereas the extract obtained from *Schinopsis balansae* has a pure tannin content of 20–21% (Venter *et al.,* 2012).

Extraction methods

The extraction process is similar to tannins extracted from chestnut. The heartwood is stripped of its bark and chipped. The obtained wood chips are extracted using hot water (100°C), with a bisulfite solution. The addition of bisulfite solution to the hot water increases the extraction rate of tannins (Venter *et al.,* 2012). The quebracho extract is composed of 95% condensed tannin or proanthocyanidins and 5% water soluble sugars on a dry basis.

The most representative CTs in quebracho tannins is proanthocyanidin, typically the red colour of CTs is due to the reaction of the high temperature and aqueous acid (Venter *et al.,* 2012). The first application of CTs tannins in leather, and more recently tannin applications as bio-based adhesives, have led to an increase in tannin use as bio-based materials in widespread industrial processes and sectors (Pizzi, 2019). Several by-products are produced during industrialized tannin extraction, such as exhausted wood biomasses, which are reintegrated into the industrial process to produce pellets for heating and energy production. In addition, chestnut wood, leaves and shells can be recycled in the food and feed industries, and in pharmaceutical and cosmetic manufacturing. Chestnuts and quebracho trees are thus a valuable source of tannins not only for industrial purposes, but also for nutrition. The renowned characteristics and properties of tannins can be applied from feed to food. These approaches are in line with the circular economy, and offer a new life and alternative prospective for recovering these by-products with beneficial effects for the environment and the economy.

Benefits and challenges of tannin supplementation in pigs

Tannins have a considerable impact on swine health and productivity. Numerous field studies have highlighted their benefits and challenges (Huang *et al*., 2018). The effects of chestnut or quebracho tannin supplementation in swine are different due to the different commercial feed additives adopted, that contain chestnut and quebracho tannin extracts (Van Parys *et al.,* 2010; Bilic-Sobot *et al.,* 2016; Bee *et al.,* 2017). The heterogeneity of commercial products is associated with the use of chestnut or quebracho individually or in mixtures with different percentages of chestnut and quebracho, and hence different amounts of HTs and CTs. The commercial products tested on pig are therefore extremely different also in terms of the concentrations of tannins in the extract which range from 54% to 82%, thus the presence of different percentages and types of HTs or CTs (Supplementary Table S1).

The beneficial effects of tannin supplementation in pig farming is related to their antimicrobial, antioxidant and radical scavenging, anti-inflammatory activities and on the immune status (Huang *et al.,* 2018). In swine farming, pathogenic bacteria such as *E. coli* are mostly associated with post-weaning diarrhoea (PWD), a multifactorial disease that occurs after weaning (Rossi *et al*., 2014a; Rossi *et al.*, 2014b). The use of tannin extracts can be a valuable alternative for the control of PWD (Smeriglio *et al*., 2017; Huang *et al*., 2018; Girard and Bee, 2020). In fact, the bacteriostatic activity of tannins has been shown in both gram-positive (*Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis and Enterococcus fecalis*) and gram-negative bacteria (*Citrobacter freundii, E. coli, Pseudomonas aeruginosa, Salmonella* *enteric ser. Typhimurium*) (Smeriglio *et al.,* 2017). Tannins have antioxidant and radical scavenger effects as also demonstrated by the study conducted by our group (Reggi *et al.,* 2020). Although HTs may enhance the antioxidant capacity of liver and plasma in animals (Huang *et al.,* 2018), the mechanism underlying the tannin action as an antioxidant compound is not fully understood.

The anti-inflammatory activity and animals' immune response is mostly associated with tannins antioxidant activities. To date, the *in vivo* study conducted by Stukelj *et al.* (2010) administered to growing pigs 0.15% HTs associated with 0.15% 4 acids which did not show differences on immune response. Nevertheless, several studies conducted on other animals' species identified a positive immune response with tannins supplementation. In particular, in both *in vivo* and *in vitro,* the response of tannins on anti-inflammatory and immune status showed promising effects when animals were exposed to stressors such as lipopolysaccharide challenge, bacterial challenge, heat stress, or intestinal cells exposed to hydrogen peroxide and Dextran Sodium Sulfate (Park et al., 2014; Liu et al., 2017; Reggi et al., 2020). Tannins, HTs and CTs, are able to modulate intestinal pro-inflammatory cytokine expression acting also on gut barrier and tight junctions (Liu et al., 2017). However, there is a lack of *in vivo* studies on the action mode of tannins in the swine gut, especially in weaning and post-weaned piglets.

The bioactive characteristics of tannins can affect the palatability, digestibility and protein use of feed. The ability to bind proteins and carbohydrates in monogastric animals is associated with the antinutritional effects of tannins in reducing feed palatability (Bee *et al.,* 2017). An increased concentration of proline in the parotid glands of animals treated with HTs from acorns may lead to the release of higher amounts of the tannin-protein complex in the saliva (Cappai *et al.,* 2013).

Proanthocyanidins, which are CTs, have a high affinity with proline-rich protein, and the strength of the interaction depends on both the nature of the protein and the proanthocyanidin molecule. An animal's ability to tolerate the antinutritional effects of HTs or CTs is thus an essential defensive mechanism that ensures its beneficial nutritional and extra-nutritional effects (Candek-Potokar *et al.,* 2015). Animals adapted to tannin ingestion, such as ruminants, have therefore shown minimized negative effects, such as regular nutrient digestibility, better tannin absorption, and minor antinutritional effects in general.

Animals not fully adapted to tannin ingestion, typically monogastric animals, are less able to tolerate the astringency effects triggered by the tannin-protein complex. Consequently, a higher astringency effect decreases the feed palatability and ingestion (Bee *et al.,* 2017). However, several studies have shown discordant findings when tannins were evaluated on amino acid and protein digestibility (Antongiovanni *et al.,* 2007; Galassi *et al.,* 2019). The capacity of tannins to bind proteins is not necessarily negative. In fact, the formation of a tannin protein-complex in the digestive tract may protect proteins, carbohydrates, and lipids from oxidative damage during digestion (Cappai *et al.*, 2013).

In general digestibility is a key factor in determining tannins' effect at gastro-intestinal level. Then, the biodegradation and the absorption of HTs along the gastro-intestinal tract has been investigated by several studies, whereas the fate and absorption of CTs seems to be more complex due to their structural complexity (Mueller-Harvey, 2006). As reported by Reggi et al. (2020) the antimicrobial and antioxidant activity of HTs and CTs were reduced after *in vitro* digestion, which may be due to a lower bio-accessibility or to the excessive degradation of antimicrobial and antioxidant molecules. The authors, however, reported a beneficial effect of *in vitro* digested HTs and CTs when administered to experimentally damaged intestinal swine cells, suggesting that a trophic effect at intestinal epithelium occurred. In light of this, the bioavailability of tannins after oral supplementation play a crucial role and should be considered and further investigated. Therefore, the mechanism of action of tannins or tannins degradation-molecules exploitation should be deeply investigated at intestinal level (tissue or cells).

Chestnut and quebracho tannin effect on growth performance and intestinal heath in pig

In the light of the activities and properties of tannins, identifying the correct application dose is essential in order to maximize the beneficial effects of tannins, and minimize the antinutritional effects on animal growth performance. The selection criteria of the studies discussed in this review were: i) studies performed on cross bred pigs from post-weaning to finishing phases; ii) studies evaluating diets supplemented with tannins from chestnut or quebracho iii) study outcomes evaluating zootechnical performance or intestinal health.

Effects of Chestnut and quebracho tannin supplementation during the post-weaning phase

Growth performances

The anti-inflammatory and antibacterial activities of tannin supplementation could enhance growth performance and alleviate post-weaning diarrhoea, one of the greatest disorders to occur during the first two weeks after weaning (Rossi *et al.*, 2013 2014a; Huang *et al.,* 2018). The studies we reviewed adopted a supplementation of tannins ranging from 0.11 to 3 %, hence the tannin dosage was considered low for < 1% of tannin inclusion, medium for ≥ 1-2%, and high for ≥ 3%.

Several studies have been performed on tannin supplementation during the post-weaning phase (Table 1). The inclusion of low tannin doses of 0.11% HTs, 0.23% HTs and 0.45 % HTs, for a period of 28 days did not influence the animal's live weight (BW) and average daily feed intake (ADFI; Biagi *et al.,* 2010). However, the same study reported a higher average daily gain (ADG) and increased feed efficiency throughout the 28 days of the trial compared with the control group (no tannin supplementation).

The inclusion in the diets of a medium tannin dose of 1% HTs/CTs, had no effect on body weight and feed efficiency ratio (Girard *et al.,* 2018). However, tannin supplementation at 2% HTs/CTs showed a positive effect on daily feed intake and average daily gain (Girard *et al.,* 2019). The use of medium tannin doses of 2% HTs/CTs may result in improved daily feed intake and average body gain, whereas doses under 1% HTs/CTs seem not to affect animals' growth performance. It is possible that the application of low tannin doses, especially a dietary inclusion of below 0.5 % HTs/CTs could have a limited effect on animals. This limitation could establish a cut-off limit for tannin efficacy in relation to the dose meriting further investigation.

The combination of tannins with other bioactive compounds could be beneficial for enhancing animal performance. The combined application of organic acids within pig feed led to better growth and affected intestinal microbiota. The administration of 0.19% HTs and 0.16% of fatty acids in the diet improved body weight, average daily gain and feed conversion ratio (FCR; Brus *et al.,* 2013b). This positive effect on growth performance could be related to the effect of the organic acids in decreasing gastric pH, improving nutrient digestion, and acting as an energy source for the gastrointestinal tract.

The synergistic effect of tannins with sodium salicylate, a non-steroidal anti-inflammatory drug, was tested in weaned-piglets (Girard *et al.,* 2019). However, the use of sodium salicylate in combination with a medium tannin dose of 2% HTs/CTs did not affect growth. Therefore, the synergistic effect of tannins and organic acids or other compounds merits further study. Organic acids may alleviate the negative effects of tannins on feed intake, growth and feed efficiency.

The medium supplementation of tannins seems to be more effective on the growth of post-weaned piglets compared to a low inclusion (Biagi *et al.,* 2010; Frankič and Salobir, 2011; Brus *et al.,* 2013b; Girard *et al.,* 2018; Girard *et al.,* 2019). However, a low dose combined with 0.16% of fatty acids showed beneficial effects on animal performance. Most of the beneficial effects of tannins are related to an improved ADG and ADFI or FCR associated with the medium tannin dose. Literature data show that low (< 1%) or medium (≥ 1-2%) doses of tannin supplementation may positively influence growth during the post-weaning phase.

Exactly how tannins improve performance is not fully understood. In the studies here considered, the duration of tannin supplementation seems to vary drastically, from day 14 to day 104. Despite the positive effect of the combination of tannins and organic acids, where the trial lasted 104 days (Brus *et al.,* 2013b), the beneficial effects were detected with a 14- or 28-day supplementation (Biagi *et al.,* 2010; Frankič and Salobir, 2011; Girard *et al.,* 2018; Girard *et al.,* 2019).

Another important factor which can modulate animal response to tannins may be the feeding regime adopted in the different studies (*ad libitum* versus restricted) and the basal diet administered which may contain different level of tannin from basal ingredients. The majority of studies during the post-weaning period adopted *ad libitum* feeding. However, Frankič and Salobir (2011) used a feed-restricted regimen, which is not common practice in piglet trials, and the obtained results may be more associated with the tannin dosage, which ranged from 0.075 to 0.30 %, rather than the duration of supplementation. The basal diets adopted during the post-weaning phase in the different studies analysed, contained different amount of basal ingredients, such as corn meal, wheat meal, barley meal, wheat starch which are intrinsically rich in tannin. The utilisation of tannin-rich feedstuffs can contribute to increase the overall concentration of tannins in the diets. The latter factor is not always considered in animal trials but it can hide or modulate animal performance and response.

Intestinal health

The optimal growth performance in pig is directly link with the health of the gastro-intestinal system. The post-weaning phase is critical in the lifetime of piglets, during which gastrointestinal morphology and physiology undergo several changes. Bioactive compounds such as tannins could positively affect these changes at physiological and microbial levels (Table 2). There are few *in vivo* studies related to the effect of tannins on intestinal health during the post-weaning. The *in vivo* administration of low tannin doses, at 0.11 % HTs, 0.23 % HTs and 0.45% HTs, did not alter the *Enterococcus spp.* count, but tended to increase the *Lactobacillus spp.* count in the jejunum and coliforms in the caecum (Biagi *et al.,* 2010). In addition, no effects of tannins were detected on villous height and crypt depth of the intestinal mucosa. The same author reported that an *in vitro* experiment did not show the effects of tannins (0.75, 1.5 and 6 HTs g/l concentrations in the medium) on coliform count, nevertheless coliforms increased with tannins at 3 HTs g/l. The *Lactobacillus* *spp.* count was significantly reduced by the highest tannin concentration (6 HTs g/l), and the *Enterococcus spp.* count increased significantly. *In vitro* fermentation experiments have shown that tannins decreased the total gas production, ammonia concentrations and total volatile fatty acids (acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and the n-butyric to iso-butyric acid ratio). The effects of tannins on intestinal morphology were not significant to induce a beneficial improvement. Thus, these findings on *Lactobacillus spp.* and coliforms seem contrasting. Girard *et al*. (2018) demonstrated that HTs/CTs did not affect *Escherichia coli* abundance in faeces on infected post-weaning piglets with *Escherichia coli* (ETEC) F4 stain supplemented with a medium 1% dose of HTs/CTs, (Girard *et al.,* 2018). Nevertheless, the supplementation of a medium 1% dosage of HTs/CTs did not prevent outbreaks of diarrhoea or ETEC shedding. However, the prevalence of diarrhoea, faecal score, and days of diarrhoea were lower in the HTs/CTs group. Similar results were found when a medium dose of HTs/CTs was used (Girard *et al.,* 2019); piglets challenged with *Escherichia coli* (ETEC) had a lower average faecal score, a lower percentage of piglets with diarrhoea, and less ETEC shedding in faeces. Both medium tannin doses of 1% HTs/CTs and 2% HTs/CTs, decreased the incidence of diarrhoea in weaned piglets. However, only 2% of HTs/CTs inclusion in the diet was able to decrease *E. coli* shedding.

The combination of a low dose of HTs with organic acids, decreased faecal *Escherichia coli, Campylobacter spp. count* and increased the lactic acid bacteria count (Brus *et al.,* 2013b). These findings could be explained by the antagonist effect of lactic acid bacteria on *Escherichia coli* and *Campylobacter spp.* growth. However, the addition of organic acids used in the Brus *et al.'s* study (2013b) and the duration of tannin supplementation (104 days) could have influenced the results. These results are interesting because they highlight the beneficial effects in reducing the bacterial count and *E. coli* shedding. The low and medium supplementation of HTs/CTs or HTs seem to reduce some bacteria populations.

The mechanisms behind the *in vivo* antimicrobial effects of tannins at the intestinal level, especially how tannins modulate intestinal health in weaned piglets, are not fully understood. Some hypotheses regarding the antimicrobial activity of tannins include the presence of ellagitatannin which showed protein binding, enzyme inhibition, substrate deprivation, complex formation with cell walls, membrane disruption and metal ions (Girard and Bee, 2020). The antimicrobial activity of tannins may be attributed to the oxidation of tannins and the liberation of hydrogen peroxide. In addition, the high affinity of tannins to bind proteins could increase the number of hydroxyl groups, subsequently increasing the antimicrobial activity (Mueller-Harvey, 2006).

The tannin dosage used during the post-weaning phase needs further investigation, since the rate that tannins move to the small intestine is not fully understood. HTs could also be hydrolysed in the stomach and in the small intestine, thus freeing several metabolites, such as ellagic acid. HTs metabolites may also be involved in the antibacterial activities. In addition, the studies adopted HTs alone or the combination of HTs and CTs, thus the synergic effect of HTs and CTs cannot be excluded.

Overall, the application of chestnut and quebracho based supplements in the intestinal health of post-weaning animals requires further studies to test the correct dosage, time of supplementation and mechanism of actions. In this regard, the use of a combination of *in vitro* experimental models, such as those described by Giromini *et al*. (2019) and Reggi *et al*. (2020), and animal trials to test the same HTs or

HTs/CTs products could provide a more a complete overview of both mechanisms of action *in vitro* and the efficacy at gastro-intestinal level in animals.

Finally, there are currently few studies on the modulation of pigs' microbiota by tannins during post-weaning. There are few *in vivo* studies on these effects and the intestinal population studied is restricted to the main pathogen bacteria in weaned piglets (Biagi *et al*., 2010; Brus *et al*., 2013b; Girard *et al*., 2018; Girard *et al*., 2020). In general, tannins' antimicrobial activity could increase the knowledge on the effects of tannins on specific pathogens. However, the effects of HTs/CTs or HTs on the entire microbiome need to be clarified which could then lead to new nutritional strategies for the best use of this promising additive.

Effects of Chestnut and quebracho tannin supplementation during the growing and finishing phases

Growth performances

In intensive pig farming, during the growing and finishing phases, nutrition plays a key role in achieving better nutrient abortion and digestibility, better feed conversion and efficiency, and improving growth performance and health. Tannins derived from chestnut and quebracho could improve animals' performance and health status due to their antimicrobial and antioxidant effects (Table 3). The use of low doses has revealed different response on animal performance. The low tannin dose adopted by Antongiovanni et al. (2007) (0.25% HTs and 0.50% HTs) did not impact on ADG and FCR. Similar results were reported by Prevolnik et al. (2012), as 0.20% of HTs supplementation did not modulate ADG, ADF or FCR in pigs. In line with this, low dose HTs effects on animal performance were found in the trial conducted by (Galassi et al., 2019). However, (Brus et al., 2013a) reported an increased ADG in the finishing phase (90-120 kg) and throughout the entire trial (30-120 kg), with a low supplementation of HTs (0.20%). Bee et al. (2017) reported that FCR was positively affected by medium, 1.5%, and high, 3%, doses of HTs/CTs; while BW, ADG and ADFI were not influenced. Conversely, a high 3% dose of HTs, decreased ADFI without modifying the BW, ADG and FCR (Candek-Potokar et al., 2015).

Other studies have reported the administration of tannins together with other compounds, such as natural extracts rich in polyphenols (Ranucci et al., 2015), acids (Stukelj et al., 2010) and PUFA (Tretola et al., 2019). However, the combination of oregano extract and HTs did not enhance pigs' performance, whereas it increased the antioxidant status and lipid oxidation (Ranucci et al., 2015). A low HTs dose combined with the supplementation of 0.15% of a mixture of four acids did not affect the growth performance of pigs (Stukelj et al., 2010). Tretola et al. (2019) reported that the use of a high HTs/CTs dose (3%) in combination with 2% PUFA did not improve animal performance. The synergistic effect of tannins with other compounds could increase the interest in view of the various beneficial activities of several compounds, especially phenolic compounds.

Most studies adopted commercial additives based on tannins extracts, however an alternative source of tannins is the direct use of dried chestnuts or chestnut meal in animal diets. Chestnut meal contains over 75% of HTs, and when used in pig diets slightly increased the tannin content in the diet. The inclusion of chestnut meal from 5 to 25 % translated into a tannin concentration in the diet ranging from 0.17 % to 0.19 % on a dry matter basis (Lee et al., 2016). Thus, supplementing dried chestnut or chestnut meal could be comparable to a low dose of tannin extract.

The supplementation of 0.3 and 0.5% (DM) chestnut meal, improved the average body weight and feed efficiency (Joo et al., 2018). Lee et al. (2016) reported an increased dry matter intake for the experimental group that received 10% of chestnut meal. In addition, the beneficial effect of tannins was highlighted by supplementing 15 % of dried chestnuts. The tannin group had a greater body weight compared to the control group (De Jesus et al., 2016). In contrast with other studies, which showed a marginal or no effect of tannins supplementation on zootechnical performance, the use of dried chestnuts or chestnut meal revealed promising results.

In the studies reported in Table 3, the duration of tannin supplementation during the growing and finishing phases varies drastically, from day 21 to day 270. Tannin supplementation during this life stage revealed dissimilar effects on growth performance. In particular, tannins extract did not increase the zootechnical parameters except for average daily gain. A 30-day study by Brus et al. (2013a) adopted a low dose of HTs, while other studies supplemented similar higher doses of HTs and HTs/CTs (from 0.15 to 3 %) for a longer period.

Results of studies conducted during the growing and finishing phases have thus shown heterogeneous responses probably related to the duration of the tannin supplementation, type of tannin product (extracted or chestnut meal) used, age of animals, and dietary basal ingredients naturally rich in tannin. In fact, tannins are present in several feedstuffs and ingredients for animal nutrition, such as corn, wheat and barley. Thus, tannin-rich feedstuffs could increase the concentration of tannins in the diet fed to animals.

It is possible that the higher 3 % dose of HTs and HTs/CTs used in some studies (Candek-Potokar et al., 2015; Bee et al., 2017; Tretola et al., 2019), in addition to the basal diet content of tannins, could increase the tannin intake resulting in no effect on growth performance.

Among the studies reported in the present review, only Lee and co-authors (2016) reported the basal diet concentration of tannins which were 0.16 % of DM. As per post-weaning phase, also in finisher studies investigating the role of HTs and CTs on growth performance and intestinal health, the concentration of HTs and CTs in the basal diet should be taken into account. Further investigations are needed to evaluate the correct administration of tannins irrespectively of the fattening and finishing period, in relation to the basal diet content of tannins.

Intestinal heath

The intestinal morphology and histological characteristics of the small intestine may be modulated by HTs supplementation (Table 4; Brus *et al.,* 2013a; Candek-Potokar *et al.,* 2015; Bilic-Sobot *et al.,* 2016). Low tannin doses of 0.20% HTs, showed no effects on the height of mucosa, height of villi, height of epithelium, and proportion of necrosis (Brus *et al.,* 2013a). In this latter study, necrosis was observed in all experimental groups, probably due to the negative correlation with daily gain. Normally, intestinal necrosis leads to a decreased feed intake and subsequent lower body gain. However, animals in the HTs group showed an equal level of necrosis as the control group, but a higher daily gain. It is not clear how HTs affect intestinal morphology, though it seems that they have a trophic effect in intestine maintenance in terms of absorbing nutrients.

Medium and high HTs supplementation from 1 to 3 % affected the morphology of the duodenum (Bilic-Sobot *et al.,* 2016). In particular, 3% of HTs supplementation increased the villus height and villus perimeter, while 1% and 3% increased mucosal thickness (Bilic-Sobot *et al.,* 2016). Only the duodenum was affected by HTs supplementation, which is an important digestive site in which an important stage of digestion takes place together with the absorption of fat and fat-soluble vitamins.

Digestion and absorption in the small intestine is the main function in which villous height and crypt depth are involved. The higher villus height and perimeter thus increased the surface area of nutrient absorption. However, Candek-Potokar *et al.* (2015) showed the positive effect of HTs supplementation on growth performance, with 1% and 2% leading to an increased daily feed intake. These results were explained by the higher villus height and perimeter, which led to an increased nutrient absorption.

There are few *in vivo* studies on intestinal morphology, and data on histological evaluations of tannin administration are not sufficient. It is therefore not possible to hypothesize what level of tannin dose could influence intestinal morphology. Van Parys *et al.* (2010) showed that *Salmonella Typhimurium* shedding and colonization were not affected by HTs in *Salmonella*-challenged growing pigs. However, the same author demonstrated *in vitro* bacteriostatic and bactericidal effects against *Salmonella Typhimurium.* The outcomes obtained *in vitro* and *in vivo* were influenced by different experimental conditions (*in vitro* condition vs. *in vivo* condition). In fact, *in vitro* experiment condition cannot fully mimic the complexity of the interactions that occur within the entire organism. In the growing and finishing phases, the effectiveness of the antibacterial activity of tannins during *in vivo* trial is still unclear.

Another important intestinal aspect is how tannins modulate intestinal microbiota. High doses of HTs/CTs (3%) were shown to modulate the intestinal microbiota of growing pigs (Tretola *et al.,* 2019). The alpha diversity analysis revealed that the bacterial community index (Chao1) and operational taxonomic units (OTUs) and phylogenetic diversity of pigs' microbiota decreased with HTs/CTs supplementation. However, HTs/CTs increased the *Oscillospira* genus of the *Ruminococcaceae* family, while reducing the *Lactobacillales* order, *Streptococcaceae*, and *Veillonellaceae* families. Thus, HTs/CTs were able to modulate some bacterial genuses and families that are considered beneficial in preventing intestinal inflammation together with other families that are usually harmful for intestinal integrity and health. However, the *Lactobacillales* order was reduced by HTs/CTs supplementation. Compare to the other studies reported in this review, considering the growing and finishing phases, Tretola et al. (2019) was the only study in which the combination of HTs and CTs was tested. Nowadays, we cannot exclude a synergistic effect of these two type of tannins. *In vitro* studies on *E. Coli* reported this possible synergistic effect (Reggi *et al*., 2020), however, the *in vitro* response it is not fully understood.

The immune effect of tannin supplementation was not deeply investigated. Considering the studies reported in this review, only Stukelj *et al.* (2010) investigated the immune effect of tannins on growing and finishing pigs. The blood concentration of neutrophils decreased, while lymphocytes and eosinophils concentration increased after 21 days of 0.15% HTs + 0.15% 4 acids supplementation. In this study, the synergistic effect of tannins with fatty acids and the effect of tannins alone should be further investigated. Hence, no reports are available on HTs or CTs effect on immune status on pigs, this lack of data should be investigated in order to better clarified the effect of HTs and CTs on immune response and in particular on expression of related genes.

The *in vitro* and *in vivo* results highlighted the promising effects of HTs or HTs/CTs on different types of bacteria, such as the *Salmonella Typhimurium, Oscillospira* genus, and the *Streptococcaceae* and *Veillonellaceae* families. Aside from the effectiveness of HTs or HTs/CTs on numerous bacteria, both gram- positive and gram- negative, there is a lack of studies on the intestinal ecology of growing and finishing pigs. Frequently the *in vitro* effects of HTs or HTs/CTs on the intestinal bacteria population are not in line with the results from *in vivo* studies. Thus, further investigations are needed in order to clarify HTs or HTs/CTs activity in pigs.

Conclusions

In conclusion, the use of hydrolysable and condensed tannins in swine nutrition could have beneficial effects on the growth performances and gastro-intestinal health status of both post-weaning and growing pigs. However, the effectiveness of tannin supplementation in weaned and finishers seems to be highly related not only to the dose administered to animals, but also to the duration of supplementation, the presence of other sources of tannins in the basal diet, the feed regimen adopted (ad libitum versus restricted) and to the synergistic effect of hydrolysable and/or condensed tannins with other supplements. Also, to date, the majority of the in vivo studies performed have not fully elucidated the properties of tannins on swine immune status and on the intestinal microbiota. All the latter points require additional investigations.

Finally, although the establishment of the effective tannin supplementation protocol in swine requires further studies, they seem to enhance productivity and animal health and therefore, they could be adopted as feed additives.

Ethics approval

This review did not require any ethical approval.

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

None

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Table 1. Effects of tannins on pigs' zootechnical performances during the post-weaning phase

Source/ Type[1]/ Feeding	Animals[2]/ Duration (day)	Dose of tannin	Zootechnical performances[3]	References
Chestnut/ HTs / ad libitum	12/ 28	Control, 0.11% HTs, 0.23% HTs, 0.45% HTs	=BW, ADG, ADFI, FCR with 0.11%, 0.23%; = BW, ADFI with 0.45%; ↑ ADG and FCR with 0.45%	Biagi et al. (2010)
Chestnut/ HTs / ad libitum	168/ 104	Control, 0.35 % 5 acids[4], 0.19 % HTs + 0.16 % 5 acids[4]	↑ BW, ADG at 82 d and 127 d; ↑ FCR at 82-127 d	Brus et al. (2013b)
Chestnut/ HTs / restricted	8/ 14	Control[5], 0.075% HTs [5], 0.15% HTs[5], 0.3% HTs [5], Vit. E[5]	=BW, ADG, ADFI, FCR	Frankič and Salobir (2011)
Chestnut and Quebracho/ HTs/CTs / ad libitum	18/ 14	Control infected[6]; Control not infected[6]; 1% HTs/CTs infected[6]; 1% HTs/CTs not infected[6]	=BW, ADG, ADFI, FCR	Girard et al. (2018)
Chestnut and Quebracho/ HTs/CTs / ad libitum	36/ 14	Control without SA[7]; 2% HTs/CTs [7]; Control with SA[7]; SA+ 2% HTs/CTs [7];	↑ BW at 7 d; ↑ ADG, ADFI	Girard et al. (2020)

[1] HTs= hydrolysable ; CTs= condensed (CTs).

[2]Number of animals per each treatment groups.

[3]BW= body weigh; ADG= average daily gain; ADFI=average daily feed intake; FCR: feed conversion ratio.

[4]Control= basal diet without supplementation; 0.19 % HTs + 0.16 % 5 acids= basal diet supplemented with 0.19% of tannins extracted from chestnut tree (Tanin Sevnica d.d., Slovenia) and overall 0.16% of five acids (formic acid, lactic acid, DLcitric acid and DL-malic acid, phosphorus acid).

[5]Control= basal diet without supplementation of linseed oil; 0,075% HTs = basal diet with the supplementation of linseed oil and 0,075% of tannins (Tanin Sevnica d.d., Slovenia); 0.15 HTs = basal diet with the supplementation of linseed oil and 0.15% of tannins (Tanin Sevnica d.d., Slovenia); 0.30 % HTs = basal diet with the supplementation of linseed oil and 0.30% of tannins (Tanin Sevnica d.d., Slovenia); Vit. E= basal diet with the supplementation of linseed oil and 100 mg/kg of vitamin E.

[6]Control infected= basal diet without supplementation of tannins and infected with *Escherichia coli*; Control not infected=basal diet without supplementation of tannins and not orally infected with *Escherichia coli*; 1% HTs/CTs infected= basal diet supplemented with 1% of tannins (Silvateam, Italy) and orally infected with Escherichia coli; 1% HTs/CTs not infected= basal diet supplemented with 1% of tannins (Silvateam, Italy) and not orally infected with *Escherichia coli*;

[7]Control without SA= basal diet without sodium salicylate and without tannins; 2% HTs/CTs = basal diet without sodium salicylate and with 2% of tannins (Silvateam, Italy); Control with SA= basal diet with sodium salicylate and without tannins; SA+ 2% HTs/CTs = basal diet with sodium salicylate and with 2% of tannins (Silvateam, Italy);

= No effect means that no differences were detected among treatments groups ($P>0.05$).

↑↓ Positive or negative effect means that the treatments with tannins showed differences compare to the control group ($P<0.05$).

Table 2. *Effects of tannins on pigs' intestinal health during the post-weaning phase*

Source/ Type[1]/ Feeding	Animals[2]/ Duration (day)	Dose of tannin	Intestinal health	References
Chestnut/ HTs / ad libitum	12/ 28	Control, 0.11% HTs, 0.23% HTs, 0.45% HTs	= ammonia (jejunum, ileum); ↓ VFA, ↑ coliforms (jejunum, caecum chime), = with 0.23%; = enterococci and clostridia (jejunum, caecum chime); ↑ coliforms with 0.45%; ↑ lactobacilli count in jejunum; = villous height (jejunum, ileum and caecum); ↓ crypt depth (Ileum); ↑ crypt depth of Ileum; with 0.11%	Biagi *et al*. (2010)
Chestnut/ HTs / ad libitum	168/ 104	Control, 0.35 % OA[3], 0.19 % HTs + 0.16 % OA[3]	↓ *Escherichia coli*; ↓ *Campylobacter spp.* with OA+T: = Lactic acid bacteria	Brus *et al*. (2013b)
Chestnut and Quebracho/ HTs/CTs/ ad libitum	18/ 14	Control infected[4]; Control not infected[4]; 1% HTs/CTs infected[4]; 1% HTs/CTs not infected[4]	=↓ average faecal score; ↓ number of days in diarrhoea; ↓ *Escherichia coli* F4 shedding; ↑ *Escherichia coli* F4 shedding with 1% (infected)	Girard *et al*. (2018)
Chestnut and Quebracho/ HTs/CTs/ ad libitum	36/ 14	Control without SA[5]; 2% HTs/CTs[5]; Control with SA[5]; SA+ 2% HTs/CTs [5];	↓ average faecal score; ↓ diarrhoea; ↓ ETEC shedding	Girard *et al*. (2019)

[1] HTs= hydrolysable ; CTs= condensed (CTs).

[2]Number of animals per each treatment groups.

[3]0.35% OA= basal diet supplemented with 0.35% of commercial acidificant FraAcidDry (Perstop Franklin; Waspik, Netherlands); 0.19 % HTs + 0.16 % OA = basal diet supplemented with 0.19% of tannins extracted from chestnut tree (Tanin Sevnica d.d., Slovenia) and overall 0.16% of five acids (formic acid, lactic acid, DLcitric acid and DL-malic acid, phosphorus acid).

[4]Control infected= basal diet without supplementation of tannins and infected with *Escherichia coli*; Control not infected=basal diet without supplementation of tannins and not orally infected with *Escherichia coli*; 1% HTs/CTs infected= basal diet supplemented with 1% of tannins (Silvateam, Italy) and orally infected with *Escherichia coli*; 1% HTs/CTs not infected= basal diet supplemented with 1% of tannins (Silvateam, Italy) and not orally infected with *Escherichia coli*;
[5]Control without SA= basal diet without sodium salicylate and without tannins; 2% HTs/CTs = basal diet without sodium salicylate and with 2% of tannins (Silvateam, Italy); Control with SA= basal diet with sodium salicylate and without tannins; SA+ 2% HTs/CTs = basal diet with sodium salicylate and with 2% of tannins (Silvateam, Italy);

= No effect means that no differences were detected among treatments groups ($P>0.05$).

↑↓ Positive or negative effect means that the treatments with tannins showed differences compare to the control group ($P<0.05$)

Table 3. *Effects of tannins on pigs' zootechnical performances during the growing and finishing phases*

Source/ Type[1]/ Feeding	Animals[2]/ Duration (day)	Dose of tannin	Zootechnical performances[3]	References
Chestnut/ HTs / n.r.	6/ 40	Control, 0.25% HTs, 0.50% HTs	= ADG, FCR	Antongiovanni *et al.* (2007)
Chestnut and Quebracho/ HTs/CTs/ ad libitum	12/ 55	Control, 1.5 HTs/CTs; 3.0 HT/CT	= BW, ADFI; ↑ FCR	Bee *et al. (*2017)
Chestnut and Oak/ HTs / n.r.	25/ 30	Control, 0.20% HTs[4], 0.20% Oak[4]	= ADG	Brus *et al.* (2013a)
Chestnut/ HTs / *ad libitum*	6/ 70	Control, 1% HTs, 2% HTs, 3% HTs	= BW, ADG, ADF, FCR; ↓ ADF with 3%	Candek-Potokar *et al.* (2015)
Chestnut/ HTs / *ad libitum*	8/ 270	Control, 15% dried chestnuts, 25% dried chestnuts	↑ BW with 25% dried chestnuts	De Jesus *et al.* (2016)
Chestnut/ HTs / *ad libitum*	8/ 49	Control, 0.30% chestnut meal, 0.50% chestnut meal, 1% chestnut meal	= BW, ADFI; ↑ ADG, ↓ FCR[5] with 0.30% and 0.50%	Joo *et al.* (2018)
Chestnut/ HTs / Restricted	14/ n.r.	Control, 0.15% HTs, 0.30% HTs	= BW, ADG, ADFI	Galassi *et al.* (2019) Exp.1
Chestnut/ HTs / Restricted	7/ 6	Control, low protein diet, low protein diet + 0.53 HT %	= BW, ADFI	Galassi *et al.* (2019) Exp.2
Chestnut/ HTs/ *ad libitum*	4/ 7	Control, 5% HTs, 10% HTs, 15% HTs	= ADFI[6]	Lee *et al.* (2016)
Chestnut/ HTs/*ad libitum*	15/ n.r.	Control, 0.20% HTs	= ADG, ADF, FCR	Prevolnik *et al.* (2012)
Chestnut + oregano oil/ HTs/ *Restricted*	30/ 155	Control, 0.1% oregano oil + 0.1% HTs	=BW, ADG	Ranucci *et al.* (2015)
Chestnut/ HTs/ *ad libitum*	12/ 21	Control[7], 0.3% FraAcidDry[7], 0.15% HTs + 0.15% 4 acids[7]	= BW, ADG	Stukelj *et al.* (2010)
Chestnut and Quebracho/ HTs/CTs/ ad libitum	11/ 97	High PUFA 2%[8], High PUFA 2% with HTs/CTs 3%[8], Low PUFA[8], Low PUFA with HTs/CTs 3%[8]	= BW, ADG, ADFI, FCR[9]	Tretola et al. (2019)

[1] HTs= hydrolysable ; CTs= condensed (CTs).
[2]Number of animals per each treatment groups.
[3]BW= body weigh; ADG= average daily gain; ADFI=average daily feed intake; FCR: feed conversion ratio.
[4]HTs= basal diet supplemented with 0.20% of tannins extracted from chestnut tree (Tanin Sevnica d.d., Slovenia); Oak= basal diet supplemented with 0.20% of tannins extracted from oak tree (Tanin Sevnica d.d., Slovenia).
[5]Joo *et al*. (2018) reported Feed efficiency parameter (gain/intake).
[6]Lee *et al*. (2016) reported dry matter intake (g/day), a quadratic effect was detected (P=0.046).
[7]Control= basal diet without supplementation; 0.3% FraAcidDry= basal diet supplemented with 0.30% of commercial acidificant FraAcidDry (Perstorp Franklin; Waspik, The Netherlands); 0.15% HTs + 0.15% 4 acids= basal diet supplemented with 0.15% of tannins (Tanin Sevnica d.d., Slovenia) and with of 4 acids (lactic acid, citric acid, orthophosphoric acid and L and R malic acid).
[8] PUFA= Polyunsaturated fatty acid; High PUFA 2%= basal diet supplemnted with 2% of soy oil; High PUFA 2% with HTs/CTs 3%= basal diet supplemnted with 2% of soy oil and 3% of tannins (Silvateam, Italy); Low PUFA= basal diet without supplementation of 2% of soy oil and without 3% of tannins (Silvateam, Italy); Low PUFA with HTs/CTs 3%= basal diet without supplementation of 2% of soy oil and with 3% of tannins (Silvateam, Italy).
[9]Tretola *et al*. (2019) reported Gain-to-feed (kg/kg).
= No effect means that no differences were detected among treatments groups ($P>0.05$).
↑↓ Positive or negative effect means that the treatments with tannins showed differences compare to the control group ($P<0.05$).
n.r. = not reported

Table 4. Effects of tannins on *pigs'* intestinal health during the growing and finishing phases

Source/ Type/ Feeding	Animals[2]/ Duration (day)	Dose of tannin	Intestinal health	References
Chestnut/ HTs / *ad libitum*	6/ 70	Control, 1% HTs, 2% HTs, 3% HTs	= villus height and perimeter (duodenum); ↑ mucosal thickness (duodenum) with 1%; = morphology of jejunum, ileum, caecum and colon ascendens; ↓ mitosis in caecum and colon descendens; ↑ apoptosis in caecum, colon ascendens and colon descendens	Bilic-Sobot *et al.* (2016)
Chestnut and Oak/ HTs/ n.r.	25/ 30	Control, 0.20% HTs[3], Oak 0.20%[3]	= height of mucosa; ↓ height of epithelium; ↑ crypt/villi ratio	Brus *et al.* (2013a)
Chestnut/ HTs/ *ad libitum*	6/ 70	Control, 1% HTs, 2% HTs, 3% HTs	↓ skatole in caecum and descending colon with 1% and 2%; ↓ skatole in caecum; ↑ skatole in descending colon with 3%	Candek-Potokar *et al.* (2015)
Chestnut/ HTs/ *ad libitum*	42/ 16	Control not infected[4], Control infected[4], 0.30% HTs infected[4]	↓ *Salmonella Typhimurium in vitro;* = *Salmonella Typhimurium* shedding *in vivo*	Van Parys *et al.* (2010)
Chestnut and Quebracho/ HTs/CTs/ ad libitum	11/ 97	High PUFA 2%[5], High PUFA 2% with HTs/CTs 3%[5], Low PUFA[5], Low PUFA with HTs/CTs 3%[5]	Without tannin: ↑ Chao1[6]; ↑ OUT[6]; ↑ OUT[6]; ↑ PD[6]; = *Oscillospira* genus of *Ruminococcaceae* family, *Treponema, Sphaerochaeta;* = *Lactobacillales* order, *Streptococcaceae, Veillonellaceae* families, *Proteobacteria* *With Tannins:* ↓ Chao1[6]; ↓ OUT[6]; ↓ Shannon; ↓ PD[6]; ↑ *Oscillospira* genus of *Ruminococcaceae*, *Treponema, Sphaerochaeta;* ↓ *Lactobacillales* order, *Streptococcaceae, Veillonellaceae* families, *Proteobacteria*	Tretola *et al.* (2019)

[1] HTs= hydrolysable ; CTs= condensed (CTs).
[2]Number of animals per each treatment groups.
[3]HTs= basal diet supplemented with 0.20% of tannins extracted from chestnut tree (Tanin Sevnica d.d., Slovenia); Oak= basal diet supplemented with 0.20% of tannins extracted from oak tree (Tanin Sevnica d.d., Slovenia).
[4]Control not infected= basal diet without supplementation of tannins and not infected with *Salmonella Typhimurium*; Control infected; dasal diet without supplementation of tannins and infected with *Salmonella Typhimurium*; 0.30 HTs infected: basal diet with the supplementation of tannins (Global Nutrition, France) and infected with *Salmonella Typhimurium*.
[5]PUFA= Polyunsaturated fatty acid; High PUFA 2%= basal diet supplemnted with 2% of soy oil; High PUFA 2% with HTs/CTs 3%= basal diet supplemnted with 2% of soy oil and 3% of tannins (Silvateam, Italy); Low PUFA= basal diet without supplementation of 2% of soy oil and without 3% of tannins (Silvateam, Italy); Low PUFA with HTs/CTs 3%= basal diet without supplementation of 2% of soy oil and with 3% of tannins (Silvateam, Italy).
[6]Chao1= Bacterial community index; OUT= Operational taxonomic units; Shannon= Shannon diversity index; PD= Phylogenetic diversity index.
= No effect means that no differences were detected among treatments groups ($P>0.05$).
↑↓ Positive or negative effect means that the treatments with tannins showed differences compare to the control group ($P<0.05$).