

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

Mahmood Nazari^{1*}, Mohamad Reza Ghorbani², Mohamad Taghi Beighi Nassiri³, Neda Fathimoghadam⁴, Razieh Sabahi⁵ and Saiedeh Taghva Mosavi⁵

¹ Associate Professor, Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran

² Associate Professor, Department of Animal Science, Shirvan Faculty of Agriculture, University of Bojnord, Bojnord, Iran

³ Professor, Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran

⁴ MSc graduate of animal nutrition, Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran

⁵ MSc graduate of animal genetic and breeding, Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran

Received: 02.08.2022

Accepted: 30.01.2023

Abstract

Estrogen consumption in women can increase the risk of breast cancer. Estrogen stimulates the growth of cancer cells through the estrogen receptor alpha (ER α). 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 ER α or ER β 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 ER β -selective.

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

* **Corresponding Author:** Mahmood Nazari, Associate Professor, Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran
E-mail: M.nazari@Asnrkh.ac.ir



© 2020 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (<http://creativecommons.org/licenses/by-nc/4.0/>).

References

- Ahangarpour, A., Najimi, S.A., Farbood, Y. (2016). Effects of *Vitex agnus-castus* fruit on sex hormones and antioxidant indices in a D-galactose-induced aging female mouse model. *J Chinese Med Ass*, 79(11),589-596.
- Ali, S., Coombes, R.C. (2000). Estrogen receptor alpha in human breast cancer: occurrence and significance. *J Mam Gland Biol Neo*, 5(3), 271-81.
- Anderson, J.L., Ashwell, C.M., Smith, S.C., Shine, R., Smith, E.C., et al. (2013). Atherosclerosis-susceptible and atherosclerosis-resistant pigeon aortic cells express different genes in vivo. *Poultry Science*, 92(10), 2668-2680.
- Anderson, J.L., Keeley, M.C., Smith, S.C., Smith, E.C. (2014). Rosiglitazone modulates pigeon atherosclerotic lipid accumulation and gene expression in vitro. *Poultry Science*, 93(6), 1368-1374.
- AOAC. (2000). Official Methods of Analysis. Vol. I. 18th Edition. Association of Official Analytical Chemists, Arlington, VA, USA.
- Arao, Y., Miyatake, N., Ninomiya, Y., Hasegawa, T., Masushige, S., Kato, S. (1994). Estrogen stabilization of the mRNA of chicken ovalbumin gene in the cultured organ from estrogen induced-chick oviduct. *Bioscience, Biotechnology, and Biochemistry*, 58(2), 261-264.
- Davis, J.E., Cain, J., Small, C., Hales, D.B. (2016). Therapeutic effect of flax-based diets on fatty liver in aged laying hens. *Poultry Science*, 95(11), 2624-2632.
- Dodgson, J.B. Romanov, M.N. (2004). Use of chicken models for the analysis of human disease. *Curr Protoc Hum Genet*. Chapter 15: Unit 15.5.
- Dougherty, D.C. Sanders, M.M. (2005). Estrogen action: revitalization of the chick oviduct model. *Trends in Endocrinology & Metabolism*, 16, 414-419.
- FASS. (2020). Guide for the care and use of agricultural animals in agricultural research and teaching. 4th ed. <http://creativecommons.org/licenses/by-nc-nd/4.0/>.
- Hrabia, A., Wilk, M., Rzasna, J. (2008). Expression of alpha and beta estrogen receptors in the chicken ovary. *Folia Biologica (Krakow)*, 56, 187-91.
- Ibrahim, N., Shalaby, A., Farag, R., Elbaroty, G., Nofal, S., Hassan, E. (2008). Gynecological efficacy and chemical investigation of *Vitex* fruits growing in Egypt. *Natural Prod Res*, 22(6), 537-46.
- Jarry, H., Spengler, B., Porzel, A., Schmidt, J., Wuttke, W., Christoffel, V. (2003). Evidence for estrogen receptor beta-selective activity of *Vitex* and isolated flavones. *Planta Med*, 69(10), 945-7.
- Johnson, P.A., Giles, J.R. (2006). Use of genetic strains of chickens in studies of ovarian cancer. *Poultry Science*, 85(2), 246-250.
- Karacolokcu, M.Z., Guclu, B.K., Kara, K., Tugrulay, S. (2016). Effect of some essential oil supplementation to laying hen diet on performance and egg quality. *Acta Vet Eurasia*, 42, 31-37.
- Katirae, F., Mahmoudi, R., Tahapour, K., Hamidian, G., Emami, S.J., (2015). Biological properties of *Vitex agnus-castus* essential oil (phytochemical component, antioxidant and antifungal activity). *Biotech and Health Science*, 2, 267-97.
- Kato, S., Tora, L., Yamauchi, J., Masushige, S., Bellard, M., Chambon, P. A. 1992. A far upstream estrogen response element of the ovalbumin gene contains several half-palindromic 5'-TGACC-3' motifs acting synergistically. *Cell*, 68(4), 731-742.
- Liu, J., Burdette, J.E., Sun, Y., Deng, S., Schlecht, S.M., Zheng, W. et al. (2004). Isolation of linoleic acid as an estrogenic compound from the fruits of *Vitex agnus-castus* L. (chaste-berry). *Phytomedicine*, 11(1), 18-23.
- Liu, J., Burdette, J.E., Xu, H., Gu, C., van Breemen, R.B., Bhat, K.P., Booth, N., Constantinou, A.I., Pezzuto, J.M., Fong, H.H., Farnsworth, N.R., Bolton, J.L. (2001). Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *Journal of Agricultural Food Chemistry*, 49(5), 2472-9.
- Liu, Z., Sun, C., Yan, Y., Li, G., Shi, F., Wu, G., Liu, A., Yang, N. (2018). Genetic variations for egg quality of chickens at late laying period revealed by genome-wide association study. *Scientific Rep*, 8, 10832.
- Lv, Z., Xing, K., Li, G., Liu, D. and Guo, Y. (2018). Dietary genistein alleviates lipid metabolism disorder and inflammatory response in laying hens with fatty liver syndrome. *Frontiers in physiology*, 9,1493.

- Mari, A., Montoro, P., D'Urso, G., Macchia, M., Pizza, C., Piacente, S. (2015). Metabolic profiling of *Vitex agnus-castus* leaves, fruits and sprouts: analysis by LC/ESI/(Qq)MS and (HR) LC/ESI/(Orbitrap)/ MSn. *Journal Pharma and Biomed Anal*, 102, 215-21.
- McKnight, G.S., Palmiter, R.D. (1979). Transcriptional Regulation of the Ovalbumin and Conalbumin Genes by Steroid Hormones in Chick Oviduct. *J Bio Chem*, 254(18), 9050-9058.
- Mosavi, S.T., Beigi Nassiri, M.T., Roshanfekar, H.R., Nazari, M. (2022). The effect of *Vitex agnus castus* fruits powder on oviduct markers gene expression of laying hens. *Research On Animal Production*, 13(38), 138-146.
- Nelson, N.A., Lakshmanan, N., Lamont, S.J. (1995). Sheep red blood cell and *Brucella abortus* antibody responses in chickens selected for multi trait immunocompetence. *Poultry Science*, 74(10), 1603-9.
- Ohler, P.O., Grimley, P.M., O'Malley, B.W. (1968). Protein synthesis: differential stimulation of cell-specific proteins in epithelial cells of chick oviduct. *Science*, 160, 86-87.
- Palmiter, R.D. (1972). Regulation of protein synthesis in chick oviduct. I. Independent regulation of ovalbumin, conalbumin, ovomucoid, and lysozyme induction. *Journal Bio Chem*, 247(20), 6450-6461.
- Pfaffl, M.W., Horgan, G.W. Dempfle, L. (2002). Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real- time PCR. *Nucleic Acids Research*, 30(9), 36.
- Rabieh, M., Rooshanfekar, H., Nazari, M., Ghorbani, M.R. (2020). Gene expression of antioxidant enzymes fed wild pistachio (*Pistachio atlantica*), purslane (*portulaca oleracea*) extract and vitamin E under in broiler chickens under heat stress condition. *Iranian Veterinary Journal*, 17(2), 51-60 (In Persian).
- Ramakers, C., Ruijter, J.M., Deprez, R.H., Moorman, A.F. (2003). Assumption-free analysis of quantitative real-time PCR data. *Neuro Lett*, 13(339), 62-66.
- Rani, A., Sharma, A. (2013). The genus *Vitex*: a review. *Pharm Rev*. 7(14): 188-98.
- SAS Institute. (2005). SAS Users Guide: Statistics. Version 9.1. SAS Institute Inc., Cary, NC.
- Sabahi, R., Nazari, M., Beigi Nassiri, M. T., ghorbani, M. R. (2020). The Effect of *Vitex Agnus Castus* fruit powder on hypothalamic GnRH gene expression in laying hens. *Research On Animal Production*, 11 (30), 92-100.
- Schweizer, G., Cadepond-Vincent, F., Baulieu, E.E. (1985). Nuclear Synthesis of Egg White Protein Messenger Ribonucleic Acids in Chick Oviduct: Effects of the Anti-Estrogen Tamoxifen on Estrogen-, Progesterone-, and Dexamethasone-Induced Synthesis. *Biochemistry*, 24(7), 1742-1749.
- Stadnicka, K., Sławińska, A., Dunisławska, A. et al. (2018). Molecular signatures of epithelial oviduct cells of a laying hen (*Gallus gallus domesticus*) and quail (*Coturnix japonica*). *BMC Developmental Biology*, 18, 9. <https://doi.org/10.1186/s12861-018-0168-2>
- Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., Seidlova-Wuttke, D. (2003). Chaste tree (*Vitex agnus-castus*)– Pharmacology and clinical indications. *Phytomedicine*, 10, 348-57.
- Yildiz, F. (2005). Phytoestrogens in Functional Foods. Taylor & Francis. Ltd. Pp. 3-5, 210-211.
- Zamani, M., Neghab, N., Torabian, S. (2012). Therapeutic Effect of *Vitex Agnus Castus* in Patients with Premenstrual Syndrome. *Acta Medica Iranica*, 50(2), 101-106.