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The effect of adding different levels of wheat germ oil with or without folic acid on sexual hormones of rabbits

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Abstract

This study was undertaken at the breeding hall of the Department of Animal Production / College of Agriculture/ University of Basrah from 1/2/2018 to 1/7/2018, to investigate the effect of different levels of wheat germ oil (WGO) with or without Folic acid treatment on sex hormones such as Follicle stimulating hormone (FSH), Luteinizing hormone (LH), estrogen and progesterone on female rabbits at different physiological stages. Eight males and 56 female rabbits, with an average weight of (810,650) grams for males and females, respectively, were randomly divided into four groups (14 females + 2 males/ group) and two replicates/ group. The groups were dosed with WGO orally for a period of five months. The first group was a control group (no WGO) and the 2nd, 3rd, and 4th groups were given WGO by 0.25, 0.50 and 0.75 ml/ kg body weight/ day respectively. After two months of giving WGO, treatments 2, 3 and 4 were divided into two secondary groups. The first group gives the same levels of (WGO) which was given at the beginning of the experiment and the second group gives folic acid (1 mg/ kg of body weight/ animal) orally. The results showed significant ($P<0.05$) increase in concentrations of FSH and LH hormones in female rabbits treated with WGO at dosage 0.75ml/ day/kg of body weight at 90 and 120 days old then the concentrations of hormones exceeded in the same treatment but with folic acid during sexual puberty and pregnancy stages in comparison with control groups. There was a significant increase ($P<0.05$) in estrogen concentration when female treated WGO at dosage 0.75ml/ day/kg of body weight with folic acid at puberty and in the last period of pregnancy (after 30 days of pregnancy) in comparison with control groups. Progesterone concentration was significant ($P<0.05$) increase in female treated at dose WGO 0.25 ml/day/ kg of body weight of at 90 and 120 days of age, Whereas,when female administrated WGO with folic acid doses 0.75 caused significantly ($P<0.05$) increase in the concentrations of progesterone in the last period of pregnancy in comparison with control groups

Keywords: Wheat germ oil, Folic acid, Sexual hormones, Puberty, Pregnancy, Female rabbits.

Introduction

There are large percentages of wheat germ produced annually as a secondary product from the production of wheat flour. In Egypt, in 2012, a total of 12,000 tons of wheat germ oil (WGO) from 120,000 tons of wheat produced, this amount was used in the production of animal feed [1]. The chemical composition of wheat germ is 10.8-11.5% moisture, 24-26.5% protein, 6.5-8.56% fats, 39.0-39.5% carbohydrates and 3.9-4.18% ash calculated on the basis of dry material, while mineral are 0.6, 9.45, 1365.1, 310.1, 13, 775.2 and 9.05mg/100 g of calcium, copper, iron, magnesium, potassium, manganese and phosphorus respectively **[2,3].**

In the wheat germ oil has a high content of tocopherol compared to all vegetable oils, which reaches about 2,500 mg/kg [4]. It is rich with vitamins such as A and D, as well as containing a group of B vitamins, these ingredients give WGO antioxidant properties **[5].**

Wheat germ oil contains some of components called phytosterols, which have the same function of natural estrogen in the body, also has the ability to bind with estrogen receptors in the body especially with Alpha and Beta estrogen- receptors [6].

The first who reported that wheat germ oil has a necessary factor to improve the reproduction is Evans and Bishop (7), when they found that mice fed on wheat germ oil at 5% did not show any effect on estrogen secretion, ovulation and fertility fertilization, while, the level at 10% of wheat germ oil increased the growth of a uterus. John et al., (8) noted that wheat and its oil, reduce the level of estrogen in the blood.

As well as, wheat germ oil plays a role in controlling the synthesis of cholesterol in blood, which thus enable to control estrogen level **[9]**. wheat germ oil may have indirect effects on the secretion of steroid hormones as it is a rich source of vitamin E, which in turn is characterized by its ability to activate and stimulate the secretion of female estrogenic hormones by the cells of the inner layer (Theca cells) of ovarian vesicles which developed in the ovary, which positively reflected on the increase of estrogen in the circulatory system **[10].** Wheat germ oil also has multiple benefits, including increment in male libido, fertility rate and fetal stabilization in female swarms especially in the early stages of pregnancy **[11].**

It was noted that the use of folic acid to increase the concentration of all sex hormones (FSH, LH and progesterone) during pregnancy except for LH in the second week of pregnancy and FSH during the third week of pregnancy of rat [12]. Additionally, the folic acid supplementation can effect on placental growth during gestation of rats [13]

The rabbits (OryctolagusCuniculus) are originally from the wild **[14]** and it is one of the mammals that was classified as a long lifespan rodent because they own a couple of front-cutters. Rabbits have many economic qualities, including that their generation is short, a high growth rate, genetic diversity and the ability to deal with a wide range of feed and by-products of field crops, it is a herbivore and can be fed with different sources of food and adapts to live in large-scale breeding and environmental conditions **[15,16].**

The aim of the present study is to find out the effect of adding different levels of WGO and folic acid on the concentration of sex hormones (follicle-stimulating hormone (FSH), Luteinizing hormone (LH), estrogen and progesterone in rabbits.

Materials and Method

This experiment was conducted in one of the breeding hall / Department of Animal Production / College of Agriculture /Basrah University from 1-2-2018 to 1-7-2018. Eight males and 56 female rabbits were purchased from the local markets, aged 4 and 2 months with an average weight (810 and 650) g for males and females, respectively. All animals were fed daily at 7 a.m. on the same diet includes yellow corn 38.25 %, wheat 30 %, soybean 15%, fish meal 10%, sunflower oil 1%, limestone 7.5% and salt 0.25%, in addition to, available green feed (weighed before given), the concentrate diet ration was adjusted on the basis of 2% of the achieved weight body every two weeks. Clean water was ad libitum in each box.

The animals were divided into four random groups (14 females + 2 males per group) with two replicates per group. Rabbits were dosed with WGO produced by the Pakistani Company Hemani by orally using sterile needle for five months. The first group was a control group (without WGO). The second group was given WGO at 0.25 ml/kg body weight/day. The third group was given 0.5 ml WGO/kg body weight/day. The fourth group dosed with 0.75 ml WGO /kg body weight/ day. After two months of giving WGO, the 2, 3 and 4 groups have been divided into two groups, the first continued to receive the WGO and the second group gives folic acid (1 mg/ kg of body weight/ day) orally. The Males remained with the females until the end of the mating period. Select the age of sexual maturity of females by the first insemination (at 150 days old).

The concentrations of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and estrogen were measured by using a chemical kit of the Germany Biochemuce Company, while the concentration of progesterone was determined by using a chemical kit of the American Monobind Inc.

The data were statistically analyzed using completely Randomized Design (CRD) for four treatments and tested the significant differences between the means were determined by using the Revised Least Significant Differences (RLSD) at $P<0.05$ levels of significance. The obtained data were analyzed statistically using the SPSS program (2013) [17].

Results and Discussion

Table (1) represents the effect of WGO and folic acid on the concentration of FSH hormone. It is observed that at 90 and 120 days old there is a significant ($P<0.05$) increase when adding WGO with or without folic acid. The third treatment (T3) showed the highest concentration of the hormone at the age of 90 days (1.32 ± 0.018 ng/ml), also, at 120 days old (1.58 ± 0.028 ng/ml). The addition of 0.75 mg/kg of WGO with folic acid (T3) showed significant ($P<0.05$) increase in the concentration of FSH at sexual puberty (4.65 ± 0.012 ng/ml) compared to control group animals. Also during pregnancy, there was a significant effect ($P<0.05$) of the T3 animals during the duration of 15 and 30 days of pregnancy and without the addition of folic acid, which (3.01± 0.031 and 2.16 ± 0.035 ng/ml, respectively), but when folic acid was added, it was 3.02 ± 0.032 and 2.33 ± 0.093 ng/ml, respectively. It is not possible to determine what causes the release of

different concentrations of FSH from 25-107 days of the life of female rabbits, but, it is known that the production of estrogen and ovum in the ovary, begin after 50 days from animal life **[18,19].**

The effect of WGO and folic acid on luteinizing hormone (LH) were observed in Table 2. The concentration of hormone at 90 and 120) days old was a significant (P<0.05) increase was in the second treatment (T2) (1.89 ± 0.017, 1,89 ± 0.13 ng/ml, respectively) and third treatment (T3) (2.31 ± 0.031, 2.39 ± 0.021 ng/ml, respectively) in comparison with the control group. The concentration of LH hormone in the female rabbit of the second and third treatment increased significantly (P<0.05) either with folic acid or without it at sexual puberty in comparison with the control group. During pregnancy, there was increased significantly (P<0.05) in T3 either with folic acid or without during 15 and 30 days of pregnancy compared with the control group. The effect of WGO on increasing of the luteinizing hormone concentration may due to the fact that the wheat germ oil contains vitamin E and phytoestrogen, the phytoestrogen have the same activity of natural estrogen in the blood, which results increase in the secretion of both FSH and. LH [20]. Plant steroids were also found to increase the excretion of gonadotropin in male rabbits and immature females **[21].**

Table (1): The effect of different levels of wheat germ oil (WGO), with or without folic acid on the concentration of FSH hormone at different physiological stages (Mean± S. E.).

Period / Treatment		The concentration of FSH hormone (ng / ml) at age				
		90 days	120 days	Sexual puberty at 150 days	15 days of pregnancy	30 days of pregnancy
Control Group		1.06 d ± 0.010	1.24 d ± 0.016	3.84 d ± 0.012	2.08 b ± 0.044	1.19 d ± 0.033
T1	WGO (0.25 ml)	1.13 c ± 0.010	1.38 c ± 0.016	4.10 c ± 0.044	1.97 b ± 0.035	1.89 c ± 0.103
	WGO (0.05 ml) +folic acid			4.32 b ± 0.022	2.93 a ± 0.017	1.90 c ± 0.039
T2	WGO (0.25 ml)	1.24 b ± 0.017	1.45 b ± 0.018	4.41 b ± 0.033	2.96 a ± 0.012	2.19 b ± 0.031
	WGO (0.50 ml)+ folic acid			4.09 c ± 0.019	1.94 c ± 0.013	2.17 b ± 0.036

T3	WGO (0.75 ml)	1.32 a ± 0.018	1.58 a ± 0.028	4.34 b ± 0.016	3.01 a ± 0.031	2.16 b ± 0.035
	WGO (0.75 ml) +folic acid			4.65 a ± 0.012	3.02 a ± 0.030	2.33 a ± 0.093
Significant level		0.05		0.05	0.05	0.05

Different small letters within a column means significant difference (P<0.05) between treatment.

Table (2): The effect of different levels of wheat germ oil (WGO), with or without folic acid on the concentration of LH at different physiological stages (Mean± S. E.).

Period / Treatment		The concentration of LH hormone (ng / ml) at age				
		90 days	120 days	Sexual puberty at 150 days	15 days of pregnancy	30 days of pregnancy
Control Group		1.65 c ± 0.004	2.04 d ± 0.039	5.57 b ± 0.055	2.19 ed ± 0.046	1.82 c ± 0.069
T1	WGO (0.25 ml)	1.82 b ± 0.019	2.19 c ± 0.021	5.60 b ± 0.046	2.24 d ± 0.049	1.87 c ± 0.079
	WGO (0.25 ml) +folic acid			5.81 a ± 0.027	2.57 b ± 0.031	2.15 b ± 0.096
T2	WGO (0.50 ml)	1.89 a ± 0.017	2.31 b ± 0.031	5.87a ± 0.028	2.26 d ± 0.106	2.08 bc ± 0.112
	WGO (0.50 ml) + folic acid			5.82 a ± 0.037	2.44 b ± 0.052	2.23 a ± 0.059
T3	WGO (0.75 ml)	1.89 a ±	2.39 a ±	5.87 a ±	2.70 a ±	2.21 a ±

		0.013	0.021	0.038	0.068	0.112
	WGO (0.75 ml) +folic acid			5.84 a ± 3.05	2.68 a ± 3.41	2.02 ab ± 0.138
Significant level		0.05		0.05	0.05	0.05

Different small letters within a column means significant difference (P<0.0) between treatment.

In table 3, which represents the effect of WGO with folic acid on estrogen.It is observed that there is no significant effect during the pre-puberty period (90-120 days). However, at the age of 150 days (sexual puberty) there was a significant effect (P<0.05) in the favor of estrogen concentration of T2 (54.04 ± 0.35 pg/ml) and T3 with folic acid (54.22 ± 0.24 pg/ml). During pregnancy, it was noted that the estrogen concentration of T3 at 30 days of pregnancy animals treated with WGO and folic acid (40.36 ± 0.76 pg/ml) was significant (P<0.05) higher in comparison with that of the control group.

The results in table 4, which represents the effect of WGO and folic acid on progesterone levels. There was a significant (P<0.05) difference during the sexual maturity of rabbit females, especially, after puberty when the rabbits used in the experiment are treated with the highest level of WGO with folic acid. The most significant increase in the level of progesterone (P<0.05) was in all coefficients and higher significant level of this hormone in blood on 15 days of pregnancy (2.97 ± 0.029 ng/ml) when WGO was taken with folic acid and then reduced during 30 days of pregnancy (1.27 ± 0.019 ng/ml) compared to the control group animals.

Table (3): The effect of different levels of wheat germ oil (WGO), with or without folic acid on the concentration of estrogen hormone at different physiological stages (Mean± S. E.).

Period / Treatment		Estrogen concentration (pg / ml) at age				
		90 days	120 days	Sexual puberty at 150 days	15 days of pregnancy	30 days of pregnancy
Control Group		17.55 ± 0.48	23.37 ± 0.31	52.28 b ± 0.67	44.08 ± 1.87	39.85 ab ± 0.44
T1	WGO (0.25 ml)	17.03 ± 0.63	22.90 ± 0.41	52.85 b ± 0.19	44.82 ± 0.99	39.80 ab ± 0.40
	WGO (0.25 ml) + folic acid			52.45 b ± 0.36	43.32 ± 1.09	37.79 b ± 0.93

T2	WGO (0.50 ml)	17.30 ± 0.37	23.64 ± 0.28	54.04 a ± 0.35	45.84 ± 1.32	39.67ab ± 0.24
	WGO (0.50 ml) + folic acid			52.94 b ± 0.25	44.87 ± 1.19	39.02 ab ± 0.73
T3	WGO (0.75 ml)	17.45 ± 0.50	23.01 ± 0.34	52.63 b ± 0.29	46.40 ± 0.96	39.39 ab ± 0.40
	WGO (0.75 ml) + folic acid			54.22 a ± 0.24	48.28 ± 1.35	40.36 a ± 0.76
Significant level		N.S	N.S	0.05	N.S	0.05

Different small letters within a column means significant difference (P<0.05) between treatment.

Table (4): The effect of different levels of wheat germ oil (WGO), with or without folic acid on the concentration of progesterone at different physiological stages (Mean± S. E.).

Period / Treatment		The concentration of progesterone (ng / ml) in serum at age				
		90 days	120 days	Sexual puberty at 150 days	15 days of pregnancy	30 days of pregnancy
Control Group		0.77 b ± 0.011	0.79 b ± 0.008	1.00 ± 0.010	2.91 b ± 0.014	1.14 c ± 0.013
T1	WGO (0.25 ml)	0.80 a ± 0.012	0.82 a ± 0.011	0.97 ± 0.007	2.90 b ± 0.005	1.21 b ± 0.015
	WGO (0.25 ml) + folic acid			0.97 ± 0.014	2.95 ab ± 0.022	1.22 ab ± 0.018
T2	WGO (0.50 ml)	0.79 ab ± 0.005	0.81 ab ± 0.004	0.99 ± 0.014	2.95 ab ± 0.038	1.16 c ± 0.014
	WGO (0.50 ml)+ folic acid			0.98 ±	3.11 a ±	1.20 b ±

				0.012	0.177	0.018
T3	WGO (0.75 ml)	0.79 ab ± 0.004	0.80ab ± 0.003	0.95 ± 0.015	2.90 ab ± 0.010	1.17 c ± 0.018
	WGO (0.75 ml) + folic acid			0.976 ± 0.016	2.97 ab ± 0.029	1.27 a ± 0.019

Different small letters within a column means significant difference ($P<0.05$) between treatment.

Conclusion

In conclusion of the current study, it can be suggested that the treatment female rabbits with 0.75 ml/kg of body weight/day of WGO with folic acid at dosage 1 mg/ kg of body weight/ daily orally from 90 days old to the last stage of pregnancy may improve the concentration of sexual hormones such as FSH, LH and estrogen.

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تاثير اضافة مستويات مختلفة من زيت جنين القمح مع او بدون حامض الفولك في الهرمونات الجنسية للأرانب

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المستخلص:

اجريت هذه الدرسة في احدى قاعات التربية في قسم الانتاج الحيواني كلية الزراعة / جامعة البصرة للفترة من 1/ 2/ 2018 ولغاية 1/7/ 2018 لدراسة تاثير مستويات مختلفة من زيت جنين القمح مع او بدون حامض الفولك في الهرمونات الجنسية (الهرمون المحفز لنمو الحويصلة المبيضة, هرمون الاباضة, الاستروجين والبروجسترون) على اناث الارانب عند مراحل فسلجية مختلفة . استخدمت في الدراسة 8 ذكور و 56 انثى ارنب , بمتوسط وزن 810 و 650 غم على التوالي, وزعت عشوائيا" الى اربعة مجاميع (14 انثى + ذكرين / مجموعة) وبمكررين لكل مجموعة. جرعت المجاميع عن طريق الفم بزيت جنين القمح لمدة خمسة اشهر. المجموعة الاولى اعتبرت كمجموعة سيطرة (بدون زيت جنين القمح) والثانية والثالثة والرابعة اعطيت زيت جنين القمح بمقدار 0.25 , 0.50 و 0.75 مل / كغم من وزن الجسم/ يوم على التوالي. بعد شهرين من التجربة قسمت المجاميع الثانية والثالثة والرابعة الى مجموعتين ثانويتين. المجموعة الاولى اعطيت ذات التركيز من زيت جنين القمح التي اعطيت في بداية التجربة والمجموعة الثانية اعطيت زيت جنين القمح مع حامض الفولك بمقدار 1 ملغم / كغم من وزن الجسم عن طريق الفم . اظهرت النتائج حصول زيادة معنوية (أ< 0,05) في تركيز هرموني FSH وLH في اناث الارانب التي عوملت بـ 0.75 مل / كغم من وزن الجسم/ يوم مع او بدون حامض الفولك عند عمر 90 و 120 يوم ثم ارتفعت تراكيز الهرمونات في ذات المجموعة مع اعطاء حامض الفوليك عند عمر البلوغ الجنسي ومرحلة الحمل بالمقارنة مع مجموعة السيطرة. كما لوحظ وجود زيادة معنوية (أ< 0,05) في تركيز هرمون الاستروجين في الاناث التي عوملت بـ 0.75 مل / كغم من وزن الجسم/ يوم مع حامض الفولك عند البلوغ والمرحلة الاخيرة من الحمل بالمقارنة مع مجموعة السيطرة. زاد تركيز هرمون البروجسترون معنويا" (أ< 0,05) في الاناث التي بـ عوملت 0.25 مل / كغم من وزن الجسم/ يوم عند اعمار 90 و 120 يوم. بينما حققت المعاملة 0.75 مل / كغم من وزن الجسم/ يوم مع حامض الفولك زيادة معنوية (أ< 0,05) في تركيز البروجسترون في المرحلة الاخيرة من الحمل بالمقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: زيت جنين القمح, حامض الفولك , الهرمونات الجنسية , البلوغ , الحمل , اناث الارانب.