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Isoflavonic Phytoestrogens – New Prebiotics for Farm Animals: a Review on Research in China

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Abbreviations for scientific terms
Dihydrodaidzein (DHD); odesmethylangolensin (ODMA); denaturing gradient gel electrophoresis (DGGE); volatile fatty acids (VFA); gastrointestinal (GI); porcine reproductive and respiratory syndrome (PRRS); acylglycoprotein (AGP); growth hormone (GH); prolactin (PRL); somatostain (SS); plant hemoagglutinin (PHA); superoxide dismutase (SOD); lipid peroxides (LPO); thyroid-stimulating hormone (TSH); insulin-like growth factor-1 (IGF-1); glutathione peroxidase (GSH-Px).

Abstract
Isoflavones are recognized to be estrogenic compounds that are often associated with a reduced risk of cancers. The estrogenic activity can be enhanced after metabolization to more active compounds such as genistein and daidzein by gut microorganisms. The direct use of these metabolites has been investigated in laboratory rats and farm animals over the last decade. This paper reviews the research progress on the effect of isoflavonic compounds including metabolites on the physiology, gut microbiology and performance of farm animals in China.

Introduction
Phytoestrogens are by definition plant-derived substances that are able to activate the mammalian estrogen receptors. Most of these plant substances are to some extent similar to the mammalian estrogen either in structure or function, in the meantime exhibiting other biological activities *in vivo* in addition to their estrogenic effect (such as anti-oxidation effects). Isoflavones are a group of phytoestrogens (Fig. 1) and can be found in many plants, particularly in the legume family. Isoflavones are often associated with a reduced risk of breast-, colon- and prostate cancer as reviewed widely (Han and Wang, 1994; Han, 1999; Setchell, 1998; Setchell and Cassidy, 1999; Cassidy *et al.*, 2000) and recently by Magee and Rowland (2004). It is widely believed that the relatively low incidence of breast and prostate cancer in China has mainly benefited from the large quantity of consumption of soy products. The estrogenic activity can be enhanced after the isoflavones are metabolized to more active compounds by the gut microbiota (Rowland *et al.*, 1999). The two major isoflavones found in soybeans are daidzin and genistin, and can be deglycosylated to daidzein and genistein, respectively, by human intestinal bacteria (Hur *et al.*, 2000). Genistein and daidzein are two of the major metabolites from isoflavones, but can be further metabolized by intestinal microorganisms (Schoefer *et al.*, 2002; Wang *et al.*, 2005). In the absence of gut microbiota in germ-free rats, daidzein and genistein could not be degraded (Bowey *et al.*, 2003). Also, the metabolic fate of isoflavones has been shown different among individuals in humans and monkeys, due to individual differences in gut microbiota composition (Fafii *et al.*, 2003, 2004; Atkinson *et al.*, 2004). Daidzein can be metabolized to equol, dihydrodaidzein (DHD) and odesmethylangolensin (ODMA), and about one third of human individuals can convert dietary daidzein into equol, others into DHD and or ODMA. Among the gut microbiota, only a few intestinal bacteria have so far been identified to metabolize isoflavones. Bacterial strains to transform daidzein into DHD and ODMA were isolated from human fecal samples (Hur *et al.*, 2000, 2002), and a human intestinal bacterium to convert DHD further into equol was also isolated (Wang *et al.*, 2005a). *Eubacterium ramulus* from the human gastrointestinal tract was shown to be able to cleave the C-ring of both daidzein and genistein (Schoefer *et al.*, 2002). A bacterium to convert the isoflavones daidzein and genistein to DHD and dihydrogenistein, respectively, was also isolated from bovine rumen (Wang *et al.*, 2005b). From human fecal samples, Decroos *et al.* (2005) obtained a mixed bacterial culture that can transform daidzein into equol, and four bacterial species were revealed by 16S ribosomal RNA (rRNA) gene-targeted PCR- denaturing gradient gel electrophoresis (DGGE) analysis, of which three could be cultured and identified as *Lactobacillus mucosae* EP12, *Enterococcus faecium* EPI1 and *Finegoldia magna* EPI3. However, the three cultured species could not produce equol from daidzein in pure culture, suggesting that some uncultured or undetectable bacterial species were responsible for the equol production. In the same report, it was also revealed that colonic fermentation products, particularly hydrogen gas and also butyrate and propionate, could stimulate equol production. This may be also one of the reasons why there was lack of equol production in pure culture.

Extensive researches have been conducted to investigate the effect of isoflavones on human health and underlying mechanisms by animal models and *in vitro* studies (King and Bursill, 1998; Setchell, 1998). Clinical studies have also demonstrated the potential of isoflavones in clinic nutrition (Setchell and Cassidy, 1999). In China, many isoflavones and their original plant extracts have been used as main components of the traditional Chinese medicine for thousands of years (Han and Shen, 1991). Over the last decade, China has also made extensive efforts to identify and understand the

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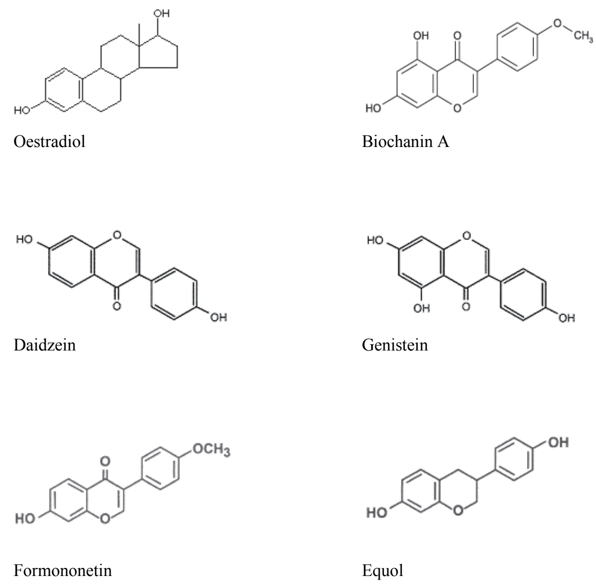

Fig. 1. Chemical structures of isoflavonic phytoestrogens.

effects of isoflavones on farm animals due to the impact of the successful application of isoflavonic compounds in clinical medicines. Many studies in our laboratories have demonstrated that isoflavones could have anabolic effects on animal metabolism and performance, and could affect neuroendocrine system and gut microbiota of animals. Of all the isoflavonic phytoestrogens, daidzein has been most extensively studied and has recently been introduced as a feed additive in farm animal nutrition in China. The aim of this paper was to summarize research progress in this regard in China.

Effects on rumen microbiota and metabolism

Natural isoflavones from plants can be easily metabolized by rumen microorganisms to various compounds with genistein and daidzein as two of the major intermediate metabolites (Van Soest, 1994). Early research in the 1980's showed that long term consumption of large amounts of legumes could cause detrimental effects on fertility of ruminants, the so called "clover disease", due to the isoflavones released from legumes (reviewed by Van Soest, 1994). Later studies with artificial rumen models showed that formononetin could increase ruminal cellulase activity, but significantly inhibited ruminal protease activity and total dehydrogenase activity (Han and Wang, 1999). With water buffalos fitted with permanent rumen- and intestine fistulas, it was demonstrated that injection of daidzein (500 mg/d, 12d) via duodenum cannulae could increase serum testosterone, rumen bacterial protein, ammonia nitrogen and total volatile fatty acids (VFA) while no apparent effect on the ratio of individual VFAs was observed (Chen *et al*., 1999). Research demonstrated that the extent of metabolism in the rumen varied depending on the fluctuation of blood testosterone levels, which could enter the rumen with saliva or via the rumen epithelium (Yang *et al*., 1998). Thus, isoflavonic compounds may affect microbial activity and their metabolism by increasing the blood and rumen testosterone levels.

Cultivation independent approaches were used to assess the effect of daidzein supplementation on microbial composition in the goat rumen. By 16S rRNA gene-targeted PCR-DGGE, Yao *et al*. (2004a) were able to detect shifts in rumen microbiota composition after the supplementation with daidzein in traditional Chinese-breed goats. Although most of the DGGE bands were common to both control and daidzein treatment rumen samples, some bands were enriched while others disappeared after daidzein supplementation. 16S rRNA gene sequence analysis further showed that most of the predominant clones either enriched or disappeared after daidzein treatment showed highest similarities to environmental sequences that in most cases had been retrieved from rumen samples. The results suggest that daidzein could indeed affect the rumen microbial composition.

The direct effect of daidzein on rumen microbial activity was also demonstrated using *in vitro* techniques (Zhu *et al*., 2002). Our research showed that daidzein at 5 and 10 mg/l could significantly increase the proportion of propionate in total VFA when rumen samples from native goats were used as the inoculum. However, this effect disappeared when the concentration of daidzein was above 20 mg/l. A time-course study with 10 mg/l of daidzein showed that the effect on VFA profiles became evident after one hour incubation. A similar pattern was observed with mixed rumen or fecal anaerobic fungi in gas production and substrate degradation. Using a pure culture of a rumen anaerobic fungus, *Neocallimastix* sp., daidzein at 10 mg/l and 20 mg/l significantly increased cumulative gas production and dry matter (DM) loss. With mixed rumen fungi, daidzein at 10 mg/l also significantly affected gas production, although no significant difference was observed for DM loss. In the same experiment, however, daidzein at higher concentrations did not show significant effects on rumen microbial fermentation. This may be due to the biphasic response, which shows estrogenic effect at low dosage, while anti-estrogenic effect is observed at high dosage. The results demonstrated for the first time the effect of daidzein on rumen microbial activity *in vitro*. As it is widely reviewed, many isoflavones and their metabolites can be absorbed and then enter in circulatory blood (Detchell, 1998; Detchell and Cassidy, 1999). Thus, it can be reasoned that isoflavones and their metabolites once circulating can also affect the rumen microorganisms in addition to their initial direct effect in the rumen. Nevertheless, clarification of the mechanism underlying the biphasic effect of daidzein on microorganisms *in vitro* requires further studies.

Effect on gut microbiota in piglets

As with rumen microorganisms, studies in our laboratories using *in vitro* fermentation approaches demonstrated that daidzein could directly affect intestinal microorganisms of the piglet gut. This was the first report of its kind with isoflavones in monogastric animals (Yao *et al*., 2004b). To investigate the effect of daidzein on the *Lactobacillus* community in the piglet gut, *in vitro* fermentation was conducted using gut digesta as inocula. Digesta from 12 conventionally raised piglets of the same litter (three on each slaughtering day) were used in following *in vitro* fermentation treatments: (a) medium with 5g/l of glucose and 50 mg/l of daidzein; (b) medium with 5g/l of glucose; (c) medium only. By using *Lactobacillus* specific primers for amplifying the V1-V3 region of the 16S rRNA gene, PCR-DGGE revealed that all samples showed similar changes in the community fingerprints after fermentation; some dominant bands disappeared, while the density of other dominant bands obviously increased, suggesting that some species may be enriched while others may not be able to grow under the culture conditions applied. Although DGGE patterns were different between digesta from different GI compartments either before or after fermentation, no apparent difference was observed between treatments for each digesta sample. However, further studies using dilution PCR for the semi-quantitative detection of lactobacilli demonstrated that daidzein treatment significantly increased the number of lactobacilli in batches inoculated with digesta samples from most of the gut compartments. Thus, daidzein may have the potential for use as a prebiotic substance in animal feed.

Immune effects

Genistein has been recognized as an inhibitor of tyrosine kinases (Setchell and Cassidy, 1999). *In vitro* studies demonstrated that high levels of genistein could reduce macrophage and natural killer cell numbers and phagocytosis rates by inhibiting tyrosine kinases (Steele and Brahmi, 1988), and decrease T and B lymphocyte production by inhibiting topoisomerase II (Chang *et al*., 1995). Low levels of genistein, however, could elicit natural killer cell activity (Zhang *et al*., 1999) and antiviral replication (Yura *et al*., 1993). In pigs challenged with porcine reproductive and respiratory syndrome (PRRS), Greiner *et al*. (2001a) demonstrated that soy genistein could enhance serum PRRS virus elimination, decrease interferon activity in the serum, and increase α_1–acylglycoprotein (AGP). The pig growth performance was also improved. The authors concluded from these results that soy genistein at 200 to 400 mg/kg can be an orally active immune modulator.

With daidzein, similar experiments were conducted in pigs by the same authors, but different effects were observed (Greiner *et al*., 2001b). Although daidzein could improve growth performance in periods of high viremia, it did not in periods when systemic virus concentrations were minimized. Furthermore, additions of 200 mg/kg and 400 mg/kg were effective, but not 800 mg/kg. Unlike genistein, dietary daidzein did neither decrease serum PRRS virus concentrations nor AGP activity. Our research with pigs, however, demonstrated that oral administration of daidzein in pregnant sows could affect immune function in the mammary organ as well as the neonate piglet (Zhang *et al*., 1995). The concentrations of antibody to swine fever vaccine in serum and colostrum were significantly increased by 41% and 44%, respectively (Fig. 2). This suggested that both the systemic and mammary gland humoral immune functions in sows were notably enhanced. The concentration of antibodies in neonate piglets from treated sows markedly increased through colostrum absorption. With daidzein administration, growth hormone (GH) and prolactin (PRL) levels of sow serum and colostrum were strikingly enhanced. GH increased 155% and 54%, and PRL increased 86% and 220%, respectively. Meanwhile, the serum somatostain (SS) level was apparently lower than that of the control animals. It appears that the immune-regulatory effect of daidzein may be involved in the decrease of the SS level and in the increase in both GH and PRL levels.

Daidzein could also improve the immune function of birds. Daidzein supplementation to diets in male chickens of 7–21 days of age could greatly increase body weight gain and feed efficiency. The relative organ weight of thymus and bursa, and the T-lymphocyte transformation were all elevated in daidzein treated animals (Gao *et al*., 2000).

In rats, daidzein was shown to increase B and T lymphocyte activity and phagocytosis rate of macrophage cells (Zhang *et al*., 1997). Our research in mice showed that daidzein and formononetin markedly enhanced the thymus weight and the phagocytosis of peritoneal macrophages. The hemolytic ability of plaque forming cells and the T-lymphocyte percentage in peripheral blood exhibited a significant increase. Formononetin and daidzein at the dose of 50 µg/mL also showed their

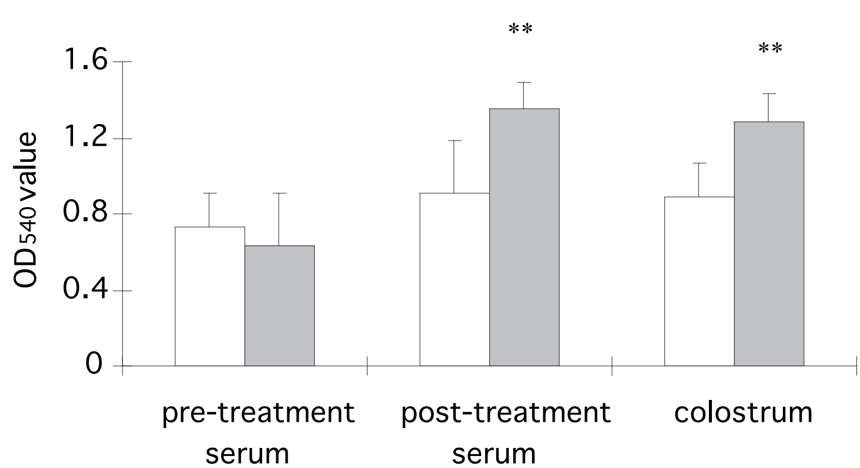

Fig. 2. Effect of daidzein on antibody levels of the swine fever vaccine in sow serum and colostrums. Antibody levels in daidzein treatment (■) and controls (☐) were expressed by OD values at 540nm as determined by spectrophotometer. Bars are standard errors. ** represents significant difference between treatment and its corresponding control. (From Zhang *et al*., 1995a.)

positive effects on the lymphocyte transformation induced by plant hemoagglutinin (PHA), which was increased by 90% and 210%, respectively (Zhang and Han, 1993). Thus, isoflavonic compounds could affect non-specific, humoral and cellular immune functions and thus could be a plant origin immune modulator.

Isoflavonic compounds also showed anti-aging effects in mice (Wang *et al*., 1999). Daidzein supplementation to the diets of male mice at 18 days of age for one month increased the SOD levels in brain, liver and red blood cells by 22%, 22% and 4%, respectively, while the LPO levels decreased in brain and liver by 36% and 13%, respectively. However, daidzein showed no effects on the SOD and LPO levels in female mice compared to that of the control group.

Effects on mammary gland development and lactation

Formononetin has been demonstrated to be effective on the development of mammary glands in ovariectomized mice (Wang *et al*., 1993). Formononetin, injected subcutaneously at a daily dose of 0.4 mg/kg for 5 days, significantly increased the relative weight and RNA content of mammary glands. The mammary ducts were well developed, and more secretion granules appeared in acinus. Radio receptor assay analysis further showed that formononetin could compete with estradiol for estradiol receptor of mammary gland cytosol. The maximum binding capacity of estrogen receptors and the prolactin concentration of plasma were markedly elevated after administration of formononetin. It seems that formononetin could enhance the development of mammary glands by directly binding to estrogen receptors of mammary gland cytosol and by promoting the secretion of prolactin from the pituitary.

Similar effects were observed with daidzein, of which the estrogenic activity is about 10-fold higher than that of formononetin. Either administrated orally or subcutaneously, both ovariectomised and conventional young rats showed markedly increase in the development of the mammary gland (Zhang *et al*., 1995).

Radio receptor assay revealed that daidzein not only greatly increased the cytosol binding sites of estradiol in mammary, pituitary and hypothalamus of rats, but also the binding sites of progesterone (Zhang *et al*., 1995). *In vitro* receptor competitive assays showed an apparent competitive binding affinity of daidzein to the mammary, pituitary and hypothalamus. Thus, the up-regulating effects of daidzein on the receptors of estradiol and progesterone and its positive effects on pituitary GH and PRL secretion may be involved in the mechanisms of daidzein in promoting mammary development.

Further experiments showed that daidzein could significantly affect lactation. Administration of daidzein in rats during late pregnancy could significantly increase milk yield, neonate rat weight gain and mammary gland development pre lactation (Zhang and Han, 1994). With sows, milk yields increased greatly when the late pregnancy sows were fed the diets containing a low dose of daidzein at 5 mg/kg (Liu *et al*., 1999). On the 10th and 20th day of the nursing period after parturition, the milk yields were 11% (P<0.05, 313.7±17.4 vs. 283.7±10.6 g/h) and 15% (P<0.05, 371.2±22.2 vs. 333.7±15.1 g/h) higher than those of the control group. Moreover, the GH, TSH and IGF-1 levels in colostrum were markedly enhanced in daidzein treatments (Table 1).

It is generally recognized that dose and duration of intake are two of the important factors that influence the biological effects of isoflavones (Setchell and Cassidy, 1999). The timing of administration may be another important factor. When daidzein was added to the diet of sows during the lactation period, a different pattern was observed. As expected, the milk yield in daidzein treatments was notably higher than that of control animals (P<0.01) at the 5th day after parturition, and remained stable until day 20 after parturition (Liu *et al*., 1997). Twenty days after parturition, however, the milk yields decreased in daidzein-treated animals as compared with the control. This may be due to the biphasic effect of daidzein. As it is well known, PRL plays an important role in the initiation and maintenance of lactation though many hormones that are involved in regulating the lactation of sows. At the initial stage of lactation when the mammary gland is not completely developed, minute dosage of daidzein acts as a weak estrogen and enhances prolactin levels, consequently increasing the milk yield. As sows ingested daidzein from the diet daily, the accumulated estrogenic effects became greater, the prolactin levels decreased, and even estrus occurred in some sows. Thus, the estrogenic effects of isoflavones can be different depending on the endogenous level of estrogens in the animal.

Growth

Although widely investigated, the effect of isoflavonic phytoestrogens varied between experiments and between animals. With conventional weaning of piglets, soy daidzein or soy genistein did not have significant effects on weight gain and feed intake (Greiner *et al*., 2001), though daidzein could slightly improve the gain/feed ratio. After challenge with PRRS virus, however, both daidzein and genistein showed a significant effect on growth performance (see also above in the section on Immune Effects). This may suggest that isoflavonic phytoestrogens may primarily work as immune-modulators rather than conventional growth promoters.

In China, investigations showed positive effects of daidzein on animal growth performance. In studies with growing castrated male pigs, daidzein supplemented to

Table 1. Effect of daidzein on metabolic hormone levels in sow colostrums (n=8).

Group	GH (ng/ml)	Insulin (μU/ml)	TSH (μIU/ml)	IGF-1 (ng/ml)
Control	7.4±2.5	319.7±13.8	8.5±2.8	684.0±95.0
Daidzein	10.6±1.4*	305.1±11.6	12.1±3.7*	897.0±115.0*

Values with * significantly differ from the corresponding control values (P<0.05) diets at 5 mg/kg significantly increased weight gain by 59% ($P<0.01$), and the blood IGF-1 and testosterone levels were elevated by 51% and 18%, respectively (Guo *et al*., 2002).

With Redbro male broilers, crude isoflavonic extracts added to the diets significantly increased the serum testosterone levels in male chicken, while serum uric acids and abdominal fat were decreased (Wang *et al*., 1994). Daidzein supplemented to the diets at 3 mg/kg significantly enhanced the daily weight gain by 10%, breast muscle and hind leg muscle weight by 6% and 7%, respectively. The ratio of RNA to DNA of muscle cells was greater than that of the control group. This suggests that daidzein could promote the muscle protein accretion of male broilers. With female broilers, however, the same dose of daidzein did not produce significant effects on average daily body weight or serum estrogen levels.

A similar effect was found in growing male rats (Wang *et al*., 1995). Compared to the control group, dietary daidzein supplementation of 3 mg/kg increased daily weight gain and feed intake by 15% ($P<0.01$) and 18% ($P<0.05$), the weights of carcass, gastrocnemius muscle and femur bone increased by 17% ($P<0.05$), 9% and 14% ($P<0.05$), respectively. The femur bone density and blood calcium concentration increased, while alkaline phosphatase activity was lowered by 15% ($P<0.05$). The blood concentration of IGF-1 and testosterone were elevated by 37% ($P<0.05$) and 17% ($P<0.05$), whereas estradiol levels were reduced by 24% ($P<0.01$). These results suggest that daidzein could improve bone metabolism and growth performance, with the effect possibly related with the secretion of the related hormones in growing male rats (Guo *et al*., 2001).

While the effects of daidzein on animal growth have been so far complex with different experiments, it appears that the effectiveness on growth is more evident for male animals, which may not be surprising when considering that daidzein has estrogenic activity. This may also remind us that animal gender should be considered for experimental design. With immune challenged animals, the isoflavonic phytoestrogens seem more effective than with conventional healthy animals. In China, where the production environment is less well controlled, the animals would be exposed to many diseases. Indeed, many of our studies demonstrated the positive effect of isoflavonic phytoestrogens on animal growth. Although solid evidence is still lacking, it may be speculated that environmental factors such as disease exposure and other stresses to the animal, can be involved in the amplitude of the isoflavonic effectiveness on growth.

Limited research also showed that daidzein had effects on the fetus growth to some extent. A dose of five and 100 mg/kg of daidzein supplemented to pregnant sows and rats, respectively, could greatly increase the offspring's birth weight (Zhang *et al*., 1993; Liu *et al*., 1996). Nevertheless, more research is needed for detailed studies.

Egg laying performance of birds

Recent studies have demonstrated that isoflavonic phytoestrogens could improve egg-laying performance in birds. Experiments with 242 and 330 day old layers showed that daidzein supplemented to the diets at 3 mg/kg significantly increased the laying rate of the old laying hens. In addition, average egg weight and feed efficiency increased to some extent (Liu YQ, 1998). This effect was also observed with 174 day old first time laying hens (Meng *et al*., 2001, 2002). Daidzein supplementation significantly increased the egg laying rate and feed efficiency by 9% and 7% in early laying period, 11% and 14% during the high laying period, and 14% and 17% during the late laying period, respectively.

Similar effects were observed with laying ducks and quails. Daidzein supplementation significantly increased the egg laying rate by 6% in laying Shaoxing ducks, with an overall increase in egg yield increasing by 8% compared to that of the control group during an experimental period of 30 days (Zhou *et al*., 2002). Daidzein treatment markedly prevented body weight loss of Shaoxing ducks and increased ovary weight compared with the control. The number of large follicles, however, did not change. Daidzein supplementation at 3 mg/kg significantly increased laying rates in quails, with values increasing by 7% ($P<0.05$) in the early and in the middle stage, and 10% ($P<0.01$) in the late stage as compared to the control (Wang *et al*., 1999).

Research has also showed that dietary daidzein could increase the feed intake of egg laying hens (Zou *et al*., 2003). By using a Feed Intake Data Acquiring System to monitor the behavior of the laying hens, Zou *et al*. (2003) were able to demonstrate that supplementation with daidzein at 6 mg/kg increased feed intake during high and late laying periods by 18% and 20% compared to the control, respectively. Meal frequency in a day also significantly increased. Obviously, the increase in feed intake could contribute to the improvement in laying performance of laying hens.

In the meantime, daidzein supplementation could affect the endocrine functions. Daidzein at 3 mg/kg significantly increased blood T3 and progesterone levels of 330 day old laying hens (Meng *et al*., 2001). In Shaoxing ducks, daidzein supplementation at 3 mg/kg significantly increased serum levels of GH, but not IGF-1, while 5 mg/kg daidzein supplementation significantly increased both GH and IGF-1, suggesting a dose-dependent effect (Zhou *et al*., 2002). With laying quails, serum T3 levels in early, middle and late laying stages were all significantly elevated. Zou *et al*. (2003) showed that while daidzein supplementation increased the feed intake, it also increased insulin and 17 β–estradiol levels in the blood.

Interestingly, the daidzein effect could be different depending on the laying stage (Ke *et al*., 2002; Meng *et al*., 2001). With 35 day old quails, daidzein supplementation at 3 mg/kg could greatly increase the laying rate and serum T3 level by 7% and 23% as compared to the control. A similar effect was observed with 7 month old quails with daidzein at the same dosage. However, daidzein supplementation at the dose of 6 mg/kg led to a decrease in laying rate of the 7 month old quails, while a significant increase was found for 12 months old laying quails. Thus, the dosage of daidzein supplementation should be applied in relation to the laying stage of the birds. Similarly, the effect of daidzein on blood 17β-estradiol was also related with the laying stage. The blood 17β-estradiol level of laying hens significantly increased compared with the control, but varied with different laying stages, with higher levels during the late laying stage (Meng *et al*., 2001). For laying quails, the daidzein effect was different, as the blood estradiol levels increased by 63% (P<0.01) in the early laying stage, but decreased by 17% in the late laying stage as compared with the control, suggesting a biphasic effect in regulation of estradiol levels.

Dietary daidzein could also affect egg content of cholesterol and its oxide (Yin *et al*., 2004). Daidzein supplemented at 40 mg/kg could significantly decrease egg cholesterol content by 19% and egg yolk cholesterol concentration by 11% as compared to the control. While supplementation at 10 and 20 mg/kg showed a similar effect, this effect was not observed with 5 mg/kg of supplementation. The same study also demonstrated that daidzein could also inhibit the formation of cholesterol oxides in cooked egg yolk, with 7-keto cholesterol and total cholesterol oxides content reduced by 27% and 35% respectively, compared to the control. Further studies showed that both daidzein and dietary tea polyphenols exhibited antioxidant effects on laying hens (Yin *et al*., 2003). LPO contents in egg yolk, liver and plasma significantly decreased with daidzein or tea polyphenols treatment (both at 40 mg/kg), while the SOD and GSH-Px levels remained relatively stable. Thus, dietary tea polyphenols and daidzein could directly improve the bird's antioxidant levels irrespective of SOD and GSH-Px activities.

The results suggested that dietary isoflavonoids at proper dosage could have beneficial effects on laying performance in birds, probably mediated by the regulation of endocrine functions. In addition, the resultant eggs from isoflavonoid supplementation could be beneficial to human health.

Conclusions

Isoflavonic phytoestrogens can exhibit weak estrogenic activity on reproduction. They can also promote male animal growth, and induce female mammary development and lactation, and improve laying performance of laying birds. These effects were usually coupled with their influence on immunity and metabolic hormones. Thus, the observed effects of isoflavonic phytoestrogens may be mediated by their modulation of immunity and endocrine. Furthermore, environmental factors that can challenge animal immune status could affect the extent to which isoflavonic phytoestrogens influence animal performance.

Intestinal microorganisms play an important role in isoflavone metabolism in the animal gut and consequently influence the metabolic fate of the isoflavones. In return, the isoflavonic phytoestrogens or their metabolites could affect gut microbial activity. This effect may be caused by the circulating isoflavonic metabolites once absorbed by the animal body. However, the *in vitro* effect on microbial activity may also suggest a direct relationship between isoflavonic phytoestrogens and gut microorganisms. Prebiotic effects of isoflavonic phytoestrogens may be involved in this relationship. Clearly, researches have suggested that isoflavonic phytoestrogens have great potential for use in animal feed supplementation in the future.

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