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Effect of Wheat (*Triticum aestivum* Linn.) Diet on the Testes of Sprague-Dawley Rats

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Wheat (*Triticum aestivum* Linn.), a member of the *Poaceae* family, is an important food crop and represents 25% of the world's grain production. There has been an increase in its daily consumption worldwide.[1] Carbohydrates, proteins, fiber, amino acids, vitamins, and phytochemicals such as phenolic and terpenoids are the nutrients present in wheat.[2] Increased wheat intake has been linked to the adoption of "western lifestyle" in industrialized and urbanized nations. For most people, wheat is the primary source of nutrients because it can be crushed into flour, semolina, and other fundamental components for pastas, bread, cookies, noodles, and other bakery products.[3] Currently, it is more widely being acknowledged that nutrition and lifestyle choices significantly impact the reproductive health.[4] However, there are several unresolved concerns regarding the relationship between wheat diet and male fertility, even though numerous studies have highlighted the health benefits of wheat.[5] Thus, given that nutrition appears to be a factor that determined fertility, we aimed to evaluate the effect of wheat diet on the histology of rat testes and reproductive hormones.

Triticum aestivum Linn. was purchased from a vendor in Jattu market, Uzairue, Edo State, Nigeria. After stones were removed, the wheat was dried in a hot furnace at 40 °C. Subsequently, the dried wheat was weighed and ground into a coarse powder using a local grinding machine. The normal rat chow was supplied by Premier Feed Mill Co. Ltd., Ibadan, Nigeria. Three wheat diets were prepared as follows: 40% wheat diet, in which 40 g of wheat was combined with 60 g of rat chow; 60% wheat diet, in which 60 g of wheat was combined with 40 g of rat chow; and 100% wheat diet, in which 100 g of whole wheat was utilized without adding any rat chow.

Twenty male Sprague-Dawley rats, weighing 200-250 g, were fed rat chow and water for two weeks during the acclimatization period before the experiment. The Edo State University Uzairue Local Ethics Committee of animal experiments, reviewed and approved all the study procedures (no: EDSU/ANA/02/16/023). The study was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.[6] The animals were grouped as follows on the basis of the feed: Group 1 (control), rat chow and water; Group 2, 40% wheat diet; Group 3, 60% wheat diet; and Group 4, 100% wheat diet. The rats were fed their respective diets for four weeks.

At the end of the experiment, blood samples were drawn via a retro-orbital puncture and placed into plain tubes. The blood was allowed to clot and subsequently centrifuged for 10 min at 3,000 rpm on a tabletop centrifuge. Using a micropipette, the serum and plasma were carefully separated and placed into separate sets of plain tubes. Following the manufacturer's instructions, the following hormone levels were measured using commercial enzyme-linked immunosorbent assay kits: testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

After the rats were sacrificed via cervical dislocation, the testes were dissected out and the surrounding tissues were removed. The testes were washed with normal saline, blotted on filter paper, and fixed in 10% buffered formalin solution for 24 h. Thereafter, the fixed tissue was cut into 5 mm-thick sections, which were stained with hematoxylin and eosin solutions. Subsequently, the sections were examined under a microscope.

The study data were analyzed using GraphPad Prism (version 6.0.; San Diego, USA). ANOVA and Bonferroni's post-hoc test were used to compare the variables between the groups. Statistical significance was set at $p < 0.05$. The data are presented as mean ± standard deviation.

There was a statistically significant increase in the body weight of the wheat-fed rats (Groups 3 and 4) when compared with the body weight of the control rats (Group 1) ($p < 0.05$; Table 1). Analysis of the hormones demonstrated that the concentrations of FSH and LH in the Groups 2, 3 and 4 did not significantly differ from those in Group 1. However, there was a significant reduction in testosterone level in Group 4 when compared with the in testosterone level in Group 1.

Histological examination of the testes in Group 1 revealed a normal architecture, with the presence of Sertoli cells, regular

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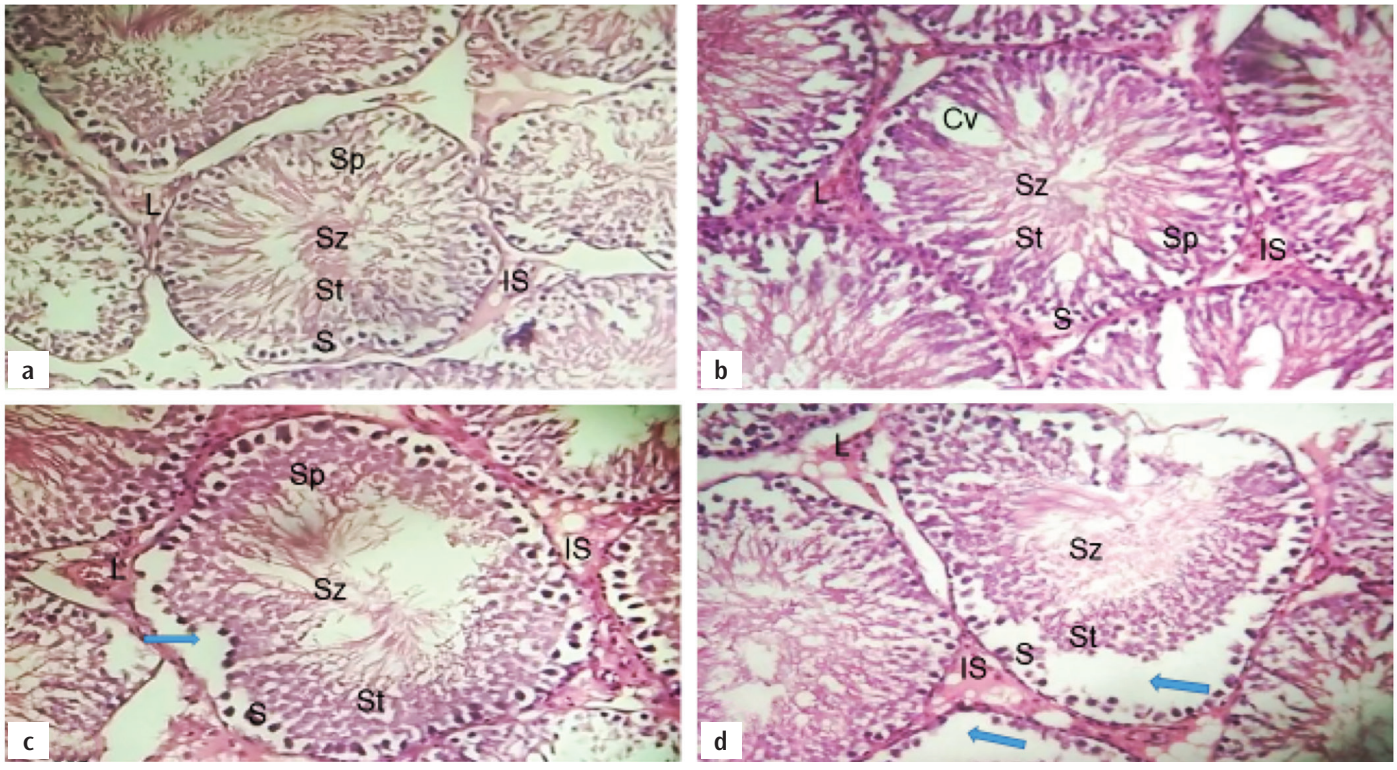

FIG. 1. Hematoxylin and eosin-stained sections of rat testes viewed under ×400 magnification. (a) In the control rat, normal histological structure of seminiferous tubules is observed with complete spermatogenic series. (b) In the rat fed 40% wheat, normal seminiferous tubules and cytoplasmic vacuoles (Cv) are observed. (c) In the rat fed 60% wheat, mild degeneration of the germ cells (arrow) of the seminiferous tubules is observed. (d) In the rat fed 100% wheat, marked degeneration of the germ cells (arrow) of the seminiferous tubules is observed.
Sertoli cell (S), interstitial spaces (IS), and Leydig cells (L). The peripheral cell layer is composed of spermatocytes (Sp) followed by a zone of spermatids (St) and spermatozoa (Sz).

seminiferous tubules, and interstitial cells of Leydig (Figure 1). The seminiferous tubules were lined by stratified germinal epithelium which represented the spermatogenic cells in different stages of development. In Group 2, the examined sections exhibited normal fairly circular seminiferous tubules, Sertoli cells, other cells of the spermatogenic series, cytoplasmic vacuole, and Leydig cells. Histological examination of the testes in Group 3 demonstrated a mild degeneration of the germ cells of the seminiferous tubules. In Group 4, the examined sections revealed seminiferous tubules that appeared distorted and dilated and marked degenerative changes in the germ cells.

The overall weight gain was greater in the wheat diet groups than in the control groups, and the amount of weight gain was diet-dependent. Gluten is a protein that makes up approximately 80-85% of the total protein in wheat. It boosts appetite, which may contribute to weight gain.[7] Additionally, wheat lectins may cause an increase in weight gain.[8] Our study results, in terms of weight gain, are consistent with those of a previous study that suggested that wheat may play a significant role in the development of obesity[9]

The major regulators of germ cell development are the gonadotropins (FSH and LH) and testosterone. The current study results demonstrated that wheat diets did not affect the FSH and LH concentrations. However, there was a decrease in serum testosterone levels (Table 1). The reduction in testosterone levels indicates that wheat may inhibit the mechanism by which Leydig cells synthesize testosterone. Hu et al.[10] demonstrated that excessive consumption of bread made from wheat flour reduces the testosterone levels in the male reproductive system. Other studies have also reported that gluten interferes with the endocrine system and lowers testosterone levels.[11] Low serum testosterone levels is associated with obesity, and nutrition plays a major role in this relationship.[12] Lignans are phytoestrogen found in wheat, and its consistent administration has been shown to reduce fertility in animals.[13] In our study, histological examination revealed that rats fed a 100% wheat diet exhibited disorganization of the seminiferous epithelium, vacuolization in the germinal epithelium, which may be as a result of germ cell loss, and degeneration of basal germ cells in the seminiferous tubules. However, the type and extent of these changes varied between the different wheat-based diet groups. Similar histological alterations were also demonstrated in another study on scrotal gamma-irradiation that was conducted by Topcu-Tarladacalisir et al.[14] Another study reported that oral administration of wheat lectin can affect sperm quality, such as reduction in sperm motility, count morphology, and serum testosterone levels. These findings indicate that excessive consumption of a modern wheat-based diet, predisposes individuals to decreased fertility.[15] Notwithstanding

TABLE 1. Effect of the Wheat Diet on the Body Weight and Hormone Levels of Rats.

Groups	Weight (g)		Testosterone, (ng/ml)	FSH, (miU/ml)	LH, (miU/ml)
	Initial	Final			
Group 1	210.4 ± 1.4	241.2 ± 6.3	7.26 ± 0.11	1.96 ± 0.11	1.58 ± 0.14
Group 2	224.2 ± 6.9	260.6 ± 5.0*	5.70 ± 0.16	2.70 ± 0.15	1.98 ± 0.12
Group 3	210.8 ± 3.6	269.8 ± 7.9*	3.60 ± 0.16	2.80 ± 0.11	2.08 ± 0.12
Group 4	213.6 ± 4.5	289.8 ± 6.2*	1.42 ± 0.07*	2.56 ± 0.12	2.02 ± 0.08

Values are presented as mean ± standard deviation. Each group: n = 5. *$p < 0.05$, when compared to Group 1. FSH, follicle-stimulating hormone; LH, luteinizing hormone; Group 1, control; Group 2, 40% wheat diet; Group 3, 60% wheat diet; Group 4, 100% wheat diet.

the health benefits of whole wheat diet,[8] the long-term effects of continuous consumption of whole wheat on the male reproductive system should be re-evaluated for the preservation of male fertility.

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Ethics Committee Approval: This study was conducted with the approval of the Edo State University Uzairue (approval number: EDSU/ANA/02/16/023, date: 06.03.2023).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

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