



Effects of phytoestrogens on the reproductive physiology of productive species. Review



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Abstract:

Phytoestrogens (PEs) are chemical compounds from the secondary metabolism of some plants, they have a potential effect on the reproductive parameters of domestic animals, acting as agonists or antagonists of estrogen receptors. The objective of this review is to know the effects produced by a diet rich in PEs on the reproductive physiology of slaughter animals. A systematic review was carried out in two databases using keywords related to the effects produced by the intake of PEs in the diet on the reproduction of animals for slaughter, only controlled studies developed *in vivo* were considered. Contradictory results were found, on the one hand, the intake of a high content of polyphenolic compounds from different fodders, in the bovine female, was related to the decrease in fertility, presence of abortions and ovarian cysts; on the other hand, the intake of the high content of PEs induced an increase in the semen quality of the males of the species: cattle, sheep and leporids, so these effects can be attributed to the concentration, the type of PEs, sex, species and even the breed of the animal.

Key words: Phytoestrogens, Slaughter animals, Reproductive effects, Coumestans, Isoflavones, Lignans.

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Introduction

In livestock activities, the selection of feed represents one of the main production costs and is one of the most technically complex aspects for the search for nutritional satisfaction in the different stages of animal production (breeding, rearing, gestation, lactation, weaning, growth and fattening), considering their physiological state, age and breed⁽¹⁻⁴⁾.

Generally, the feed of slaughter animals (SAs) is based on grains of sorghum and corn, since they provide a rich source of energy, in addition to other feeds that provide protein, such as soybeans, canola, alfalfa and clover⁽²⁻⁴⁾. It is essential to highlight that it has been suggested that changes be made in the feed or forage source in the event of alterations in the reproduction of SAs⁽⁴⁾.

Plant-based feeds are considered a rich source of phytoestrogens (PEs), nonsteroidal polyphenolic compounds derived from plant metabolism with conformational structure

similar to 17- β estradiol (E2)^(3,5). When ingested by animals, they can act as selective modulators of estrogen receptors (ERs) and act as endocrine disruptors in an agonistic or antagonistic manner, depending on the dose ingested^(5,6), thus interfering with the synthesis, secretion, transport and metabolism of reproductive hormones, during embryonic development and in adult life⁽⁶⁻¹¹⁾.

Nearly 100 PEs have been recognized; these are categorized, according to their chemical structure, into four classes: isoflavonoids (genistein, daidzein, formononetin); flavonoids (naringenin, kaempferol); coumestans (coumestrol “COU”), sativol, COU diacetate, 4-methoxycoumestrol) and lignans (enterolactone and enterodiol)^(12,13). Soybeans are the most abundant source of isoflavones, as it is one of the feeds with the highest content of genistein and daidzein, while alfalfa and clover contain a high amount of coumarins. It is worth mentioning that PEs are naturally present in plants, as glycosides, which are hydrolyzed to aglycone (which is the active form) catalyzed by enzymes present in the digestive system^(14,15).

Through several studies, it has been shown that the intake of PEs causes reproductive alterations in animals of both sexes, as well as temporary infertility syndromes^(16,17). However, there is also evidence that PEs favor reproduction because they increase the concentration, motility and volume of spermatozoa necessary for fertilization⁽¹⁸⁻²⁰⁾.

Due to the economic and productive importance that reproductive alterations represent in the context of animal production, the objective of this work is to know the potential effect that feeds rich in PEs have on the reproduction of SAs.

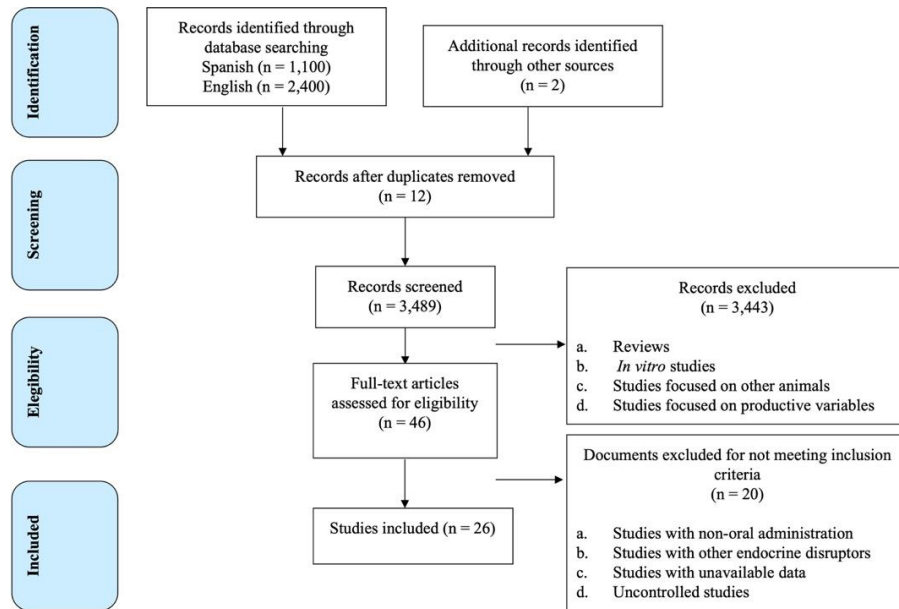
Method

This document was developed in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)⁽²¹⁾ statement.

Literature search

A systematic review was conducted on the Web (Figure 1); Google Scholar and PubMed were used as specialized information search engines with the aim of identifying studies that explored the effects of PEs present in the diet on the reproductive physiology of SAs, which have been published in blind peer-reviewed journals, extended abstracts in specialty

congresses, and in graduate theses between 1996 and 2019. The keywords were established according to the PICO (Participants, Interventions, Comparisons and Outcomes) principle: P as slaughter animals (cattle, sheep, goats, equines, pigs, leporids and poultry); I as feeding with a diet rich in PEs; C as control groups, or groups of animals with low phytoestrogen diet, or reproductive variables of the same experimental group reported before or after exposure to the high-phytoestrogen diet; and R as reproductive variables in males and females. The statement of the topic of the review question was developed as: Do phytoestrogens present in the diet produce adverse effects on reproductive variables in SAs? According to the keywords, Google Scholar was first searched and, subsequently, PubMed, dated until June 2020. Generally, the following words and Boolean operators were used, in order to identify the studies available on the web: {'Reproduction' AND 'Animals' AND 'Phytoestrogens' AND/OR 'Fertility' AND/OR 'Feed' AND/OR 'Isoflavones' AND/OR 'Coumestans', AND/OR 'Lignans' AND 'Cows' OR 'Heifers' OR 'Bulls' OR 'Ewes' OR 'Small Ruminant' OR 'Sheep' OR 'Ovine' OR 'Goat' OR 'Nanny-goat' OR 'Equine' OR 'Mare' OR 'Horses' OR 'Porcine' OR 'Sows' OR 'Pigs' OR 'Leporids' OR 'Rabbits' OR 'Hens' OR 'Rooster' OR 'Chickens'} {'Reproducción', AND 'Animales', AND 'Fitoestrógenos' AND/OR 'Fertilidad' AND/OR 'Alimentación' AND/OR 'Isoflavonas' AND/OR 'Coumestanos' AND/OR 'Lignanós' AND 'Vacas' OR 'Vaquillas' OR 'Toros' OR 'Pequeños rumiantes' OR 'Ovejas' OR 'Ovinos' OR 'Cabras' OR 'Equinos' OR 'Yeguas' OR 'Caballos' OR 'Porcinos' OR 'Cerdas' OR 'Lepóridos' OR 'Conejos' OR 'Aves' OR 'Gallinas' OR 'Gallos' OR 'Pollos'}. All the articles were searched in English and Spanish, were initially examined by reading their abstracts. The full texts of the documents included preliminarily were reviewed again to select the material to be used. Figure 1 shows a flowchart that details the selection of studies.

Figure 1: Flowchart of study search progress

Inclusion and exclusion criteria

The studies considered were those on controlled experiments *in vivo*, in SAs fed a diet rich or supplemented with PEs (soybeans, clover, alfalfa) for more than seven days, and that included the determination of the amount of PEs ingested or sufficient information to calculate it, in that case, the amount of PEs in the diet (mg/kg of DM [dry matter]) was obtained from the database of the United States Department of Agriculture^(22,23); likewise, to obtain the amount of daily feed, the ratio of the animals' weight and the amount of feed consumed containing PEs (reported in the article) was obtained. Those studies in which the administration of PEs was not related to feeding, or was administered orally through an excipient, or when reproductive parameters were not assessed, were excluded.

Data extracted from eligible studies were recorded as follows: species, breed, daily diet, main effects on reproductive variables, exposure time in days, PEs present in the diet and amount of PEs (mg/kg of DM).

Risk of bias analysis

The included papers were evaluated by two examiners in order to clarify emerging doubts, a third party was consulted to identify the presence of bias of randomization, blinding, results reporting and others (Table 1). To represent the bias analysis, a color scale was proposed: if the study met the described criterion, the green color was used; if the study was unclear or there was not enough information for the evaluation of bias, the yellow color was used.

Table 1: Summary of bias analysis of each included study

Reference	The random selection of the animal sample is clearly described	The researchers who selected the sample did NOT know to which treatment it would be assigned	Blinding of evaluators to the assigned treatment was ensured	The results are complete	The study is free of other sources of bias
García <i>et al.</i> , 2018 ⁽²⁴⁾					
Woclawek-Potocka <i>et al.</i> , 2005 ⁽²⁵⁾					
Hashem <i>et al.</i> , 2016 ⁽²⁶⁾					
Rodríguez <i>et al.</i> , 2013 ⁽²⁷⁾					
Piotrowska <i>et al.</i> , 2006 ⁽²⁸⁾					
Yurrita <i>et al.</i> , 2017 ⁽²⁹⁾					
Cantero <i>et al.</i> , 1996 ⁽³⁰⁾					
Pace <i>et al.</i> , 2006 ⁽³¹⁾					
Hashem <i>et al.</i> , 2018 ⁽³²⁾					
Pace <i>et al.</i> , 2011 ⁽³³⁾					
Aragadvay-Yungán <i>et al.</i> , 2018 ⁽²⁾					
Sierra <i>et al.</i> , 2015 ⁽³⁴⁾					
Domínguez <i>et al.</i> , 2014 ⁽¹⁹⁾					
Ferreira-Dias <i>et al.</i> , 2013 ⁽³⁵⁾					
Gentao <i>et al.</i> , 1999 ⁽³⁶⁾					
Yuan <i>et al.</i> , 2012 ⁽³⁷⁾					
Cardoso <i>et al.</i> , 2007 ⁽³⁸⁾					
Cardoso <i>et al.</i> , 2009 ⁽³⁹⁾					
Yousef <i>et al.</i> , 2004 ⁽¹⁸⁾					
Hashem <i>et al.</i> , 2008 ⁽⁴⁰⁾					
Saleh <i>et al.</i> , 2019 ⁽⁴¹⁾					
Ni <i>et al.</i> , 2007 ⁽⁴²⁾					
Lu <i>et al.</i> , 2017 ⁽⁴³⁾					
Wistedt <i>et al.</i> , 2012 ⁽⁴⁴⁾					
Arija <i>et al.</i> , 2006 ⁽⁴⁵⁾					
Heng <i>et al.</i> , 2017 ⁽⁴⁶⁾					

The green color indicates low risk of bias, the yellow color indicates that it is not clear or there is not enough information for the evaluation of the criterion, according to the authors' judgment.

Results

In the systematic search, 26 specialized documents were finally collected: six on cattle (one article in Spanish and three in English, a master's thesis in Spanish and a doctoral thesis in English); seven on sheep (one in Spanish and four in English, a master's thesis in Spanish, a conference abstract in Spanish); one on equines in English; two on pigs in English; four on leporids in English and six on poultry in English (Table 2). All articles were published between 1996 and 2019.

Cattle

In a study conducted on 11-month-old Bradford heifers (n=15 per group) fed, six months before their first service, with 0.8 % of soybeans, calculated based on live weight (LW), (feed that was considered by the authors to be high in PEs when compared to the usual feeding of 0.3 % LW), a percentage of gestation (%G) of 93 % and an abortion were observed, compared with the control group, where a 100 % success rate in pregnancy and no cases of abortion were reported; the authors mention that it is not possible to affirm that feeding with 0.8 % LW was the cause of abortion, and that this feeding did not affect reproductive parameters⁽²⁴⁾. In another study, was evaluated the %G in Holstein/Polish heifers (75 and 25 %) that were constantly fed 2.5 kg of soybeans, it was observed that the pregnancy rate was not significantly different compared to the control group, although equol and p-ethyl-phenol (compounds derived from the metabolism of PEs) were detected in the serum of the heifers⁽²⁵⁾. On the other hand, it has been reported that, in Holstein heifers fed clover for five months, the %G decreased (61.5 %), compared to heifers fed corn silage (92.3 %); the percentage of heifers that did not become pregnant after several inseminations was higher (38.46 %) in the group of cows fed clover compared to its control (7.7 %)⁽²⁶⁾ (Table 2).

In adult Holstein/Polish cows (75 and 25 %) that were fed 2.5 kg of soybeans from a lactation period prior to mating, they had a %G of 60 % compared to the control group, which showed 100 % gestation⁽²⁵⁾. In the lactation stage of Holstein cows fed for 60 d with alfalfa or red clover meal, alterations in the concentrations of E2, progesterone (P4) and luteinizing hormone (LH) were reported. A significant decrease in E2 was documented in the groups of cows fed alfalfa (2.32 ± 0.12 pg/mL) or red clover (2.25 ± 0.67 pg/mL) compared to control cows (4.24 ± 0.31 pg/mL); the concentration of P4 decreased at the end of the supplementation period in cows fed alfalfa (1.586 ± 0.27 ng/mL) or red clover (0.988 ± 0.3 ng/mL) compared to control animals (2.82 ± 0.34 ng/mL); LH also decreased in cows in the groups fed alfalfa (3.82 ± 0.22 IU/mL) or red clover (3.7 ± 0.26 IU/mL) compared to control

cows (6.66 ± 0.39 IU/mL)⁽²⁷⁾. In another study, it was observed that the concentrations of P4 decreased throughout the estrous cycle in Holstein/Polish black cows when they were fed 2.5 kg of soybeans for 21 d, compared to the control group, this suggests that PEs contained in soybeans can alter the function of the corpus luteum (CL), it is worth mentioning that the effects began to be observed between d 15 and 18 after its intake⁽²⁸⁾ (Table 2). In the only available study conducted on Angus bulls, with a diet with 10 % of soybeans from weaning to pre-puberty, an improvement in scrotal growth and semen quality in adulthood was observed⁽²⁹⁾.

The documented differences between sexes, derived from the effects produced by PEs, are biologically plausible, given that PEs are known xenoestrogens that alter the endocrine system depending on the availability of ERs and target organs of both sexes. In the studies reviewed, the age of exposure to PEs and the type of PEs determine their biological effect, observing a greater harmful effect in adult cows kept with feed rich in PEs than in heifers.

Small ruminants

In Manchega ewes that consumed alfalfa *ad libitum* for 10 mo and that contained COU at the rate of 25 ppm in autumn, 30 ppm in winter and 17 ppm in spring, it was found that 43 % of them showed alterations in the genital tract: cysts or microcysts in the endometrium accompanied by petechiae and ecchymosis in the uterine mucosa, increased glandular activity and paraovarian cysts⁽³⁰⁾. There are studies in which no harmful effects on the reproductive parameters of ewes and males of the Comisana breed are documented, it has even been suggested that prolonged administration of subterranean clover, with low formononetin content (less than 10 % of the total isoflavones on a dry basis), induces a significant improvement in the weight gain of animals and, in males, good carcass and meat characteristics⁽³¹⁾. In another study conducted on pregnant Rahmani ewes, which were fed for two months prior to calving and until the induction of the next heat (3.5 mo postpartum) with 849.4 g/kg of DM of *Trifolium alexandrinum*, no significant differences between groups in ovarian activity and ovulation in induced heat were documented, but females fed *Trifolium alexandrinum* showed shorter estrus duration (20 h) compared to ewes fed corn (34 h), in which P4 concentrations in the luteal phase of the induced estrus were significantly higher, compared to treated ewes⁽³²⁾.

In another work conducted on female lambs of the Sarda and Comisana breeds, the effects of feeding *ad libitum* with alfalfa or subterranean clover were evaluated, in these animals, no alterations were observed in the development of the reproductive system, fertility, fecundity, reproductive performance and calving interval, although animals fed clover had a higher

weight at puberty, the authors suggest that some clover varieties do not negatively affect the reproduction of ewes and appear to improve the growth rate of animals⁽³³⁾.

In Creole rams fed 1.1 kg/d of alfalfa contaminated with *Pseudopeziza medicaginis* at 10, 40 and 70 % for 45 d, a significant decrease in sperm concentration was documented in the rams fed alfalfa contaminated with the fungus at 40 % and 70 %, compared to the group with 10 %⁽²⁾. It should be mentioned that fungal infestations caused by *Pseudopeziza medicaginis* increase the synthesis of phytoestrogenic substances such as coumarins and isoflavones⁽²⁾. Other studies do not report differences between sperm characteristics, for example, in a study with hybrid Hampshire/Suffolk sheep, which were fed about 1 kg of alfalfa (2.5-6.5 mg of COU/100 g of alfalfa^(22,23)) or 200 g of extruded soybeans (57 mg of genistein/100 g of extruded soybeans; 31 mg of daidzein/100 g of extruded soybeans^(22,23)), daily for 90 d, no alterations were reported in sperm volume, color, motility and concentration, evaluated in fresh or cryopreserved semen⁽³⁴⁾. Likewise, in three-month-old hybrid Katahdin/Pelibuey lambs fed 23 % of alfalfa for 90 d, no differences were observed in sperm volume, sperm membrane integrity, total or progressive motility and acrosome status⁽¹⁹⁾ (Table 2). In sheep, contradictions were found regarding the deleterious or beneficial effects attributed to the intake of PEs on reproductive variables.

Equines

The effects of PEs due to feeding on equines are poorly described. Conjugate and free (active) forms of COU and its metabolites have been identified in the plasma of mares fed for 14 d with alfalfa pellets in increasing concentrations of up to 1 kg/d, during the time of the experiment, all mares cycled, on day zero half of the mares were in the follicular phase, and the other half in the luteal phase, on d 13 and 14 of the experiment, all the mares had the luteal phase⁽³⁵⁾ (Table 2). The results suggest that PEs affect the length of the estrous cycle and can prolong luteal function in the mare, due to the induction of the persistence of CL⁽³⁵⁾.

Pigs

In pregnant sows fed a regular diet supplemented with 0.005 mg of daidzein/kg of LW, in the peripartum period (from 30 ds prepartum to d 7 postpartum), they showed an increase in the weight of the litter compared to the control group, which did not consume daidzein; in addition, the production of milk, colostrum proteins and growth hormone increased by about 12 % in those sows fed that isoflavone⁽³⁶⁾. In minipigs fed low concentration of isoflavones

(250 ppm), no negative effects on reproduction were observed; in contrast, when the concentration increased (500 ppm), the testicular index ((bilateral testicular weight/total body weight) x 100 %) LH and testosterone (T4) decreased; in addition, there was an increase in apoptotic germ cells, indicating testicular peroxidation⁽³⁷⁾ (Table 2).

In sows, feeding supplemented with daidzein favors the weight of the offspring at calving and milk production, in the case of males, the effect of PEs in the diet depends on the amount administered, low amounts of PEs favor testicular function.

Leporids

In a study conducted on pregnant does fed a diet of 18 % of soybean meal (13 mg/kg of isoflavones/body weight) during the stages of gestation, lactation and until the offspring were 33 weeks old, it was observed that males were earlier in the time of onset of puberty compared to the control group, no significant differences were reported in the morphology of the reproductive organs, semen quality and sexual behavior⁽³⁸⁾. In another study, the effect of exposure to a commercial rabbit diet with 18 % of soybean meal during perinatal period (intrauterine and lactation) on the morphology of the reproductive organs of males at 26 wk of age was evaluated. No alterations were observed in the reproductive tract of the male progeny⁽³⁹⁾.

In 7-mo-old adult New Zealand male rabbits fed 30 % of Berseem (*Trifolium alexandrinum*) hay and supplemented with 2.5-5 mg of isoflavones/kg of body weight every third day for 13 wk, improvements in semen and libido characteristics were documented, since there was an increase in sperm volume, concentration and percentage of sperm motility⁽¹⁸⁾. When the effect of a diet rich in soybeans (80 g of soybeans/kg of feed) and flaxseed (100 g of flaxseed/kg of feed) in adult rabbits was evaluated, increases in the occurrence of abnormalities in spermatozoa, decrease in libido and in the process of steroidogenesis were observed, however, when they were crossed with untreated females, pregnancy rate, litter size and fertility were not affected⁽⁴⁰⁾ (Table 2).

The results of the previous studies suggest that the administration of commercial feed, with a concentration of up to 18 % of soybeans, to pregnant does, does not produce, in the offspring, alterations in the morphology of the reproductive organs in F1 males. Nor do diets with percentages less than 80 g of soybean meal or 100 g of flaxseed/kg of feed cause effects on the reproductive organs of males, the volume of the ejaculate or fertility. No studies evaluating the effects of PEs directly on the reproductive physiology of females were found (Table 2).

Poultry

In a study conducted on 65-wk-old Bovans Brown laying hens⁽⁴¹⁾ fed diets supplemented with 1 g of flax seeds or fenugreek/kg of feed (rich in PEs, 0.20 mg of isoflavones/kg of DM) for 6 wk, an increase in the concentrations of E2 and LH, weight and an improvement in shell thickness at the end of the egg laying cycle were observed, both with the supplementation of the seeds separately and with their combination (flax and fenugreek)⁽⁴¹⁾. The increase in eggshell thickness documented in the previous study was also present in another, in which 445-d-old ISA laying hens were fed a diet supplemented with 10 mg of daidzein/kg of feed⁽⁴²⁾. In addition, in this last study, a significant increase in the proportion of oviduct weight to body weight was reported⁽⁴²⁾. There were no alterations in the width and length of the egg, nor in the serum concentrations of E2⁽⁴²⁾. On the other hand, it has been observed that in 44-wk-old Rugao laying hens fed supplemented diets from 60 to 248 mg of daidzein/kg of feed for 12 wk, it did not generate significant differences in egg quality or fertility, although an increase in the hatchability of egg laying was observed⁽⁴³⁾. Wistedt *et al*⁽⁴⁴⁾ also reported the absence of effects on reproductive variables in 15-wk-old Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) laying hens on the morphology and size of ovaries and oviducts after being supplemented with 50 mg of daidzein/kg of feed. A difference in the sensitivity of the breeds to daidzein was observed, since the eggshells were thicker in LB hens than in LSL hens⁽⁴⁴⁾. In 1-d-old Cobb broilers, fed 100, 200 or 300 mg of extruded or raw beans/kg of feed for 21 d, a decrease in T4 and androstenedione concentrations was observed with raw bean feeding, in contrast, the administration of extruded beans increased the same variables⁽⁴⁵⁾.

Finally, in 70-d-old young breeding roosters fed a commercial product added with isoflavones, it was observed that, with 5 mg/kg, testicle weight, concentration of gonadotropin-releasing hormone (GnRH) and expression of the mRNA of the StAR enzyme⁽⁴⁶⁾ were increased (Table 2). The results of the studies suggest that PEs cause favorable effects on reproductive variables of male and female poultry.

Bias

In none of the studies included in the present review, the evaluators were blinded to the assigned treatment, nor in the selection of the sample. In contrast, complete results were identified in all the papers, and they were free from other sources of bias. More than half of the included studies used some randomization technique for their experimental units^(18,27,30,31,36,37,38,39,40,41,42,43,45,46).

Discussion

In ruminants, isoflavones are metabolized in the rumen, which generates estrogenic or non-estrogenic compounds. Secoisolariciresinol and matairesinol are the precursors of COU, enterodiol and enterolactone; biochanin A and genistein can be broken down into p-ethylphenol (non-estrogenic); formononetin is metabolized to daidzein and finally to equol, a more estrogenic compound that is absorbed through the rumen wall^(47,48).

The main effects produced by PEs in cattle include hormonal alterations, decrease in %G and increase in abortions, this depends on the reproductive stage, the time of consumption and the type of PEs involved. Early embryonic mortality and the increase in the abortion rate can be explained by the ability of PEs to inhibit the secretion of P4 stimulated by LH^(49,50). In cows, the release of prostaglandins (PG) with luteolytic action is regulated by E2 and P4⁽⁵¹⁾. PEs and their active metabolites alter the PGE2-F2 α ratio, which leads to the non-physiological production of luteolytic agents during gestation and the estrous cycle⁽²⁵⁾. In gestation, the balance between PGE2-F2 α is crucial for the maintenance and function of CL, the recognition of pregnancy, embryo implantation and development, so the stimulation of PGF2 α production can lead to interfere with this balance and embryonic development⁽⁵²⁾. PEs as agonists, in non-pregnant animals, reduce the length of the estrous cycle, since during luteolysis and ovulation, stimulation of PGF2 α secretion can accelerate positive feedback between PGF2 α and oxytocin^(51,52). On the other hand, PEs, as antagonists, induce alterations in follicular development and therefore, the absence of estrus^(53,54). In addition, a possible positive relationship between the concentration of isoflavones in blood plasma and the incidence of silent heat in dairy cattle fed soybeans has been reported⁽⁵⁵⁾.

Being structurally similar to E2, PEs act as agonists or antagonists of ERs^(7,56). The biological effects attributed to PEs occur with concentrations around 1,000 times higher than endogenous E2 concentrations (1-10 nM). This is based on the fact that Woclawek-Potocka *et al* reported $1.6 \pm 0.3 \mu\text{M}$ of p-ethyl phenol and $1.2 \pm 0.28 \mu\text{M}$ of equol⁽²⁵⁾; Piotrowska *et al* reported $1.28 \pm 0.10 \mu\text{M}$ of equol and $6.24 \pm 0.30 \mu\text{M}$ of p-ethyl phenol⁽²⁸⁾; Zdunczyk *et al* reported daidzein, genistein, equol and p-ethyl-phenol in a range of 0.1-3.6 $\mu\text{mol/L}$ in cows that showed reproductive alterations⁽⁵⁵⁾. In another study, it was calculated that the intake of 66.8 mg/kg of COU (present in alfalfa) produced plasma concentrations of 13 ng/mL of this compound, which also turns out to be 1,000 times higher than the concentration of E2 during estrus⁽²⁵⁾. Even considering that the biological activity of COU is 160 times lower compared to that of E2, the amount would be equivalent to six times the effective concentration of E2 in estrus, and therefore, sufficient to induce estrogenic changes similar to those found in the cow during this phase of the estrous cycle⁽⁵⁷⁾. The above data suggest that cows that are continuously exposed to a diet that includes PEs may show reproductive alterations, contrary

to what is reported in males, in which feeding with 10 % of soybeans as a source of protein improves sperm formation and concentration⁽²⁹⁾.

In sheep, the effects of PEs on reproductive variables are contradictory. In some studies, it has been suggested that these compounds do not cause alterations as in other species^(19,20); in others, their deleterious effect is evident, even the “Clover Syndrome” has been described, which consists of infertility, prolapse of the uterus and dystocia^(58,59). The main effects reported in females are morphological alterations in reproductive organs and increased activity of the endometrial glands, which leads to quantitative and qualitative changes in cervical mucus, which can hinder fertilization^(30,60). It is reported that PEs sensitize the cervix to estrogenic action, in addition to occupying ERs in the cervix, they also stimulate the appearance of new binding sites⁽⁶¹⁾. In addition, PEs alter the secretion of the follicle-stimulating hormone (FSH)⁽⁶²⁾. In ewes, the number of recruitable follicles depends on FSH concentrations⁽⁶³⁾, therefore, PEs are likely to interfere with follicular recruitment.

It has been reported that the factors related to the absence of effects caused by PEs in some studies are seasonality, the dose of PE ingested and the species and conditions of the plant used, since it has been documented that, in a single plant species, more than one type of PEs can be found at different concentrations; for example; under normal conditions, the concentrations of COU in alfalfa are 1 to 2 mg/kg and in circumstances of defense of the plant, the concentration of that PE can increase up to 100 mg/kg⁽⁶⁴⁾. The concentration of PEs also depends on the presentation and organ of the plant, for example: the isoflavone content in raw green soybeans is 48.9 mg/100 g of DM; that of extruded soybeans is 91 mg/100 g of DM and textured soybeans contain 172.6 mg/100 g of DM^(22,23). With respect to flavonoids, mature soybeans contain 37.41 mg/100 g of DM and green soybeans contain 1.23 mg/100 g of DM^(22,23).

The transition of photoperiods contributes to the regulation of estrous behavior, ovulation, ERs availability and concentration of endogenous steroids⁽³²⁾. In sheep, it has been suggested that prolonged ingestion of subterranean clover with PE levels below 0.3 % or ~10 mg/g DM of alfalfa does not produce infertility or reproductive disorders, it even significantly increases body growth⁽³³⁾, probably because PEs also stimulate growth hormone^(42,65). It has been suggested that the differences in deleterious effects on reproduction may be due to the type of isoflavones administered, the difference in the number of ERs and the type of metabolism between the different species⁽⁶⁶⁾. In male sheep, contradictory results were also found, Aragadvay *et al*⁽²⁾ describe reproductive alterations with the feeding of alfalfa contaminated with *Pseudopeziza medicaginis* at 40 and 70 %; other studies do not report harmful effects^(19,34). The reason why this difference in results exists is not described yet, probably due to the difference in the sensitivity of steroidogenic compounds between breeds of the same species, as reported in other animal models⁽⁶⁷⁾, it is even likely that male sheep are less

susceptible to the effect of phytoestrogens⁽²⁰⁾ or that some plant varieties do not negatively affect the reproduction of ewes^(20,33).

According to the criteria established in this review, no published studies related to goats were found. However, it has been reported that goats constantly fed 30 % of dehydrated alfalfa had an incidence of 20 % of rectal or vaginal prolapses in the last month of gestation⁽⁶⁸⁾.

In a case report of mares fed 5-8 kg/d of mixture of alfalfa and clover hay for at least 5 mo, uterine edema, absence of ovulation and accumulation of uterine fluid and return to normal ovarian cyclicity within 2 to 3 wk after withdrawal of the feed rich in PEs, were observed⁽³⁵⁾. The mechanism of absorption of PEs is not fully described; in this species, digestion is very fast and feed can pass through the stomach and small intestine within the first 5 h⁽⁶⁹⁾. This was confirmed in a study in which active forms of COU reached their highest level between 1 and 3.5 h after ingestion⁽³⁵⁾. The amount of PEs in the pasture depends on the season of the year, since a decrease in COU and its metabolites was found when evaluated from November to March and it is known that its synthesis increases under adverse conditions for plants⁽³⁵⁾. It is worth mentioning that the dehydration of alfalfa to produce pellets could reduce estrogenic activity⁽⁷⁰⁾. Therefore, the exposure time, the presentation of PEs and the season of the year are fundamental for the effects to occur.

The effects of PEs, present in the diet of pigs, on reproductive variables are few, in contrast to the amount of data on estrogenic effects of feed contaminated with mycotoxins, or works in which the effect of isoflavones on meat quality and growth is studied⁽⁷¹⁻⁷³⁾. Daidzein and genistein are the main isoflavones contained in soybean meal, which is the basic protein ingredient in the diet of pigs, and it is known that both represent 88 % of the isoflavones circulating in the blood⁽⁷³⁾. One of the effects of feeding sows with diets containing PEs in the last third of gestation is an increase in the weight of animals, this may be due to the positive effect produced by PEs on growth hormone concentrations⁽⁷⁴⁾. In males, the effect is much more evident, since, in the fetal stage, the concentrations of circulating estrogens are low, and the presence of PEs would stimulate growth due to a change in metabolism, proliferation and differentiation of skeletal muscle⁽⁷⁵⁾. The increase in milk production and better milk quality in sows fed isoflavones could be due to their agonist effect on insulin-like growth factor-1 (IGF-1) and prolactin reported in periparturient sows⁽⁷⁶⁾ or to a better balance of antioxidants⁽⁷⁷⁾. In males, a positive effect on reproduction has been identified when low concentrations of PEs are administered⁽³⁷⁾. This has been attributed to the binding of isoflavones to ERs in the hypothalamus, pituitary gland and testicles, thus stimulating spermatogenesis, sperm maturation and gonadal growth⁽⁶⁾.

As for leporids, it is reported that females are more sensitive to the effect of environmental estrogens⁽³⁸⁾. In male rabbits, intrauterine and lactational administration of PEs do not induce deleterious effects on semen production or sexual behavior, it has even been suggested that

soybean meal can be used as part of the normal diet in pregnant females without reproductive damage to the progeny^(38,39). The beneficial influence of isoflavones on semen parameters has been attributed to their antioxidant effect because they reduce the production of hydrogen peroxide and protect spermatozoa against oxidative damage⁽¹⁸⁾. In addition, in adult males, estrogen plays a major role in the prevention of the apoptosis in germ cells through its paracrine or autocrine actions in the testicles⁽³²⁾. The type of PEs consumed by rabbits also influences the final effect, for example, lignans suppress T4 synthesis more strongly during spermatogenesis and libido, compared to isoflavones⁽³²⁾.

In poultry, as in other domestic animals, PEs can act as agonists/antagonists, depending on the dose, tissue type, ER subtype and presence of endogenous hormones, therefore they are considered selective modulators of ERs⁽⁷⁸⁾. In poultry, the effects produced by the consumption of PEs are related to the genetic component, since lines of LB and LSL hens showed different sensitivity to these compounds⁽⁴⁴⁾; this could be due to the fact that there is a difference in the location and expression of ERs β in uterine capillaries between genetic lines, which could influence the obtaining of greater weight of the oviduct⁽⁴⁴⁾.

In bird males, it was observed that a diet rich in PEs promotes testicular growth due to the increase in hormonal secretion, probably due to the regulatory participation of isoflavones in the feedback mechanism of the hypothalamic-pituitary-gonadal axis⁽⁷⁹⁾, which in turn regulates the secretion of FSH, LH and T4 that promotes the growth and maturation of Sertoli and Leydig cells. The administered dose also has an important role, it is documented that genistein has partial agonist effects in roosters at doses of 50-200 mmol/kg, and acts as an antagonist at doses of 400-500 mol/kg⁽⁷⁸⁾; in addition, PEs have the ability to inhibit the activity of steroidogenic enzymes, and influence the viability of sex hormones through the regulation of their binding proteins; they alter brain centers related to sexual behavior as they cross the blood-brain barrier and bind to ER α and β ⁽⁸⁰⁾.

Finally, the metabolism of dietary PEs is predominantly determined by gastrointestinal bacterial metabolism and depends on the reproductive stage, for example, during gestation, genistein has the potential to influence fetal metabolism and growth; in the colon, genistein can be metabolized to dihydrogenysteine or 6'-hydroxy-O-desmethylangolensine, while daidzein can be reduced to dihydrodaidzein and converted into O-desmethylangolensin or equol, these metabolites can be absorbed or metabolized into phenols in the colon lumen⁽⁸¹⁾. After the consumption of PEs, these are deconjugated by the intestinal bacterial flora, reabsorbed, re-conjugated in the liver and excreted in the urine^(25,61). The demethylation of these compounds occurs in the intestine by acetogenic bacteria and in the liver⁽⁸¹⁾. Therefore, the intestine microbiome present in each species will influence the final effect of PEs on productive and reproductive variables.

Conclusions

Based on the papers reviewed and discussed, it is concluded that phytoestrogens cause alterations in the reproductive physiology of slaughter animals considering four factors: 1) The plant species consumed; 2) The season of the year in which the plant species is consumed; 3) The particularities of the animals (metabolism, age, breed, species and sex) and 4) The processing conditions of the plant. Future studies should be carried out to elucidate the endocrine mechanisms of the actions of PEs in the reproduction of animals. It is necessary to reevaluate the ingredients that make up the feeds of the different productive animals.

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Table 2: Summary of the main effects of phytoestrogens (PEs) on productive species

Species	Condition and breed	Daily intake	Main effects	Exposure (days)	Amount of PEs according to the species and presentation of the plant (mg/kg of DM)	Authors and year
Cattle	Holstein/Polish cows	2.5 kg of soybeans	Decrease in PR	300	1900 of GEN and DAI	Woclawek-Potocka <i>et al.</i> , 2005 ⁽²⁵⁾
	Holstein cows	~5 kg of alfalfa	Increase in E2, decrease in P4 and LH	60	~25-65 of COU	Rodríguez <i>et al.</i> , 2013 ⁽²⁷⁾
	Holstein cows	~5 kg of red clover	Increase in E2, decrease in P4 and LH	60	~100 of GEN and 110 of DAI	Rodríguez <i>et al.</i> , 2013 ⁽²⁷⁾
	Holstein/Polish cows	2.5 kg of soybeans	Decrease in P4	21	1900 of GEN and DAI	Piotrowska <i>et al.</i> , 2006 ⁽²⁸⁾
	Bradford heifers	2.22 kg of soybeans	Decrease in PR, increase in abortions, not significant	300	~570 of GEN and 310 of DAI	García <i>et al.</i> , 2018 ⁽²⁴⁾
	Holstein/Polish heifers	2.5 kg of soybeans	No difference in PR	21	1900 of GEN and DAI	Woclawek-Potocka <i>et al.</i> , 2005 ⁽²⁵⁾
	Holstein heifers	~7 kg of clover	Decrease in PR and P4 Increase in E2	150	6.60 of GEN, 8.05 of DAI, 2.85 of FOR and 282.5 of BI	Hashem <i>et al.</i> , 2016 ⁽²⁶⁾
Angus male calves	~800 g of soybean meal	Increase in sperm concentration	360	~135 of GEN and 6.32 of DAI	Yurrita <i>et al.</i> , 2017 ⁽²⁹⁾	
Sheep	Manchega ewes	Alfalfa pasture <i>ad libitum</i>	Endometrial and paraovarian cysts	300	17-30 of COU	Cantero <i>et al.</i> , 1996 ⁽³⁰⁾
	Rahmani ewes	800 g of subterranean clover	Shorter estrus duration	180	6.60 of GEN, 8.05 of DAI, 2.85 of FOR and 265 of BI	Hashem <i>et al.</i> , 2018 ⁽³²⁾

	Comisana female lambs	Subterranean clover pasture <i>ad libitum</i>	No effects on reproduction	60	797 of GEN, DAI, FOR and BI	Pace <i>et al.</i> , 2006 ⁽³¹⁾
	Sarda female lambs	Alfalfa pasture <i>ad libitum</i>	No alterations in reproductive system, fertility and fecundity	60	900-10210 of GEN, BI and FOR	Pace <i>et al.</i> , 2011 ⁽³³⁾
	Sarda female lambs	Subterranean clover pasture <i>ad libitum</i>	No alterations in reproductive system, fertility and fecundity	60	900-10210 of GEN, BI and FOR	Pace <i>et al.</i> , 2011 ⁽³³⁾
	Comisana female lambs	Subterranean clover pasture <i>ad libitum</i>	No alterations in reproductive parameters	600	810-880 of GEN, BI and FOR	Pace <i>et al.</i> , 2011 ⁽³³⁾
	Creole rams	~1 kg of alfalfa	Sperm concentration inverse to the dose	45	~25-65 of COU	Aragadvay-Yungán <i>et al.</i> , 2018 ⁽²⁾
	Hampshire/Suffolk rams	1 kg of alfalfa meal	No alterations in sperm parameters	90	~25-65 of COU	Sierra <i>et al.</i> , 2015 ⁽³⁴⁾
	Hampshire/Suffolk rams	200 g of extruded soybeans	No alterations in sperm parameters	90	~570 of GEN and 310 of DAI	Sierra <i>et al.</i> , 2015 ⁽³⁴⁾
	Katahdin/Pelibuey male lambs	~230 g of alfalfa	No alterations in sperm parameters or morphology	90	~25-65 of COU	Domínguez <i>et al.</i> , 2014 ⁽¹⁹⁾
	Comisana male lambs	Subterranean clover pasture <i>ad libitum</i>	No effects on reproduction	60	797 of GEN, DAI, FOR and BI	Pace <i>et al.</i> , 2006 ⁽³¹⁾
Equines	Lusitano mares	1 kg of alfalfa pellets	High concentrations of COU in serum	14	~25-65 of COU	Ferreira-Dias <i>et al.</i> , 2013 ⁽³⁵⁾
Pigs	Large White/Erhualian pregnant sows	~1.5 mg of DAI	Increase in milk production	37	-	Gentao <i>et al.</i> , 1999 ⁽³⁶⁾

	Minipig boars	~6.75–27 mg of soybean isoflavones	At low doses, increase in testicular index; at high doses, decrease in testicular index, LH and T4	60	125-500 of ISO	Yuan <i>et al.</i> , 2012 ⁽³⁷⁾
Leporids	New Zealand pregnant does	~20 g of soybean meal	No effect on reproductive organs, semen quality and sexual behavior of F1	~256	~135 of GEN and 632 of DAI	Cardoso <i>et al.</i> , 2007 ⁽³⁸⁾
	New Zealand bucks	~32 g of soybean meal	No alterations in reproductive organs	60	~135 of GEN and 632 of DAI	Cardoso <i>et al.</i> , 2009 ⁽³⁹⁾
	New Zealand bucks	7.13-14.25 mg of ISO*	Improvement in the characteristics of semen and libido	91	-	Yousef <i>et al.</i> , 2004 ⁽¹⁸⁾
	Line V bucks	~15.44 g of soybeans	It does not affect semen fertility, decrease in T4	84	240.4 of DAI and 131 of GEN	Hashem <i>et al.</i> , 2018 ⁽⁴⁰⁾
	Line V bucks	~19.3 g of flaxseed	It does not affect semen fertility, decrease in T4	84	368 of SECOI and 52.8 of DAI	Hashem <i>et al.</i> , 2018 ⁽⁴⁰⁾
Poultry	Bovans Brown hens	~120 mg of flax	Increase in LH, FSH, E2	42	-	Saleh <i>et al.</i> , 2019 ⁽⁴¹⁾
	Bovans Brown hens	~131 mg of fenugreek	Increase in LH, E2	42	~0.1 of DAI and 0.1 of GEN	Saleh <i>et al.</i> , 2019 ⁽⁴¹⁾
	Bovans Brown hens	~128 mg of flax and 128 mg of fenugreek	Increase in LH, FSH, E2	42	~0.1 of DAI and 0.1 of GEN	Saleh <i>et al.</i> , 2019 ⁽⁴¹⁾
	ISA hens	~1.2 mg of DAI	Increase in oviduct weight	63	-	Ni <i>et al.</i> , 2007 ⁽⁴²⁾
	Rugao hens	~5.4-22.57 mg of DAI	No effect on fertility	84	-	Lu <i>et al.</i> , 2017 ⁽⁴³⁾

Lohmann Brown hens	~5 mg of DAI	No changes in morphology and size of ovaries and oviducts high sensitivity to DAI	84	-	Wistedt <i>et al.</i> , 2012 ⁽⁴⁴⁾
Selected Leghorn hens	~5 mg of DAI	No changes in morphology and size of ovaries and oviducts	84	-	Wistedt <i>et al.</i> , 2012 ⁽⁴⁴⁾
Young breeders	~0.75 mg of ISO	Increase in testicle weight, GnRH	63	-	Heng <i>et al.</i> , 2017 ⁽⁴⁶⁾
Cobb chickens	~2.38-7.14 mg of extruded beans	Increase in T4 and androstenedione	21	-	Arija <i>et al.</i> , 2006 ⁽⁴⁵⁾
	~2.38-7.14 mg of raw beans	Decrease in T4 and androstenedione	21	-	Arija <i>et al.</i> , 2006 ⁽⁴⁵⁾

BI= Biochanin A; COU= Coumestrol; DAI= Daidzein; E2= 17-β estradiol; FOR= Formononetin; F1= Filial generation 1; GEN= Genistein; GLY= Glycitein; GnRH= Gonadotropin-releasing hormone; ISO= Isoflavones; LH= Luteinizing hormone; MCOU= Methoxycoumestrol; PEP= Para-ethyl-phenol; P4= Progesterone; SECOI= Secoisolariciresinol; T4= Testosterone; PR= Pregnancy rate; -= Not applicable; *= Every 3 days; ~= approximate calculation.