Histological and Hormonal Studies of the Effect of Wheat-Based Diet (*Triticum Aestivium L*) on the Ovaries of Adult Sprague-Dawley Rats

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ABSTRACT.

Background: The public is familiar with wheat as a common dietary source. It is undeniable that wheat contributes to the nourishment of both humans and animals. **Objectives:** This study looked at the effects of wheat-based diets on the histology of the ovary and the female reproductive hormones. **Materials and Methods**: Fifteen mature female Sprague-Dawley rats were used in this investigation; they were divided into three groups (A-C), each with five rats. For four weeks, the animals in groups A to C received the following diets; group A had regular rat chow, group B was fed a diet made entirely of wheat (100%), groups C were fed 60% wheat (60g of wheat mixed with 40g of rat chow). The levels of follicle-stimulating hormones, luteinizing hormones, estrogen and prolactin were assessed using hormonal tests. The ovary was carefully dissected out and quickly fixed in 10% formal saline for routine histological study after the H&E method. **Results:** Comparing the wheat-based diet groups to the control, there was a significant increase (P>0.05) in body weight. However, there was no difference in the ovarian weights. The follicle-stimulating hormone, luteinizing hormone and estrogen levels were unaffected by the wheat-based diets, except prolactin levels that increased in the 100% wheat diet group B. There was no histological changes observed in the ovary of the wheat groups; as follicles at different stages of development, corpora luteum and few cytoplasmic vacuoles are present. **Conclusion:** The research indicated that a diet high in wheat should be consumed in moderation.

Keywords: Histology; Hormones; Wheat, Ovaries; *Triticum aestivium L.*

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INTRODUCTION

Triticum aestivum Linn, also known as wheat, is a hexaploid species of flowering monocotyledonous plants in the Poaceae (grass family) and is one of the most frequently grown crops in the world [1]. People eat it as a staple food since it is readily available and reasonably priced [2]. Wheat contains other healthy ingredients including minerals, iron, protein, and vitamins like riboflavin, thiamine, niacin, and alpha-tocopherol. It is a significant source of soluble fibre, trace minerals, and phytochemicals [2], as well as starch and gluten protein, both of which offer enormous amounts of energy [3]. Dietary fibre, phosphates, and other mineral salts present in wheat aid with bowel movement; while muscle tissue growth and repair are aided by wheat protein, vitamins B and E [4]. Because of this, wheat contributes significantly to both the human diet and animal feed in emerging nations [5]. Wheat consumption has increased as a result of urbanization and industrialization in Sub-Saharan African countries, primarily Nigeria. Wheat flour is highly necessary for the production of bread, noodles, pasta, and cereals [2]. Whole wheat contains wheat bran and wheat germ, which protect a number of diseases, such as appendicitis, obesity, diabetes, diverticulum in the colon, ischaemia, and heart disease [6,7]. According to studies, whole wheat grain is enriched with essential nutrients that have several medicinal properties, including the ability to treat chest pain, boils, scars, tonsil pain, tooth disorders, acne or pimples, and internal rejuvenation [7].

In female species, the ovary is a pair of reproductive organs that produces eggs. It produces a variety of steroid and peptide hormones, including progesterone and estrogen, which perform a variety of reproductive tasks [8]. Despite the many advantages of whole wheat, very few research have looked into how the wheat diet affects the female reproductive system. According to studies, diets can have a significant impact, either beneficial or detrimental, on the physiology of female reproduction [9]. This study sought to determine the impact of a wheat-based diet on ovarian histology and female reproductive hormones.

MATERIALS AND METHODS

Triticum aestivum Linn. (Poaceae) was obtained from a vendor at Mile 12, a commercial market in Lagos State, Nigeria. The taxonomic identification was done by wheat breeders at Lake Chad Research Institute (LCRI) Maiduguri, Borno State, Nigeria. The specimens with accession number LUH 6150 were deposited in the herbarium of the Department of Botany of the University of Lagos.

Experimental animals

Fifteen female Sprague-Dawley rats weighing between 150–170 g were used. The experiments complied with the Guidelines for the Care and Use of Laboratory Animals [10]. The rats used in the experiment were bred in the animal facility at the Department of Anatomy, Madonna University Elele River State. The rats were allowed to feed *ad libitum* and housed in good ventilated metal cages in a temperature-controlled environment (22 ± 2 °C) with 12 h light/12 h dark cycles before the commencement of the experiment.

Preparation of Study Diet

Wheat kernels were picked, cleaned, dried at 40°C in a hot air furnace, weighed, and ground in a London-made Thomas Wiley mill, Model ED-5. While the regular rat food was provided by Premier Feed Mill Co. Ltd., Ibadan, Nigeria, the ground wheat was sieved with an 80 mesh grid to form the wheat diet. Whole wheat of 100g was used as 100% wheat, without adding any rat chow. To make 60% of the wheat-based diet; 60g of wheat was weighed and mixed with 40g of standard rat chow. These test diets were pelleted in a feed mill in Agege, Lagos, Nigeria, and then dried at room temperature.

Study Design

At random, the rats were divided into three groups of five rats each. Group A (the control) were fed regular rat chow (48.8% Carbohydrate, 21% Protein, 3% fat). Rats in groups B and C were fed diets; 100% and 60% composed of wheat respectively. The rats were kept in cages and had free access to water. For four weeks, diets were offered to the animals in their cages, within hoppers, each day at 7 a.m. The weight of the food still present 24 hours before it was replaced with a freshly weighed diet was subtracted from the fresh food put into the cages every morning to determine how much food was consumed each

day. The body weights of the rats were taken once per week using a Camry weighing balance (model JI801170752 China) until euthanization.

Body and ovary weights, with hormonal assay

The weight of the rats was measured after the investigation. The beginning body weight was deducted from the final body weight to determine the changes in body weight. By cervical subluxation, the rats were put to death, and then blood was drawn to assess the hormone parameters using a heart puncture. Using Accu-bind enzyme-linked immunoassay (ELISA) kits from Monobind Inc. Lake Forest, CA 92630, USA, the levels of luteinizing hormone, follicle-stimulating hormone, estrogen, and prolactin were assessed. The manufacturer's instructions served as the tests' guiding principles and included the steps required in the various testing methodologies. At the time of sacrifice, both ovaries were painstakingly removed, freed of adipose tissues, and their average weights were measured in each animal using electronic weighing balance (Mettler-Toledo model AL 204 Switzerland).

Histological processing and staining

The samples of ovaries were rapidly preserved in a 10% neutral buffered formalin solution for regular histological procedures. By passing through a graduated series of alcohol and being embedded in paraffin, the tissues were dehydrated. A semi-automated rotator microtome was used to prepare the tissues, which were then cut into 5-µm slices, deparaffinized with xylene, and rehydrated with alcohol and water. Haematoxylin and eosin (H&E) were used to stain the slices of fixed ovarian tissue. A light microscope (Leica DM500, Leica Switzerland) was used to examine the slides.

Statistical analysis

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

RESULTS

Body and ovary weight

As indicated in Figure 1, there was a statistically ($P<0.05$) significant increase in the body weights of the rats fed with 100% wheat (Group B) when compared to the group receiving standard rat food (Group A). Compared to the results obtained for the standard rat chow group (RT= 0.346 ±0.014g and LT= 0.344±0.015g), the average weight of the ovarian tissue in the 100% wheat-based diet was RT= 0.346±0.017g and LT= 0.356±0.021g and in the 60% wheat-based diet was RT= 0.348±0.01g and LT= 0.346±0.015g. Hence, there were no statistically significant differences in the ovarian weight of rats from the wheat diet groups when compared to the standard rat chow group (Figure 2).

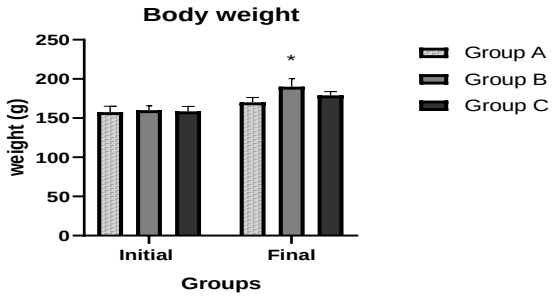

Figure 1: The weight of animals during the experiment. *Statistically significant, $P<0.05$ compared with the control. Data were recorded as (Mean ±SD). Group A: regular rat chow, Group B: 100% wheat diet; Group C: 60% wheat diet.

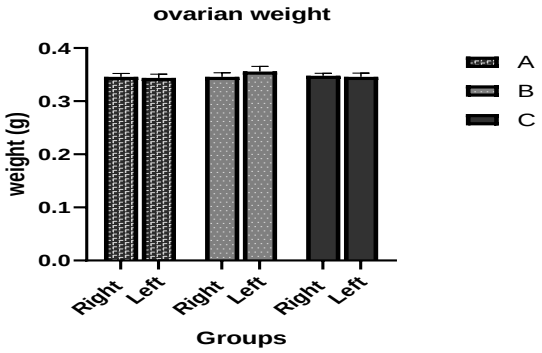

Figure 2: The weight of the ovaries of animals during the experiment. Data were recorded as (Mean ±SD). Group A: regular rat chow, Group B: 100% wheat diet; Group C: 60% wheat diet.

Hormonal Assay

The prolactin levels were significantly increased *(P*< 0.01) in the 100% wheat diet (Group B) when compared to groups A and C. However, there were no statistically significant difference in the serum concentrations of FSH, LH and Estrogen in the wheat diet groups B and C when compared to the standard rat chow group A (Figure 3).

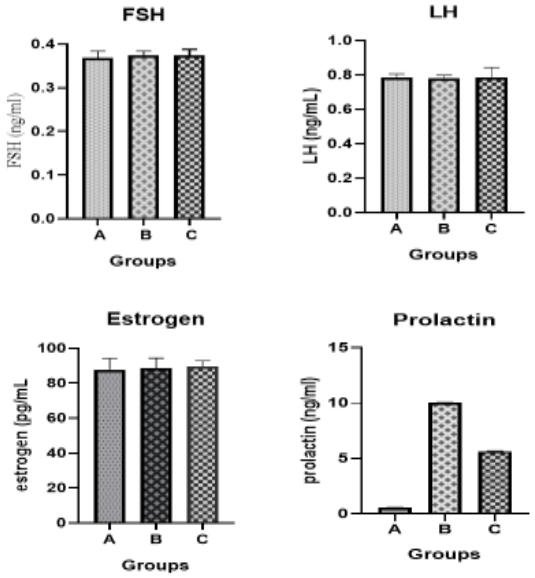

Fig 3: Effect of wheat-based diet on FSH, LH, Estrogen and Prolactin after 4 weeks of consumption. Values represent Mean± SD, (n=5); ** represents a significant difference when group B is compared with group A ($p < 0.01$), * represents a significant difference when group C is compared with group A *(P*< 0.05).
Group A: regular rat chow, Group B: 100% wheat diet; Group C: 60% wheat diet.

Histological findings

The photomicrograph of the ovaries of the rats following the 4 weeks of the experiment. Figure 4 shows the standard rat chow ovary of the rats in Group A with an outer cortex and the inner medulla consisting of connective tissue and blood vessels. It shows ovarian follicles at different stages of development; primordial, primary follicle and secondary follicle with the presence of the corpus luteum. Groups B and C photomicrographs showed follicles at different stages of development, such as primordial follicles; corpus luteum and few vacuoles.

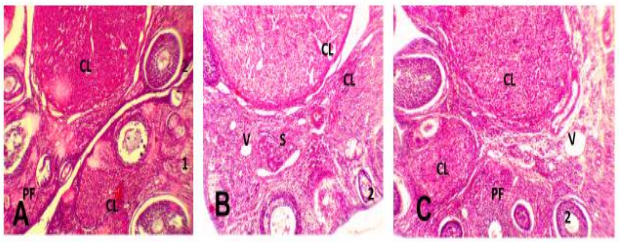

Figure 4. Histological section of the ovaries in groups A, B and C displays follicles at varying stages of development. 1= primary follicle, 2= secondary follicles, primordial follicles (PF), corpora lutea (CL), vacuolation (V), stroma (S). Micrographs of haematoxylin and eosin (H&E X 400). *Group A: regular rat chow, Group B: 100% wheat diet; Group C: 60% wheat diet.*

DISCUSSION

There was an increase in the body weight of the wheat diet groups in the current study. According to reports, the breaking down of gluten releases opioid peptides that are taken into the blood, go to the brain, and then cause pleasurable feelings and an increase in appetite, leading to wheat gluten increasing food consumption [11]. Increased weight gain after gluten inclusion in the food was shown in recent research in mice; this effect was thought to be brought on by changes in metabolic rate [12,13]. These findings are consistent with those of Zhang *et al*. [14], who found a positive correlation between the risk of overweight/obesity and increased wheat intake. However, there was no statistical difference in the ovarian weight of the wheat groups when compared to the control.

Endocrine hormones like FSH, LH, estrogen, and prolactin regulate female reproductive function; the female reproductive system is a complicated system that involves a feedback process in its hormonal control [15]. Gonadotropin-releasing hormone (GnRH), which is emitted by the hypothalamus, acts as a catalyst for the release of FSH and LH from the anterior pituitary gland [16]. The ovulatory process starts when follicular tissue is triggered by an increase in pituitary gonadotropins. The key hormone that initiates ovulation is follicle-stimulating hormone, which is well-known for its function in follicular development [17].

Progesterone and testosterone are stimulated to be secreted by LH; testosterone is then converted to estrogen by the nearby granulosa cells [18]. The hypothalamic-pituitary-ovary axis is named as such because GnRH is also controlled by estrogen found in the ovary [19]. To maintain ovarian granulosa,

differentiate cells, create follicles, expand oocytes, and even induce ovulation, the estrogen that is produced must bind with estrogen receptors [20]. Whole wheat is a great source of bioactive phytochemicals such as phytoestrogens, phenolic acids, alkylresorcinols, carotenoids, tocopherols [21]. Lignans are recognized as phytoestrogens and are present in wheat [22]. Phytoestrogens have been shown to imitate the effects of estrogen hormones in the body and occasionally have estrogenic or antiestrogenic effects [23]. The lignans help to balance the circulating level of estrogen by binding to estrogen receptor sites in the body, thereby controlling the level of estrogen in the blood [24,25]. This may explain why there were no statistically significant differences in estrogen levels between the wheat diet groups in the current study. Our findings also indicated that there was no statistically significant difference in FSH and LH levels between the wheat diet groups. Consequently, wheat has little impact on hormone levels. These findings contrast with those of [26], who found that serum FSH levels were significantly elevated (P<0.05) while ovarian tissue LH levels were significantly lowered.

The anterior pituitary gland produces the polypeptide hormone known as prolactin (PRL), which serves a variety of physiological purposes [27]. Prolactin secretion was enhanced by the 100% wheat diet to an estimated 10.01± 0.048 ng/ml, which was substantially higher (P <0.0001) than the control diet's result of 0.566± 0.021 ng/ml. The increase in prolactin found in this study may be due to extended gluten exposure, which may have damaged the prolactin receptors on the target organs; since gluten is known to be a water-soluble storage protein of wheat and is known to be an endocrine disruptor [28]. In addition, earlier animal studies suggest that gluten exorphins may contribute to an increase in serum prolactin levels [29], providing yet another method by which wheat may interfere with prolactin levels. These results are in line with earlier studies that reported a rise in prolactin levels following wheat ingestion [28].

In mammalian species, the ovarian follicle is a crucial component of the reproductive system. The main functional units of the ovaries are called follicles, and each follicle contains an oocyte [30]. The development of ovarian follicles is influenced by the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are crucial for ovarian follicle growth [31]. The histological examination of the ovary of the wheat-based diets in the current study showed cortical stroma containing ovarian follicles in various stages of development and corpus luteum (indicating that ovulation has occurred); and an inner medullar comprising richly vascularized loose connective tissue. There was no difference in the ovary of the rats across the wheat-fed groups when compared to the control.

Increased ovulation rates result in more corpus luteum, and the existence of follicles suggests that folliculogenesis has occurred [32]. It may be possible that folliculogenesis was not reduced in wheat. Similar observations have been made, according to [33]. Furthermore, because wheat contains a variety of chemical compounds with antioxidant capabilities [34]; these antioxidants are crucial for regulating the redox balance in the ovaries and maintaining normal ovarian function [35]. Due to their impact on the ovaries, antioxidants found in wheat, such as phenolic acids, ferulic acid, alkylresorcinols, lignans, lutein, and other nutritional substances, may have aided in ovulation [2,21,36]. Wheat is a significant source of several trace elements as Zn, Cu, Mn, and Ni [37]. Trace elements are also important antioxidants because they can provide the active sites in cells that antioxidants require to function properly or regulate antioxidant enzymes as cofactors; because they can offer the active sites in cells that antioxidants need to function correctly or act as cofactors to regulate antioxidant enzymes [35]. These findings suggest that wheat has no effect on FSH, LH, estrogen and histology of the ovary of the rats, except on prolactin and the body weight.

Strengths and limitations of the study

The rats were able to adjust to the wheat diets. However, the estrous cycle was not examined, which would have provided additional insight into how wheat diets affect the estrous cycle and how it relates to the hormone findings from the study.

CONCLUSION

In summary, this study demonstrated that wheat diets have no effect on FSH, LH, estrogen, or the histology of the ovary, other than the prolactin and

weight gain. Hence, wheat consumption should be taken with moderation.

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Author contributions
ICA and IIO designed the research. UED and ED performed the experiment. The analysis and interpretation of the data was done by ICA. All the authors did the proofreading of the manuscript, drafting and revision of the manuscript. All authors read and approved the final manuscript.

Data availability
The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest
The authors declare that they have no competing interests.

Ethical approval
The study was reviewed and approved by the Institutional Ethics Committee of Madonna University Elele, Rivers State

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