Research Article

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Reproductive Performance Status of Adult Female Rabbits Administration Spirulina and Combination of Folic acid, B6 and B12

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Abstract

The current study aimed to evaluate the effects of oral administration of spirulina (as a food supplement) on the reproductive performance of adult female rabbits. Female rabbits (16 to 18 weeks old) were divided into three groups (10 rabbits/ group). These groups are: (Gr.I) as a control group and (Gr. II) spirulina was given at a dose (0.5 g / kg of body weight) and (Gr. III) was given a complex of folic acid, B6 and B12 (2.5 + 50 +1 mg / kg body weight) all groups were treated for four weeks. At the end of the treatment period, 2 untreated males (two males/ group) were naturally mated with treated females. The results showed that there was no significant difference in the estrogen concentrations among all groups, while the progesterone concentration showed a significant decrease in the third compound group compared to the control group and DXN-spr. Also, the testosterone hormones did not show significant differences in all groups. FSH and LH concentrations appeared to be significantly decreased in the third group than in the other two groups. The fertility rate and the number of female fertilities increased in the second group (DXN-Spr.) compared to the other groups. There was also a significant increase in birth weight and fertility rate in this group compared to the control and group No. 3 groups. We conclude that oral dosed spirulina (0.5 g/kg body weight) acts as a natural antioxidant that improves reproductive efficiency and reproductive hormones.

Key words: Spirulina, Mice, Folic acid.

Introduction

Spirulina commonly known as arthrosporic is a small, multicellular, blue, green, spiral, filamentous, photosynthetic alga that naturally grows in alkaline water. Spirulina which means "small spiral". Its high protein content (about 65-70%), this is the added value of biochemical components, essential fatty-acids (for example: 9T-Linolenic-Acid), carotenoids, Phycobilin-proteins, polysaccharide, Vitamins-(except Vit-B12), also it contains: Minerals-and attractive compounds made it a lower for fat, calorie, and free cholesterol origin exporter for-protein (1,2). Spirulina has one of the main features of high protein content can be considerable as a traditional kind of protein (3,4). Algae due to lack of cellulose in the cell walls is easily digested may be due to that greater than 85% of this protein is digestible and absorption after (18 h.) (5). Decreased natural-resources, high price of fishmeal and fluctuations in world market supply have led to suitable-alternatives-(such-as plant-sources) for replacement of fishmeal in the used aqua-culture feed. However, vegetarian diets usually contain highly-levels of-fiber, -starch, insoluble carbohydrates (CHO), and anti-nutrients that affect the growth and digestibility of fish (6, 7); For these-reasons, replacing fish meal with plant proteins is limited. Thus, partial or complete replacements of fish or soybean diets from algae meals in aquaculture systems have gained an increasing-trend in recent years (8). This research aims to study the effect of DXN-spirulina and combination of folic acid +VitB6+ Vit.B12 on reproductive efficiency in female rabbits.

Materials and Methods

In this experimental, rabbits for this study were included: 30 adult female rabbits are randomly divided into three groups as the following:

Gr. I (control): 10 Adult female rabbits orally administered distilled water (5 ml/animal) by gavage daily for 30 days. Gr. II (DXN-spirulina): 10 ADULT female rabbits were orally administered DXN-spirulina (0.5 gm/ kg BW) dissolved in 5ml distilled water by gavage daily for 30 days. Gr. III (folic acid+B6+B12): 10 Adult female rabbits were orally administered of folic acid+B6+B12 (2.5 +50 +1 mg/Kg BW) dissolved in 5 ml distilled water by gavage daily for 30 days. At the end of the treatment period, 2 untreated males (two males/ group) were naturally mated with treated females.

Hormones assay (Enzyme-Linked Immunosorbent Assay "ELISA"):The basic principle of this method according to (9).

Estimation of Follicle Stimulating Hormone concentration (FSH): The FSH enzyme immunoassay principle is acted upon a solid phase enzyme linked immunosorbent test. The estimation of FSH concentration by using an enzyme test kit (Human GmbH.53020 Wiesbaden. Germany gesellschafts for biochemical and diagnostic mbH). (10).

Estimation of Luteinizing Hormone (LH) concentration (ng/ml): The direct antigen ELISA detection by using the high affinity

of biotin for streptavidin which has been covered on the surface of microtiter wells. This method done by used a LH enzyme test kit (Human GmbH.53020 Wiesbaden. Germany gesellechalf for biochemical and diagnostic mbH). The procedure is similar to described in the procedure method of FSH-test kit except LH- enzyme conjugate (11).

Estimation of Estradiol (E2) Concentration (pg./ml): The measurement of serum concentration is generally regarded; kit was used (Monobind Inc. lake forest CA 92630, USA) (12). **The Estimation of Progesterone Concentration (ng/ml)**: The measurement of serum progesterone concentration was used Kit (Monobind progesterone ELISA Kit, Inc. Lake Forest CA 92630, USA) (12). Experimental II- Reproductive efficiency: The remainder female rabbits (4 females' groups) from experiment I (12 female rabbits) were mated with healthy mature male rabbits (6-8 months age), one male rabbit was mated with two female rabbit to evaluate the fertility rate. Statistical Analysis: Expressed the results as mean ± standard deviation (M±SD), the experiments analyzed by using One-way ANOVA by SPSS (Special Program for Statistical System) version 22.0. The least significant difference test (LSD) was used to determine the differences between groups in ANOVA-test, the level significant set on ($p< 0.05$) (13).

Result:

Effect of DXN-spr. and comb. of (FA +B12 +B6) on the reproductive hormone in mature female rabbits: The effect of DXN-spr. and combination of (F. A+B6+B12) on female reproductive hormone represented in table (1). The estrogen concentrations appeared no significant difference between all groups, while the progesterone concentration showed significant ($P<0.05$) decreased in combination Gr. (GIII) as compared to the control group and DXN-spr. group when showed no significant differences between it. Also, the testosterone hormones appeared no significant differences in all groups. Also, the data about the FSH and LH concentration results showed reported in table (1), that appears significant ($P<0.05$) decreased in group GIII more than the other two groups, as showed no significant differences between Gr. I (control) and group that administration DXN- spr. (GII).

Effect of DXN-spr. and combination of (FA. +B6+B12) on reproductive efficiency in mature female rabbits of experimental: The data of reproductive efficiency after mating of adult female rabbits with adult healthy males, fertility rate and number of fertility female increased in Gr. II (DXN-Spr.) compared with other groups. Also showed increased significantly ($P<0.05$) in the weight of newborn and % of fertility in this group compared to control and combination groups (Table 2).

Table (1) Effect of DXN-spr. and combination of (FA +B12 +B6) on the reproductive hormone in mature female rabbits.

Groups Parameters	Gr. I (Control)	Gr.II (DXN-spr.)	Gr.III (FA+B12+B6)	LSD
Estr.	2612.83 A	2700.50 A	2897.66 A	N. S
(pg/ml)	± 351.71	± 334.97	±420.58	
Prog.	9.65 A	8.616 A	6.71 B	3.90
(ng/dl)	± 1.77	± 1.39	± 0.59	
Testost.	0.32 A	0.28 A	0.28 A	N. S
(ng/dl)	± 0.068	± 0.05	± 0.037	
FSH	0.16 A	0.16 A	0.11 C	0.04
(IU/L)	± 0.04	± 0.007	± 0.02	
LH	0.17 A	0.16 A	0.10 B	0.06
(IU/L)	±0.008	± 0.01	± 0.01	

Values expressed in capital letters mean significant differences at (p<0.05) levels (M±SD)

Table (2) Effect of DXN-spr. and combination of (FA+B6+B12) on reproductive efficiency in adult female rabbits

Groups Parameters	Gr. I (Control)	Gr. II (DXN-Spr.)	Gr.III (FA. +B6+B12)	LSD
No. of female	10	10	10	-------
No. of male	2	2	2	-------
No. of new born	6.00 B	8.25 A	6.75 B	1.5
	±0.81	±0.95	±0.90	
weight of new born	23.05 B	30.70 A	25.93 AB	7.67
	±2.50	±5.20	±5.67	
Fertility rate	50%	100%	75%	-----

Values expressed in capital letters mean significant differences at (p<0.05) levels (M±SD).

Discussion:

Effect of DXN spr. and combination of (F+ B6+ B12) on reproductive hormones in female rabbits: It has been showed that reproductive hormones in female rabbits in this study is influences by administration of DXN- Spr. and combination of (F+B6+ B12) positively, we can be showed in table (1). The level of reproductive hormones ; estrogen and progesterone were significantly higher in group of DXN-spr. than to Gr.III this similarly that reported by El-Ratel (14) who observed these hormones increased in group of spr. than other groups due to attributed to spr. that directly effects on hormones secretion from follicles and corpus letum effect on ovaries and also indirect effects of spr. on Gnrh secretion from hypothalamus and also may be due to the spr. contain all essential amino acid, essential fatty acids, vitamins, minerals, essential polyunsaturated fatty acid and photo synthetic pigments (15- 19).

The natural sources for biological activity substances which increase the reproductive potential of male and female animals and also poultry due to improvement the gametogenesis and ovulation quality in female (20). (21) whom reported that spr. treatment to arsenic poising group which effect on ovary by altering the regulation of the pituitary –ovarian axis, this may result in improve and increased ovarian mass and follicular regression whereas stimulation of estradiol signaling in female rats of arsenic group.

The effects of folic acid combination with B6 and B12 on reproductive hormones showed in table (1) many researchers' indication to the role of folic acid in reproductive system, showed folate deficiency and homocysteine can lead to decreased cell division, increased inflammation of cytokine production, elevated apoptosis, altered nitric oxide metabolism and disturbed methylation reactions and subsequently affect oocyte development (22, 23). Effects of DXN-spr. and combination of (FA+ B6+ B12) on reproductive efficiency in female rabbits: The results of reproductive efficiency of female rabbits' administration DXN-spr. and combination (FA+B6+B12) documented in table (2) that showed significant increase in number of females pregnancy, number of fetus and also % of fertility increased in group of spr. similar to that reported in group of control. The results of these efficiency may indicate to that spr. have enhance in protein metabolism, lipid profile, hormonal regulation and secretion especially for reproductive system and decreased. the oxidative stress (it occurs in the body for any changes which play role in formation of free radicals that effect directly and indirectly on reproductive system). El-Ratel (14) reported that spr. enhanced and regulation the concentration of reproductive hormones (estrogen and progesterone) while the ratio of E2/ P4 decreased, these results and conclusion supported by a result of reproductive hormones in this study when showed enhancement in the reproductive hormones, these sign to the spr. have vital role on efficiency of reproductive system due to its contents essential CHO, fatty acid and amino acid also protein, vitamins and minerals (24, 25). In some studies, showed

the spr. elevation the iron content in blood of pregnancy rats during the first 10 days from pregnancy (26) and also other observation by other researchers who that reported the blood volume and red cell volume of material which increment significantly at the end of pregnancy period to meet the elevation iron demand of fetus development (27). Spr. have good hematopoietic nutrients were adequate to meet the higher demands of lactation and pregnancy in group of spr. due to its content of essential component (26). In the present study, the results showed positively effect of supplementation spr. on reproductive efficiency by increased number of pregnancy female rabbits and number of fetus and fertility% (table 2), these agreement with that reported by (28), the reproductive performance parameters increased but not significantly but increased in total and live litter size and also weight of birth treated with spr. Also (29) whom reported that fertility of rabbits significantly affected in treated with spr. and also with lycopene addition but enhance in litter weight and size of birth fed algae compared to group of control. Fertility efficiency: After 30 days of treatment, the mating of remaining mature female rabbits with healthy male rabbits for study the reproductive efficiency. The results appeared significant improvement in number and percentage of female fertility and also in number of new born and increment weight of new born in group of DXN-spr. these results occurred may be due to enhance in hormonal reproductive concentration in table (1). The DXN-spr. positively efficiency on reproductive by increased fertility of female and number of fetuses after administration of spr. these results agreement with result reported by James *et al*. (30) Which feeding different levels of spr. to fish and showed increased in fertility and growth performance and also who reported that these parameters positively increased when increased the level of spr.

conflict of Interest

The author(s) declared that there is no conflict of interest.

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تقييم الحالة التناسلية للأناث البالغة المجرعة بالسبيرولينا ومزيج من حمض الفوليك وب 6 وب 12

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الخلاصة

هدفت الدراسة الحالية إلى تقييم تأثيرات إعطاء السبيرولينا عن طريق الفم (كمكمل غذائي) على الكفاءة التناسلية لإناث الارانب البالغة وتم تقسيم إناث الأرانب (بعمر 16 إلى 18 أسبوعًا) إلى ثلاث مجموعات (10 أرانب / مجموعة). هذه المجموعات هي: (المجموعة الأولى) كمجموعة سيطرة و (المجموعة الثانية) تعطى سبيرولينا بجرعة (0.5 جم / كجم من وزن الجسم) و (المجموعة الثالثة) أعطيت مركب من حمض الفوليك، وب 6 وب 12 (2.5+ 50+ 1 ملغم / كجم من وزن الجسم) عولجت جميع المجاميع لمدة أربعة أسابيع. في نهاية فترة العلاج، تم التزاوج بشكل طبيعي من قبل 2 من الذكور الذين لم يتم علاجهم (ذكران / مجموعة) مع اناث المجاميع المعالجة. أظهرت النتائج عدم وجود اختلاف معنوي في تراكيز هرمون الاستروجين بين جميع المجاميع، بينما أظهر تركيز البروجسترون انخفاضًا معنويًا في المجموعة المركبة المجموعة الثالثة مقارنة بمجموعة التحكم وDXN-spr. كما أن هرمونات التستوستيرون لم يظهر فروق ذات دلالة إحصائية في كل المجاميع. يبدو أن تركيزات FSH وLH انخفضت بشكل ملحوظ في المجموعة الثالثة أكثر من المجموعتين الأخريين. وارتفع معدل الخصوبة وعدد خصوبة الإناث في المجموعة الثانية (DXN-Spr) مقارنة بالمجاميع الأخرى. كما ظهرت زيادة معنوية في اوزن المواليد ونسبة الخصوبة في هذه المجموعة مقارنة بمجموعتي السيطرة والمجموعة رقم 3. نستنتج بان سبيرولينا المجرع عن طريق الفم (0.5 جم / كجم من وزن الجسم) يعمل كمضادات أكسدة طبيعية يحسن الكفاءة التناسلية والهرمونات التناسلية.

الكلمات المفتاحية: سبرولينا' فئران' حامض الفوليك.