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Article

Evaluation of wheat (*Triticum aestivium L*) diet on reproductive functions of adult female rats

Evaluación de la dieta de trigo (*Triticum aestivium L*) sobre las funciones reproductivas de ratas hembras adultas

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ABSTRACT

Introduction: The relationships between wheat diet, sex hormone and the estrous cycle are important from the perspective of fertility.

Aim: This study aims to investigate the effect of wheat diet on weight, estrous cycle, reproductive hormones and histology of the ovary of female Sprague-Dawley rats.

Methods: Prior to the test, vaginal smear was monitored daily for 16 days period to select females with normal (regular) estrous cycle. A total of twenty rats with regular estrous cycle weighing between 170-200 g were randomized into four sets of 5 animals each. Group A is the female control and was fed with rat chow, group B, C and D was given 40%, 60%, 100% of wheat diet respectively. The wheat diet was giving respectively for a period of 4 weeks. Estrous cycle pattern was monitored using the vaginal smear for 16 days. At the end of the experiment, the rats were sacrificed and the blood sample were taken for follicle stimulating hormone, luteinizing hormones, estradiol, and progesterone analysis. The histology of the ovary were carried out.

Results: The result showed that there was an increase in the weight of the rats that was fed with wheat and the increase was diet dependent. The wheat diets disrupt the order of appearance of normal estrous cycle stages, hence; there was no constancy in the frequency of all cycle stages in the wheat groups, mostly the 100% wheat group. No significant endocrine or histological differences noticed between control and wheat diets groups. Therefore photomicrograph of wheat diet shows large corpus luteum, thick germinal epithelium, zona granulosa and oocyte.

Conclusion: Wheat diets disrupt the estrous cycle of the rats; however no adverse effect was noticed on the circulating reproductive hormones and histology of the ovary. However, high consumption of wheat should be taken with precaution.

Keywords: Diet, Wheat (*Triticum aestivium L*), Estrous cycle, Sex hormones, Histology

1. Introduction

The economic importance of Wheat (Triticum aestivum L.) and its contribution to the diets of humans and livestock cannot be disputed (Shewry & Hey, 2015). About 21% of the world's food depends on the wheat crop, which grows on more than 200 million hectares of farmland worldwide. Wheat is consumed worldwide on a daily basis and represents 25% of the world's cereal production; it constitutes one of the main food source of carbohydrates; and it contains also proteins, fibers, amino acids, vitamins, phytochemicals such as phenolics and terpenoids (Giraldo et al., 2019). Wheat increased consumption have being associated with the adoption of a "western lifestyle" in countries undergoing urbanization and industrialization (Del Coco et al., 2019).

Nutrients are substances required by the body to perform its basic functions. Nutrients are used to produce energy, detect and respond to environmental surroundings, move, excrete wastes, respire (breathe), grow, and reproduce (Kim et al., 2019). Hence, the nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources (Grote et al., 2021). The importance of wheat is mainly due to the fact that its seed can be grounded into flour, semolina, etc., which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients to the most of the world population (Sanadya et al., 2021). The nutritional composition of wheat are as follows; 12% water, 70% carbohydrates, 12% protein, 2% fat, 1.8% minerals, and 2.2% crude fibers (Iqbal et al., 2022). Thiamine (B1), riboflavin (B2), niacin, pantothenic acid, inositol, P-aminobenzoic acid, folic acid and vitamin B6 and small amounts of vitamin A are also present. Minerals contained in wheat include calcium, phosphorous, potassium, iron, magnesium and sodium (Iqbal et al., 2022)

Diet quality can severely influence an individual's reproductive success (Duxbury & Chapman, 2020). Hence, the normal physiological principle guiding female reproductive efficiency involves an interplay between the hypothalamus that secretes gonadotropin-releasing hormone (GnRH) in a pulsatile manner and the pituitary gland which is stimulated by the hypothalamus via the hypothalamo-pituitary-adrenal axis to regularly the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), in addition to the ovary that has both methodical enzymatic system and steroidogenesis for producing sex steroid hormones such as; estrogen and progesterone (Stamatiades & Kaiser, 2018). The estrous cycle is a recurrent process that describes changes in reproductive hormone levels caused by ovarian activity under the influence of pituitary hormones. Estrous cycles are characterized by morphological changes in ovaries, the uterus and the vaginal cells (Paccola et al., 2013). The length of the estrous cycle and the frequency of the reproductive cycle in each organism are different. In mice, lasts for about 4-5 days. The estrous cycle consists of four phases, namely proestrus, estrus, metestrus and diestrus (Ajayi & Akhigbe, 2020). During this period, the vaginal mucosa undergoes tremendous structural changes and is affected by hormones such as FSH (stimulates the growth and development of ovarian follicles), LH (regulates estrogen and progesterone hormones), progesterone and estradiol (Lovick & Zangrossi, 2021)

Furthermore, there are many unanswered questions with regard to the consumption of wheat in regard to fertility. Therefore, since diet and lifestyle seem to be significant factors influencing fertility, it is valid to expand knowledge in this area. Therefore, the study is aim to demonstrate how the estrous cycle, reproductive hormone, and ovarian histology of female rats given a wheat-based diet are related to one another.

2. MATERIALS AND METHODS

Preparation of Study Diet

Triticum aestivum Linn. (Poaceae) was purchased from a vendor in Jattu market, Uzairue, Edo State, Nigeria. The wheat were carefully picked for stones, dried at 40°C in a hot furnace, weighed and were grinded into a coarse powdered form using a local grinding machine. While the regular rat food (70% carbohydrates, 12% protein, 2% fat) was provided by Premier Feed Mill Co. Ltd., Ibadan, Nigeria, the ground wheat (Carbohydrates 78%, protein 14%, fat 2%) was sieved with an 80 mesh grid to form the wheat diet. To make 40% of the wheat-based diet; 40g of wheat was weighed and mixed with 60g of rat chow. To make 60% of the wheat-based diet; 60g of wheat was weighed and mixed with 40g of rat chow. Whole wheat of 100g was used as 100% wheat, without adding any rat chow.

Experimental Animal and Housing

Thirty female rats weighing 170 - 200g were obtained from the Animal Housing of Edo State University, Edo State, Nigeria. The rats were kept under standard laboratory condition and fed regular rat chow and water. They were acclimatized for two weeks before starting the experiment. All procedures relating to animal care and use were implemented in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals (Guide for the Care and Use of Laboratory Animals., 2011) and were approved by the Ethics Review Committee of the of Department of Anatomy, Edo State University Uzairue. Edo State. Nigeria.

Sample collection

Prior to the test, vaginal smear was monitored daily for a 16 -days period to select females with regular estr0us cycle (Bend et al., 2022). Twenty rats with regular estrous cycle were randomized into four groups of 5 animals each.

Group A- control animals consisting of 5 rats received only distilled water orally.

Group B- consisted of 5 rats and were given 40% wheat

Group C- consisted of 5 rats and were given 60% wheat

Group D- consisted of 5 rats and were given 100% wheat

The wheat diet was giving respectively for a period of 4 weeks. At the end of the 4 weeks of feeding, the estrous cycle was checked for the female rats in the next 16 days (Monima et al., 1970). At the end of the experiment, the rats were sacrificed by cervical dislocation; the blood sample were taken for hormonal analysis. The ovaries were harvested after abdominal incision, and then fixed in 10% buffered formalin. Tissue sections were prepared using routine histological tissue preparation.

Monitoring the Estrous Cycle:

Estrous cycle stages (proestrus, estrus, metestrus, and diestrus) were determined by daily observations of vaginal smear (epithelial cell cytology). Vaginal smears were collected daily using a small suction pipette and normal saline (0.9% NaCl) between 9 am and 10am., then the smears were placed on slides and examined using the light microscope (Ajayi & Akhigbe, 2020). Rats with a 4 to 5-day estrous cycle of proestrus-oestrus-metestrus-diestrus were classified as normal, whereas any deviation from this pattern in duration and sequence was classified as abnormal. Cell morphology was then used to determine cycle stages with metestrus characterized by a mix of lymphocytes, cornified cells and epithelial nucleated cells; metestrus typically lasts from 12 to 24 hours. Diestrus was classified by lymphocytes/ neutrophils, and a little to no epithelial nucleated cells and a low cell density; diestrus is the longest phase of the cycle and typically lasts for 48 to 72 hours. Proestrus was classified by nucleated cells (this cells are rounded in shape, with a dark nucleus and deeply stained cytoplasm that form sheets) and some anucleated epithelial cells with very little to no neutrophils present; it last form 14 to 24 hours, and estrous was classified by masses of large cornified cells that lack nuclei; estrus typically lasts approximately 48 hours (Alonso-Caraballo & Ferrario, 2019).

Hormonal assay

At the conclusion of the experiment, blood samples were taken via retro-orbital puncture and placed into plain tubes. The blood were allowed to clot and were centrifuged for 10 minutes at 3000 rpm on a tabletop centrifuge. Using a micropipette, the serum and plasma were carefully separated and placed into separate sets of plain tubes. Using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits, the following hormones were measured: progesterone (P), estrogen, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The manufacturer's instructions were followed for the hormonal assay.

Haematoxylin and Eosin (H & E) staining

The rats were sacrificed by cervical dislocation, the ovary dissected out and surrounding tissues removed and washed with normal saline. The ovaries were blotted on filter paper fixed in 10% buffered formalin solution for 24 h. The paraffin-embedded tissues were cut at 5 mm thickness and stained with hematoxylin-eosin solution. The sections were examined microscopically for histological observation.

Statistical analysis

Data obtained from this study was analysed using GraphPad Prism software, (Version 6.0. San Diego, USA). One way analysis of variance (ANOVA) was done followed by post hoc- Bonferroni test. The confidence limit of statistically significance was set at $P<0.05$. The result therefore was presented as mean ± standard deviation (SD).

Results

As indicated in table 1, there were a statistically ($P<0.05$) significant increase in the body weights of the rats fed with wheat diets (Groups C and D) when compared to the group receiving standard rat food (Group A).

Table 1: Effect of the wheat diet on body weight of female rats

Groups	Initial weight	Final weight
Group A	174.4±8.24	195.8±8.86
Group B	170.0±9.74	209.4±7.01
Group C	179.6±6.9	230.8±3.9*
Group D	170.0±2.6	238.7±6.91*

Values represented as M± SD. N=5; *(P<0.05) when compared to control. Group A= Control; Group B= 40% wheat diet; Group C=60% wheat diet; Group D=100% wheat diet.

Effects of wheat on the estrous cycle

Rats in different phases of estrous cycle during the 16 days of the experiment with wheat diets. A significant difference was seen between control and other treated groups (p>0.05) (Table 2).

Table 2: Estrous cycle tracking of control and wheat diet groups (duration is expressed in terms of day)

		Groups																			
Days	Time	A					B					C					D				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	AM	D	P	E	E	D	E	D	D	E	E	M	D	D	M	M	D	E	D	P	E
2	AM	E	E	D	M	E	M	P	D	M	M	D	D	D	D	D	D	M	P	E	M
3	AM	D	M	P	M	M	D	E	D	D	D	D	D	P	D	D	E	D	E	M	M
4	AM	P	E	E	D	E	E	M	M	D	D	D	P	E	P	M	M	D	M	D	D
5	AM	E	M	M	P	M	E	D	D	E	P	E	E	M	E	P	D	D	M	D	M
6	AM	M	D	M	E	E	M	D	E	E	E	M	M	M	E	E	D	P	D	D	D
7	AM	E	D	D	E	M	M	E	D	M	M	D	D	D	M	E	E	M	D	P	D
8	AM	E	P	E	M	D	D	M	E	D	M	D	D	D	D	E	M	D	D	E	E
9	AM	M	E	M	D	P	P	D	D	D	D	P	E	E	D	M	E	D	P	M	M
10	AM	D	M	D	D	E	E	P	E	E	D	E	M	M	E	M	E	E	E	D	D
11	AM	D	D	D	E	M	M	E	E	M	D	M	M	D	M	M	M	E	M	D	D
12	AM	D	E	P	M	E	D	M	D	D	P	D	D	D	D	D	M	M	M	D	P
13	AM	P	E	E	M	D	P	M	D	D	E	D	D	D	D	D	D	M	M	E	E
14	AM	E	M	M	D	P	E	D	P	E	M	E	M	E	P	D	D	D	D	M	E
15	AM	M	M	D	P	P	E	D	D	M	D	E	M	M	E	P	P	D	D	D	M
16	AM	D	D	D	E	D	M	D	M	M	D	M	D	D	E	E	E	D	D	D	D

Estrous cycle analysis samples from individual female rats are organized into columns for 16 days (4 cycles). Group A= Control; Group B= 40% wheat diet; Group C=60% wheat diet; Group D=100% wheat diet. Abbreviations: proestrus (P), estrus (E), metestrus (M), diestrus (D).

Serum FSH, LH, progesterone and estradiol level

The analysis of the changes in the concentration of sex hormones in the females - gonadotropins (follicle-stimulating hormone, FSH, luteinizing hormone, LH; Progesterone, Estradiol) in the groups of the wheat diets showed that the concentration of FSH. LH, Progesterone and Estradiol in the groups B, C and D did not change significantly when compared to group A. (Table 3).

Table 3: Effects of the wheat diet on reproductive hormones in female rats

Groups	FSH (mlU/ml)	LH (mlU/ml)	Progesterone (mlU/ml)	Estradiol (pg/ml)
Group A	2.21±0.05	4.64±0.02	23.1±1.46	88.36±0.11
Group B	3.34±0.17	2.40±0.16	20.16±0.09	95.1±0.59
Group C	3.22±0.12	2.40±0.01	19.26±0.12	89.08±0.48
Group D	2.70±0.07	2.20±0.11	17.16±0.08	90.04±1.59

All values are expressed as Mean ± SD, N= 5. Group A= Control; Group B= 40% wheat diet; Group C=60% wheat diet; Group D=100% wheat diet

The effect of wheat diet on the histology of ovary

Group A photomicrograph of ovary reveals not so prominent large corpus luteum, a thick germinal epithelium and zona granulosa with corona with varying eccentrically located Graafian follicles. Group B photomicrograph reveals large corpus luteum, a thick germinal epithelium and a prominent oocyte devoid of luteinized theca cells. Group C photomicrograph showing visible corpus luteum, a thick germinal epithelium and small eccentrically located oocyte of varying sizes. Group D photomicrograph reveals oocytes with oedematous changes, visible corpus luteum with cystic accumulation and fluid (Figure 1).

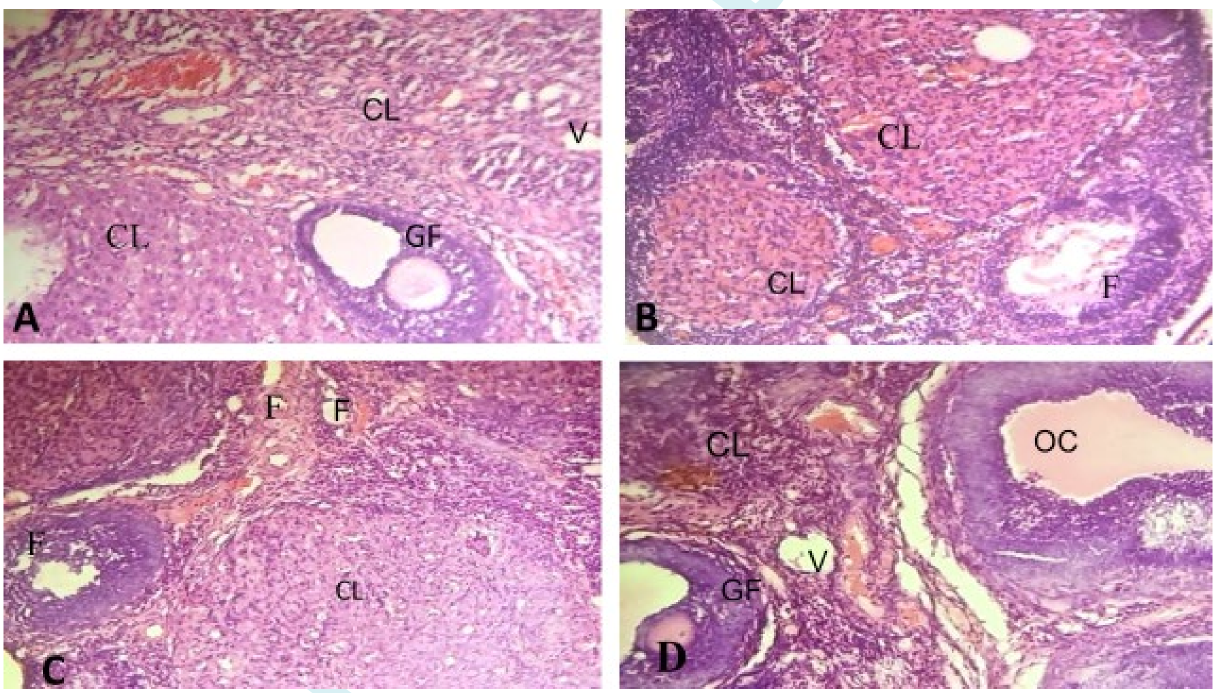

Figure 1. Histological section of the ovaries in groups A, B, C and D. Graafian follicles (GF), corpora lutea (CL), vacuolation (V). Follicles (F), oedematous changes (OC) Group A: rat chow, Group B: 40% wheat diet; Group C: 60% wheat diet and Group D: 100% wheat diet/ Micrographs of haematoxylin and eosin (H&E X 400).

3. Discussion

The wheat diet groups gained more weight overall, however; the weight that was seen was diet-dependent. One possible explanation for the increased body weight can be attributed to too many calories in wheat that may have led to the increase in the weight. This finding is in agreements with a study that reported, that wheat might be a major cause of obesity (Brouns & Shewry, 2022) Glutens are present in wheat, however; anecdotal evidence have led to the hypothesis that peptides showing opioid action present in gluten induce weight gain. Another theory is that gluten peptides lead to a favourable energy balance by reducing resting energy expenditure. Completed dietary peptides need to attach to receptors involved in hunger, satiety, and energy control, be absorbed in adequate amounts, and stay intact in the circulation for long enough to have long-lasting biological activity in order to cause such effects in vivo. Moreover, a wealth of observational data in humans indicates that the levels of gluten consumption are unrelated to BMI or daily calorie intake, and the degree of overweight seen in different nations appears to be independent of the amount of wheat consumption (Brouns & Shewry, 2022).

The hormones of the ovaries (progesterone, estradiol, and inhibins), the anterior pituitary (follicle-stimulating hormone and luteinizing hormone), the hypothalamus (gonadotrophin-releasing hormone), and the uterus (prostaglandin) control the rat estrous cycle (Aritonang et al., 2017)the uterus and the vaginal cells. The length of the estrous cycle and the frequency of the reproductive cycle in each organism are different. In mice, lasts for about 4-5 days. The estrus cycle consists of four phases, namely proestrus, estrus, metestrus and diestrus. During this period, the vaginal mucosa undergoes tremendous structural changes and is affected by hormones such as FSH (stimulates the growth and development of ovarian follicles. The estrous cycle is regulated by these hormones through a system of positive and negative feedback. Acute hormonal alterations and ovulation take place during proestrus and estrus, which are crucial periods for ovulation and fertility (Lenert et al., 2021). Fertility may be hampered by the prolonged diestrus and metestrus phases of the estrous cycle, as shown in wheat groups in the study. Wheat contains lignan, which has been recognized as a phytoestrogen (Rodríguez-García et al., 2019)partly attributable to their abundant micronutrient (e.g., polyphenol. Phytoestrogens has the ability to produce "estrogen-like" effects because of their structural resemblance to the female hormone estradiol (17-β-estradiol) (Canivenc-Lavier & Bennetau-Pelissero, 2023). When lignan is continuously administered to animals, it has been observed to decrease fertility (Ward & Thompson, 2006). These may be the likely causes of the disturbance of the estrous cycle phases seen in this study.

The process by which ovarian follicles grow, develop, and mature is essential to successful reproduction. In the ovary, primordial follicles are formed during embryonic development (Simon et al., 2020)paracrine, and endocrine factors together with tightly regulated interactions between granulosa cells and oocytes for the growth and survival of healthy follicles. Culture of ovarian follicles is a powerful approach for investigating folliculogenesis and oogenesis in a tightly controlled environment. This method has not only enabled unprecedented insight into the fundamental biology of follicle development but also has far-reaching translational applications, including in fertility preservation for women whose ovarian follicles may be damaged by disease or its treatment or in wildlife conservation. Two- and three-dimensional follicle culture systems have been developed and are rapidly evolving. It is clear from a review of the literature on isolated follicle culture methods published over the past two decades (1980-2018. During fetal development, a fixed number of primordial follicles is established. Throughout the female's life, primary follicles enter a developing pool. Gonadotrophic hormones are not necessary for the early phases of follicle growth; instead, antral follicles respond to and eventually become dependent on FSH (Zhang et al., 2023). A corpus luteum (CL) is created upon ovulation and secretes progesterone during the luteal phase of the cycle. Progesterone secretion is the CL's primary job. Both throughout a typical estrous cycle and during pregnancy, progesterone is produced from CL. The primary luteotrophic hormone, LH, stimulates the luteinization of the theca and granulosa cells of the preovulatory follicle's into luteal cells (Przygrodzka et al., 2021).

In comparison to the control group, there was no statistically significant rise in the levels of follicle-stimulating hormone, luteinizing hormone, or progesterone in the female rats fed wheat (Table 3). This may be related to the report that any disruption to the pituitary-gonadal axis can have detrimental effects since it is crucial for the upkeep of the reproductive system (Nwogueze et al., 2019). Additionally, a study has shown that neither wheat flour nor wheat bran impacted the levels of progesterone or FSH (Arts & Thijssen, 1992). Moreover, there was no significant rise in estradiol concentrations across the wheat diet groups. Therefore, this could suggest that the rats' levels of FSH, LH, progesterone, and estrogen are unaffected by the wheat diet.

Thus, the histology of the ovary fed wheat diets did not exhibit any discernible pathologic change; instead, the ovary displayed oocytes, thick germinal epithelium, normal cortical stroma containing ovarian follicles and visible corpus luteum (signaling that ovulation has occurred), and an inner medullar made up of loose connective tissue that was richly vascularized. Moreover, 80%–85% of the total protein in wheat is made up of a kind of protein called gluten (Wu et al., 2021). There have been reports that the gluten included in wheat may work as a prebiotic, influencing key physiological and metabolic processes (Njoku et al., 2023). Gluten may enhance the metabolic and physiological processes involved in reproduction in rats by means of this mechanism. Furthermore, wheat includes tocols that increase vitamin E activity, zinc, magnesium, and selenium—all of which are crucial for reproductive processes (Yahaya et al., 2020). The results of this investigation improved our knowledge of the relationships between the wheat diet, ovarian histology, the estrous cycle and reproductive hormones.

4. Conclusion

The study's findings demonstrate that wheat diet, which was dose dependent had a negative effect on the estrous cycle. However, FSH, LH, progesterone, estradiol, and the histomorphology of the female rats' ovaries, on the other hand, were unaffected. This implies that the animals' long-term fertility might be an issue. Therefore, it is advised to use caution when consuming wheat diet.

Ethical aspects

All procedures relating to animal care and use were implemented in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals (*Guide for the Care and Use of Laboratory Animals.*, 2011) and were approved by the Ethics Review Committee of the of Department of Anatomy, Edo State University Uzairue. Edo State. Nigeria

Declaration of Interests

The authors declare that there is no conflict of interests that could prejudice the impartiality of the research reported.

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Contribution by Authors

ICA conceived and designed the research, interpreted the data, drafted the initial manuscript. AG collected and assembled data. ICA and AG both critically revised the article for important intellectual content.

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RESUMEN

Introducción: Las relaciones entre la dieta de trigo, la hormona sexual y el ciclo estral son importantes desde el punto de vista de la fertilidad.

Objetivo: Este estudio tiene como objetivo investigar el efecto de la dieta de trigo sobre el peso, el ciclo estral, las hormonas reproductivas y la histología del ovario de ratas hembras de Sprague-Dawley.

Métodos: Antes de la prueba, se monitoreó diariamente el frotis vaginal durante un período de 16 días para seleccionar mujeres con ciclo estral normal (regular). Un total de veinte ratas con ciclo estral regular que pesaban entre 170 y 200 g fueron asignadas al azar en cuatro grupos de 5 animales cada uno. El grupo A es el control femenino y fue alimentado con comida para ratas, los grupos B, C y D recibieron 40%, 60%, 100% de dieta de trigo respectivamente. La dieta de trigo se administró respectivamente durante un período de 4 semanas. El patrón del ciclo estral se controló mediante frotis vaginal durante 16 días. Al final del experimento, las ratas fueron sacrificadas y se tomó la muestra de sangre para el análisis de la hormona estimulante del folículo, las hormonas luteinizantes, el estradiol y la progesterona. Se realizó la histología del ovario.

Resultados: se observó un aumento en el peso de las ratas que fueron alimentadas con trigo y el aumento fue dependiente de la dieta. Las dietas de trigo alteran el orden de aparición de las etapas normales del ciclo estral, por lo tanto, no hubo constancia en la frecuencia de todas las etapas del ciclo en los grupos de trigo, principalmente en el grupo de trigo 100%. No se observaron diferencias endocrinas o histológicas significativas entre los grupos de dietas control y de trigo. Por lo tanto , la fotomicrografía de la dieta de trigo muestra un gran cuerpo lúteo, epitelio germinal grueso, zona granulosa y ovocito.

Conclusión: Las dietas de trigo alteran el ciclo estral de las ratas; sin embargo, no se observaron efectos adversos sobre las hormonas reproductivas circulantes ni sobre la histología del ovario. Los resultados sugieren que el alto consumo de trigo debe tomarse con precaución.

Palabras clave: Dieta, Trigo (*Triticum aestivium L*), Ciclo estral, Hormonas sexuales, Histología