Immunomodulatory potential of dietary soybean-derived isoflavones and saponins in pigs[1]

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ABSTRACT: In this review, the potential for use of soy-derived bioactive compounds as immunomodulatory feed additives in pigs is discussed. Soy is a major component of the modern U.S. swine diet in today's commercial industry, providing the bulk of dietary AA necessary for growth and production. However, soy use has generally been limited in early growth phases, during which the risks of immunological insult and disease are among the highest. Improvements of soybean processing and development of soy protein products with little to no antinutritional factors have made soy more appropriate for use in young pigs but additional processing may affect bioactive compound levels in the feed. The bioactive compounds of interest for this review are soy isoflavones and soy saponins. Soy isoflavones are flavonoid compounds with a range of biological activity including moderate estrogenic effects at low biological concentrations. Although estrogenic effects are of more interest in human medical research, isoflavones are also known for their anti-inflammatory, antioxidative properties at cellular levels, engaging several receptors and pathways including inhibition of NF-κB activation and inducible-nitric oxide synthase enzymes, thereby ascribing antiviral properties. Saponins, amphipathic glycoside compounds, also engage anti-inflammatory pathways, though their biological activity in pigs has not been well investigated and seem to mainly be observed on the mucous membrane in the gastrointestinal tract. Regarding use as an immunomodulatory feed additive, supplemental soy isoflavones have been shown to improve immunological status of pigs and produce mild improvements of growth performance under certain disease challenges including porcine reproductive and respiratory syndrome virus. Although more in vivo research in pigs is needed to fully understand biological activity of these compounds in the live animal, soy-derived bioactive compounds show great potential as a health promoting feed additive for the modern swine industry.

Key words: disease, immune system, isoflavones, saponins, soybean, swine

J. Anim. Sci. 2018.96:1288–1304
doi: 10.1093/jas/sky036

INTRODUCTION

With implementation of the U.S. Veterinary Feed Directive, a government mandate seeking to cease long-term applications of in-feed antibiotics as growth promotants, livestock producers, and nutritionists are tasked with the transition of feeding livestock with greater restrictions on the use of subtherapeutic antibiotic interventions (FDA, 2015). This has generated significant interest in development of feed additives and utilization of value-added feed ingredients in diets fed to livestock in an attempt to improve animal growth performance and subsequent product quality. Of the numerous feed additives currently being investigated or employed, soybean-derived bioactive compounds

[1]The authors gratefully acknowledge funding provided by the United Soybean Board for this literature review. No conflicting or professional interests exist for these authors.

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Received October 13, 2017.
Accepted February 15, 2018.

have peaked the interest of human and animal nutritionists. Soybean meal (**SBM**) and further processed soybean products are included in a majority of commercial diet formulations for swine and poultry, making incorporation of these bioactive compounds in current feeding regimens a readily attainable opportunity for swine producers and nutritionists.

In regard to soy-derived bioactive compounds, isoflavones have garnered much attention due to their diverse range of biological activity including estrogenic and inhibitory actions on a variety of target cell populations. The aim of this review is to discuss soybean use in the industry today, with specific emphasis on biological actions of isoflavones, the mechanisms by which they exert these actions, and biological and processing effects on the bioavailability of dietary isoflavones. Saponins, another soy-derived bioactive compound, will also be discussed in a similar manner, though to a lesser extent due to a general lack of available information. Finally, this review will compare applications of soy-derived compounds in the swine industry; specifically, in pigs experiencing disease challenge, and discuss what implications their action has on animal productivity and performance, and potential applications in a commercial swine production setting.

FEEDING SOY IN CURRENT SWINE PRODUCTION SYSTEMS

Soybeans have a rich history of use as valuable protein and fat sources in both human and animal diets. By 1982, SBM became the most commonly used protein source in livestock diets in the United States, over other popular sources including cottonseed meal and fish meal by a wide margin (Shurtleff and Aoyagi, 2007). According to the USDA National Agricultural Statistics Service, ~30,035 metric tons of soybeans were used domestically for livestock feed in 2016 alone (USDA-NASS, 2017). SBM is created through the collection of soy oil, a process by which soybeans are cleaned, cracked, de-hulled, and heat-treated before being crushed into flakes. Flakes are then washed with a volatile solvent, such as hexane, to extract crude oil, thereby leaving behind a high-quality protein ingredient. Oil-extracted flakes (i.e., defatted soybeans) undergo an additional heating step (i.e., toasting) to remove residual solvent and, in many cases, are mixed back in with soybean hulls and ground to form SBM, which can be fed to livestock. Toasting soybean flakes towards the end of processing also contributes to the removal of certain antinutritional factors, mainly urease and lipoxygenase enzymes (Shurtleff and Aoyagi, 2016).

SBM is the most commonly used plant-based protein source in swine diets throughout the post-weaning growth period in the United States. Its popularity stems from a relatively high digestible protein fraction, high lysine content to complement dietary corn inclusion, and the ability to extract antinutritional factors through processing (Pettigrew et al., 2017). And while SBM remains a widely used protein source in swine diets, advancements in soybean processing techniques and applications have resulted in development of value-added feedstuffs. Soy protein concentrate (**SPC**) is produced from defatted soybean flakes, as is SBM, but undergoes additional processing steps to remove the soluble carbohydrate fraction. Two methods are employed for this step, extraction or enzymatic digestion, with extraction being more common. Extraction is the process of solubilizing carbohydrates with an aqueous alcohol solution, removing not only vulnerable carbohydrates but also additional antinutritional factors including oligosaccharides, as well as estrogenic and antigenic factors (Peisker, 2001). Soy protein isolate (**SPI**) is essentially the same as SPC, with the residual nonsoluble carbohydrate fraction left behind after alcohol extraction removed. Further processing of soybeans into SPC and SPI results in products with a higher protein composition compared with SBM (65% to 90% and ≥90% vs. 40% to 50% for SPC, SPI, and SBM, respectively; Shurtleff and Aoyagi, 2016). It is important to note that processing steps involved in the manufacture of SPC and SPI do affect available isoflavone concentrations, which is discussed later in this review.

Despite the popularity of using SBM in swine diets, among the different growth phases of current production systems, diets of early-weaned pigs traditionally contain minimal amounts of soy. This practice of low SBM inclusion is largely driven by immaturity of the weanling pig's digestive capacity and effectiveness, in addition to presence of dietary antinutritional factors. However, developments in soy processing and treatment of SBM-containing diets have made feeding soy protein to early-weaned pigs more appropriate. In addition to removal of antinutritional factors through production of SPC and SPI, microbial fermentation of soy feedstuffs can be utilized during this sensitive period for weaned pigs. Early-weaned pigs provided fermented liquid diets during the initial post-weaning period typically show improved growth performance (~22% improvement for average daily gain) and reduced disease level with respect to enteric

pathogens such as *Escherichia coli* and *Salmonella* due to unfavorable acidic gastrointestinal environment (Stein, 2002). Early-weaned pigs that receive microbial-fermented soy protein sources in more traditional dry diets similarly show improved growth performance, increased nutrient digestibility, and, under certain experimental conditions, reduced diarrhea scores, an important indicator of improved disease status in young pigs (Cho et al., 2007; Kim et al., 2007; Kim et al., 2010; Zhang et al., 2013).

SOY ISOFLAVONES—METABOLISM AND BIOAVAILABILITY

Soy Isoflavone Structure and Metabolism

Isoflavones are naturally occurring flavonoid compounds found at high concentrations in the soybean plant. They are characterized as phytoestrogens due to their structural similarities to 17β-estradiol (E2), though their biological action is not exclusively estrogenic in nature. Genistein and daidzein comprise the majority of isoflavones found in soybeans and are the subject of most ongoing research. Isoflavones exist in the plant in nonbioavailable glycoside forms (e.g., genistin and daidzin). For isoflavones to be absorbed and biologically available, they must be hydrolyzed within the digestive tract by host-derived β-glycosidases to their aglycone forms (e.g., genistein and daidzein). β-Glycosidases are present as brush border enzymes in the small intestine, but are more prevalent as microbial enzymes in the hindgut of the gastrointestinal tract of monogastric species like the pig (Cassidy et al., 2006). Genistein and daidzein are either directly absorbed in the intestine or undergo further metabolism via hydrogenation to other bioactive compounds, including equol, 5,7,4′-rihydroxyisoflavan, 4,7,4′-trihydroxyflavan, dihydrodiadzein (**DHD**), dihydrogenistein (**DHG**), and others. These hydrogenation reactions are nonspecific, resulting in nonuniform mixtures of these compounds (Chang et al., 1995). In humans, isoflavones absorbed from the gastrointestinal tract are present in plasma for 5 to 8 h post-prandial (Cassidy et al., 2006). Only a small fraction (<2%) of isoflavones enter circulation with a majority of isoflavones being directly metabolized by enterocytes, which may indicate that isoflavones exert maximum functionality locally in the gastrointestinal tract (Masilamani et al., 2012). Similar plasma concentrations of isoflavones have been observed in humans and pigs receiving soy-containing diets, though the proportions of different isoflavones and their metabolites excreted in urine differ, suggesting that individual isoflavone biological activity and cellular consumption may also differ between species (Gu et al., 2006). In addition to differences in metabolism of dietary isoflavones, the bioavailability of isoflavones in a given feedstuff can vary significantly.

Factors Affecting Bioavailability of Soy Isoflavones

There are several factors that affect bioavailability of dietary isoflavones, the first of which influence isoflavone content in the soybean plant itself. Factors such as plant variety, location, and growing conditions can all affect isoflavone contents of any given yield and variations of these factors been considered for some time. Studies have demonstrated that total glycoside isoflavone content of a single soybean plant variety can vary as much as 200 mg/g when the same variety is grown at different regional locations. On a year-to-year basis, variation in isoflavone concentrations of up to 50% has been reported within the same growing location (Eldridge and Kwolek, 1983). Additionally, differing patterns of individual isoflavone distribution among soybean crops maintained in different countries (including United States, Brazil, Argentina, and Japan) suggest that plant genetics have an effect on isoflavone development and metabolism (Wang and Murphy, 1994; Goerke et al., 2012). Although plant variation contributes to total isoflavone content within the raw soybean, soybean processing has an even greater influence on final product concentrations.

Processing techniques greatly affect the final isoflavone content in soy products and, subsequently, their bioavailability. Although most of the data available for soy processing effects are from products for human consumption, the principles remain the same for livestock feedstuffs. Roasted soy products, including SBM, contain the highest bioavailable content of glycoside isoflavones, whereas SPC and SPI contain the lowest concentrations (Erdman et al., 2004). This difference is due to ingredient processing methods, and while the defatting process of full fat soybeans in the generation of SBM does not affect isoflavone concentrations, aqueous alcohol extraction utilized to generate SPC removes >95% of total isoflavones (Kuhn et al., 2004). Manufacturing processes for SPI, a product mostly devoid of fats and carbohydrates, result in over a 50% reduction in isoflavone content due

to the alkaline extraction methods used. A summary of typical isoflavone concentrations in SBM, SPC, and SPI can be found in Table 1. Thus, isoflavones are lost into the alkaline-insoluble fraction, which is typically discarded in the processing procedure. Alternatively fermented soy products contain a higher bioavailable content due to microbial enzyme hydrolysis of isoflavones from their glycoside to aglycone forms (Wang and Murphy, 1996).

Food matrix may also affect isoflavone metabolism kinetics. In a human trial, liquid diets resulted in faster isoflavone absorption rates and higher peak plasma concentrations compared with solid diets (Cassidy et al., 2006). As mentioned previously, fermented liquid feeding and fermented SBM have been suggested to improve nursery pig performance. Although no confirmatory evidence exists, it may be possible that the isoflavone fraction of diets containing fermented soy products may be more bioavailable, resulting in faster absorption and delivery to target cells, thereby contributing to beneficial performance effects.

SOY ISOFLAVONES—BIOLOGICAL ACTIVITY

Estrogenic Properties of Soy Isoflavones

Due to their structural similarities to 17β-estradiol, isoflavones have the ability to act as weak agonists or antagonists for endogenous estrogen, depending on the concentrations present at the cellular level. Isoflavones, especially genistein, preferentially bind estrogen receptor-β (ER-β) vs. estrogen receptor-α (ER-α); alternatively, soy saponins bind more strongly to ER-*a*, as discussed later in this review. Estrogen receptor-β is expressed on a variety of cell types, including uterine epithelial cells and immune cells such as blood monocytes and tissue macrophages, and is the most prevalent estrogen receptor found within the gastrointestinal tract. Although isoflavones may act as estrogen receptor modulators, their potency is 1,000-fold lower than that of endogenous estrogen, so it is unlikely physiological effects induced by isoflavones, especially in regard to response to disease, is through estrogenic activity alone (Andres et al., 2009). There is evidence that ER-β modulates ER-α activity during uterine development in mice via antiproliferative effects, which could indicate that circulating isoflavones may influence reproductive development (Weihua et al., 2000). In pigs, there is less information available on the effects of soy isoflavones on the reproductive tract of intact females. One study evaluated the effect of oral genistein administration on hormonal patterns of gilts during estrus and following artificial insemination (AI). Gilts exposed to genistein via oral administration (1 mg/kg BW twice daily) demonstrated increased plasma concentrations of oxytocin and prostaglandin E_2 (**PGE_2**) and a more frequent pulsatile pattern in prostaglandin F2α concentrations around administration of AI. Alternatively, genistein decreased plasma luteinizing hormone (LH) concentrations after AI was administered compared with control gilts. These results suggest that hormonal release may be altered by soy isoflavones, likely through interactions with estrogenic receptors, but the impact of these alterations on gilt reproductive performance is not known (Norrby et al., 2011).

Anti-inflammatory and Anti-oxidant Properties of Soy Isoflavones

Soy isoflavones possess both anti-inflammatory and antioxidant activities, which has generated a lot of attention for applications in both humans and animals. These activities are mainly observed through isoflavone inhibitory effects on tyrosine-specific protein kinases and nuclear factor-κβ (**NF-κβ**) transcription. Tyrosine-specific protein kinases act as regulators for a broad spectrum of cellular functions. These protein kinases respond to several cell-signaling molecules including growth factors (i.e., epithelial growth factor, IGF-1) and cytokines, regulating cell proliferation and transformation properties (Akiyama et al., 1987). They are also the target of several virus types and are likely the main mechanism of action of reducing viral infectivity, though several effects have been reported and thus the reduction in infectivity is

Table 1. Isoflavone concentrations (mg/kg of product) of common soybean-derived products

Item (mg/kg)	Soybean meal	Soybean protein concentrate	Soybean protein isolate
Genistein	1,147	52.6	573
Daidzein	808	57.8	308
Glycitein	161	15.7	85.4
Total isoflavones	2,096	115	911

Values obtained from the USDA Agricultural Research Service Nutrient Data Laboratory (USDA-ARS, 2016).

likely a combination of several pathways (Andres et al., 2009). For this review, effects of isoflavones on the antigen-specific immune response, antioxidative cellular pathways, and viral infectivity will be discussed in more detail.

Soy isoflavones have been found to suppress antigen-specific immune responses in addition to more broad anti-inflammatory activities. Antigen-specific immune responses are highly involved in sensitization to dietary antigens, establishment of allergic responses, and autoimmune reactions. Of the antigen-specific immunomodulatory effects mediated by isoflavones, interactions with dendritic cells seem to be involved in the mechanism of action. Mice fed genistein- and daidzein-enriched diets that were orally sensitized and challenged with peanut-derived proteins exhibited reduced anaphylactic symptoms, with 35% to 40% having no or significantly less severe symptoms compared with control mice, and 25% to 30% less mast cell degranulation. Isoflavones also decreased the synthesis of peanut-specific IgE and IgG_{2a} humoral antibodies, which suggests that these isoflavones may also mediate B-cell activity. However, since these effects were not maintained when peanut-derived proteins were injected intraperitoneally, the authors suggested that diet-derived isoflavones may be more likely to elicit local inhibition of dendritic cells, such that supraphysiological concentrations would be necessary for total systemic suppression of allergic sensitization (Masilamani et al., 2011). In an in vivo collagen-induced arthritis model, representative of an autoimmune reaction, mice injected with genistein had suppressed delayed-type hypersensitivity reactions to oxazolone (cell-mediated) and granulocyte-mediated (non-cell-mediated) responses compared with control animals. The suppression of cell-mediated responses was likely not through estrogen-receptor binding since coinjection with ICI 182,780, an estrogen-receptor antagonist, did not prevent inhibitory action of genistein. More likely, the mechanism of action for cell-mediated immune inhibition is through protein kinase inhibition in B-cells, which reduces receptor-mediated signaling, antigen processing, and subsequent antibody production (Verdrengh et al., 2003). Although these findings are relevant, especially for human medical researchers, the antioxidant potential for isoflavones is of stronger interest for animal nutritionists.

The antioxidant activity of isoflavones is mainly through their inhibition of NF-κβ, a protein complex that controls the transcription of many proinflammatory genes. Based on in vitro findings, antioxidative effects via NF-κβ inhibition include reduction in inducible nitric oxide synthase (**iNOS**) expression, nitric oxide (**NO**) production, cyclooxygenase-2 (**COX-2**) expression, and PGE_2 production (Dia et al., 2008). In LPS-induced RAW 264.7 macrophages, genistein decreased NO production in a dose-dependent manner (IC_{50} = 69.4 μ*M*) without adverse effects on cell viability, suggesting that genistein was acting through inhibition of tyrosine kinase receptors. In the same experiment, 50 and 100 μM concentrations of genistein-reduced accumulation of thiobarbituric acid reactive substance (**TBARS**), an indicator of lipid oxidation, increased concentrations of glutathione, an important antioxidant, and increased activities of antioxidative enzymes, superoxide dismutase and catalase (Choi et al., 2003). When provided individually, genistein and daidzein decreased NO production and iNOS expression in a dose-dependent manner (genistein at 10 μ*M* vs. daidzein at 100 μM), decreased PGE_2 production, and decreased COX-2 expression (genistein at 100 μM vs. daidzein at 300 μM). However, the magnitude of inhibitory effects was highest when a mixture of soy isoflavone glycosides was used, suggesting interactions among the isoflavone forms (Dia et al., 2008). Similarly, in lipopolysaccharide (**LPS**)-induced murine J774 macrophages, genistein and daidzein were shown to elicit moderate inhibitory effects via NF-κβ on iNOS expression (57% to 72% inhibition, IC_{50} for genistein and daidzein was ~30 and ~70 μM, respectively) (Hämäläinen et al., 2007). Many of these pathways are employed directly during bacterial and viral insults, but there is additional evidence that isoflavones may also affect specific viral infectious pathways that may lead to reduced viral infectivity.

Anti-viral Properties of Soy Isoflavones

Building upon the backdrop of broad immunomodulatory actions of isoflavones, soy isoflavones may benefit the immune response under viral-challenged conditions. Although many of the findings discussed in this section were gathered from studies involving in vitro human cell lines or mice, most of the viruses are of the same classes as those commonly present in the swine industry. Swine-specific viruses and immunomodulatory effects of isoflavones in such disease models will be discussed subsequently.

Rotavirus, a virus that causes acute enteritis in neonates of several mammalian species, is one such virus whose infectivity is modulated by

soy isoflavones. In a study by Andres et al., genistein and daidzein inhibited infectivity of human rotavirus in cultured macrophages (MA-104 cell line). Reductions by genistein alone reduced rotaviral infectivity by 33% to 62% and a mixture of isoflavones resulted in a reduction of 66% to 72%. Mixtures not containing genistein lost antiviral activity, indicating that genistein is likely the most active isoflavone in mediating rotaviral inhibition. This particular inhibition was thought to be through inhibition of tyrosine-specific protein kinase-induced activation of α2-β1 binding of the virion (Andres et al., 2007).

In an in vitro assay evaluating flavonoid modulation on infectivity of another common virus, herpes simplex virus types-1 and -2 (HSV-1 and HSV-2), genistein exhibited high inhibitory activity against cytopathic effects (**CPE**; i.e., structural changes caused by viral infection in host cells that result in host cell death). These effects were considered moderate (50% to 80% CPE inhibition at 5 µM concentrations), but were observed for both viral types (Lyu et al., 2005). Reductions in herpes virus infectivity by genistein were also demonstrated for *Bovine herpesvirus* (BHV-1), where genistein reduced BHV-1 viral replication by 90% by 18 h post-inoculation when infected cells were treated with 25 µM at 0 and 12 h (Akula et al., 2002). Potential future applications of these findings in the swine industry would be investigations into antiviral effects of genistein on *Suid herpesvirus* (SuHV1), the causative agent of pseudorabies, a disease which causes respiratory infections in pigs under 2 mo of age and abortions in pregnant sows.

Modulation of Epithelial Tight Junctions by Soy Isoflavones

Along with modulation of cellular pathways through cell surface and nuclear receptors, isoflavones have direct effects on cell membranes and their function. Soy isoflavones modulate mucosal barrier function between intestinal epithelial cells through interactions with tight junction multiprotein complexes that regulate paracellular transport. The main proteins that comprise this complex include occludins, zonula occludins, and claudins. Zonula occludins-1 (ZO-1) binds transmembrane occludins and claudins, anchoring them to cytoskeletal actin, permitting alterations of junction size between cells. The activity of these proteins are under the influence of endocrine and immune stimuli via activation of protein kinases, altering intestinal permeability during times of stress or illness (Turner, 2009). This can allow increased passage of large molecules, such as bacteria or dietary antigens, across paracellular junctions increasing mucosal inflammatory reaction (Lambert, 2009).

It has been demonstrated that occludins undergo tyrosine phosphorylation during times of tight junction disruption, a process that is inhibited by genistein in vitro. Cultured Caco-2 cells (i.e., a heterogenous line of human colonocytes) subjected to oxidative stress resulted in rapid increases in tyrosine phosphorylation of occludins, ZO-1, E-cadherin (an adherence junction protein), and β-cantenin, resulting in movement of these proteins away from intercellular junctions. Moreover, a decrease in transepithelial electrical resistance (**TER**; measure of intestinal permeability) was observed, and application of 300 µM of genistein prevented all observed effects (Rao et al., 2002). In an independent study, Caco-2 cells cultured with daidzein and genistein consistently expressed increased TER responses over a 24-h period without the treatment with a cellular stressor, which suggests that isoflavones may have effects on barrier function even in the absence cellular insult (Noda et al., 2012). Additionally, in an experiment using cultured HT-29/B6 colonic epithelial cells stimulated with tumor necrosis factor-α (**TNF-α**; a proinflammatory cytokine), genistein treatment (185 µM) increased TER (Schmitz et al., 1999).

In addition to effects on total gastrointestinal mucosal permeability, genistein has also been demonstrated to prevent uptake of enteric bacteria by enterocytes. Treatment of cultured Caco-2 and HT-29 enterocyte cell lines exposed to *Salmonella typhimurium* and *E. coli* with 300-µM genistein reduced bacterial internalization and prevented reductions in TER, suggesting that genistein supports intestinal barrier stability (Wells et al., 1999). The relevance of these effects on intestinal barrier function is not clear because it is unlikely dietary concentrations of soy isoflavones would reach concentrations used in these in vitro assays; however, it could indicate potential applications of pharmacological administration. A summary of all physiological effects by soy isoflavones discussed in this review can be found in Table 2.

SAPONINS—STRUCTURE, METABOLISM, AND BIOAVAILABILITY

Saponins, another biologically active compound found in soybean plants and others, are classified as amphipathic glycosides. They earned this classification with their natural detergent characteristics,

Table 2. Summary of physiological effects by soy isoflavones and saponins (includes in vitro and in vivo findings)

Soy bioactive	Interact with estrogen receptors	Anti-inflammatory properties	Anti-oxidative properties	Anti-viral properties	Anti-protozoal properties	Interact with intestinal epithelial junctions
Isoflavones	Yes; primarily estrogen receptor β[a]	Yes; inhibit tyrosin-specific protein kinase pathways[b]	Yes; inhibit NFκB activation, decrease lipid oxidation, and increase anti-oxidative enzyme activity[c]	Yes; reduce infectivity of rotavirus and herpes simplex virus *in vitro*[d]	No results found for soy isoflavones	Yes; decrease intestinal epithelial permeability[e]
Saponins	Yes; primarily estrogen receptor α[f]	No results found for soy saponins	Yes; inhibit NFκB activation, act as superoxide scavenger[g]	No results found for soy saponins	No results found for soy saponins	Yes; increase intestinal epithelial permeability[h]

[a]Andres et al., 2009; Weihua et al., 2000.
[b]Akiyama et al., 1987; Verdrengh et al., 2003.
[c]Choi et al., 2003; Dia et al., 2008; Hämäläinen et al., 2007.
[d]Akula et al., 2002; Andres et al., 2007.
[e]Noda et al., 2012; Rao et al., 2002; Schmitz et al., 1999.
[f]Andres et al., 2009.
[g]Francis et al., 2002; Kang et al., 2005.
[h]Johnson et al., 1986.

possessing surfactant-type properties. The physical structure of saponins includes a steroid or triterpenoid nucleus with one or more carbohydrate side chains, including oligosaccharides and monosaccharides with D-glucose and D-galactose being the most common (Anderson and Wolf, 1995; Cheeke, 2000). The *Yucca schidigera* and *Quillaja saponaria* plants are the most well-known and researched saponin-containing plants, although soy-derived saponins have also been investigated (Cheeke, 2000). Similar to isoflavones, saponins are metabolized from their glycone form to a more biologically active aglycone form via the action of β-glycosidases. Saponins enhance their own absorption by enterocytes through a process called self-micellation, which involves saponins forming a micelle-like structure which increases saponin solubility (Gao et al., 2012). This lipid-like behavior allows saponins to interact with cholesterol molecules as well, which has significant implications on biological activities of saponins. Due to similar structural traits with isoflavones, the bioavailability of saponins is influenced by many of the same factors including plant type, growing conditions, and soybean processing conditions. It may be important to note that though concentrations of these bioactive compounds are affected by variations in growth conditions, the concentration of saponins does not appear to be correlated to the total biologically available isoflavone content in the soybean (Vasantha Rupasinghe et al., 2003). As with isoflavones, applications of heat or acidic environments such as fermentation result in more conversion of saponins to their aglycone form, thus increasing bioavailability. Additionally, though there may be concern that saponins bind oligosaccharides, an antinutritional factor for swine, both are contained within the same plant fraction that is susceptible to extraction or enzymatic digestion processing, in which case both compounds would be removed (Peisker, 2001). Although the focus of this review is specifically bioactive compounds derived from soy, the direct research of soy saponins is somewhat limited and thus biological actions of saponins in general will be discussed in Soy Saponins—Biological Activity.

SOY SAPONINS—BIOLOGICAL ACTIVITY

Antiprotozoal Properties of Saponins

Although potentially more relevant to ruminant and poultry species, Yucca saponins have been shown to exhibit antiprotozoal activity. Saponins form complexes with cholesterol molecules within protozoal membranes, resulting in membrane disruption and ultimately protozoal cell death. The ability to form these complexes relies on intact saponin structure, which requires a steroid or triterpenoid nucleus attached to carbohydrate side chains. This becomes a limitation for feed applications of saponins in ruminants as antiprotozoal agents are exposed to subsets of rumen microbial populations possessing saponin-hydrolyzing enzymes. Alternatively, applications for saponins in nonruminant and ruminant species are modulators of protozoal gastrointestinal disease. Research into the effects of Yucca saponins on giardiasis

indicates that saponins may be effective at killing giardia tropozites within the intestine, particularly within the distal small intestine (Cheeke, 2000; McAllister et al., 2001). It is these effects within the intestine that makes saponins compounds of interest for potential anticoccidial applications, as giardia and coccidia species share similar life cycles. There is also some evidence that glycoside forms of saponins have high toxicity effects on some strains of fungi, though saponins with disaccharide side chains or aglycone forms lack this activity (Francis et al., 2002).

Modulation of Intestinal Permeability by Saponins

It has been well demonstrated that saponins have high biological activity in the intestine, resulting in generally negative effects on enterocyte function. Similar to interactions with cholesterol moieties in protozoal cellular membranes, saponins interact with enterocyte membranes, affecting mucosal barrier function and nutrient absorption. These effects include changes in cellular permeability, uptake of larger molecules, and disruption of active transport channels. In vitro, some saponin forms have been shown to inhibit mucosal transport channels and allow uptake of larger molecules not typically absorbed by enterocytes by lowering the transmural potential difference, the electrochemical gradient that drives active transport (Francis et al., 2002). It is important to note that soy-derived saponins have been demonstrated to have approximately 10-fold lower activity in vitro, which could indicate that they may not induce these negative effects in the animal (Johnson et al., 1986). In rats fed Gysophylla saponins, there was histological evidence of damage to intestinal villi, though this appeared to be counteracted by increased enterocyte proliferation (Francis et al., 2002). Saponins also cause rapid depolarization of mucosal membranes, even at very low concentrations, which may also contribute to poor nutrient absorption, decreased growth, and decreased feed conversion ratio in animals. The mechanism of action causing these changes is not well known, though it could be due to saponins binding integral membrane cholesterol (i.e., as occurs in protozoal species) or direct binding of numerous ligands within the intestine that increase or decrease functional association with mucosal membranes. It is also not clear whether saponins found in peripheral tissues are exclusively absorbed via intestinal cells or through increasing enterocyte permeability, and it is important to note that biological activity is not observed for all saponin types. Finally, there exists evidence that saponins can reduce absorption of certain nutrients, including iron and proteins, through complexation within the intestinal lumen (Francis et al., 2002; Southon et al., 2017).

Immunological Activities of Saponins

Beyond direct interactions with cellular membranes, of both native and non-native origin, saponins possess many immunomodulatory activities. Some of these activities are shared with isoflavones, including antioxidant functions. Saponins with triterpenoid nuclei, like those found in soybean plants, have α and β ester groups that complex with cysteine residues on transcription factor NFκB, rendering this transcription factor nonfunctional and thereby dampening the proinflammatory response and reducing the release of reactive oxygen species (Francis et al., 2002). These findings were corroborated in an experiment evaluating the effects of soy saponins on responses of peritoneal macrophages stimulated with LPS in vitro (Kang et al., 2005), where soy saponins inhibited the release of PGE_2, NO, TNFα, and monocyte chemotactic protein-1 (MCP-1) in a dose-dependent manner (range from 30 to 100 μg/mL) without affecting cell viability. Researchers concluded the reduced inflammatory response occurred via suppression of NFκB activation by sequestering this transcription factor in the cytosol and preventing nuclear translocation (Kang et al., 2005). In addition to preventing release of oxidative factors, a particular subset of soy saponins (group B) contain sugar residue 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**DDMP**) that acts as a superoxide scavenger and can effectively reduce oxidative damage (Francis et al., 2002). It is important to mention that the sugar residue DDMP remains intact through conventional processing procedures, so soy saponins have high potential to serve as candidate antioxidant targets for inclusion in foods and feeds.

Along with antioxidant activity, saponins have also been indicated as potential enhancers of passive immunity due to their permeability effects on intestinal mucosa. Quillaja-derived saponins tended to increase serum IgA concentrations and rate of growth in low birth weight piglets (<1.0 kg) when administered as a 5 mg/kg BW oral bolus on 3 and 4 d of age. These researchers speculated that an advancement in passive immunity by saponins may be more important for piglets receiving limited access to immunoglobulin-rich colostrum (Garcia et al., 2004). The mechanism of these findings would

need to be further investigated, but they suggest that saponins could potentially be employed with supplemental or artificial colostrum administration to improve immunoglobulin absorption in at-risk piglets through increasing enterocyte permeability.

Owing to their immunomodulatory potential, saponins have long been utilized as vaccine adjuvants, thereby increasing the immunogenicity, or ability to provoke an immune response, of proteins or peptides within vaccines. To date, most research focusing on the adjuvant applications for saponins has evaluated Quillaja-derived saponins, which are potent inducers of antibody production to soluble T-dependent antigens. As such, saponins have been shown to improve total antibody titers following vaccine administration and increase CD8+ cytotoxic T lymphocyte (**CTL**) responses to subunit antigens, which are both integral parts of a cell-mediated immune response (Powell and Newman, 1995). Soy-derived saponins may possess especially strong adjuvant activity, as measured via the passive hemagglutination assay, with little hemolytic effects on red blood cells, a commonly observed adverse reaction to intramuscular administration of saponins (Oda et al., 2000). These effects could be a result of several proposed mechanisms of action including saponin interactions with antigen presenting cells, thereby causing secretion of immune signals such as cytokines and membrane-permeabilizing activity. Increased cell permeability would allow antigens to gain access to the endogenous pathway for antigen presentation, which subsequently promotes strong CTL responses (Rajput et al., 2007). A summary of all physiological effects by soy saponins discussed in this review can be found in Table 2.

APPLICATIONS FOR SOY BIOACTIVES IN THE SWINE INDUSTRY

Soy Bioactive Compounds as a Swine Growth Promotant

As have many feed additives, soy-derived bioactive compounds such as isoflavones and saponins have been evaluated for their use as growth-promoting compounds in swine diets. Although it appears that these compounds have some effects of growth performance, the findings are inconsistent and often provide conflicting results.

In an early study, sows were provided isoflavone-enriched diets containing 8 mg of daidzein per kg of feed from day 85 of gestation to farrowing and developmental effects on piglets were quantified. Gestational daidzein supplementation resulted in greater birth weights for male piglets (females were unaffected) and increased the number of live born piglets. Daidzein supplementation of sow diets also increased expression of IGF-1 receptors in skeletal muscle of piglets. Because IGF-1 direct contributes to the regulation of skeletal muscle growth, this may indicate that gestational exposure to soy isoflavones could have impacts on offspring growth patterns. Alternatively, daidzein supplementation reduced expression of ER-β in the hypothalamus, indicating gestational daidzein ingestion may also influence the neuroendocrine system of offspring (Ren et al., 2001). In contrast, dietary daidzein provided at 1 mg/kg from day 85 of gestation to farrowing had no effects on litter size or birth weights in a subsequent study (Rehfeldt et al., 2007). While providing conflicting evidence, these two experiments suggest that dietary soy isoflavones likely cross-placental membranes, but subsequent effects of exposure during gestation may depend on dose and isoflavone types. When applied to cultured porcine skeletal muscle cells, genistein and daidzein do not appear to change mRNA expression of epidermal growth factor (**EGF**) and IGF-1 receptors at typical dietary concentrations (1 to 10 µM), though mRNA expression decreased for these receptors at high concentrations (100 µM) (Kalbe et al., 2008). Collectively, the available evidence indicates that high concentrations of isoflavones may be required to affect autocrine or paracrine functions of skeletal muscle cells, but it cannot be confirmed whether circulating isoflavones would reach those concentrations at the level of myocytes, so in vitro findings should not necessarily be extrapolated to in vivo conditions.

Although in vitro studies introduce some speculation of the true effects of soy isoflavones on growth, there are live animal studies that have investigated the effects of dietary isoflavones with production-focused aims including influence on growth performance, carcass traits, and meat quality. In a series of experiments, barrows and gilts receiving control or isoflavone-supplemented diets based on practical ingredients for the U.S. swine industry were studied from approximately 30 to 115 kg BW. These experiments examined both SBM and SPC within their experimental diets, so results regarding particular protein sources will be identified as such. In barrows, no overall differences in growth performance were observed between control (SBM only) and treatment groups (SPC and SPC with supplemental isoflavones), though pigs receiving diets containing SPC with supplemental

isoflavones exhibited improved ADG and ADFI compared with control and SPC only pigs during the late-finishing phase. Isoflavone supplementation of SPC diets also resulted in increased dressing percentage, carcass length, lean-to-fat ratios, and percent fat-free lean in ham and the carcass overall. In gilts, ADFI decreased linearly during the growing and late finishing phases as isoflavone supplementation concentrations increased, but no overall growth performance or carcass trait effects were observed (Payne et al., 1997). Collectively, these studies suggest that considerable variation exists for soy isoflavone supplementation on growth performance, and that these effects may be sex-dependent (positive influence in barrow performance, but no effects in gilts). Thus, isoflavone supplementation above concentrations inherent to corn-SBM diets does not likely improve growth performance and product quality parameters in healthy growing and finishing pigs.

A similar study investigated growth performance and product quality when weanling pigs were fed SBM or SPC, which differ substantially in naturally occurring isoflavones (782 μg/g vs. 125 μg/g, respectively) without manipulation via supplementation. As mentioned previously, differing processing methods for SBM and SPC result in different bioavailable concentrations of isoflavones. Between experimental groups, there were no differences in growth performance, carcass composition, meat quality, or IGF-1 receptor mRNA expression in the LM. Pigs fed SBM did maintain higher circulating isoflavone concentrations compared with SPC-fed pigs (10-fold increase for genistein and 20-fold increase for daidzein). Suggested reasoning for lack of effects included potential interactions between dietary soy isoflavones and other dietary constituents (barely-triticale-soy–based diets), differential effects based on growth phase, or the growth-stimulating (i.e., estrogenic) and inhibitory (i.e., non-estrogenic) activities of isoflavones may have neutralized each other, resulting in no overall effects (Kuhn et al., 2004). These findings are in line with those reported by others in that increased isoflavone content in practical diets for healthy growing pigs may not provide additional benefits for growth performance.

Soy Bioactive Compounds and Swine Respiratory Disease

Respiratory disease contributes to significant levels of morbidity and production loss in several livestock species, including swine. Stressful events like weaning can make young animals more vulnerable to a variety of pathogens, thus making it an important production stage in which to apply nutritional interventions. Swine influenza, porcine circovirus, and porcine reproductive and respiratory syndrome virus (**PRRSV**) are all respiratory viruses that cause morbidity resulting in decreased growth performance, but less commonly mortality in young pigs. Mycoplasmal pneumonia is more likely to cause severe morbidity and potentially mortality, especially with concurrent viral infections, but more typically affects growing and finishing pigs. Economic importance of these respiratory diseases is influenced by population density on farm, production type, and herd movement (Dee, 2018). Of these respiratory diseases, PRRSV is one of the most costly to the industry with estimated annual losses in excess of $664 million (Holtkamp et al., 2013). Due to severity and pervasiveness of PRRSV in the industry, there are several studies using PRRSV as the disease challenge model to assess the effects of feeding soy isoflavones on disease reactivity and performance and, thus, will be the focus of this section.

Prior to investigation of isoflavones as immunomodulatory supplements, an experiment was performed to establish the influence of systemic PRRSV infection in weanling pigs on growth performance and immune responses. When inoculated at 29 d of age, weaned pigs naïve to PRRSV exhibited rapid increases in serum viral concentration and serum interferon (**IFN**) concentrations by 4-d post-inoculation (**DPI**). Both ADG and ADFI declined for the first 8 DPI by 47.5% and 22.4%, respectively. These authors went on to demonstrate a negative relationship between serum viral concentration and growth performance measures, indicating that as serum virus concentrations increased, pig performance decreased. The implications of these early findings suggested that immunomodulators with potential to decrease systemic viral concentrations could improve weaned pig performance under disease challenge conditions (Greiner et al., 2000).

After establishing the growth and immune responses to PRRSV infection in weanling pigs, further research sought to investigate whether supplementation with the genistein and daidzein could improve growth performance in the face of an active PRRSV infection. Pigs received purified dietary genistein at 0 to 800 mg/kg and were inoculated with live PRRSV at 29 d of age. Graded dietary intake of genistein caused a linear reduction in serum virus concentrations and a quadratic

reduction is serum IFN concentrations, thereby indicating faster viral clearance. Additionally, dietary genistein ingestion improved ADG and ADFI, though the magnitude of this response was greatest during the early post-inoculation period (4 to 12 DPI) and only when pigs were fed 400 to 800 mg/kg genistein. These improvements in ADG were logarithmically related to serum virus concentration, with each log reduction of serum viral concentration resulting in an improvement of 0.034 kg in pigs averaging 5.3 kg BW and 0.004 kg in pigs averaging 11 kg BW. Two mechanisms of action were suggested, including effects on function and intracellular signaling pathways of immune cells, and interruptions in viral replication or attachment (Greiner et al., 2001a). Under the same experimental conditions, dietary daidzein fed at 0 to 800 mg/kg alone did not result in changes in serum virus concentrations. These authors also reported that 200 to 800 mg/kg of dietary daidzein resulted in increased serum IFN concentrations during early periods of high serum virus concentrations (0 to 4 DPI), which is opposite from what was observed for genistein. Admittedly, 200 or 400 mg/kg of dietary daidzein did improve ADG, ADFI, and feed efficiency during periods of high serum virus concentrations (4 to 16 DPI), though these effects were lost as serum virus concentrations decreased (Greiner et al., 2001b). It is important to note that graded ingestion of genistein and daidzein during the preinoculation period resulted in linear decreases in ADG, suggesting that supplemental soy isoflavones may not benefit the performance of non-disease-challenged animals. However, the lack of a genistein supplementation and day interaction indicates that feeding genistein prior to inoculation may have aided in the pigs' ability to resist viral infection initially or reduce viral infectivity, as suggested above. That could imply that while supplemental isoflavones do not appreciably benefit growth performance, they may help protect against prolonged performance reduction if that same population were to be exposed to a live pathogen.

After evaluating the effects of supplementing individual soy isoflavones in disease-challenged pigs, a natural next step was to investigate potential effects of natural soy isoflavone-containing feedstuffs. One study examined the effect of dietary SBM concentration, a product with moderate-to-high levels of naturally occurring soy isoflavones, on the growth and immune responses of PRRSV-infected weanling pigs. Following a similar experimental design, weanling pigs receiving either a low SBM diet (**LSBM**, 17.5% SBM, 22.8% CP, 700 mg/kg total isoflavones) or high SBM diet (**HSBM**; 29.0% SBM, 26.7% CP, 1,246 mg/kg total isoflavones) were inoculated with active PRRSV. Post-inoculation, PRRSV-infected pigs receiving HSBM diets did not experience a decrease in ADG, whereas infected pigs receiving LSBM diets did. There were no effects of SBM inclusion on serum viral concentrations at 3 or 7 DPI, but the HSBM-fed pigs did exhibit higher cycle threshold values (i.e., reduced viral concentrations) at 14 DPI compared with LSBM-fed pigs. Most inflammatory parameters were not affected by SBM concentration under PRRSV challenge, though HSBM-fed pigs did have higher hematocrit concentrations than LSBM-fed pigs at 14 DPI, possibly indicating lessened stress on red blood cell reserves throughout the infection process. Although higher concentrations of SBM in the diet improved growth in PRRSV-infected pigs, these study outcomes are limited by not being able to differentiate beneficial effects from biologically active compounds like isoflavones and increased crude protein or AA availabilities (Rochell et al., 2015).

This unclear relationship has set the foundation of much of the work moving forward for application of dietary soy isoflavones and disease challenge. Our laboratory has attempted to elucidate whether isoflavones were responsible for beneficial effects observed in pig performance under PRRSV challenge in an unpublished study. Pigs receiving 1 of 2 dietary protein sources, standard SPC or SPC marketed as low in antinutritional factors, with or without the presence of supplemental soy isoflavones (provided by Novasoy 400, a soy-based isoflavone concentrate; ADM, Decatur, IL) were subjected to PRRSV challenge at 28 d of age. Within this model, isoflavone supplementation resulted in only minimal effects on growth. However, there was evidence that isoflavone supplementation did affect the systemic immune response, specifically through an increase in proportion of peripheral helper T lymphocytes. Additionally, isoflavone supplementation improved overall measures of peripheral red blood cell status (e.g., RBC count, hemoglobin, and hematocrit) over the course of infection, which could be an indicator that the systemic response to infection was dampened. Additional studies are necessary to understand how these effects on the immune response could influence the recovery period and subsequent growth performance following a PRRSV infection. A summary of the

Table 3. Summary of PRRSV challenge models using soybean protein or soy isoflavones as nutritional intervention

Reference	Pig sex and weaning age	Soy isoflavone source	Dietary inclusion levels	Disease model	Age of pig at inoculation	Growth performance outcomes under disease challenge	Immune performance outcomes under disease challenge
Greiner et al. (2001a)	Mixed sex weaned at 8–12 d of age	Soy genistein (88.8% pure)	0, 200, 400, and 800 mg/kg	PRRSV	29 d	↑ ADG, ADFI for 400, 800 mg/kg	↓ serum virus concentrations ↓ serum interferon concentrations
Greiner et al. (2001b)	Mixed sex weaned at 8–12 d of age	Soy daidzein (93.7% pure)	0, 200, 400, and 800 mg/kg	PRRSV	29 d	↑ ADG, ADFI, G:F for 200, 400 mg/kg during 4–16 DPI	↑ serum interferon concentrations
Rochell et al. (2015)	Mixed sex weaned at 21 d of age	Soybean meal	Low SBM (17.5%, 700 mg/kg total ISF) and High SBM (29.0%, 1,246 mg/kg total ISF)	PRRSV	28 d	↑ ADG, G:F during 7–14 DPI for high SBM diet	↓ serum virus concentrations at 14 DPI for high SBM diet
Smith et al. (unpublished)	Barrows weaned at 21 d of age	Novasoy 400[a]	0 and 1,500 mg/kg	PRRSV	28 d	No overall growth effects	↑ % Peripheral helper T Cells ↑ RBC count ↑ Hemoglobin concentration ↑ % Hematocrit

PRRSV = porcine reproductive and respiratory syndrome virus; DPI = d post-inoculation; ISF = isoflavones.
[a]ADM; Decatur, IL.

swine respiratory disease models discussed and their findings can be found in Table 3.

Soy Bioactive Compounds and Swine Gastrointestinal Disruption

Gastrointestinal disruption and disease is another leading cause of morbidity and mortality for weaned pigs in today's industry. Although the stress of weaning, group mixing, and transition to a solid diet causes alteration in eating behavior and gut function that contribute to gastrointestinal distress, bacterial and viral diseases are also prevalent. Of the gastrointestinal diseases affecting weaned pigs, enteric colibacillosis caused by infectious *E. coli*, porcine epidemic diarrhea virus (**PEDV**), rotavirus, and transmissible gastroenteritis virus (**TGE**) are all very common in the United States (Hank, 2018). These bacterial and viral enteric diseases cause extensive damage to the cells lining the gastrointestinal tract, thereby reducing nutrient absorption, limiting animal growth, and, in severe cases, leading to death. Regarding studies where soy protein or soy isoflavones were used as a dietary intervention, there are few that incorporated a specific gastrointestinal disease model. For that reason, the research discussed in this review are studies that focused on the effects of feeding soy protein or isoflavones on the physiological response to administration of bacterial endotoxins or metabolites that are produced by several pathogenic bacteria that cause gastrointestinal disease in swine.

With regard to studies involving soy and gastrointestinal disease models, more findings focus on soy as a protein source than isoflavone activity directly. Currently, there is evidence that under inflammatory conditions, diets high in SBM improve ADG, FCR, and whole-body and carcass lean tissue accretion. This is in contrast to utilizing crystalline AA in diets of growing pigs, which has been reported to improve growth performance of healthy pigs when compared with intact protein sources. The practical implication is that crystalline AA may be economical and effective to feed to healthy pigs, but SBM should be utilized in pigs or herds under high inflammatory stress. The benefits of SBM in this context are confounded, however, with CP content of the diet. Benefits could be due to anti-inflammatory properties of compounds such as isoflavones and saponins or could be due to increased availability of AA in the diet to support immune tissues (Boyd et al., 2010).

In an inflammatory bowel disease model, dextran sodium sulfate (**DSS**) was used to induce acute colitis in pigs. Pigs receiving supplemental soy-derived dipeptides and tripeptides (150 to 500 kDa in

size, estimated to be 84% of total dietary concentration) exhibited decreased macroscopic histological signs of colonic inflammation and decreased gut permeability in response to DSS exposure compared with nonsupplemented pigs, though there were no effects on clinical symptoms or growth performance. Based on these findings, researchers concluded that feeding soy peptides to pigs experiencing acute gastrointestinal inflammation had a protective effect on colonocytes and supported a more productive immune response (Young et al., 2012). In another weanling pig trial evaluating intestinal function, researchers concluded that dietary CP level (19.0% vs. 23.7% for low and high CP, respectively) had a greater capacity to improve growth performance and diarrhea scores than protein source (fish meal vs. SPC). Regardless of protein source, these authors speculated that decreased growth performance of pigs fed high CP diets may have been associated with disruption of intestinal integrity and function by stimulating antigenic proinflammatory responses at the level of the enterocyte, thereby altering mucosal permeability (Wu et al., 2015).

Although soy has been investigated more closely in gastrointestinal disease models, researchers have begun to identify applications for dietary soy isoflavones. In a study evaluating effects of dietary soy isoflavones on the response of gastrointestinal tissues to LPS challenge, weaned pigs received either a control diet without supplemental diets or a treatment diet containing 40 mg/kg supplemental soy isoflavones. On days 7 and 14 of the study, half of the pigs in each group received intraperitoneal injections of LPS. The LPS challenge resulted in overall reductions of ADG, ADFI, and G:F during days 7 to14 of the study (0 to 7 DPI) in control animals. During that same time period, LPS-challenged pigs receiving supplemental isoflavones displayed increased ADG and ADFI over challenged control animals and a near statistical difference ($P = 0.06$) for G:F. Isoflavone supplementation decreased plasma endotoxin concentrations under LPS challenge compared with positive control animals, again suggesting that isoflavones may improve intestinal barrier function under inflammatory conditions. Potential mechanisms for this improved barrier function include interactions with ZO-1 and occludins at the site of the tight junction and inhibition of the NF-κB signaling pathway (Zhu et al., 2015). These early understandings of immunomodulatory actions of dietary soy isoflavones, in both gastrointestinal and respiratory disease models, have generated novel research interests and questions for what these findings could mean for the swine industry moving forward. A summary of the swine gastrointestinal disease models discussed and their findings can be found in Table 4.

FUTURE DIRECTIONS

Reflecting on what is known about the biological actions of soy isoflavones and saponins and their impacts on pig growth and disease models, we can discuss the implications of their use as feed additives. Due to inconsistent growth performance responses in pigs, soy isoflavones and saponins are likely not strong candidates as exclusively growth promoting feed additives. However, the growth performance studies referenced in this review largely reflect growth performance responses in healthy animals, which is not a guarantee in a production setting. When dietary soy isoflavones and saponins are provided to animals under disease challenge, there is evidence that they aid in facilitating disease clearance while reducing overall declines in animal performance. With

Table 4. Summary of swine gastrointestinal inflammatory challenge models using soybean protein or soy isoflavones as nutritional intervention

Reference	Pig sex and weaning age	Soy/isoflavone type	Soy/isoflavone inclusion level	Disease model	Age of pig at challenge	Growth performance outcomes under disease challenge	Immune performance outcomes under disease challenge
Young et al. (2012)	Mixed sex weaned at 5–7 d of age	Soy di- and tripeptides	250 mg·kg BW^{-1}·day^{-1} (starting at 5 DPI)	Dextran sodium sulfate-induced colitis	11–13 d	No effects	↓ colonic inflammation ↓ gut permeability
Zhu et al. (2015)	Barrows weaned at 14 d of age	Soy isoflavone mixture	40 mg/kg	Bacterial infection mimetic (LPS-induced inflammation)	21 d	↑ ADG, ADFI	↓ plasma endotoxin concentrations ↓ incidence of diarrhea (nonsignificant trend) ↑ abundance of ZO-1 and occluding mRNA in jejunal mucosa

DPI = d post-inoculation; LPS = lipopolysaccharide.

regard to PRRSV infection models, while ADG and/or G:F responses were lessened in nondisease periods for animals receiving moderate-to-high levels of isoflavones, average ending weights were typically the same (i.e., mild overall effects). This could suggest any negative effects on growth in healthy animals may be worthwhile from a health management perspective during high-stress periods like weaning as it could improve the reaction of those populations to impending disease challenge. However, in order to realize soy isoflavone and saponin immunomodulatory potential in an at-risk group of pigs, special consideration must be given when feeding soy to young pigs. As discussed, fermented soy sources may be included in diets of young pigs with less gastrointestinal distress along with potential for higher aglycone (i.e., the biologically active form) isoflavone levels. Additional soy protein sources including SPC and SPI also pose lower risk when fed to young pigs, but are largely devoid of soy isoflavones and saponins due to processing methods. If these protein sources were to be used, then supplementation with isolated soy isoflavones or saponins may elicit benefits. Nutritionists considering incorporation of supplemental soy isoflavones and saponins, regardless if presented in a whole food matrix (e.g., SBM) or as an isolated product, should ideally have validated analytical data for these compounds due to significant variations caused by soybean variety, growing conditions, and processing effects.

With nutritional mediation of disease in weaned pigs as the potential application for soy isoflavones and saponins in mind, there are several questions and limitations in current research that should be addressed. One of the most limiting aspects of current soy isoflavone and saponin research is that a majority of the available evidence comes from in vitro experiments. Although these experiments have allowed researchers to elucidate specific cellular receptors and pathways that isoflavones and saponins target, translating effects observed in culture to the live animal are difficult. Cell cultures are typically exposed to concentrations of isoflavones or saponins that far exceed physiological concentrations. This may lead researchers to overestimate in vivo responses due to differences in metabolic kinetics or, conversely, underestimate in vivo effects if certain cellular factors are absent in the live animal that help potentiate biological activity (Erdman et al., 2004). Isoflavone concentrations are highest within the intestinal lumen, and, thus, have potential to exert highest biological activity on enterocytes. However, less is understood about the maximum circulating isoflavone concentrations that are achievable upon delivery to specific tissue types (e.g., hepatocytes vs. skeletal myocytes).

Although this need for understanding isoflavone and saponin supplementation on a whole animal level appears to be recognized by swine researchers, many of the trials that have been conducted thus far are of shorter duration, especially in regard to swine disease models. These experiments demonstrate how isoflavones and saponins modulate the immune and cellular response during the acute disease period; however, much may be gained from extending these trials over a longer feeding period. Doing so would allow researchers to monitor animals for changes during the recovery period, influences on compensatory performance following immunological insult, and whether soy isoflavone or saponin treatment would have effects on overall growth performance and quality of product.

Beyond limitations of previous experimental parameters and design, there are additional interests in soy isoflavone metabolites beyond what was discussed in this review. In humans, there is some in vitro evidence that isoflavone metabolites are able to modify the numbers of key bacterial species in the gut, which may contribute to beneficial effects observed from dietary isoflavone supplementation (Vázquez et al., 2017). Whether pig gastrointestinal microbial populations would be affected similarly could be of future interest, especially in regards to pathogenic gastrointestinal bacteria affecting swine. Of isoflavone metabolites, equol is one of great interest due to its biological activity. Equol is a metabolite of daidzein, and is an isoflavandiol, nonsteroidal estrogen compound produced through hydrogenation by bacterial enzymes. As derived from in vitro studies, equol maintains high biological activity, out competing both genistein and daidzein for growth inhibition of several fungal and common bacterial types and demonstrating the greatest antioxidant capacity among isoflavones and their metabolites (Chang et al., 1995; Setchell et al., 2002). Equol also appears to be relatively stable and able to tolerate peripheral circulation in its biologically active form better than soy isoflavones. However, the ability to synthesize equol is highly variable between species and individuals within a species, largely owing to differences in composition of the commensal microbiota. In humans, only 30% to 50% of the population studied were considered "equol-producers" (Setchell et al., 2002). Within swine populations, it appears that the microbiota of pigs may support some synthesis of equol. It has been demonstrated that feces from Erhualian pigs, a Chinese pig breed, contain bacterial strains that

are able to metabolize daidzein to equol. Whether the equol levels produced were physiologically relevant to the animal was not discussed, though researchers suggested their findings could support equol-production being a contributing factor to the beneficial effects seen in isoflavone- or daidzein-supplemented livestock (Yu et al., 2008). In more common U.S. swine breeds, equol production may be more variable. A study evaluating equol production and contents in feces and urine from large white sows showed results similar to what has been demonstrated in humans with high interindividual variability. Like humans, individual sows that had larger detectable levels of equol in their feces or urine had similar microbial population phenotypes to each other. However, across all large white sows evaluated, the amount of daidzein in feces or urine was higher than that of equol, though levels of equol and daidzein were positively correlated (Zheng et al., 2017). Future research continuing to identify equol-producing bacteria in the swine gastrointestinal tract, especially for common commercial breeds, is merited moving forward. Additionally, trials utilizing isolated equol as a dietary supplement to observe effects on growth and productivity could direct future work in the field of soy bioactive feed additives.

CONCLUSION

Bioactive compounds derived from soybeans, including isoflavones and saponins, possess a multitude of applications within both the human and animal health sectors. Regarding animal agricultural species, as the industry moves away from prophylactic antimicrobial use in livestock diets, maintaining adequate herd health falls back to sound nutritional and management practices, and it creates opportunities for value-added feedstuffs to be considered. Soy-derived isoflavones and saponins hold great potential as immunomodulatory compounds that may serve to benefit the efficiency and sustainability of pork production when used as health-promoting feed additives. Although there remain many limitations and unanswered questions, soy isoflavones and saponins could prove to be a valuable health management tool for future swine producers.

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