



Iron nanoparticles as feed additives: Effects on performance, muscle and histopathology in Broiler

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Abstract

Nanoparticles are widely recognized for their remarkable efficiency across various scientific fields especially nutrition. Poultry feed costs almost 70 percent of total economy for poultry production. Iron is an important trace mineral element of poultry feed. In this study, commercially used iron salt (FeSO_4) in poultry feed was replaced with its oxide form (Fe_2O_3) in nanostructures. The nanoparticles were synthesized and incorporated into experimental groups at different concentrations (5mg, 10mg and 15mg/kg of feed) alongside a negative control (no iron in feed) and a positive control group (with commercial iron value, 80 mg FeSO_4 /kg of feed). The feed, water, bird weight, and feed conversion ratio (FCR) data were collected during the trial. Furthermore the iron analysis and histopathology was conducted on muscle samples, and liver respectively. The results revealed that feed containing 10mg of iron oxide nanoparticles per kg of feed and commercial feed yielded same results of FCR (1.96). Conversely, feed with 15 mg/kg of nanoparticles has improved FCR as compared to any group and it was 1.87. Iron analysis indicated, that birds group fed with commercial feed had highest iron level (2.517 $\mu\text{g}/\text{ml}$ in chest and 3.141 $\mu\text{g}/\text{ml}$ in thigh samples) whereas birds fed 15mg exhibited lower iron levels (1.646 $\mu\text{g}/\text{ml}$ and 2.533 $\mu\text{g}/\text{ml}$ in chest and thigh muscle samples). Histopathological examination showed hemosiderin contents and sign of toxicity in both commercial and experimental group. In conclusion iron oxide nanoparticles have the potential to improve FCR with lower quantities than commercially available iron, offering an economically viable alternative in poultry production.

Introduction

Nanotechnology is emerging and promising, with vast potential to revolutionize the global agriculture and livestock sectors (Marappan et al., 2017). This technology focuses on reducing the larger molecules to nanometer

particles (1 and 100 nm), this transformation alters the inherent innate physical and chemical characteristics of the material (Loghman et al., 2012, Ognik et al., 2016). The subsequent product has enhanced permeability, reactivity, and quantum features, that can applied in multiple scientific domains (Ogles and Yalcin, 2008). Broiler production, as a

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significant sector of the poultry industry, faces challenges such as maximizing growth rates, improving nutrient utilization, and reducing environmental impacts (Maharjan et al., 2021). These nanoparticles has been efficiently used in livestock breeding (Mohammadi et al., 2015, Farag et al., 2017). The incorporation of iron-based nanostructures into broiler feed represents a promising avenue to address these challenges and optimize production outcomes (Bunglavan et al., 2014).

The broiler's diet is routinely supplemented with Iron (Fe), which is an essential micronutrient for poultry, playing a crucial role in various physiological processes, including oxygen transport, energy metabolism, and immune function Iron (Xu et al., 2020). Traditional iron sources, such as inorganic salts and organic complexes, have limitations regarding bioavailability and stability in the gastrointestinal tract of birds. In contrast, iron-based nanostructures offer advantages such as enhanced solubility, increased surface area, and improved absorption kinetics, potentially leading to better utilization by broilers (Anwar et al., 2019). Several studies have explored the use of iron-based nanostructures in broiler feed, examining their effects on growth, nutrient utilization, and immune function (Bunglavan et al., 2014). These properties make them attractive candidates for supplementation in poultry diets to meet the bird's nutritional requirements more effectively. Therefore, the aim of this study was to evaluate the impact of nano-iron dietary supplementation on growth performance, muscle, and the histopathology in broiler chickens.

Materials and Methods

Nano-particle synthesis

The current research was conducted at International Islamic University, Islamabad (IIUI) and National Veterinary Laboratories (NVL). Iron oxide nanostructures (Fe_2O_3) were synthesized at Nanotechnology Lab at "Department of Physics" in IIUI and poultry trial was conducted at Room no. 1 in Animal House at NVL at National Agriculture Research Centre Islamabad (NARC).

Feed Preparation

First of all feed ingredients were purchased from local markets of Rawalpindi according to requirements of feed formulation. All ingredients were mashed and then thoroughly mixed. Feed was prepared at feed mill, Animal Sciences Institute (ASI) at NARC. The mineral mixture was used as an extender for nanostructures to be mixed in feed. The feed was formulated with different concentration of nanoparticles and categorized into five groups labelled as X, C, 5, 10 and 15 groups (Table 1).

Experimental design

From a local hatchery, 100 day old broiler chicks were purchased and were thoroughly checked for quality. All chicks were of average size and weight of 40 grams. They were divided into 5 groups, 20 birds/group. 2 of these groups were controlled and three groups were experimental. Feeding and watering was performed on daily basis, while body weight and feed conversion ratio (FCR) were monitored weekly (Chen et al., 2014).

Table 1. Feed formulation and group categorization

Group	Consideration
X	Fed with no iron additive in feed
C	Fed with the commercial value of the iron additive in feed
5	Fed with 5mg iron nanostructures/kg of feed
10	Fed with 10mg iron nanostructures/kg of feed
15	fed with 15mg iron nanostructures/kg of feed

Iron Analysis in Muscles

The sampling was performed 2 times, first at 35th day and second at 42nd day of trial. After slaughtering, muscles samples from chest, liver and right thigh were taken from each bird. Iron contents in muscles were analyzed by Atomic Absorption Spectrophotometer (AAS) in Central Lab, PMAS, AAUR using wet digestion method (Nikonov et al., 2011).

Histopathology

The liver samples histopathology was performed at Disease Section, PRI, Rawalpindi using methodology (Bancroft and Gamble, 2008). Briefly, the tissue were sectioned in suitable size (not more than 1 cm in thickness) by scalpel blade in a surgical tray and put them in tissue cassettes. Then fixed in 10% neutral buffered formalin (NBF) for 24 hrs. After that tissue were dehydrated in 70%, 85%, 95% and 100% isopropyl alcohol (IPA) solutions each for one hour step by step and lastly 1 hours more in 100% solution. The slide is rehydrated at 100%, 85% and 75% IPA for 30 seconds each step by step. Then slides were stained by hematoxylin for 2 minutes and for eosin Y for 1 minute and were observed.

Statistical analysis

All data were analyzed using the statistical software package Minitab (version 16, USA) employing one-way ANOVA and Chi-Square tests. Results are presented as Mean $\pm$ Standard deviation, with a significance level set at $P < 0.05$.

Results

Growth performance

Data analysis of feeding record from 1st to 4th week of trial showed that X group had utilized least amount of feed while

10 group and 15 group utilized same quantity of feed and it was more than any other group. The trend for feed consumption remained as X group > 5 group > C group > 10 group = 15 group (Fig.1). Alternatively, the trend of water consumption was in increasing order as X group > C group > 5 group > 10 group > 15 group and its same for each week of trial (Fig. 2).

The weight record of broiler exhibited that during first week the average weight of all groups was same. The difference in weight was started from 2nd week of age with an increasing order as X group > 5 group > C group = 10 group > 15 group and trend remained consistent in later weeks (Fig. 3)

The comparison of the FCR record of each group revealed that at the end of 1st week X group had highest value of FCR but in latter week it began to reduce as compared to other groups (Fig. 4).

Iron Analysis in Muscles and Histopathology

The concentrations of iron achieved after analysis indicated that samples from the commercial group (3.201 µg/ml in thighs and 2.517 µg/ml) showed highest value as compared to other groups (Table 2).

Histopathology of liver samples showed liver sample were more fragile and had blackish appearance. Furthermore, gross lesions with hemorrhages on borders were observed in each sample collected on 35th and 42nd day respectively (Fig. 5).

Discussion

Nanoscale nutrient incorporation in feed formulations, represent promising opportunity for advancing research and development in poultry nutrition. In this research Iron salts (Fe₂(SO₄)₃) has been replaced by iron nanostructures Fe₂O₃ in different quantities with the objective to improve the production potential of broiler birds. The results showed that iron nanoparticles had no toxic effect on broiler chickens but they showed more production as compared to control groups. Similar findings were reported indicating that nano-Fe supplemented in broiler diets improved body weight without impacting the composition of liver and muscle (thigh, breast) (Sizova et al., 2016). Similarly in this research it was resulted that birds fed with 15mg of iron nanostructure per kg of feed had best FCR and weight gain but at same time they utilized more amount of feed and water than other experimental and controlled groups. Here we can postulate that nanoparticles would have triggered the potential of birds but being less in iron quantity and availability, birds might not be able to gain their potential as compared C group which has 80mg/kg of iron in feed. Another study was performed on nano-iron

supplementation. reported increased FCR in broiler (Abdel-Rahman et al., 2022).

Table 2. Iron Concentration µg/ml in Muscle

Group	Sample 1	Sample 2	Sample 3
XC	0.436	0.433	0.442
XL	0.581	0.572	0.581
CC	2.512	2.492	2.517
CL	3.141	3.051	3.201
5C	1.307	1.289	1.293
5L	2.103	2.112	2.115
10C	1.389	1.364	1.382
10L	2.197	2.172	2.191
15C	1.653	1.633	1.646
15L	2.533	2.514	2.531

The elevated concentration of iron in muscle aligned with the previous research on organically complex trace minerals in broilers. The findings suggest that supplementation with Cu (4 mg), and Fe, Mn, and Zn (40) from organic sources may be sufficient for normal broiler growth. This approach of using lower levels of organic trace minerals in broiler diets aim to mitigate excessive excretion of trace mineral (Anwar et al., 2019).

Histopathology of liver samples was performed to find the effects on nanoparticles on tissues. Its results showed hemosiderin contents in C group more than 15 group so sign of toxicity were observed more in C group than any other group. Gross lesions confirmed that C group exhibited pronounced hemorrhages along the borders in liver samples sampled on 42nd day compared to those on 35th day. Additionally, in the 15 group, liver sample were fragile with blackish appearance. Several in vitro studies have linked cytotoxicity with exposure of nano-iron (Manke et al., 2013).

Conclusion

The research suggests that iron in the form of nanostructures have the potential to improve growth weight and feed conversion ratio in broiler, as compared to the commercially available iron offering an economically viable alternative for improving poultry production.

Contribution

SAIB designed this study. JIS participated in sample preparation. MA performed the experiments, conducted data analysis and drafted the manuscript.

Source of funding

N/A.

Conflict of interest

The authors declare no conflict of interest with anyone.

Ethical statement

All the procedures for animal handling and lab protocols were approved by the ethical committee of International Islamic University, Islamabad (IIUI), Pakistan.

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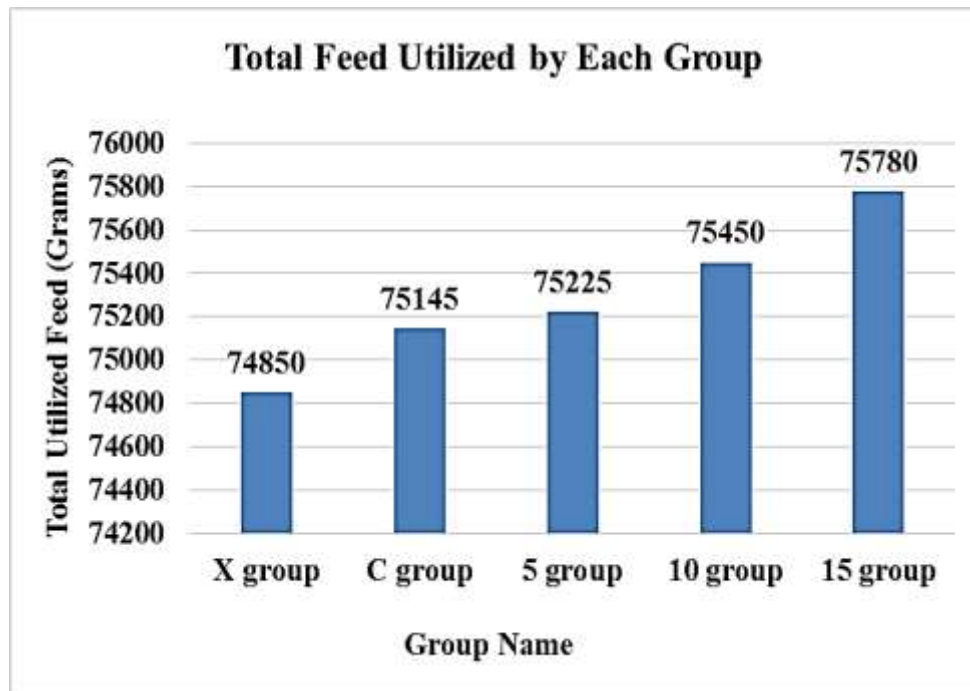


Figure 1. Total feed consumed by each group in 42 days

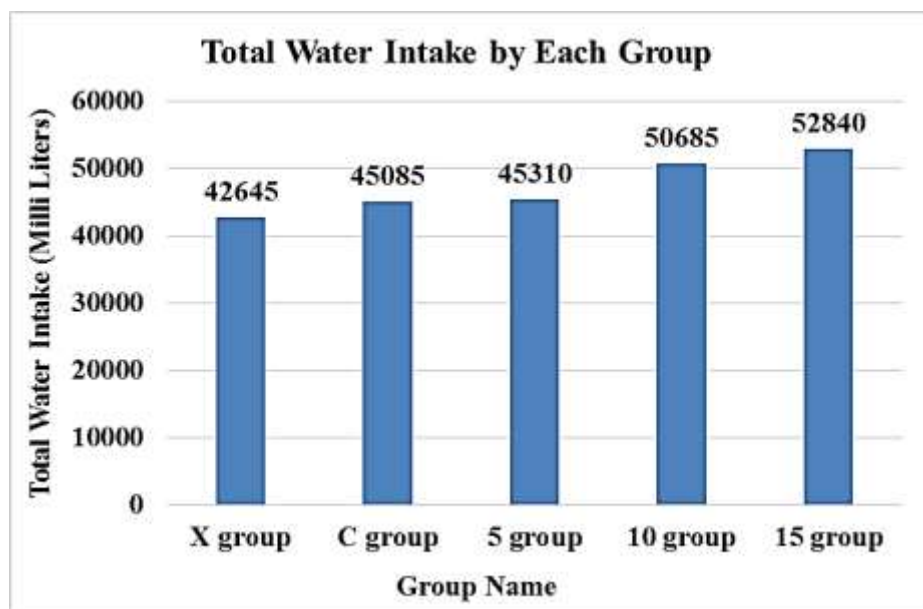


Figure 2. Total water intake by each group in 42 days of Trial

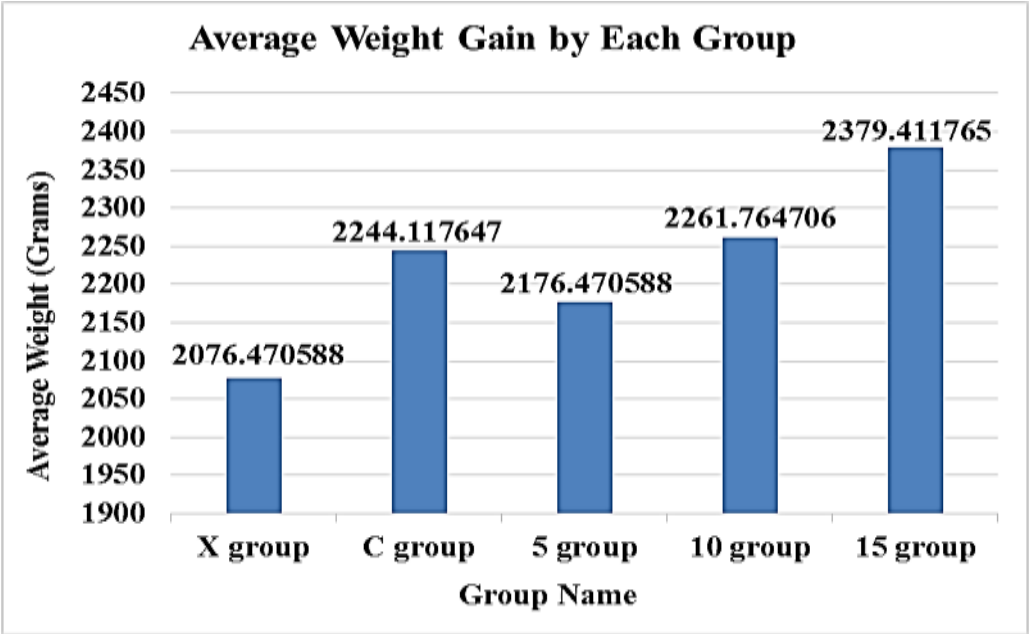


Figure 3. Average weight gain by each group in 42 days of trial

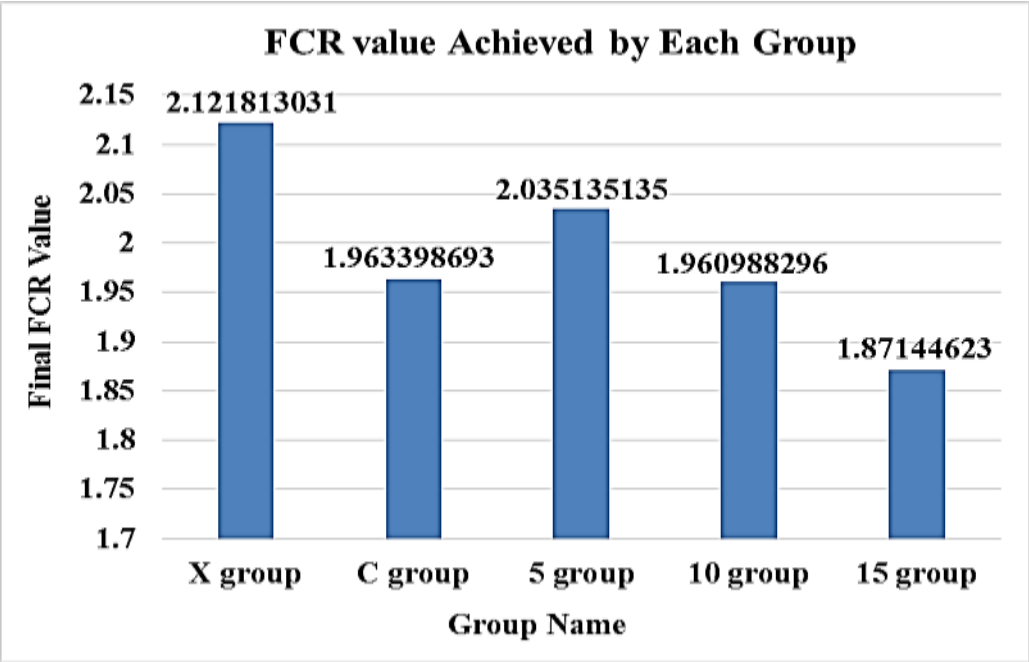


Figure 4. Weekly comparison of FCR in different groups

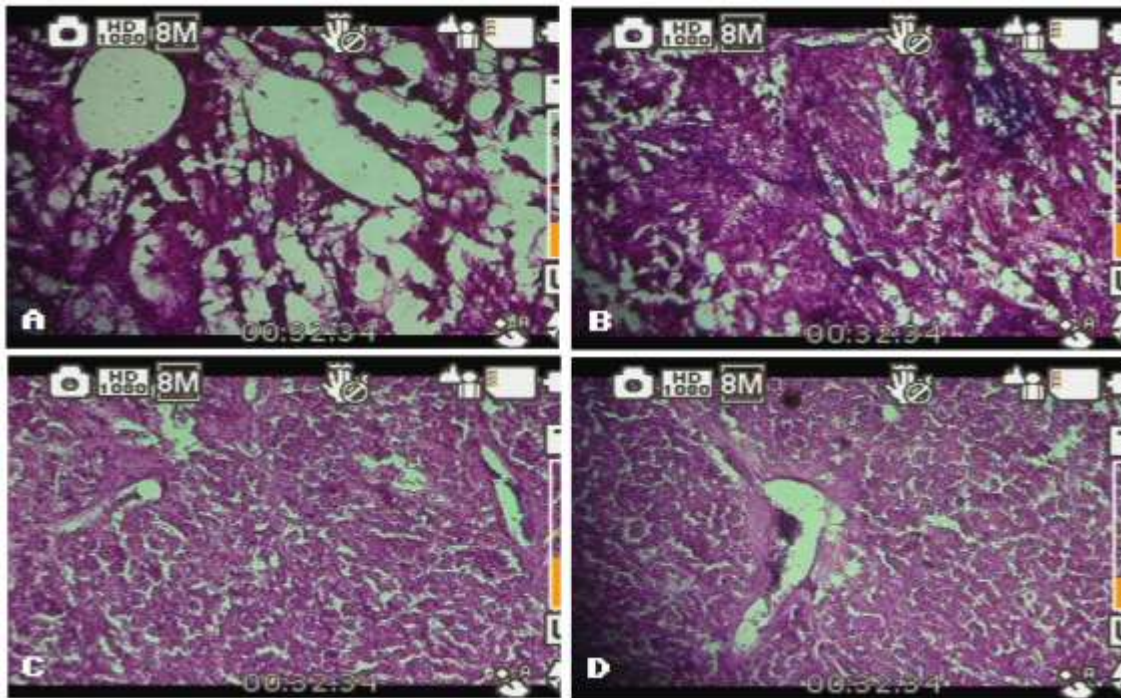


Figure 5. Histopathology of two liver sample at 35th labeled as (A and B) and 42nd day (C and D)