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Effects of alfalfa on reproductive hormones in male rats

Godratollah Mohammadi[*1], Seyed Reza Fatemi Tabtabaei[2] and Shaghayegh Zanganeh[3]

[1] Associate Professor, Derpartment of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] DVM Graduated, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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Abstract

Alfalfa is one of the most important phytoestrogenic plants which contains epigenine, comsterol and coumarin and it has a variety of effects on reproduction in domestic animals. Some of the studies have shown alfalfa has negative effects on the fertility of domestic animals. Given that reproductive hormones play major role in fertility, "in this study, the effects of alfalfa were investigated on reproductive hormones in male adult rats". For this purpose, 30 rats were divided in two groups of treatment and control. Treatment groups received alfalfa during 30, 45 and 60 days. Based on the results, level of Blood plasma FSH in the first treatment group (30 days) was significantly higher than the control group, but in the second (45 days) and third (60 days) treatment groups, insignificantly increased. Rate of Blood plasma LH in first and second groups were insignificantly increased, but in the third treatment group significantly increased. Testosterone level of blood plasma in the first treatment group showed a significant decrease and in the second treatment group insignificantly decreased, but in the third treatment group were significantly increased. The levels of estradiol in all treatment groups increased. Conclusion: the results of this study showed that use of alfalfa for up to 60 days did not have negative effects on reproductive hormones in male rats.

Key words: Alfalfa, reproductive hormones, phytoestrogen

Introduction

Phytoestrogens are nonsteroidal estrogenic compounds which are activating alpha (ERα) and beta (ERβ) estrogen receptors (Kuiper, Lemmen et al. 1998). Phytoestrogens are structurally similar to 17 beta-estradiol and are linked to estrogen receptors and produce their biological effects. However, it has been observed that phytoestrogens also have anti-estrogenic properties (Yildiz 2005). It is possible that the estrogenic effects of phytoestrogens observe in domestic animals, but their anti-estrogenic properties observe in humans. The plasma estradiol level of livestock is low (15 pg/ml), but in human is high (50 to 400 pg/ml) (Adlercreutz, Honjo et al. 1991).

Alfalfa is one of the most important phytoestrogenic plants that contains the plant estrogens of epigenin, comestrol and coumarin, and has various effects on reproduction in domestic animals (Samuel 1967, Assinder, Davis et al. 2007). Some researchers believe that alfalfa has stimulating effects, and others believe that it has a negative effect on reproduction. For example, Casanova et al. (1999) showed that phytoestrogens in rodents accelerate puberty and growth of the genital tract

[*] **Corresponding Author**: Godratollah Mohammadi, Associate Professor, Derpartment of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran, E-mail: ghmohammadi9@yahoo.com

(Casanova, You et al. 1999) but Weber et al (2001) demonstrated that alfalfa in male rodents decreases the size of the prostate and sexual organs. In addition, phytoestrogenic compounds have been found to cause temporary infertility in domestic animals (Adams 1995). Activity of alfalfa phytoestrogens is similar to female hormones that are responsible for the growth of the genital tract. Phytoestrogens reduce menopausal symptoms in women, prevent osteoporosis, reduce breast and prostate cancer (Rishi 2002, Zgorka and Glowniak 2008). Also, isoflavones in older men can improve memory and perception of the brain, increase bone density, and prevent breast, large intestine and prostate cancer (Anklesaria 2011). In addition, it has been observed that alfalfa has varied effects on reproduction, especially reproductive hormones (Al-Yawer 2011). Since the effects of alfalfa on male reproductive hormones have not been studied, therefore, the aim of this study was to investigate the effects of alfalfa on reproductive hormones such as FSH, LH, testosterone and estradiol in adult male rats.

Materials and Methods

In this study, 30 adult white Wistar male rats with age of 15-16 weeks and weight of 200-220gr were randomly divided into three treatment groups (each 5 rats) and three control groups (each 5 rats). The first, second, and third treatment groups daily were fed with 80 grams of solid food (barley 26.5%, corn 11%, wheat bran 30%, cottonseed meal 4.7%, beet pulp 8%, soybean 12%, beet molasses 1.5%, vitamin premix 7%, calcium carbonate 1.5%, salt 5%) and 300 gr of fresh alfalfa for 30 (T1), 45 (T2) and 60 (T3) days, respectively. The control groups were fed daily with 140 grams of solid food for 30 (C1), 45 (C2) and 60 (C3) days, respectively. The water was available freely and animals kept at temperature of 23±1° C with 12-hours daylight and 12 hours in darkness. After the end of each treatment, the rats were anesthetized with intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine (5 mg/kg body weight) After weighing, blood sampling was done by a capillary tube from the back vein of the eyes. Then serum isolated by centrifugation at 4000 rpm for 10 minutes and transferred to 1.5 ml tube and immediately transferred to freezer (-20). The amount of FSH and LH hormones were evaluated by ELISA method and using ideally diagnostic kits (Ideally Co., Iran), by a non-shaking technique at the well surface, with interaction between the plate-coated Streptavidin and special monoclonal antibody. The levels of testosterone and estradiol were measured by ELISA method and using testosterone and estradiol test kit (DRG, Germany). All used kits were human kind.

Data were analyzed using the SPSS 16. To determine the normality of the data distribution, Kolmogorov–Smirnov test was used. for analysis of parametric data, independent sample t-test was used to calculate statistical significance between the control group and each treated group. The level of significance was set as $P< 0.05$ for all statistical tests.

Results

The results of the comparison of plasma levels of FSH, LH, testosterone and estradiol in the studied groups are shown in Table 1.

The results of this study showed that blood plasma testosterone levels in rats in the first and second treatment groups were insignificantly lower than the control groups ($P>0.05$). However, in the third treatment group there was a significant increase in comparison to control group ($P<0.05$).

The levels of estradiol in the first and third treatment groups showed an insignificant increase in comparison to control group ($P>0.05$) but in the second

group, there was a significant increase compared to the control group (P<0.05).

The FSH level of the first treatment group was significantly higher than the control group (P<0.05), however, in the second and third treatment groups, insignificant increase was observed (P>0.05).

The LH levels of blood plasma of rats in first and second treatment groups were insignificantly increased in comparison to the control group (P<0.05). However, there was a significant increase in the level of plasma LH in the third treatment group compared to the control group (P<0.05).

Table 1. Effects of alfalfa on reproductive hormones (Mean ±SD)

Groups	LH µIU/ml	FSH mIU/ml	Estradiol pg/ml	Testosteron eng/ml
Control 1	0.412±0.06	0.28±0.02	5.19±1.24	4.8±1.41
Treatment 1	0.45±0.045	*0.39±0.02	6.56±0.89	*1.03±0.07
Control 2	0.51±0.18	0.39±0.06	6.31±1.15	1.98±0.37
Treatment 2	0.524±0.08	0.42±0.01	*12.73±6.69	1.45±0.74
Control 3	0.49±0.10	0.44±0.05	7.06±0.52	2.51±1.06
Treatment 3	*0.65±0.08	0.55±0.17	8.22±1.24	*4.88±1.59

* Significant difference between Treatment and control groups (P<0.05). Treatment groups 1, 2, and 3 were fed by normal diet plus alfalfa for 30days, 45 days, and 60 days, respectively while all the control groups were consumed normal diet during the study.

Discussion

The results of the present study showed that alfalfa can change the level of sex hormones (estradiol and testosterone) and gonadotropin hormones (FSH and LH) in treated rats.

The changes of effects of phytoestrogen such as alfalfa on gonadotropin maybe due to the type of phytoestrogen components (Assinder et al. 2007), dose of phytoestrogen, during of consumption (Pan et al. 2007 and Retana-Márquez, Hernández et al. 2012) and gender of animals (Adaay et al. 2013).

The difference between the effects of alfalfa and other phytoestrogen such as soybean on gonadotropins may be due to the kind of phytoestrogen components, as alfalfa contains epigenine, cumestrole and coumarin (Adaay et al. (2013) but soybean contains genistein, Daidzein and beta-glycosides phytoestrogens (Assinder et al. 2007).

In the present study there is a decreasing in testosterone level in the first treatment group but there is an increasing in testosterone level in third treatment group. This difference may be due to the duration of consumption of phytoestrogen (Retana-Márquez et al. 2012). Because the first treatment group received alfalfa for 30 days, but third treatment group received alfalfa for 60 days. In other hand used of phytoestrogens cause reproductive disruption in males (Retana-Márquez et al. 2012). May be long term use of alfalfa can reduce fertility in male rats. Because long term use of phytoestrogen can reduce fertility of animal (Pan et al., 2007, Retana-Márquez et al. 2012).

In the present study there is a coordination between increasing of LH and testosterone levels. Leydig cells can produce testosterone under control of LH. So the increasing of LH can increase testosterone production in Leydig cells (Mohammadi 2015).

The results of hormone profile in this study showed an increase in estradiol serum level in all treatments. This observation might be related to increase in estrogens

synthesis due to increase of FSH, because estrogen is produced in Sertoli cells by aromatization of androgens under control of FSH. The LH stimulates biosynthesis of testosterone from cholesterol by Leydig cells; the testosterone then transfers to Sertoli cells then convert to estradiol and dehydrotestesteron (Mohammadi & Barati 2009).

Changes due to the different effects of phytoestrogens on the amount of sex hormones in various studies can be depended on the type, plant species, and amount of ingested (Retana-Márquez et al. 2012).

Although phytoestrogens can produce estrogenic or antiestrogenic effects on reproduction, but in present study consumption of alfalfa for 60 days had positive effects on rat hormones. The mechanism of alfalfa on reproduction is not clear, but alfalfa may be bond to estrogen receptors (Navarro 2005) and display estradiol-like effects and mimic estradiol effects on reproduction (Yildiz 2005). In other hand alfalfa has antioxidant effects so this phytoestrogen can directly or indirectly change levels of reproductive hormones in rats.

In conclusion consumption of alfalfa for up to 60 days not only did not have negative effects on serum FSH, LH, testosterone and estradiol but also increased these reproductive hormones in male rats.

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Conflict of interest

The authors declare no conflict of interest.

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اثرات یونجه بر هورمون های تولید مثلی موش صحرایی

قدرت‌الله محمدی[1]*، سیدرضا فاطمی‌طباطبایی[2] و شقایق زنگنه[3]

[1] دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[2] استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[3] دانش‌آموخته‌ی دکتری عمومی دامپزشکی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران

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چکیده

یونجه یکی از مهمترین گیاهان فیتواستروژنی است که دارای اپیژنین، کومسترول و کومارین می‌باشد و دارای آثار متعدد بر تولید مثل حیوانات اهلی است. برخی از مطالعات نشان داده‌اند که یونجه دارای آثار منفی بر باروری حیوانات اهلی است. با توجه به این که هورمون‌های تولید مثلی نقش مهمی در باروری دارند، در این مطالعه تاثیر یونجه بر هورمون‌های تولید مثلی موش‌های صحرایی نر مورد بررسی قرار گرفت. به این منظور ۳۰ موش صحرایی به دو گروه درمان و کنترل تقسیم شدند. گروه درمان در طول ۳۰، ۴۵ و ۶۰ روز یونجه دریافت کرد. بر اساس نتایج، سطح FSH پلاسمای خون در گروه درمان اول (۳۰ روز) به صورت معنی‌داری بیشتر از گروه کنترل بود ولی در گروه‌های درمان دوم (۴۵ روز) و سوم (۶۰ روز) افزایش غیرمعنی‌دار داشت. میزان LH پلاسمای خون در گروه‌های درمان اول و دوم به شکل غیرمعنی داری افزایش یافت ولی در گروه درمان سوم افزایش معنی‌دار داشت. سطح تستوسترون پلاسمای خون در گروه اول درمان کاهش معنی‌دار نشان داد و در گروه درمان دوم کاهش غیرمعنی‌دار داشت ولی در گروه درمان سوم افزایش معنی‌دار داشت. سطح استرادیول در تمامی گروه‌های درمان افزایش یافت. نتایج این مطالعه نشان داد که استفاده از یونجه تا ۶۰ روز اثر منفی بر هورمون‌های تولید مثلی موش‌های صحرایی نر نداشت.

کلمات کلیدی: یونجه، هورمون‌های تولید مثل، فیتواستروژن

*نویسنده مسئول : قدرالله محمدی، دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
E-mail: ghmohammadi9@yahoo.com