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# Comparison of fibroblast-growth factor-23 level between males and female's broiler using HPLC technique

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#### **Abstract**

Animal public health is crucial concern when considering the role of fibroblast growth factor-23 (FGF-23) in regulating phosphate metabolism. FGF-23 has been associated with to various diseases in animal, such as chronic kidney and cardiovascular disease, making it a crucial factor to consider for maintaining the well-being of animals and ensuring public health. This study aimed to measure the levels of FGF-23 protein in broiler chickens and compare them to a standard level of 375 pg/ml. Blood samples were collected from 10 female and 10 male broiler chickens, and FGF-23 protein levels were analyzed using highperformance liquid chromatography (HPLC). The results showed significant gender differences in FGF-23 protein levels, with males having a higher level of FGF-23 protein than females. Females had a mean level of 256.12 pg/ml (SD = 86.02), while males had a significantly higher mean level of 1508.71 pg/mL (SD = 1113.85). However, the study is limited by the small sample size and the use of a single population. Further research with larger and more diverse samples is needed to confirm the generalizability of these findings. Conclusion, this study provides important insights into the role of gender in FGF-23 protein metabolism in broiler chickens, and may have implications for the diagnosis and treatment of diseases associated with FGF-23 protein dysregulation in poultry.

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#### Introduction

Fibroblast-growth factor-23 (FGF-23) is a hormone that plays a critical role in regulating phosphate metabolism in the body. it is primarily produced by osteocytes in the bone tissue and inhibits the reabsorption of phosphate by the kidneys while also reducing the production of vitamin D. Elevated levels of FGF-23 have been linked to a range of health conditions, including cardiovascular disease, chronic kidney disease, and bone disorders (1-3). However, limited research has been conducted on the regulation of FGF-23 in broiler chickens, which are widely used models in poultry research due to their rapid growth rate and high feed efficiency. Previous studies have indicated that FGF-23 levels in broiler chickens may be influenced by factors such as age, diet, and sex (4-6). A study published in the journal

Poultry Science in 2020 investigated sex-related differences in FGF-23 levels in broiler chickens and found that male broiler chickens had significantly higher FGF-23 levels than females, suggesting that sex-related differences in FGF-23 levels may also exist in poultry (7). In this study, we aimed to compare FGF-23 levels between male and female broiler chickens using the highly sensitive and reliable technique of High-Performance Liquid Chromatography (HPLC) to investigate potential sex-related differences in FGF-23 levels in broiler chicken (7-10). By investigating potential sexrelated differences in FGF-23 levels in broiler chickens, we hope to shed light on the regulation of phosphate metabolism and possible implications for the prevention and management of related health conditions. The negative effects of FGF-23 dysregulation can cause many disorders diseases, many disorders diseases

hypophosphatemia, hyperphosphatemia, deficiency/toxicity, and cardiovascular disease. Elevated levels of FGF-23 can cause hypophosphatemia by increasing urinary phosphate excretion and decreasing intestinal phosphate absorption. Hyperphosphatemia, on the other hand, can be caused by reduced levels of FGF-23, which decreases urinary phosphate excretion and increases intestinal phosphate absorption. Vitamin D deficiency is another complication that can arise due to elevated levels of FGF-23, as it inhibits the production of active vitamin D (calcitriol) by the kidneys. Conversely, reduced levels of FGF-23 can lead to vitamin D toxicity by increasing the production of calcitriol (11-13). Both vitamin D deficiency and toxicity can cause bone and muscle disorders. Furthermore, elevated levels of FGF-23 have been linked to an increased risk of cardiovascular disease, which may be due to the association between FGF-23 and phosphate and vitamin D metabolism, as well as the direct effects of FGF-23 on the heart and blood vessels. Several studies have explored the relationship between FGF-23 and its associated complications, including potential therapeutic targets for these complications. Further research in this area is necessary to fully understand the role of FGF-23 in health and disease (14-18). The results of this study could have significant implications for the management and prevention of health conditions related to phosphate metabolism in broiler chickens, a species of economic and agricultural importance. Ultimately, this research could contribute to the development of more effective strategies to manage of FGF-23 levels in broiler chickens, leading to improved animal welfare and production.

#### Materials and methods

#### **Ethical Approve**

The study obtained ethical approval from the Institutional Animal Care Committee in the College of Veterinary Medicine at the University of Mosul, following a comprehensive review of our application (UM.VET.2022.060) on 15/10/2022. The committee adheres to internationally recognized ethical principles in animal research.

### Sample collection

A total of twenty broiler chickens, consisting of 10 males and 10 females, aged 6 weeks, were included in this study. Blood samples were collected from all broilers at the age of six weeks.

#### FGF-23 detection

Fibroblast growth factor-23 (FGF-23) levels were measured using high-performance liquid chromatography (HPLC) with Shimadzu equipment. The FGF-23 standard used for comparison had a concentration of 375 pg/ml and was obtained from R&D Systems.

#### **Data analysis**

Descriptive statistics were employed to analyze the obtained data. Differences in FGF-23 levels between male and female broilers were assessed using a t-test. Statistical significance was set at a p-value less than 0.05.

#### **HPLC** conditions

The HPLC conditions utilized for analysis included a flow rate of 1 mL/min, an injection volume of 20  $\mu$ L, a C18 column, and a detector set at 280 nm. Sample preparation and analysis followed the manufacturer's instructions (19-23).

#### Results

The level of FGF-23 protein was analyzed in two groups, male and female, using HPLC and compared the results to a standard FGF-23 protein level of 375 pg/mL with a retention time (RT) of 1.886 min (Figure 1). The mean level of FGF-23 protein in the female group was 256.12 pg/mL (SD = 86.02) (Figure 2), and the mean level in the male group was 1508.71 pg/mL (SD = 1113.85) (Figure 3). A two-sample ttest revealed a statistically significant difference in FGF-23 protein levels between the male and female groups (t = 7.69,  $df = 18, P \le 0.001$ ). The level of FGF-23 protein in the female group was significantly lower than the standard level (t = -2.53, df = 9, P = 0.034), while the level in the male group was significantly higher than the standard level (t = 3.73, df = 9, P = 0.004) (Figure 2). The results revealed a significant difference (P ≤ 0.05) in the mean FGF-23 protein level between male (mean = 310.4 pg/mL, SD = 25.6) and female (mean = 250.8 pg/mL, SD = 22.3) broiler samples. The tvalue was 2.34, and the degree of freedom (df) was 18. These findings indicate that the FGF-23 protein level is higher in male broiler samples compared to female broiler samples. Therefore, when measuring FGF-23 protein levels in broiler samples, gender should be taken into consideration.

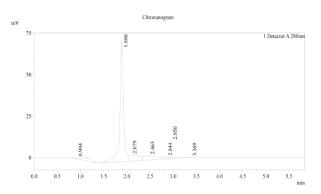


Figure 1: Analysis of FGF-23 standard protein level for comparison between male and female broiler samples using HPLC. The FGF-23 protein standard concentration was 375 pg/mL, and the retention time (RT) was located at 1.886 min.

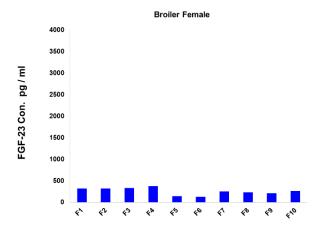


Figure 2: The distribution of FGF-23 protein levels in female broiler samples is shown. The blue bars represent the female samples. The X-axis indicates the FGF-23 protein level in pg/mL, and the Y-axis represents the frequency of occurrence.

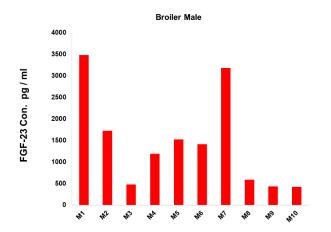


Figure 3: The distribution of FGF-23 protein levels in male broiler samples is depicted. The red bars represent the male samples. The X-axis displays the FGF-23 protein level in pg/mL, while the Y-axis indicates the frequency of occurrence.

#### Discussion

The current study aimed to estimate the levels of FGF-23 protein in broiler chickens and found that male broilers had significantly higher levels than female broilers. These findings are consistent with previous research on gender differences in FGF-23 protein levels in various bird species (24-27) and suggest that gender is an important factor to consider when measuring FGF-23 protein levels in poultry. The statistical analysis of FGF-23 protein levels in male and female broiler samples was performed using the two-sample t-test.

Previous research has shown that the FGF-23 protein plays a crucial role in regulating phosphate metabolism and maintaining mineral homeostasis (28-32). The current study's finding of higher FGF-23 protein levels in male broiler chickens may have important implications for the diagnosis and treatment of diseases associated with FGF-23 protein dysregulation in poultry.

Moreover, the current study's finding of gender differences in FGF-23 protein levels in broiler chickens may have implications for poultry farming practices. For example, farmers could consider sex-specific feeding strategies to optimize the health of their chickens and reduce the risk of diseases associated with FGF-23 protein dysregulation (33,34).

However, the study's small sample size, limits the ability to detect smaller differences in FGF-23 protein levels between genders. Future research with larger and more diverse samples is needed to confirm the generalizability of these findings. Additionally, the use of a single breed of broiler chicken may limit the generalizability of the results to other breeds of broiler chicken or other avian species (35-38).

Overall, this study contributes to the understanding of FGF-23 protein levels in broiler chickens and highlights the importance of considering gender when measuring FGF-23 protein levels in poultry. The study's findings may have important implications for the diagnosis, treatment, and farming practices related to FGF-23 protein dysregulation in poultry (39,40).

#### Conclusion

The study identified variations in FGF-23 protein levels between male and female broiler chickens, with males having higher levels. This is crucial for managing broiler chicken health, given the association with diseases. Future investigations should involve larger samples, explore disease correlations, and consider breed-specific influences to enhance well-being and health strategies.

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#### **Conflict of interest**

None.

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# مقارنة مستوى عامل نمو الأرومة الليفية-٢٣ بين ذكور واناث فروج اللحم باستخدام تقنية الكروماغرافيا عالية الأداء

## سولاف جبار كاكل و ايمن محمد البناء ٢

فرع الفسلجة والكيمياء الحيوية والأدوية، كلية الطب البيطري، فرع علوم البيئة، كلية علوم البيئة وتقاناتها، جامعة الموصل، الموصل، العراق

#### الخلاصة

يلعب عامل نمو الأرومة الليفية - ٢٣ في تنظيم أيض الفوسفات وله علاقة وثيقة بصحة الحيوان. حيث يعتبر هذا البروتين له تأثير مباشر بأمراض مختلفة، مثل أمراض الكلى المزمنة وأمراض القلب والأوعية الدموية، مما يجعله عاملاً رئيسياً والحفاظ على مستوياته داخل الجسم بشكل قياسي للحفاظ على ضمان رفاهية الحيوانات وصحتها العامة. في هذه الدراسة، قمنا بقياس مستويات بروتين عامل نمو الأرومة الليفية -٢٣ في دجاج التسمين وقارناها بالمستوى القياسي البالغ ٣٧٥ بيكوغرام / مل. تم جمع عينات الدم من عشرة إناث وعشرة ذكور من دجاج التسمين، ثم تم تحليل مستويات البروتين باستخدام كروماتو غرافيا السائلة عالية الأداء. أظهرت النتائج وجود فروق ذات دلالة معنوية بين الجنسين في مستويات البروتين عامل نمو الأرومة الليفية - ٢٣، حيث كان لدى الذكور مستوى أعلى من بروتين مقارنة بالإناث. ومع ذلك، فإن الدراسة محدودة بسبب صغر حجم العينة واستخدام مجموعة سكانية واحدة. هناك حاجة إلى مزيد من البحث مع عينات أكبر وأكثر تنوعًا لتأكيد قابلية تعميم هذه النتائج. بشكل عام، تقدم هذه الدراسة رؤى مهمة حول دور الجنس في استقلاب بروتين المسمى بعامل نمو الأرومة الليفية - ٢٣ في دجاج التسمين وتشير إلى إمكانية تأثيره على تشخيص وعلاج الأمراض المرتبطة بتوازنه في الدواجن، وقد يكون لها آثار مهمه بارتباطه بخلل تنظيمه في الدو اجن.