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# Effect of Dietary Supplementation of Ferrous Sulphate on the Blood **Biochemical Attributes and Development of Digestive Organs of Turkey Poults**

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#### **ABSTRACT**

The present study was conducted during February–April, 2020 in the Department of Poultry Science, Pandit Deen Dayal ▲ Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, Uttar Pradesh, India to assess the effect of dietary supplementation of ferrous sulphate on the blood biochemical attributes of turkey poults. Nighty six straight run day old turkey poults were placed into four treatment groups, each with three replicates and eight turkey poults in each replicate. The poults were kept in a system with deep litter. The experiment was carried out on turkey poults aged 0 to 8 weeks. In the experiment, (control) basal diet, supplemented with FeSO<sub>4</sub> @ 80 mg kg<sup>-1</sup> diet, supplemented with FeSO<sub>4</sub> @ 120 mg kg<sup>-1</sup> diet, and supplemented with FeSO<sub>4</sub> @ 160 mg kg<sup>-1</sup> diet were provided to the poults. Blood was collected at the end of the experiment. Length and weight of different digestive organs were measured separately at 8 weeks of age after sacrificing birds. Plasma was separated and stored in refrigerator (-20°C) until analyzed. The data obtained showed that there was no significant difference in plasma total proteins, uric acid, ALT, AST, ALP, plasma cholesterol, plasma HDL, SOD, ROS, LPO values and development of digestive organs among different treatment groups. Further studies can be done by assessing the effect on blood biochemicals and digestive organ development of poultry birds by supplementing organic Fe chelates.

KEYWORDS: Iron, poults, FeSO<sub>4</sub>, biochemical, digestive organs

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

#### 1. INTRODUCTION

Tron (Fe) is a trace mineral that is required by all living **⊥**organisms and is involved in oxygen and electron transport as well as DNA synthesis (Von and Adamson, 2013). Physiological function is degraded, Fe deficiency anaemia and other disorders are more common when chickens are fed Fe-deficient diets or it is poorly absorbed, with serious consequences on animal productivity (Taschetto, 2017). Fe, a vital part of haemoglobin in erythrocytes, is essential for oxygen transport throughout the body, with haemoglobin and myoglobin (Strube et al., 2002) being used for oxygen delivery, storage, and usage in muscles (Anderson and Vulpe, 2009). Hemoglobin and myoglobin are essential for maintaining appropriate meat colour, which is the most visible sign of meat quality (Craig et al., 2017).

Sulfates, oxides, and carbonates are commonly utilised in commercial poultry diets as supplemental iron (inorganic form) (Jarosz et al. 2016). Fe is found in the ferrous and ferric forms in mineral supplements such as limestone and phosphates (Abbaspour et al., 2014). Ferrous oxide is not regarded as a source of Fe capable of meeting animal needs. Dietary recommendations for Fe supplementation varied from 20 to 100 ppm depending on bird age and responses measured, with no Fe source stated and insufficient information regarding Fe availability differences between sources (Anonymous, 1994; Rostagno et al., 2011; Abbasi et al., 2015).

The most prevalent and pervasive nutritional condition in the world, iron deficiency anaemia (IDA), is responsible for 90,000 fatalities worldwide in 2013 (WHO, 2014). Food fortification with various types of iron is the most popular strategy for preventing iron deficiency (WHO, 2007). The iron bioavailability of chicken protein sources is higher than that of other dietary sources of Fe (Pizarro et al., 2016). Iron should be given every day in readily accessible forms because it is rapidly depleted in chicken. Commercial chicken diets frequently use supplemental iron (inorganic form), such as sulphates, oxides, and carbonates (Jarosz et al., 2016).

The expression of iron-containing enzymes in the liver or heart has been shown to increase with a Fe requirement of 97-136 mg Fe/kg dry matter (DM) in a broiler diet (Ma et al., 2016; Liao et al., 2017). However, extra Fe accumulates in the liver when dietary consumption exceeds nutritional needs. Fe overload can cause oxidative stress, weaken immunity by generating inflammatory cytokines, hasten hepatocyte death, and seriously harm the liver, heart, or intestines structurally and functionally (Zhang et al., 2020; Luo et al., 2021). Fe sulphate heptahydrate, which has a high dietary Fe concentration of 500 mg Fe/kg DM, is given to male Ross 308 broilers to minimise the build-up of liver triglycerides and abdominal adipose fat (Bai et

al., 2021). Additionally, since excess Fe is directly linked to the production of reactive oxygen species (ROS), the oxidation of lipoproteins, and the activation of platelets in mice, Fe overload may also contribute to the pathogenesis of atherosclerosis (Wan et al., 2021; Marques et al., 2019). Therefore, broilers should be evaluated for any potential harmful effects of high dietary Fe.

Fe is found in all materials used in poultry diets and is widely distributed in nature (Anonymous, 1994). Plant Fe content varies depending on soil composition, agricultural system, and environment (Gupta et al., 2008). Fe is primarily linked to phytate in cereals and oilseeds (Yu et al., 2000), which limits its availability for poultry when diets are not supplemented with phytase (Gibson et al., 2010). Fe concentration and availability in poultry diets vary depending on the source and concentration (e.g., plant-based ingredients, mineral supplements or animal by-products). The objective of the study was to see the effect of dietary supplementation of ferrous sulphate on the blood biochemical attributes and development of digestive organs of turkey poults.

# 2. MATERIALS AND METHODS

The experiment was carried out at the Department of ▲ Poultry Science, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, Uttar Pradesh, India (27.4655° N, 77.7076° E) during the months of February– April in the year 2020. Nighty six straight run day old turkey poults were placed into four treatment groups, each with three replicates and eight turkey poults in each replicate. The poults were wing banded, weighed individually, and randomly assigned to treatment groups based on their body weight. The poults were kept in a system with deep litter. Water was provided ad lib. There were four different dietary treatments; T<sub>1</sub>- control, basal diet (turkey starter ration; Anonymous, 1994), T<sub>2</sub>- supplemented with FeSO<sub>4</sub> @ 80 mg kg<sup>-1</sup> diet, T<sub>3</sub>- supplemented with FeSO<sub>4</sub> @ 120 mg kg<sup>-1</sup> diet, and T<sub>4</sub>- supplemented with FeSO<sub>4</sub>@ 160 mg kg<sup>-1</sup> diet were provided to the poults.

# 2.1. Biochemical parameters

Blood was drawn from the wing vein of eight turkey poults from each group at the end of the 8 week of biological experiment using a heparinized syringe and placed into a sterile tube. The blood samples were centrifuged at 2500 rpm for 10-15 minutes. Plasma was isolated and kept at -20°C until it was examined. Standard diagnostic kit (Span Cogent Diagnostics product) was used to assess plasma cholesterol, HDL cholesterol, protein, uric acid, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphate (ALP) according to

manufacturer's instructions. With some modifications, the activity of superoxide dismutase (SOD) in serum was tested using the method published by Madesh and Balasubramanian (1998). According to Placer et al. (1966), lipid peroxidation (LPO) in plasma was determined by reacting thiobarbituric acid (TBA) with Malondialdehyde (MDA). Reactive oxygen species (ROS) was estimated in terms of H<sub>2</sub>O<sub>2</sub> in test sample as developed by Alberti et al. (2000) for a 96-well plate format.

# 2.2. Development of digestive organs

To evaluate the length and weight of the various digestive organs (proventriculus, small intestine, large intestine, and caecum) at 8 weeks of age, two male and two female birds from each group, or a total of 16 birds, were selected at the time of slaughter.

### 2.3. Statistical analysis

Statistical Package for the Social Sciences (Anonymous, 2011) was used to do one-way analysis of variance in a completely randomised design (Snedecor and Cochran, 1994). Homogenous subsets were separated using multiple range test described by Duncan (1955).

# 3. RESULTS AND DISCUSSION

# 3.1. Chemical composition of turkey starter feed

The chemical composition of turkey starter feed has been presented in Table 1. The feed was adequate in all nutrients as per nutritional requirements of turkey (Anonymous, 1994).

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Table 1: Chemical composition of feed								
Cat- egory		Total Ash %			Ca %		Fe (mg kg <sup>-1</sup> )	
Turkey starter feed	89.50	8.51	27.84	2.73	2.24	1.25	78.84	

# 3.2. Biochemical parameters

The data obtained showed that there was no significant difference in plasma total proteins, uric acid, plasma cholesterol, plasma HDL values among different treatment groups (Table 2). The results showed that there was no adverse effect of dietary supplementation of FeSO, at different levels in turkey poults. None of previous studies were according to our result for few parameters. Kwiecien et al. (2015) also found no statistically significant differences in total cholesterol, triglycerides, LDL cholesterol, or the Chol/HDL ratio between broiler groups supplemented with FeSO<sub>4</sub> @20 and 40 mg/kg of feed. Broilers fed FeSO<sub>4</sub> @20 mg kg<sup>-1</sup>, on the other hand, showed significantly lower (p<0.001) plasma total protein levels than those fed FeSO @40 mg kg<sup>-1</sup> in the same trial. In addition, broilers fed FeSO4 @20 mg kg<sup>-1</sup> exhibited significantly higher plasma HDL cholesterol levels (p<0.05) than those fed FeSO, @ 40 mg kg<sup>-1</sup>. Our result is not according to result found by Kwiecien et al. (2015) in case of total protein and HDL cholesterol.

The data obtained showed that there was no significant difference in plasma ALT, AST, ALP, SOD, ROS, LPO values among different treatment groups. The level of activity of enzymes including ALT, AST, ALP, and lactate dehydrogenase (LDH) was also found to be unaffected by the form and level of Fe in the feed (Kwiecien et al., 2015). The rate of enzyme responses did not deviate from reference values and was at a same level regardless of the dose of iron employed, whether in an organic form or as sulphate (Kaneko et al., 2008).

#### 3.3. Development of digestive organs

The data obtained showed that there was no significant difference in weight of proventriculus (%), small intestine (SI) weight (g 100 g<sup>-1</sup>), large intestine (LI) weight (g 100 g<sup>-1</sup>), cecal weight (g 100 g<sup>-1</sup>), SI length (cm 100 g<sup>-1</sup>), LI length (cm 100 g<sup>-1</sup>) and cecal length (cm 100 g<sup>-1</sup>) among the treatment groups (Table 3). However, Yousefi

Table 2: Effect of dietary supplementation of ferrous sulphate on blood biochemical parameters of turkey poults at 8 weeks of age										
Treatment	Protein (g dl <sup>-1</sup> )	Uric acid (mg dl <sup>-1</sup> )	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Cholesterol (mg dl <sup>-1</sup> )	HDL Cholesterol (mg dl <sup>-1</sup> )	SOD (units ml <sup>-1</sup> )	ROS (nM ml <sup>-1</sup> )	LPO (nM ml <sup>-1</sup> )
T1	3.83	9.68	5.19	3.31	229.84	136.04	73.22	308.74	0.89	0.024
T2	3.86	9.80	4.19	3.20	236.62	135.49	72.15	309.08	0.89	0.023
T3	3.80	9.65	4.53	3.32	226.62	135.21	72.77	311.58	0.88	0.024
T4	3.83	9.87	4.30	3.09	231.19	135.08	71.78	312.14	0.89	0.025
Pooled SEm±	0.02	0.08	0.25	0.20	3.082	0.67	0.36	0.65	0.004	0.0002
Sig. level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NS p=0.05										

Table 3: Effect of dietary supplementation of ferrous sulphate on development of digestive organs of turkey poults at 8 weeks of age

Treatment	Proventriculus %	SI weight (g 100 g <sup>-1</sup> )	LI weight (g 100 g <sup>-1</sup> )	Cecal Wt. (g 100 g <sup>-1</sup> )	SI length (cm 100 g <sup>-1</sup> )	LI length (cm 100 g <sup>-1</sup> )	Cecal length (cm 100 g <sup>-1</sup> )
$T_1$	0.53	3.19	0.46	0.85	10.09	0.84	1.61
$T_2$	0.55	3.44	0.45	0.84	10.82	0.86	1.59
$T_3$	0.56	2.91	0.44	0.81	09.85	0.84	1.55
$T_{_4}$	0.56	2.91	0.42	0.88	10.02	0.79	1.54
Pooled SEm±	0.008	0.13	0.006	0.03	0.21	0.013	0.014
Sig. Level	NS	NS	NS	NS	NS	NS	NS

p=0.05

and Saki (2019) reported that broilers receiving dietary supplementation with FeSO<sub>4</sub> resulted in significant increase in ratio of Bursa of fabricius and spleen compared to control. In our study, the experiment was carried out in turkeys unlike the aforesaid experiment and the non-significant effect of dietary supplementation of iron could be attributed to the species difference.

### 4. CONCLUSION

There was no significant difference in plasma total 👃 proteins, plasma uric acid, ALT, AST, ALP, plasma cholesterol, plasma HDL, SOD, ROS, LPO values among different treatment groups when ferrous sulphate supplemented in diets of turkey poults. Also, there was no significant difference in weight of proventriculus (%), small intestine (SI) weight (g 100 g<sup>-1</sup>), large intestine (LI) weight (g 100 g<sup>-1</sup>), cecal weight (g 100 g<sup>-1</sup>), SI length (cm 100 g<sup>-1</sup>), LI length (cm 100 g<sup>-1</sup>) and cecal length (cm 100 g<sup>-1</sup>) among the treatment groups.

#### 5. FURTHER RESEARCH

Purther studies can be done by assessing the effect on blood biochemicals of turkey birds by supplementing organic Fe chelates in the diet. With the help of external research fund or supports further studies may be done to evaluate the effect of ferrous sulphate @ 160 mg kg-1 of diet on expression of growth and immunity genes.

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