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The effects of Chaste-berry fruits on hypothalamic-pituitary-ovarian markers gene expression and immune response of laying hens: Phytoestrogens in Chaste-berry are ERβ-selective

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Abstract

Estrogen consumption in women can increase the risk of breast cancer. Estrogen stimulates the growth of cancer cells through the estrogen receptor alpha (ERα). One of the strategies that has recently been considered is the use of phytoestrogens. Previous studies have shown that Chaste-berry contains high levels of phytoestrogens. Scientists disagree on whether the phytoestrogens in Chaste-berry are used to treat many diseases in women, which are ERα or ERβ selective. In the present study, laying hens were used as a model to find the answer because only alpha estrogen receptor is expressed in the oviduct. In this study, the effect of Chaste-berry fruit powder on performance, egg quality, immune response, and the expression of GnRH, LH, ovalbumin (OVAL), and ovomucoid (OVM) genes in laying hens were evaluated. A total of 90 leghorns (Hy-Line, W-36) laying hens (at 72 to 80 weeks old) were used in a completely randomized design with three treatments and five replicates (n=6). The treatments were various levels of Chaste-berry fruit powder including zero, 1, and 2% levels of Chaste-berry fruit powder per kg of diet. Our results showed that performance parameters, egg quality factors, and immune responses were not significantly affected by various levels of Chaste-berry fruit powder. Moreover, the results indicated that the various levels of Chaste-berry did not have a significant effect on LH, OVAL, and OVM gene expression. However, GnRH gene expression was significantly increased in treatment 3 (a diet containing 2% Chaste-berry) compared to the control and 1% Chaste-berry groups. Moreover, the addition of 1% Chaste-berry fruit powder to the diet had no significant effect on GnRH gene expression. Therefore, Chaste-berry supplementation is not recommended in laying hens. Furthermore, our data reinforce this theory that phytoestrogens in Chaste-berry fruits are ERβ-selective.

Key words: Chaste-berry, Estrogen receptor, Phytoestrogen, Cancer

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Introduction

Some types of breast cancer are affected by hormones such as estrogens. Studies have shown that estrogens are important for stimulating the growth of breast cancers. It has been indicated that estrogens are carcinogenic in breast cancer. Estrogens are considered to play a key role in promoting the proliferation of breast epithelium. Estrogens action is mediated by two receptors: estrogen receptors alpha (ERα) and beta (ERβ). Now it is recognized that ER-alpha plays a role in breast cancer cell proliferation. Estrogen by binding to the estrogen alpha receptor increases the proliferation of cancer cells in breast cells (Ali and Coombes, 2000). One solution that has recently been considered is the use of phytoestrogens that bind to the estrogen receptor beta. Phytoestrogens are considered less dangerous than chemical estrogens. Chaste-berry (Vitex) is a multifunctional herb belonging to the Verbenaceae family with phytoestrogenic properties. Also, Vitex is known for its antifungal, anti-androgenic, antibacterial, anticancer, antiseptic, and various biological activities such as antimicrobial and antioxidant properties (Zamani et al, 2012). Vitex is a native plant of the middle Asian, southern European, and Mediterranean countries (Mari et al, 2015). This plant has numerous uses in traditional medicine and is traditionally used to treat many diseases in women, including infertility, premenstrual syndrome, hyperprolactinemia, abnormal menstrual cycles, endometriosis, relief of menopausal symptoms, and insufficient breast milk (Katiraee et al, 2015; Rani and Sharma, 2013). Previous studies showed that Vitex fruits increase the estrogen level and the progesterone levels in mice (Ahangarpour et al, 2016) and decrease LH without affecting FSH in rats (Ibrahim et al, 2008).

Some researchers have claimed that the phytoestrogens in Vitex fruits bind only to the estrogen receptor beta (Wuttke et al, 2003; Jarry et al, 2003), while others have shown that Vitex fruits contain phytoestrogens that can also bind to the estrogen receptors alpha and beta (Liu et al, 2001; Liu et al, 2004). The researchers indicated that the reason for this discrepancy was probably due to the origin and extraction of the drug and the treatment of the extractions. The mentioned experiments were all performed in Vitro conditions and were not confirmed in Vivo conditions. Examining the effect of Vitex on organs that produce only one of these receptors (ERα or ERβ) can help to understand which type of estrogen receptor bind to phytoestrogens in Vitex.

The chicken was used as an experimental animal model for human genetics and disease because of its advantages since early in the last century (Dodgson and Romanov, 2004). Several human diseases such as cancer, Atherosclerosis, and nonalcoholic fatty liver disease (NAFLD) were investigated by using poultry (Anderson et al, 2014; Anderson et al, 2013; Davis et al, 2016; Johnson and Giles, 2006). It has been well documented that in all parts of the laying hen oviduct only the estrogen receptor alpha (ERα) is produced (Stadnicka et al, 2018). Therefore, laying hens can be a good model for this experiment. In the same line, the purpose of this study was to evaluate the effect of Vitex fruits on performance, egg quality, immune response, and genes expression of hypothalamic GnRH, luteinizing hormone (LH) from the anterior pituitary, and oviduct markers (ovalbumin (OVAL) and ovomucoid (OVM)) of laying hens to determine if phytoestrogens in Vitex can affect oviduct estrogen receptor alpha.

Materials and methods

Plant material

Vitex fruits were prepared by a local producer. The parts of the plant suitable for consumption were dried in dark and powdered by an electric mill. The chemical composition of Vitex powder was

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determined by the Association of Official Analytical Chemists methods (AOAC, 2000). The results showed that Crude protein (CP), ether extract (EE), crude fiber (CF), and crude ash were 10.5%, 5.6%, 56.0% and 12.6% of dry matter, respectively.

Birds and sample collection

All procedures involving experimental animals and poultry welfare were performed in accordance with FASS guidelines (FASS, 2020). In short, a total of 90 Leghorn (Hy-Line, W-36) laying hens in the second cycle of production were used in a completely randomized design with sampling (equal number of samples per experimental unit) with three treatments and five replicates (n = 6) for eight weeks according to the following model equations: $Y_{ijk}= \mu + T_i + e_{ijk} + \delta_{ijk}$

In the above equations, Y_{ijk} is the dependent variable, μ; population mean, T_i; the effect of the i^{th} treatment, e_{ijk}; the random error, and δ_{ijk} is the sampling error effect.

Treatments were various levels of Vitex including zero (control; T1), 1.00% (T2), 2.00% (T3). The laying hens received a corn-soybean meal-based diet supplemented diet (corn and soybean-based diet with 15.05 % crude protein and 2802 kcal/ kg metabolizable energy) in a mash form and formalized according to the Hy-Line W-36 (2016) nutrient requirements (Table 1). Diet and water were prepared ad-libitum during the experiment. The birds were exposed to 16-hour light and 8-hour darkness (in 24 hours) throughout the experimental periods.

Egg weight (EW, gr), egg production (EP, %) and egg mass (EM, gr/hen/day) were registered daily. Feed intake (FI, gr) was measured weekly and feed conversion ratio (FCR, grams of feed: grams of egg mass) was calculated weekly. Two eggs were randomly collected from each replicate and egg quality (Haugh unit, shell strength, shell percentage and shell thickness) was determined weekly.

At the end of the experiment, one bird was sacrificed by cervical dislocation from each replicate, and their magnum, hypothalamus, and hypophysis were immediately separated. The tissues were washed with phosphate buffered saline (PBS) and transferred to the genetic laboratory with liquid nitrogen and stored at -70 ºC until total RNA extraction.

Real-time quantitative PCR

Total RNA was extracted from magnum tissue using Invitrogen TRIzol^R Reagent. The quality and integrity of the extracted RNA were assessed using 1% denaturing gel electrophoresis and a NanoDrop TM 2000C spectrophotometer (A260/A280). In the following, cDNA was synthesized using the Sinnaclon First Strand cDNA Synthesis Kit (Sinnaclon, Cat. No: RT5201). Eventually, the expression of OVAL, OVM, and Beta-actin genes was quantified by real-time quantitative PCR (RT-qPCR) technique. The primer information for these genes is listed in Table 2 .

In this study, cDNA was amplified by the polymerase chain reaction (PCR) technique. DNA products were quantitated using a Step One Plus real-time PCR (Applied Biosystems). The real-time qPCR cycling programs were as follows: A denaturation step (5 min at 95 ºC) was followed by an amplification step with 40 cycles of 15s at 95 ºC, 45s at 60 ºC, and 30 s at 72 ºC. A melting curve was plotted to investigate the formation of a non-specific product. The melting curves were produced by gradually increasing the reaction temperature from 55 to 95 ºC (at a 0.1 ºC/s incremental increase).

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Table 1. Composition and calculated analyses of the basal diet T1, T2 and T3

Ingredients (%)	T1 (control)	T2 (1.00% Vitex)	T3 (2.00% Vitex)
Corn	60.19	59.00	57.60
Soybean meal	22.30	22.30	22.40
Vitex powder	0	1	2
Vegetable oil	3.05	3.34	3.74
Di-calcium phosphate	1.6	1.6	1.6
Oyster shell	6.5	6.5	6.5
Limestone	5.15	5.15	5.15
Sodium bicarbonate	0.23	0.23	0.23
Salt	0.23	0.23	0.23
L-lysine hydrochloride	0.01	0.01	0.01
DL-Methionine	0.14	0.14	0.14
Vitamin premix1	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25
Calculated analysis			
Metabolizable energy (kcal kg^{-1})	2802	2798	2798
Crude protein (%)	15.05	15.03	15.01
Calcium (%)	4.85	4.84	4.84
Available phosphorus *(%)*	0.41	0.41	0.41
L- Lysine (%)	0.77	0.77	0.77
Methionine + Cystine (%)	0.64	0.64	0.64

[1]Vitamin premix provided the following per kilogram of diet: vitamin A: 8,000 IU; vitmin D3: 33000 IU; vitamin E: 20.00 mg; vitamin K3: 2.20 mg; vitamin B1: 1.50 mg; vitamin B2: 4.00 mg; vitamin B3: 8.00 mg; vitamin B5: 35.00 mg; vitamin B6: 2.50 mg; vitamin B9: 0.50 mg; vitamin B12: 10 µg; choline chloride: 468.70 mg.
[2] Mineral premix provided the following per kilogram of diet: Mn: 80.00 mg; Fe: 75.00 mg; Zn: 64.00; Cu:6.00 mg; I: 0.87 mg; Se: 0.30 mg.

In this method, the Beta-actin gene, as a reference gene, was used to normalize the data. The $2^{-\Delta\Delta CT}$ method was applied for quantitative real-time PCR data analysis (Pfaffl et al., 2002). This method is a convenient way to analyze the relative changes in gene expression from real-time quantitative PCR experiments. The efficiency corrected calculation models is:

$$ratio = \frac{(E_{target})^{\Delta CT target\ (control-sample)}}{(E_{ref})^{\Delta CT ref (control-sample)}}$$

In this model, E_{target} and E_{ref} are efficiency of target and reference gene respectively. PCR efficiency per amplicon was calculated using the LinRegPCR program. The program calculates PCR efficiency for each amplicon by linear regression on the Log (fluorescence) per cycle number data for each sample (Ramakers et al, 2003).

Antibody response to challenge with sheep red blood cells (SRBC)

To determine immune response, at 6 and 8 weeks of experiment, 0.5 mL of 20% sheep red blood cells (SRBC) was injected to the breast muscle of 2 hens per replicate. Then blood samples were taken from brachial vein 7 days after each injection. Blood samples were collected in EDTA vials stored at -20 °C. Antibody titer against SRBC was determined by the hemagglutination assay described by Nelson (1995).

Statistical analysis

The data were statistically analyzed by one-way analysis of variance (ANOVA), General Linear Model procedure, using SAS 9.1 software (SAS, 2005). Significant differences between treatments were detected using Duncan's multiple range test at the $P<0.05$ level of significance.

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Table 2. The sequence and characteristics of primers used for RT-PCR and real time quantitative PCR

Gene	Forward (F) and reverse (R) primers (5′➔ 3′)	Annealing temperature (ºC)	Amplicon size (bp)	Reference
GnRH	F:5´-ATTTTCCAGCGGGAAGAGTTG -3´ R:5´-TGGGTTTGTTGATGGTGTTGT-3´	62	350	Sabahi et al. 2020
LH	F:5´-GTTGGTGCTGATGACCCTTT-3´ R:5´-TGGTGGTCACAGCCATACAT-3´	62	194	Sabahi et al. 2020
Ovalbumin	F: CGTTCAGCCTTGCCAGTAGA R: AGTATTCTGGCAGGATTGGGT	60	60	Mosavi et al. 2022
Ovomucoid	F: TATGCCAACACGACAAGCGA R: CCCCCTGCTCTACTTTGTGG	60	133	Mosavi et al. 2022
Beta-actin	F: TGCTGTGTTCCCATCTATCG R: TTGGGACAATACCGTGTTCAT	60	150	Rabieh et al. 2020

Results

Egg production and quality

The effect of dietary Vitex fruits on egg production traits of laying hens is presented in Table 3. Performance parameters such as FI, EP, EM, EW and FCR were not significantly affected by various levels of Vitex fruit powder (P>0.05). The effect of dietary Vitex fruits on egg quality parameters of laying hens is illustrated in Table 4. The data indicate that egg quality parameters such as haugh unit, shell strength, shell percentage, shell thickness, albumen weights percentage, and yolk weights percentage were not affected significantly by Vitex supplementation (P>0.05).

Immune response

Effects of dietary Vitex fruits on immune parameters of laying hens are summarized in Table 5. Primary and Secondary AntiSRBC antibody levels were unaffected by dietary Vitex fruits treatment.

Table 3. The effect of dietary *Vitex* fruits on egg production and quality traits of laying hens on the second cycle of production (at 72 to 80 weeks old)

Treatments	Egg weight (g)	Egg mass (g)	Egg production (%)	Feed intake (g)	FCR (g/g)
T1 (Control)	64.39	53.22	82.55	107.98	2.04
T2 (1.00% Vitex)	64.90	53.67	82.80	107.41	2.01
T3 (2.00% Vitex)	64.84	51.05	79.50	111.62	2.23
Standard errors of means (SEM)	0.22	1.40	1.83	2.28	0.11
p-value	0.96	0.70	0.79	0.48	0.25

No significant differences were observed among treatments ($p > 0.05$).

Table 4. The effect of dietary *Vitex* fruits on egg quality traits of laying hens on the second cycle of production (at 72 to 80 weeks old)

Treatments	Shell Strength (kg/cm2)	Shell thickness (mm/100)	Haugh unit	Shell (%)	Yolk weight (%)	Albumen weight (%)
T1 (Control)	1.49	39.66	85.07	10.37	28.45	61.18
T2 (1.00% Vitex)	1.47	39.65	86.35	10.50	27.79	61.71
T3 (2.00% Vitex)	1.3	39.62	86.21	10.44	27.61	61.91
Standard errors of means (SEM)	0.05	0.17	0.69	0.08	0.36	0.30
p-value	0.14	0.96	0.77	0.98	0.60	0.83

No significant differences were observed among treatments (p>0.05).

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Table 5. The effect of dietary *Vitex* fruit powder on immunity response of laying hens

Treatments	Groups			SEM	P-value
	(Control)	(1.00% Vitex)	(2.00% Vitex)		
Primary AntiSRBC antibody level (log2)	4.00	5.33	4.60	0.21	0.34
Secondary AntiSRBC antibody level (log2)	4.50	5.50	5.25	0.19	0.23

No significant differences were observed among treatments ($p>0.05$).

Total RNA quality assessment

RNA quality includes two factors: RNA purity and RNA integrity. A common technique for assessing the quality of RNA is optical density (OD) measurement. A 260/A280 ratio between 1.8 and 2.1 is usually considered an acceptable indicator of good RNA quality. Isolating total RNA using the TRIzol reagent resulted in a high-quality product. The 260 / 280 absorbance ratios showed that all RNAs were of high quality (between 1.85 and 2.05). The RNA integrity was evaluated by the sharpness of rRNA bands visualized on a denaturing agarose gel. For all of the RNA samples tested, two strong bands (28S and 18S) without degradation were observed (Figure 1).

Oviduct markers amplification

To determine whether GnRH, LH, OVAL, OVM, and Beta-actin mRNA are expressed in the oviduct of laying hens, total RNA was isolated from magnum cells and analyzed using real-time qPCR. Agarose gel electrophoresis confirmed the expression of Beta-actin mRNA (Figure 2A), OVM (Figure 2B), OVAL (Figure 2C), GnRH (Figure 2D) and LH (Figure 2E) of laying hens. The GnRH, LH, OVAL, OVM, and Beta-actin appeared as a single band on 2% (w/v) agarose gels. Electrophoresis of the PCR products showed the 350, 194, 60, 133, and 150-bp fragments for GnRH, LH, OVAL, OVM, and Beta-actin, respectively.

Validation of real-time qPCR

Conventional PCR amplification and real-time PCR melting curve for OVAL, OVM, and Beta-actin genes were given. Melting peaks analysis on the PCR products for all primers confirmed minimal primer-dimers and primer specificity as shown by single peak melting curves for individual genes (results not presented). To determine the linearity of the reaction and the PCR efficiency of amplicons, the LinRegPCR program is used to calculate linear regression on the Log (fluorescence) per cycle number data for each sample (Ramakers et al., 2003). The mean PCR efficiency and coefficient of determination (R^2) calculated 99%, 0.9938 for Beta-actin, 102%, 0.9998 for OVAL, and 103%, 0.9990 for OVM.

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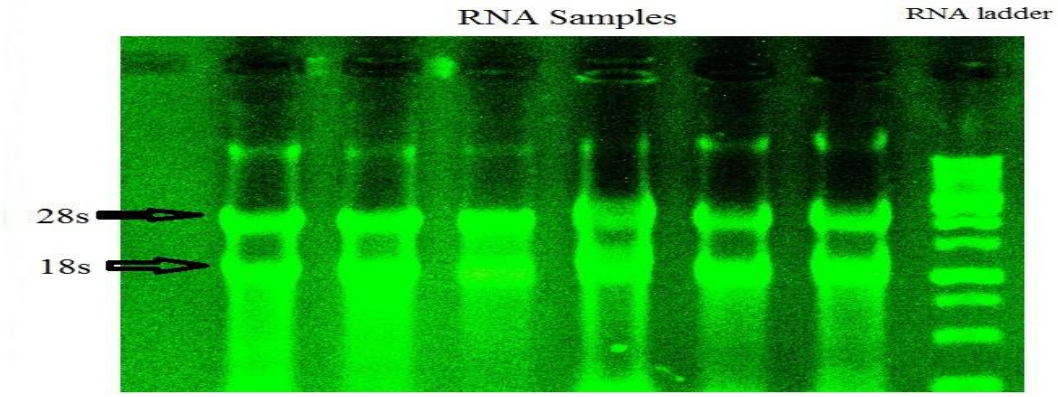

Fig. 1 Agarose gel electrophoresis and RNA integrity analysis of total RNA samples extracted from magnum cell of laying hens isolated by the TRIzol reagent.

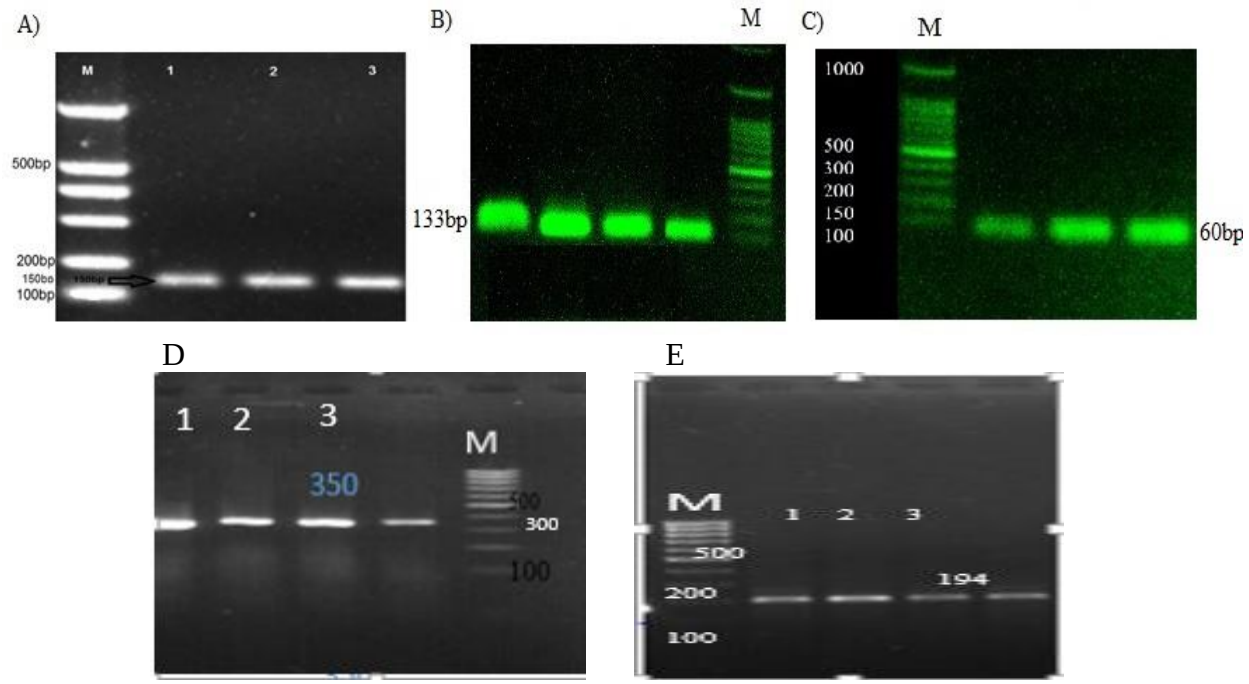

Fig. 2 A sample of electrophoresis of PCR products for (A) Beta actin (B) Ovomucoid (C) Ovalbumin (D) GnRH (E) LH of laying hens on the 2% Agarose gel. M: size marker 100bp

Gene expression analysis

Results of real-time quantitative PCR for GnRH, LH, OVAL and OVM mRNA expression are summarized in Table 6. The amount of mRNAs was normalized to the amount of Beta-actin mRNA. There were no significant changes in OVAL and OVM mRNA concentrations compared to the control. The results showed that the various levels of Vitex did not have a significant effect on OVAL and OVM genes expression ($P>0.05$). The ANOVA results revealed that GnRH gene expression was significantly increased in treatment 3 (diet containing 2% Vitex) compared to the control and 1% Vitex groups ($P<0.01$). While, addition of 1% Vitex fruit powder to diet had no significant effect on GnRH gene expression ($P>0.05$). Furthermore, addition of 1 or 2% Vitex fruit powder had no significant effect on LH gene expression ($P>0.05$).

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Table 6. The effects of dietary *Vitex* fruit powder on genes expression of laying hens

Target genes	Groups			SEM	P-value
	(Control)	(1.00% Vitex)	(2.00% Vitex)		
GnRH	1.000 [b]	1.396 [b]	5.206 [a]	0.85	0.017
LH	1.000	1.1000	1.273	0.15	0.162
Ovalbumin	1.000	1.401	1.465	0.090	0.497
Ovomucoid	1.000	1.360	1.580	0.239	0.587

The amount of mRNAs was normalized to the amount Beta actin mRNA. Different letters superscripts (a, b) among treatments differ significantly ($p \leq 0.05$), according to analysis of variance (ANOVA) using a general linear model (GLM).

Discussion

Vitex is traditionally used to treat many diseases in women (Katiraee et al, 2015; Rani and Sharma, 2013). Previous studies reported that the fruits of Vitex contain high amounts of phytoestrogens. As a result, consumption of Vitex fruits increased estrogen and progesterone levels (Ahangarpour et al, 2016). Due to their structural similarity with estradiol, phytoestrogens play an estrogenic and/or anti-estrogenic role. The structural similarities between phytoestrogens and estrogens cause them to act as an antagonist of estrogen (Yildiz, 2005).

Estrogens are produced by the theca cells of the ovarian follicles in birds. Estrogens regulate reproductive functions, reproductive behavior, synthesis of egg yolk proteins, and egg white proteins in birds (Hrabia et al, 2008). Estrogens are responsible for the growth of the follicle and yolk (Dougherty and Sanders, 2005). Moreover, estrogen stabilizes the mRNA of the ovalbumin gene in laying hens (Arao et al, 1994). Studies indicated that estrogen plays a role in the transcriptional and post-transcriptional regulations of ovalbumin (Palmiter, 1972; McKnight and Palmiter, 1979; Schweizer et al, 1985; Kato et al, 1992). Tubular glands induced by estrogen synthesize large numbers of egg-white proteins, such as ovalbumin, ovomucoid, lysozyme, and conalbumin (Ohler et al, 1968). Two oviduct markers OVAL and OVM, which are known as molecular signatures of oviduct cells, are expressed only in the oviducts of laying hens (Stadnicka et al, 2018). Moreover, research has shown that estrogen plays an important role in regulating Gonadotropin-releasing hormone (GnRH). GnRH is a key regulatory molecule of the hypothalamus–pituitary gonadal axis which induces transcription of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary (Lv et al. 2018).

Egg quality and egg production rapidly decrease at the end of the laying cycle. Because the levels of ovarian steroid hormones decrease in aged birds, egg quality and egg production rapidly decrease at the end of the laying cycle (Liu et al, 2018). It was hypothesized that supplementation of Vitex fruits containing phytoestrogens could increase estrogen at the end of the laying hen production period, thus overcoming the decline in production. In this study, Vitex fruits powder was added to the laying hen diet at three levels of 0, 1, and 2% in the late phase of the second production cycle.

RT-qPCR results indicated that the various Vitex levels did not significantly affect the expression of LH, OVAL, and OVM genes. Egg quality and egg production confirmed the RT-qPCR results. The results revealed that egg production and egg quality (egg weight, egg mass, albumen, and yolk weights percentage) were not significantly affected by various levels of Vitex powder. Consistent with the results, Karacollokcu et al. (2016) reported

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that supplementing Myrtus and Vitex volatile oil (alone or combined) in the laying hen diet did not affect the performance and internal and external quality traits of the eggs during the peak of the egg production period.

It should be noted that Vitex fruit powder supplementation up to 2% significantly increased the expression of hypothalamic GnRH gene compared to the control group and 1% Vitex ($P<0.01$). While the addition of 1% Vitex fruit powder to the diet has no significant effect on GnRH gene expression ($P>0.05$). It seems that adding Vitex to the poultry diet cannot be effective. Perhaps the reason must be sought in how the active phytoestrogens in the Vitex and estrogen receptors (ERα and ERβ) interact. Vitex consists of compounds such as vitexin, apigenin, and pendoltin, which are the most active phytoestrogen in Vitex fruits and mostly affect estrogen receptor beta (ERβ) (Wuttke et al, 2003; Jarry et al, 2003). It also contains small amounts of linoleic acid (0.0056 µµ% in 90 g of a defatted methanol extract), which can bind to alpha and beta estrogen receptors and stimulate ERβ mRNA expression (Liu et al, 2004). Of course, these results are all obtained in vitro conditions. The results of our experiments revealed that this small amount of linoleic acid (compared to other phytoestrogens in Vitex fruits) could not be effective. The Vitex supplementation in laying hens diet could increase GnRH gene expression in the hypothalamus where both ERα and ERβ are present but in the oviduct which only expresses the ERα (Stadnicka et al, 2018), phytoestrogens were not effective. The results of this research reinforce this theory that the phytoestrogens in Vitex fruits are ERβ-selective.

In the current study, considering the phytoestrogenic properties of Chaste-berry (Vitex), the effects of Chaste-berry fruits on laying hens were investigated. According to our results, supplementation of Chaste-berry fruits at levels 1 and 2 in the diet of laying hens had no effect on performance, and immune response. Therefore, Chaste-berry supplementation is not recommended in laying hens. RT-qPCR results indicated that the various Vitex levels did not significantly affect the expression of LH, OVAL, and OVM genes. However, Vitex fruit powder supplementation up to 2% significantly increased the expression of hypothalamic GnRH gene compared to the control group and 1% Vitex. Also, our results confirmed that phytoestrogens in Vitex fruits are ERβ-selective.

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Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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اثر تغذیه میوه ویتکس بر بیان نشانگرهای هیپوتالاموس-هیپوفیز-تخمدان و سیستم ایمنی مرغ تخمگذار: اتصال فیتواستروژن‌های میوه ویتکس به گیرنده استروژن بتا

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چکیده

مصرف استروژن در زنان می‌تواند خطر ابتلا به سرطان سینه را افزایش دهد. استروژن رشد سلول‌های سرطانی را از طریق گیرنده استروژن آلفا تحریک می‌کند. یکی از استراتژی‌هایی که اخیرا مورد توجه قرار گرفته است، استفاده از فیتواستروژن‌ها است. مطالعات قبلی نشان داده است که گیاه ویتکس حاوی سطوح بالایی از فیتواستروژن است. در مورد این که آیا فیتواستروژن‌های موجود در گیاه ویتکس برای اتصال کدام گیرنده (استروژن آلفا یا بتا) را انتخابی می‌کنند اختلاف نظر وجود دارد. با در نظر گرفتن این موضوع که در اوویداکت مرغ تخمگذار تنها گیرنده استروژن آلفا حضور دارد، در این آزمایش از مرغ‌های تخمگذار به عنوان مدل برای یافتن پاسخ استفاده شد. در این مطالعه اثر پودر میوه ویتکس بر عملکرد، کیفیت تخم مرغ، پاسخ ایمنی و بیان ژن‌های GnRH، LH، اوموئید و اووآلبومین در مرغ‌های تخمگذار بررسی شد. تعداد ۹۰ قطعه مرغ تخمگذار لگهورن (Hy-Line, W-36) در سن (۷۲ تا ۸۰ هفتگی) در قالب طرح کاملاً تصادفی با سه تیمار و پنج تکرار مورد استفاده قرار گرفت. تیمارها سطوح مختلف پودر میوه ویتکس شامل سطوح صفر، ۱ و ۲ درصد پودر میوه ویتکس به ازای هر کیلوگرم جیره بودند. نتایج تحقیق حاضر نشان داد که پارامترهای عملکرد، کیفیت تخم‌مرغ و پاسخ‌های ایمنی تحت تأثیر سطوح مختلف پودر میوه ویتکس قرار نگرفتند. نتایج واکنش زنجیره‌ای پلیمراز در زمان واقعی نشان داد که سطوح مختلف پودر میوه ویتکس تأثیر معنی‌داری بر بیان ژن‌های LH، اوموئید و اووآلبومین ندارد. با این حال، بیان ژن GnRH در تیمار ۳ (جیره غذایی حاوی ۲ درصد ویتکس نسبت به گروه شاهد و ۱ درصد ویتکس به طور قابل توجهی افزایش یافت. علاوه بر این، افزودن ۱ درصد پودر میوه ویتکس به جیره تأثیر معنی‌داری بر بیان ژن GnRH نداشت. در نتیجه، مصرف مکمل میوه ویتکس در مرغ‌های تخمگذار توصیه نمی‌شود. علاوه بر این، داده‌های ما این نظریه را تقویت می‌کند که فیتواستروژن‌های موجود در میوه‌های ویتکس، گیرنده استروژن بتا را برای اتصال یافتن انتخاب می‌کند.

کلمات کلیدی: ویتکس، بیان ژن، گیرنده استروژن، سرطان

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