EFFECT OF FEEDING OF HEAD LETTUCE AND ALFALFA ON GROWTH PERFORMANCE AND REPRODUCTIVE PARAMETERS IN ADULT MALE RABBITS

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ABSTRACT

The present objective was done for investigate the ameliorative feeding effect of *Lactuca sativa* and alfalfa on growth performance and reproductive parameters (concentration of hormones, characteristic of sperm epididymal in adult male rabbits). Twelve adult male rabbits were divided randomly in to two groups. Control group (G1) animals feeding on alfalfa (1kg/day/animal) for 6 weeks. Group Two (G2) this group feeding on head lettuce (1kg/day/animal) for 6 weeks. The result revealed significantly increase in serum testosterone, LH and FSH concentration and significantly differences were observed in sperm concentration, viability, abnormality and decrease significantly in motility of sperm in group feeding on head lettuce compared to the group feeding on alfalfa.

INTRODUCTION

The plants and their derivatives played a key role in world health. They have possessed biological activity, great nutritional and medicinal important and all modern drugs about thirty percent are derived from plants (1; 2 and 3).

Lactuca sativa was classified as an annual plant of the aster or family of the sunflower as Asteraceae, Lettuce firstly cultivated by the ancient Egyptians who turned it from a weed, whose used to produce oil from seeds, lettuce spread to Greeks and Romans as to a plant grown for its leaves, the name " Lactuca " gave it from the English "Lettuca" is ultimately derived, it residues from grading of head lettuce used in feeding of animals (1 and 4).

Alfalfa (*Medicago sativa* L.) is the plant rich with highest level of calcium and calories and should be sparingly fed. The people liken taste between alfalfa and Timothy as chocolate versus lettuce. Alfalfa palatable for most rabbits, also it is cheaper and available readily at most feed farms or stores (5).

MATERIALS AND METHODS

Head of *Lactuca sativa* was purchased from local markets in Basrah Province/ Iraq. Voucher specimens of plants were identified and authenticated at College of Agriculture/University of Basrah.

Experimental animals:

The experiment was conducted in the animal house, Faculty of Veterinary Medicine, University of Basra, in controlled environment rooms (24°C).

Twelve adult male rabbits (6-8 weeks) were used in this study. The rabbits were weighted (1.33-1.66 Kg) and allotted randomly into 2 groups (6 male rabbits for these groups). The rabbits were housed in cages (1.5m X 0.5m). The diets were fed three times daily at 7.00 AM.; 12.00 PM. and 6.00 PM and water was available *ad libitum*. Artificial light was provided for 12 hours /day in the experiment.

Experimental design: In this experimental the animals were weighted at 0, 2, 4 and 6 weeks.

Blood and specimens collection:

Collection of blood samples was done at the end of the experiment; the animals were anesthetized by using diethyl ether and sacrificed. The collection of blood samples directly done by cardiac puncture and putting into test tube, then separation the serum and stored at -20 ˚C until used for analysis of hormonal.

The two testes with the epididymides removing from animal and weighted by usage electrical balance after removing the fat and tissues surrounding it. Caudal epididymis was put in a watch glass containing normal saline, and then minced into small tiny pieces with surgical scissors until it became homogenization solution that contains the spermatozoa suspension which used for the studying of the epididymal sperm characteristics.

Concentration of sperm epididymal:

According to the method of (6) the sperms were counted by using hemocytometer of Neubauer chamber.

Procedure: The epididymis putting in 5 ml of 0.9 % normal saline contained in Petridish. And then the epididymis was cut into (6 – 10 pieces) by using scalpel sharp. Filtered the suspension resulted from the previous step by a clean gauze piece into a test tube. After this one drop of filtrate was dropped on the Neubauer chamber which covered with cover slid. The sperms found in the five squares which used in the counting of RBC_S and by using the objective lens (40 X). The calculation of sperms in one mm^3 as follows:

Sperms/cm = sperm No. in 5 squires X 10000

Percentage of sperm motility (%):

Motility of the individual sperms epididymal measuring depending upon the graduation basis as described by Chemineau *et al*. (7) as follows:

Dropping of diluted sperm epididymal was on a clean and warm slide at 37°C and covered by cover slip. Examination of sperm was done under light microscope by using 40X power. Depending upon the progressive, forward sperm's movement and the strength and speed of their motion are converted into the percentage.

Table (A) Sperm's movement strength and speed (7):

Motion Types	***Degree***	***Percentage***
Moving of Sperms (rapidly and straightly).	5	90 – 100**%**
Sperms are move fast and rapidly but some of it circle move .	4	75 – 85**%**
Sperms are move straight slope and without motion shivering.	3	45 – 65%
Simple moving in an irregular shivering motion.	2	20 – 40**%**
Sperms shiver with swinging tail and movement slowly.	1	10**%**
No sperm movement.	0	0**%**

Sperm abnormality:

The abnormal spermatozoa percentage were counted in the same slide that used for measurement of the epididymal sperm viability by using account 200 sperms under a light microscope under 100X power (8).

Diluted of semen dropping on a clean and warm slide. Warm stain of eosin – nigrosine was dropping on the semen and mixed together carefully with the use of a rod glass. A smear doing by used clean slide which put angularly on the slide and was dragged horizontally. The slide was leaved drying. And then the slide examinations under the light microscope by using 40X power. White color appeared for live sperms and red color for the dead sperms. Live sperms appeared in white color and the dead sperms appeared in red color.

Stain of Eosin – Nigrosine which consist from the following: Eosin (1.67 gm), Nigrosine (10 gm), Sodium citrate (2.9 gm) and Distal water (100 ml).

Hormonal assay:

ELISA Kit of Testosterone: The concentration of total testosterone determined quantitatively in serum was done by a microplate enzyme immunoassay (9).

Measurement of Concentration of Follicle Stimulating Hormone (FSH) and Concentration of Luteinizing Hormone (LH) (ng/ml): determination of FSH concentration was done by using kit of enzyme test (Human GmbH.53020 Wiesbaden. Germany Gesellechalf for Biochemical and diagnostic mbH) (10).

RESULTS

Growth performance: The growth performance data of the rabbits are presented in (table 1). The body weight was significantly ($p<0.05$) increased in G1 when compared with G2 at 4 and 6 weeks of treatment.

Table (1): Feeding effect Alfalfa and head lettuce on body weights (kg) of male rabbits:

Groups	0 w.	2w.	4w.	6w.
G1 (alfalfa)	1.66±0.089 ^{Aa}	2.00±0.025^{Aa}	2.33±0.009^{Ab}	2.77 ± 0.048^{Ac}
G2 (head lactuca)	1.33± 0.099 ^{Aa}	1.51±0.028^{Aa}	1.5 ± 0.047 ^{Bb}	1.61± 0.008^{Bc}

Capital letters= denote to the different between groups and small letters= denote to the different within groups

Hormones levels Assay:

The levels of serum hormone levels were measured at the end of experiential, there was significantly increase ($p<0.05$) in all levels of hormones in G2 when compared with G1.

Table (2): Feeding effect Alfalfa and head lettuce on testosterone, FSH and LH hormones of male rabbits:

Groups	Testosterone (ng/ml)	FSH (mIU)	LH (mIU)
G1 (alfalfa)	2.74±0.22^{B}	4.16± 0.24^{B}	1.16±0.11^{B}
G2 (head lactuca)	3.79± 0.21^{A}	5.27±0.32^{A}	1.86± 0.04^{A}

Capital letters=denote to significant differences ($p< 0.05$) between groups.

Effect of feeding Alfalfa and head lettuce on testis weight:

There was highly significant increase ($p<0.05$) in weight of testis per body weight in G2 comparative to G1.

Table (3): Feeding effect Alfalfa and head lettuce on testis weight of male rabbits:

groups	Testis weight(gm)
G1 (alfalfa)	2.52 ± 0.050^{B}
G2 (head lactuca)	3.55 ± 0.057^{A}

Capital letters=denote to significant differences ($p< 0.05$) between groups.

Feeding effect Alfalfa and head lettuce on sperm account, motility, viability and abnormal sperm in male rabbits:

The sperm account, motility %, viability%, and abnormal sperm % are shown in table 4. All these parameters increase significantly ($p<0.05$) in G2 compared to G1.

Table (4): Feeding effect of Alfalfa and head lettuce on sperm account, motility %, viability%, and abnormal sperm % of male rabbits:

Groups	Sperm account	Motility %	Viability%	Abnormality%
G1	**55.05 ± 0.82^{B}**	**69.00 ± 4.02^{B}**	**80.37 ± 4.02^{B}**	**33.50 ± 2.36^{A}**
G2	**67.35 ± 0.47^{A}**	**83.37 ± 2.01^{A}**	**85.62 ± 0.47^{A}**	**29.50 ± 3.31^{B}**

Capital letters=denote to significant differences ($p< 0.05$) between groups.

DISCUSSION

The body weight of animals significantly increases in alfalfa when compared to head lettuce feeding on adult male rabbits, may be due to the Alfalfa contain high percent of fiber and high dry matters (DM %) while the head lettuce used for human consumption as vegetable, low content of DM in it (about 40 gm/kg) (4). The diets were fed three times daily at 7.00 h. 12.00 and 6.00 h. (Pm) to increase the offer level and intake of feed because the Lactuca sativa residue had low concentration of dry

matter (DM) content and fermented quickly if offered in large amounts at the same time and had free access to water

Poor fiber and low DM in lettuce head leaves may be factors negatively effects this may be due to deficiency of fiber. Yin *et al.* (11) signs to the diets containing fiber with high digestibility which causes diarrhea. It is not clear what the minimum fiber intake for prevention of diarrhea in rabbits. In this project, the fiber intake from head lettuce about 8 gm and DM intake about 10%. The fiber requirement in growing rabbits for 4 to 12 weeks of age about 11.2 gm/kg BW/day (12). These almost requirements are three times of fiber intake in this study. The *Lactuca sativa* haven't side effect. It has been found that there were no toxic affects and the fiber present in enough percentage in head lettuce was for the cellulolytic bacteria for the population increment and for the control the balance of the caecum micro-organisms (4). For these causes that suggest that this plant has no general toxic effect on body weight and causes losses in body weight with good healthy opposite to that feed on alfalfa. These results disagreement with that reported by Khalifa, *et al*. (3) that showed the body weight for treated animals with the alfalfa have no changes for firstly 2 weeks from experiment but after third week, the alfalfa in high dose which induced significantly decrease in the body weights. In addition Adedapo *et al*. (13) reported that consumption of leaves of Moringa in doses (up to 2000 mg/kg) which are saved. In 21 days of the study, a dose-dependent decrease in body weights of the rats occurred over after this time. Cajuday and Pocsidio (14) have been found the body weight of the leaves extract of alfalfa gives to animals for 21 days remained unchanged, that showed the doses selected didn't exert any harmfully effect and the normal processes of metabolic in the treated animals.

Testes weights increase significantly; the testes, epididymis and other reproductive organs are structured and physiologically dependent upon the testosterone and other androgens hormones (15). Testosterone stimulates growth and secretory activity of the reproductive organs (16), so these hormones in this study increase significantly could increase the number and function of germinal and somatic cells of testis and also increase the testis and epididymis weight.

The sperm account, motility and viability increase significantly. In male rabbits (mammals), these parameters are regulation by the FSH and LH. The spermatogenesis

stimulates by FSH, which binds with receptors in Sertoli cells. While the production of testosterone are stimulates in leydig cells by LH, that may act on peritubular cells and sertoli cells in seminiferous tubules and indirectly to stimulates spermatogenesis by testosterone (17), for this purpose the LH increase significantly leads to increase in testosterone secretion from leydig cells. The treatment with flavones resulted in an increase in a count of sperm and antioxidant activity in male rabbits (18). The FSH and the hormones LH are inducing the sex hormones synthesis such as testosterone and 17ß-estradiol (E2) that control spermatogenesis development and ovulation. The E2 operates on negative and positive feedback roles on the gonadotropin or synthesis and secretion of its upstream hormone, by direct interaction with estrogen. Taken together, study revealed that the feeding on head of lactuca up regulated the expression of fertility genes (CYP19, LH and FSH).

Conclusion: Head lettuce can be fed to rabbits in on-farm conditions and result in good reproductive performance in male rabbits. These results can be introduced to farmers to encourage them to replace or supplement head lettuce to improve reproductive performance. And from results the head lettuce can be used in human and animals for looser body weight

تأثير التغذية على الجت و رؤوس الخس على أداء النمو والصفات التناسلية في ذكور الأرانب البالغة

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الخـــــلاصـــــة

اجريت هذه التجربة في البيت الحيواني لكلية الطب البيطري ـ جامعة البصرة، لتشخيص تأثير التغذية بالخس على أداء او كفاءة النمو والمعايير التناسلية. أثنى عشر ذكر أرنب قسمت الى مجموعتين (٦ أرانب في كل مجموعة)، المجموعة الأولى G1 غذيت على الجت وبمعدل كيلو يومياً لمدة ستة أسابيع والمجموعة الثانية G2 غذيت على رؤوس الخس (أوراق الخس) وبمعدل كيلو لكل ارنب يومياُ لمدة ٦ اسابيع ايضاً.

النتائج اظهرت وجود اختلاف معنوي كبير ($p<0.05$) في وزن الجسم النهائي ، نقصان معنوي في وزن الجسم للمجموعة الثانية المغذى على الخس مقارنة مع المجموعة الاولى المغذى على الجت. لكن هناك زيادة

معنوية عالية في هرمون التيستوستيرون والمحفز للجريبات اللوتيني وكذلك زيادة معنوية في وزن الخصى وصفات النطف البربخية في المجموعة المغذى على رؤوس الخس مقارنة مع مجموعة الجت.

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