THE EFFECTS OF SUBSTITUTION BARLEY BY 10, 30% HYDROPONIC BARLEY IN DIET OF AWASSI MALE RAMS ON SEXUAL BEHAVOIUER AND REPRODUCTIVE PERFORMANCE

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ABSTRACT

The objective of this study is to cultivated and produce sprouted barley under local conditions to investigate the incorporation effects at a levels of 10% and 30% in diet of 16 weaned lambs of Awassi male lambs, on reproductive traits and sexual behavior. The experimental plan was designed for 135 days period, started from 1/12/2016 to 15/3/2017 using hydroponic steel chamber Barley grain were cleaned, washed, soaked and spread on the trays to implantation by hydroponic system and water irrigation, lambs is with an average body weight of 19.25±0.25 kg, was aged four month and was split into 3 groups 6 to each, All groups were daily feed with 2% of body weight at concentrate diet, the chemical analysis of sprouts were recorded, pen test of male rame were done to estimate the sexual behaviors and body weight at puberty were recorded. The chemical analysis revealed significantly higher value and improve nutritional alteration in sprouts comparative to grains. In blood serum sex hormones evaluation, both treated groups particularly 30% group recorded higher values compare to control. In pen test and sexual estimation treated groups particularly 30% group recorded high improvement with earlier and higher puberty weight than both 10% and control groups. According to these results it could be recommend to replacement of sprouted barley different level to the diet of ruminants and lambs for more improvements to reproductive traits.

INTRODUCTION

Hydroponic fodder production is an advanced technology in agriculture use a technique of growing grain, in a hygienic environment free of chemicals, and artificial growth promoters (26). Green fodder is an essential component of the livestock ration to enhance their reproductive and productive performance, Consequently, for improving livestock products, green fodder should be fed more often to animals (16). Sprouted barley has high feed quality that is rich with proteins, fibers, many vitamins, and minerals (6, 13) with health beneficial effects on animals (9). Hydroponic sprouting barley has 23 times more Vitamin A than carrots, 22 times more Vitamin B than lettuce, 14 times more Vitamin C than citrus, contains many sugars. Sucrose, fructose, fructans, glucose, and maltose are all present and produce more energy, more efficiently for the animal, (21) also, sprouts are a tremendous source of (plant) digestive enzymes. Enzymes act as biological catalysts needed for the complete digestion of protein, carbohydrates and fats lead to increase the animal growth. The physiology of vitamins, minerals and trace elements is also dependant on enzyme activity and Sprouts can be a rich source of antioxidants, in the form of Beta-Carotene (a precursor of Vitamin-A), Vitamin-E, Vitamin-C and related trace minerals such as Selenium and Zinc (38).

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The majority of sheep raised locally are of the Awassi breed (15). Nutrition regard an important factor affecting puberty of ram lambs such as Awassi lambs and quality of Nutrition is the main limiting factor for reproduction system in animal (10). In tropical environment, where the influence of photoperiod, good nutrition appears to be the major modulators of reproduction and sexual activity in small ruminants, since improving the nutritional status cannot reverse a seasonal reduction in testicular size once it has started , as demonstrated in rams (7). Increasing feeding quality and its levels leads to be heavier weight (8) when there is an indicating a link between better nutrition and higher sperm producing parenchyma in the testes of the animals. However sprouted barley feed enhance puberty and many researchers found that adding sprouted barley to rams diet enhances the puberty. A limited research has been done on feeding value of hydroponic fodder for improve mint of reproductive traits in small ruminants, (34). Therefore; this study was aimed to determine the improvements of reproductive trait by feeding hydroponic grown barley fodder for Awasssi male lambs.

MATERIALS AND METHODS

THE EXPERIMENTAL DESIGN

Current study was conducted for 135 days period, at Animal Farm, College of Veterinary Medicine, Baghdad University, Eighteen Awassi male lambs purchased from alocal trusted farms with an average body weight of 19.25±0.25 kg and four months old at the start of the experiments. Experimental Lambs treated with anti ecto-and endo-parasites and vaccinated against enterotoxaemia, they were weighed and grouped according to live weight. Herd split into 3 groups based upon their weight at the start of the trial 6 Lambs in each group after two weeks of adaptation period. All groups were daily feed with 2% of body weight of concentrated diet, the control Group were daily fed only this amount, the second, third groups of lambs were fed with same amount of concentrated diet included 10 and 30% of fresh sprouted barely respectively, (Table 1). The amount of feed offered to the lambs and the remaining were weighed . all groups supplied with water and mineral blocks.

Table 1: The Formulation of experimental concentrated diet (%)

Ingredients%		Sprouted Barely	
item	control	10%	30%
Barley grain	40	30	10
Fresh sprouting barely	0	10	30
Wheat bran	38	38	38
corn	20	20	20
Mineral &vitamin	2	2	2
Total	100	100	100

Each kg of vitamin and mineral mixture contained 0.30 g $CoSO_4$, 20.1 g $CuSO_4$, 10 g $FeSO_4$, 50 g ZnO_2, 40.2 g $MnSO_4$, 0.75 g KI, 878 g NaCl, 500,000 IU vitamin A, 500,000 IU vitamin D and 10,000 IU vitamin E, according to AOAC. 1995 (14).

Fresh sprouted barley daily feed intake for concentrate diets.

Each lambs has daily feed by diet, calculated such as following :

$$\frac{\text{Body weight (kg} \times 2\%}{100} = \text{(N) kg consent. diet daily feed}$$

$$\frac{\text{(N)} \times 10\% \text{ or } 30\%}{100} = \text{(x) kg of dry weight of sprouts}$$

SPROUTS BARLEY PRODUCTION

Hydroponic cultivation and the chemical analyses of sprouted grain used according to (36). The forages production plan was conducted using one of hydroponic steel chamber, with dimensions of 2.0 m in length X 2.0 m in height X 1.0 m in width ,designed to carrying 21 perforated steel trays by seven shelves three trays to each, with capacity of seven growth stages by rely of seven days, trays were used to germinate barley seeds with dimensions of 100cm in length X 40cm in width and 5 cm in height to each, equipped with manual sprayer irrigation water and electric air ventilation, the condition inside the Rome chamber was controlled to get a range of temperature 18-20 °C and relative humidity about 72% by air circulating. Fluorescent lighting with watertight appliance were provide on the wall in the vertical position to growing leaves in which supply about 1000 microwatt/cm 3 during 9-12 hours of daily light. Barley seeds (*Hordeum vulgare* L.) were got from a local supplier, which were cleaned, washed and soaked by in tap water, placed in container for 24 h, then spread on the trays with 1.1 kg and 2cm thickness, for each ,and stayed, for 7 days. At end of this period. grass of barley seedling growth reaches about 16-18 cm in height. It has carpet like appearance with dark green color and thick roots. Three trays contain green grass carpets were removed every day, thus, they required seventh- days for each one cycle of seeds germination, by continues daily harvesting of green forages. The removal carpets exposed to air ventilation for 24 hours in for extra more drying, weighing, and calculated before threaded to small pieces and mixing with other components of their concentrated diets.

CHEMICAL ANALYSIS

Samples of green forage diets was collected weekly and kept frozen until chemical analysis for protein, crude fiber, crude fat, nitrogen free extract (4) (Table 1). The feed sampled and composted daily taken until the end of the collection period, grounded through a 1 mm-screen hammer mill and analyzed for DM (at 70ºC) and nutrients composition were determined (5, 23) .

Blood serum analysis

Blood samples were collected from jugular vein, serum was obtained after centrifugation at 3000 r.p.m., stored at (–200) C° till analysis and used to determine, spermatogenesis stimulating hormone and total testosterone concentration hormone in serum protein by The Enzyme-Linked immunosorbant assay (ELISA) (17) Kit which produced from (ABECKMAN COULTER COMPANY IMMUNOTECH)(FRANCE),

ESTIMATION OF THE SEXUAL ACTIVITY

Pen test (27) was done to estimate sexual behavior of the lambs once, at biweekly intervals after a week of semen collection period it was done, by introducing one estrous female stimulated by 5mg Osteradiol Benzoate drug i/m injected, (24 hours before test time) and allowing, each tested male to mount and recording the number of the mounts within 20 minutes. The second test was done by introducing 3-4 estrous females to that tested male and recorded the number of successful mating within 20 minutes (12).

STATISTICAL ANALYSIS

One way anova tests were used to compare the means of each treatment, and data were recorded, (39), data were analyzed using SPSS, statistical analysis -version 19 (2010).

RESULTS AND DISCUSSION

Table (2) showed that there were a significant ($p<0.05$) higher values in most nutrient components of sprouts compared to origin grains during germination period, including fresh weight, dry matter weight, crude protein, ether extract, NDF, ADF, NFC, and WSC. In Fresh weight of sprouted barley recorded a significantly ($p<0.05$) higher values than raw grains, in which, from one kilogram of grains yielded more than four times weight of sprouts. such chemical alteration is occur during germination where protease enzymes are activated and convert the protein polymers into amino acids and small peptides (37). There is an agreement in obtained results with that declared in other studies (16, 19, 20, 40). These changes occur due to some enzymes, that convert complex compounds of protein into albumin and globulin, leading to, improve the protein quality of sprouts and the lysine contents (11). Also, activation of amylase and lipase enzymes, increases the sugar and essential fatty acids content of sprouts (29) Such method of fodder production consider a suitable simple technique to germinate the seeds for improvement of their nutritive value (11). However, there are many reports revealed that all vitamins content in sprouts were increased during sprouting of cereal grains, as B-complex (11), carotene, vitamin E, biotin and free folic acid (14), increases in Essential Fatty Acids and increase in lipase activity has been also reported in sprouted barley (29). All these positive nutritional change and improve feeding by such fresh fodder cause improvement of digestion and absorption by using less energy, enabling the animal to save and use energy for other important activities as reproduction, wool production, weight gain as a refluxes of these nutritional improvements (18).

Table 2: Effect of sprouted grains on nutrient contents of grains (21)

item	barley	Sprouted barley	Significances
Fresh weight	1000.00	4220.20	4.22*
DM weight	903.70	815.07	0.90 *
Organic matter	887.69	806.40	0.90 ns
Crude protein	95.8	130.66	1.36 *
True protein	64.98	65.22	1.05 ns
Ether extract	13.21	26.32	1.99 *
Neutral detergent fiber	213.15	274.36	1.28 *
Acid detergent fiber	85.55	133.40	1.55 *
Non fiber carbohydrate	512.96	437.90	0.85 *
Water soluble carbohydrate	38.49	68.33	1.77 *

SEX HORMONAL ASSAY

Spermatogenesis stimulating hormone (SSH)

As shown in table (3) 30% group recorded significantly ($p< 0.05$) higher levels than other groups particularly in the latest weeks of the study, the 10% group showed significantly ($p<0.05$) higher values than the control group in late period of the experiment.

Testosterone hormone

Testosterone hormone values increased of all groups up to the latest weeks of the experiment (Table 4), but 30% group established significantly ($p<0.05$) higher values than those of the 10% and control groups in most studied periods, also the 10% group showed significantly ($p<0.05$) higher values than the control group in the last three weeks of the study.

Table 3: The effect of in replacement barley by 10% and 30% hydroponic barley the diet on Spermatogenesis stimulating Hormone (SSH) (U /L) of Awassi male lambs(means ± SE).

Period of experiment	Treatment			
	10% group	30% group	Control	LSD Value
Two weeks	0.57±0.06	0.52±0.06	0.45±0.08	Ns
four weeks	0.73±0.07	0.97±0.08	0.83±0.08	Ns
Six weeks	1.00±0.07	1.06±0.05	1.03±0.04	Ns
Eight weeks	1.12±0.01	1.17±0.01	1.13±0.01	Ns
Ten weeks	1.43±0.02 b	1.54±0.02 a	1.26±0.02 c	0.10
Twelve weeks	2.39±0.06	3.78±0.18	2.59±0.20	0.31
Fourteenth weeks	2.89±0.04 b	3.47±0.08 a	2.35±0.14 c	0.12

Different letters show significant differences among treatments at one period at5% level .

Table 4: The effect of replacement barley by 10% and 30% hydroponic barley in the diet on Testosterone Hormone (n.mol/L) of Awassi male lambs (means ± SE)

Period of experiment	Treatment			
	10% group	30% group	Control	LSD Value
Two weeks	0.75±0.12	0.73±0.06	0.86±0.11	ns
four weeks	2.24±0.20	2.600.16	1.90±0.15	ns
six weeks	3.37±0.09	3.56±0.07	3.12±0.20	ns
eight weeks	4.60±0.04 ab	4.72±0.06 a	3.95±0.08 b	0.33
ten weeks	4.85±0.06 a	5.15±0.07 a	4.25±0.10 b	0.54
Twelve weeks	5.55±0.06 ab	5.87±0.11 a	5.12±0.11 c	0.22
Fourteenth weeks	5.87±0.04 a	5.95±0.21 a	5.17±0.21 b	0.17

Different letters show significant differences among treatments at one period at5% level .

Fayed (29) revealed that addition of sprouted barley to the diet of lambs improved the performance including productive and reproductive traits, that might be reflected in increasing in the level of SSH hormone due to the hydroponic barley feeding, mostly, because of these feed additives contain high (33, 35) quality of nutritional compounds and high content of vitamins such as B6, E, C, which act as antioxidant as well as the volatile fatty acids which stimulate an increasing in the testes size and weights. These nutrients alterations have a vital role, affecting biological process, within the testes, concerning the main tissues of high efficiency for steroids production and semen secretion due to SSH hormone. This was observed in this study, and the increase of SSH of those lambs fed sprouted barley might be due to accumulation effect of such plants nutrients as an antioxidant and affect on the activity of male reproductive system or might refer to the direct effect of hypothalamus/pituitary for SSH secretion (24). However, Kilgour et al. (27) showed that sertoli cell numbers duplication many times from birth to puberty, the testes increase in the size duribg this period. Hochercan de Reviser and Seck (25) confirmed the increase in the function of the sertoli cells to produce and secret the androgen binding protein (ABP). This acts to transport androgen and interstitial cells to semineferous tubules. The SSH effects on interstitial cells by increasing receptor and its response to ICSH on interstitial cells SSH also acts to stimulate the germinal

epithelial cells to create sperms (maintain the spermatogenesis) and to enhance their maturation, with the same causes stimulate of semineferous tubules growth and prepare to form sperms and it also acts to stimulate the sertoli cells to produce the inhibin and activtivin hormones (22). The level of testosterone in both treated groups were significantly ($p<0.05$) increased in comparison with the control group, this might be due to effect of good nutrient contents in sprout to act as an antioxidant defensive system and during its direct effect on interstitial cells in the testes proliferation and produce sex hormones.

BODY WEIGHT AT PUBERTY AND THE DATE OF FIRST EJACULATE

Body weight at puberty of all lambs was 34.67 kg, the 30% group showed significantly ($p<0.05$) higher body weight than other groups (table 5), while the puberty weights in 10% group showed significantly higher body weight than the control group. Animals in 10% group showed their puberty at early period of eleventh weeks, but the animals of 30% group showed their puberty earlier at the tenth weeks experimental period. while the animals in control group show their puberty later at the thirteenth weeks of experimental period.

Table 5: The effect of replacement barley by 10% and 30% hydroponic barley in the diet on the puberty age of Awassi male lambs (means ± SE)

Trait/date	Treatment			
	10% group	30% group	Control	LSD Value
Puberty Weight	37.25±1.08 a	39.33±0.24 a	33.77±0.88 C	2.55
date of first ejaculate obtained	Eleventh weeks of experiment (4) animals showed first ejaculateand other (2)after couple days	Tenth weeks of experiment (4)animals showed first ejaculate and other (2) after couple days	Thirteenth weeks of experiment (4)animals showed first ejaculate and other (2after couple days	-

Different letters show significant differences among treatments at one period at5% level

The mean of body weight at puberty of all groups for Awassi sheep was 36.78 kg which was lower than those found by Hamdani (47.20kg) while Madhat (30) showed lower body weight (31 kg) for Awassi sheep, Kirdli etal. (28) showed higher body weight at puberty for different breeds, Ramanov x Awassi (38.8 kg), Chaerollais x Awassi (44.2 kg) and Awassi (42.3 kg). The higher performance in the Body Weight gain of lambs supplemented with hydroponic fodder in this experiment might be due to the ability of the Hydroponic Barley to supply most necessary nutrients. This was in line with the concept of (32) who revealed that hydroponic sprouts is a rich sources of nutrient components and bioactive enzymes and active ingredients substances that improves the performance of livestock include reproductive traits. However 30% groups showed higher body weight at puberty than those of 10% and control groups, also 10% group showed significantly higher body weight than the control group and this is in agreement with Hocherean de Reviers and seek (24) who reported that testosterone secretion accompanied with an increase in testes weights, with age, the testosterone level increased then enhanced the puberty, hydroponic barley feeding is rich in nutrients, high levels of enzymes and vitamins especially vit E which consider one of earliest sexual activator, this might be explain that all animals in 30% group, showed their first ejaculation earlier than 10% and control groups respectively, while, 10% group enhancing the puberty age, earlier than the control group which reach it at thirteenth week.

SEXUAL BEHAVIOR

Number of mounts/ successful mating

The control group recorded significantly ($p<0.05$) higher numbers than all other treated groups. 30% group recorded significantly ($p<0.05$) lower values than 10% group (table 6).

Table 6: The effect of replacement barley by 10%and 30% hydroponic barley in the diet of pens test (number of mount/successful mating) of Awassi male lambs (means ±SE)

Period of the test	Treatment			
	10% group	30% group	Control	LSD
First test (At thirteenth weeks of experiment)	1.80±0.50 b	1.60±0.22 c	2.23±0.66 a	0.33
Second test After 3 days of first test	1.70±0.12 b	1.50±0.12 c	2.77±0.73 a	0.77
Third test After 3 days of second test	1.50±0.18 b	1.30±0.16 c	2.56±0.89 a	0.11

Different letters show significant differences among treatments at one period at5% 2.

Number of successful mating

The number of successful mating was increased in all groups with time, but treated groups recorded significantly ($p<0.05$) higher numbers than the control group in test periods. In the mean time 30% group recorded the higher number followed the 10% group (table 7).

Table 7: The effects of replacement barley by 10% and 30% hydroponic barley in diet on the number of successful mating of Awassi male lambs on (means ± SE)

Period	Treatment			
	10% group	30% group	Control	LSD Value
First test At thirteenth week of experiment	1.62±0.08 a	1.45±0.05 b	0.69±0.06 c	0.29
Second test After 3 days of first test	1.82±0.05 a	1.58±0.06 a	0.88±0.07 b	0.28
Third test After 3 days of second test	2.22±0.05 a	2.47±0.04 a	1.55±0.04 c	0.22

Different letters show significant differences among treatments at one period at5%.

The mounts per successful mating of both treated groups significantly ($p< 0.05$) showed lower times than those of the control group. This might be due to the good nutrition with rich vitamins contents and nutrient components and enzymes activators of hydroponic barley in diet, (2, 31) causing an increase in SSH and testosterone hormone which consider as a measurements could be used as indices for puberty induction and increased in their sexual behavior and got the lowest mounts per successful mating which could increase in their sexual behavior. However 30% was significantly ($p<0.05$) higher than 10% on sexual behaviors. The same trend was shown in the number of successful mating , the animals in the 30% groups showed significantly ($p<0.05$) more successful mating times than 10% and control group. While group 10% also showed significantly ($p<0.05$) higher successful mating attempts than the control group. Hydroponic sprouting barley eliminates enzyme inhibitors which in turn helps digestion of other feed and so cauing less stress on the whole digestive system, such green fodders has a high benefit in which keeping a large number of sheep alive in extreme weather conditions to improvements the productive and reproductive

features, increased fertility, conception rates, improved birth rate and lower infant mortality, (21) .

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تأثير احلال الشعير المستنبت بنسبتين 10و30% بحبوب الشعير في أعلاف ذكور حملان الأغنام العواسية في الأداء التناسلي والسلوك الجنسي

ماجد جودة لعيبي الساعدي

الملخص

ان الهدف من هذه الدراسة هو زراعة واستنبات الحبوب في ظروف محلية ملائمة وتقديمها اعلافاً طازجة بمستويين 10 و30% ضمن العلف المركز اليومي المقدم للحيوان بنسبة 2% لـ 16 حملاً من حملان الذكور العواسية ودراسة تأثيراتها في الصفات التكاثرية والسلوك الجنسي. تم تصميم التجربة لمدة زمنية تبلغ 135 يوماً ابتداءً من 2016/12/1 لغاية 2017/3/15 وهيئت غرفة الإستنبات مع تصميم منظومه متكاملة لري وتدوير مياه السقي. تم تنظيف حبوب الشعير وغسلها وثم غمرها بالماء لمدة 24 ساعة وثم نشرها على صواني خاصة بالزراعة بواقع 1 كغم لكل صينية لتنتج ما يقارب من خمسة كغم من العلف الاخضر في أثناء مدة سبعة أيام لكل وجبة. أختيرت الحملان بمتوسط وزن مقداره 19 كغم تقريباً وبعمر أربعة أشهر قسمت الى ثلاث مجاميع، ستة لكل مجموعة، غذيت المجاميع جميعها بالعلف المركز بنسبة 2% من وزن الجسم يومياً واضيف الشعير المستنبت بنسبتين 10 و30% على التوالي لكل واحدة من المجموعتين المعاملتين في حين غذيت مجموعة السيطرة فقط على العلف المركز، أجرى التحليل الكيميائي للشعير المستنبت وأجري إختبار الحضيرة للمجاميع المعالجة لتقويم السلوك الجنسي لكل مجموعة، كما سجل وزن الجسم عند البلوغ ،استخدمت طريقة انوفا لتحليل البيانات إحصائياً وللمقارنة بين المجاميع المعالجة: أسفر التحليل الكيميائي عن قيم عالية بالمحتوى الغذائي خاصة بالبروتينات مقارنة بمجموعة السيطرة وفي قيم مصل الدم و الهرمونات الجنسية أظهرت مجاميع المعالجة وخاصة مجموعة الـ 30% قيم أعلى مقارنة بمجموعة السيطرة وفي اختبار الحضيرة الخاص بالكفاءة الجنسية سجلت مجاميع المعالجة أيضاً تحسناً واضحاً متزامناً مع قصر في وقت البلوغ الجنسي مع تسجيل زيادة في الوزن عند البلوغ خاصة لمجموعة الـ 30% مقارنة بمجموعة السيطرة ومجموعة الـ 10% ووفقا لهذه النتائج يمكن ان نوصي بإجراء دراسات موسعة لاستبدال حبوب الشعير بالشعير المستنبت وعلى مستويات مختلفة في النظام الغذائي للمجترات وكذلك الحيوانات وحيدة المعدة لاستحداث المزيد من التحسينات على الصفات التكاثرية والإنتاجية.

كلية الطب البيطري، جامعة بغداد، بغداد، العراق.