

# Effect of Hot Melt Processed Nano Iron and Its Effects on Growth Performance, Digestibility, and Blood Biochemical Profile in Weanling Pig

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## Abstract

*The objective of this study was to investigate the influence of dietary nano-iron on growth performance, nutrient utilization, and blood biochemical profile in grower pigs. An experiment was conducted considering twenty-four (N=24) weaned pigs of HDK-75 having an average body weight of 21.50±0.38 kg selected from AICRP pig farm, and allotted into four treatment groups, each group with 6 pigs on a body weight basis. The treatment groups were T0 (Control), T1 (100 mg inorganic iron), T2 (75 mg organic iron), and T3 (50 mg nano iron). Results of the 90-day feeding trial showed that there was a significant difference (P<0.05) found in body weight gain and average fortnightly body weight change, which was observed from 75th to 90th day. However, FCR showed a significant difference (P<0.05) from 60th to 90th day among the different treatment groups. The feed intake and digestibility of nutrients did not show any significant effect except NFE digestibility which was significantly (P<0.05) higher in the T3 group. In hematological profiles, Hb, PCV, and RBC values were significantly (P <0.05) higher in the T3 group as compared to other treatment groups and there was no significant difference (P> 0.05) observed for platelet, WBC, lymphocyte, monocyte and granulocyte count. In blood biochemical parameters, serum protein and serum iron were found significantly (P<0.05) higher in the T3 group and there was no significant difference (P>0.05) observed for blood glucose, albumin, globulin, A:G, AST, ALT, and BUN. So, it can be suggested that supplementation of nano-Fe (50 mg per kg of diet) improves overall growth performance, nutrient digestibility, and hematological profile of the experimental pig to prevent the occurrence of piglet anemia.*

**Keywords:** Digestibility, HD-K75 Pig, Inorganic Iron, Nano-iron, Organic iron, Serum Profile.

## Introduction

Iron (Fe), an essential trace element for pigs, is needed for proper blood hemostasis and count of hemoglobin (Hansen, 2009; Peri *et al.* 2016). Iron deficiency anemia in suckling piglets is caused by a low supply of this iron below the daily requirements; it mainly affects newborn piglets due to low iron transfer from the sow to the progeny through the placenta and also due to the low mineral content in milk (Liu *et al.* 2014). Therefore, weaning piglets eventually suffer from the carry-over effects of iron deficiency from the suckling period. In the wild, newborn piglets absorb sufficient iron by rooting in the soil. In conventional pig farming, iron must be supplemented to ensure animal vitality, health, and performance. The common cause of iron deficiency in newborn piglets is a striking imbalance between high iron demand and inadequate iron supply. Huge iron requirements during the first few weeks after birth (7–16 mg Fe/piglet/day) (Svoboda and Drabek, 2005; Svoboda, 2018) result from the selection of piglets for a large litter size, high birth weight, rapid growth and in consequence greater blood volume, and increased red blood cells (RBC) count. Considering that red blood cells are the largest reservoir of iron in the body (Svoboda, 2018), meeting iron requirements for erythropoiesis from natural iron-deficient sources such as hepatic iron stores. Iron is required as a component of hemoglobin in red blood cells which is also a constituent of several metabolic enzymes. As the bioavailability of inorganic iron is poor in comparison to organic and nano iron (because of its nano properties) so could be useful with the following objective of this study is to evaluate the effects of dietary nano iron for optimum health and production performance of weaned piglets to prevent from piglet anaemia.

## Materials and Methods

### *Preparation of Nano Iron*

Nano-Fe standards are prepared from ferric chloride anhydrous in the laboratory under manual control and system and their standardization is checked in atomic absorption spectrophotometry (Chatterjee *et al.*, 2007). To determine the particle size and potential, a Zeta sizer (Malvern Zetasizer Nano, ZS90) was used under room temperature, and the peak was observed. Transmission electron microscopy (TEM) analysis was performed to determine the shape and size of synthesized nanoparticles.

### *Experimental Animal and Diet*

A total of 24 weaned pigs (HDK-75) of above two months of age irrespective of sex were randomly allotted with an average initial body weight (BW) of  $21.5 \pm 0.36$  kg based on initial body weight according to a randomized block design (RBD) in All India Coordinated Research Project (AICRP) pig farm College of Veterinary Science, Khanapara, Assam. There are four treatments and each treatment has 6 pigs. The treatment included: control (no iron), inorganic iron (100 mg/kg of diet), organic iron (75 mg/kg of diet), and nano iron (50 mg/kg of diet). The experimental diets were fed for 90 days. The body weights were recorded on the first day of this experiment and subsequent fortnightly intervals till the end of the experiment. Feed intake was recorded and the remaining feed was measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). A digestibility trial was conducted at the end of the feeding trial to investigate nutrient digestibility. Four pigs from each group were selected randomly and placed in a digestibility cage. The measured feed was offered feed residue and feces voided were measured and representatives of each sample were dried in a hot air oven. The proximate analysis for experimental diets of feed and faeces was followed according to (AOAC, 2006). Blood was collected at the start, middle, and end of the trial from each pig from the anterior vena cava. For biochemical analysis, blood was collected in a red top clot activator vial, kept slanting with an ice pack for 30 minutes, and centrifuged at 1500 rpm for 15 minutes to separate serum for analysis of glucose, total protein, albumin, globulin, A: G, AST, ALT, serum iron and BUN. For hematological analysis, it was collected in a purple top EDTA vial and analyzed in a CBC machine. Data were analyzed by using the SPSS (Statistical Package for Social Science, version 20, Chicago, USA) with both one-way and two-way ANOVA at a 5% level of significance.

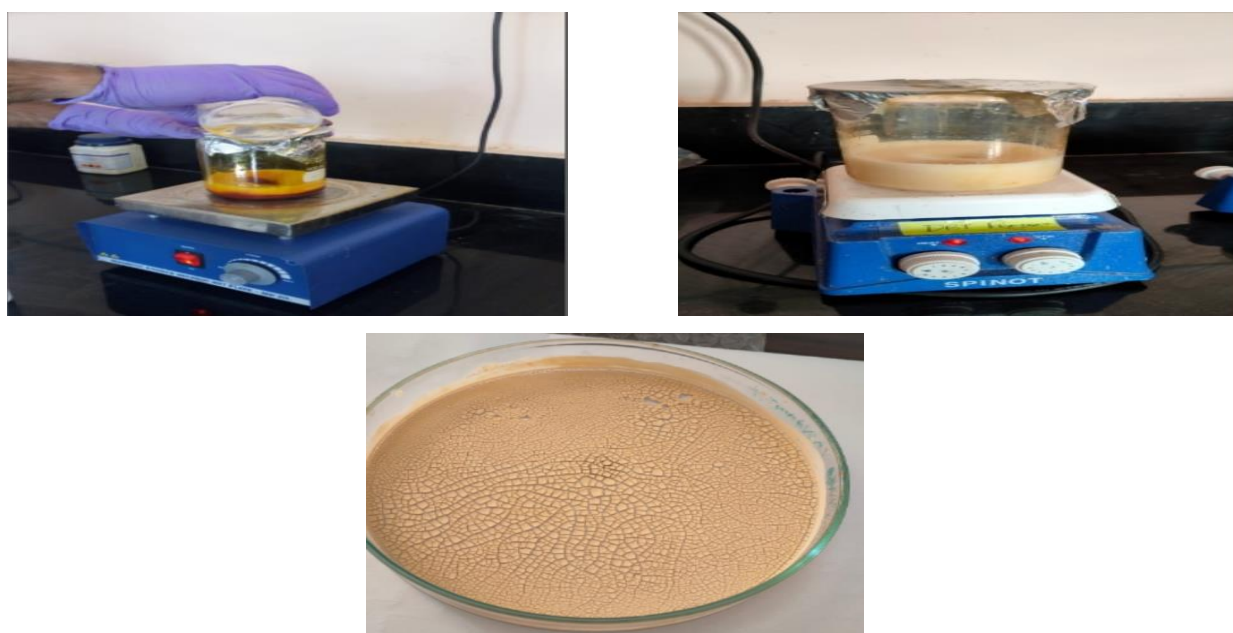
**Table 1:** Chemical composition of the experimental basal diets (% on air dry basis)

Attributes	%DM basis
<b>Ingredient composition</b>	
Maize	59
Wheat bran	13.5
Groundnut cake	11
Soyabean meal	15
Mineral mixture	1.5
<b>Chemical composition</b>	
OM	92.5
CP	18.59
CF	5.1
EE	3.74
NFE	64.99
Total ash	7.5
Calcium	1.11
Phosphorus	0.23

## Results

### *Micro-Analytical Characterization of Synthesized Nano-Dimensional Particles*

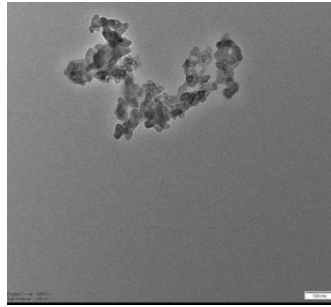
An attempt has also been made to synthesize different varieties of iron nano-particles (i.e. nano-dimensional ferric phosphate and nano-shaped ferric caseinate) by adopting the soft chemistry route (Fig.1) and their nano properties studied under field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). Under this study, the particle shape of nano-iron revealed their structural homology and bundled fashion appearances and size also ranged within nano-scale (1-100 nm).



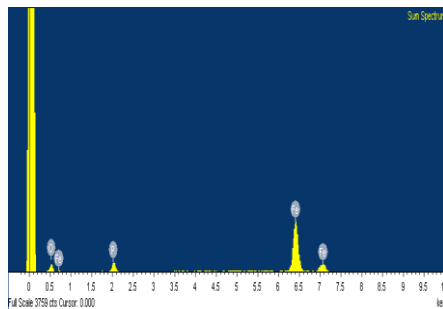
**Fig.1:** Green synthesis of nano iron by using ferric chloride anhydrous



**Fig.2:** Different size image under transmission electron microscopy



The EDx study (Fig.3.) of nano-structured FePO<sub>4</sub> ensured its elemental composition and purity.



**Fig. 3:** Depicting the EDx study of FPNP (ferric phosphate nano-particles)

### ***Growth Performance, Structural Measurement, and Nutrient Digestibility***

**Table 2:** Effect of dietary iron Concentration and Source on growth performance in Grower pigs

Attributes	T <sub>0</sub> (Control)	T <sub>1</sub> (Inorganic100mg/kg of diet)	T <sub>2</sub> (Organic75mg/kg of diet)	T <sub>3</sub> (nano-Fe 50mg/kg of diet)	P- value
Initial body weight	8.50 ± 0.066	8.65 ± 0.070	8.40 ± 0.045	8.65 ± 0.095	1.000
Final body weight	29.04 <sup>b</sup> ±0.34	28.08 <sup>b</sup> ± 0.321	27.37 <sup>b</sup> ± 0.325	26.13 <sup>a</sup> ± 0.430	<0.01
Total gain (kg)	29.17 <sup>b</sup> ±0.004	29.72 <sup>a</sup> ±0.003	30.26 <sup>a</sup> ±0.001	32.75 <sup>c</sup> ±0.004	<0.01
ADG (g/day) *	324.15 <sup>b</sup> ±0.360	330.26 <sup>a</sup> ±0.350	336.19 <sup>a</sup> ±0.110	363.89 <sup>c</sup> ±0.408	<0.01
Total feed intake (kg)	111.47±3.20	110.81 ± 3.12	110.66 ± 3.13	108.38 ± 2.84	>0.01
Feed Conversion Ratio*	3.72 <sup>a</sup> ± 0.16	3.67 <sup>b</sup> ± 0.10	3.56 <sup>c</sup> ± 0.19	3.30 <sup>d</sup> ± 0.12	<0.01

\*T<sub>0</sub>, basal diet; T<sub>1</sub>, basal diet with 100 ppm inorganic iron (w/w %); T<sub>2</sub>, basal diet with 75 ppm organic iron (w/w %); T<sub>3</sub> basal diet with 50 ppm (w/w %) of nano- iron

Table 2 results for final body weight and total gain significantly (P<0.05) affected from the 60<sup>th</sup> day onwards among the different treatment groups. Whereas, the T<sub>3</sub> group was significantly (P<0.05) different in respect of total gain in

body weight and daily gain in body weight. The feed intake increased along with an increase in body weight. However, a significant ( $P < 0.05$ ) difference existed from the 60<sup>th</sup> day onwards to the 90<sup>th</sup> day among the different treatment groups. No influence ( $P > 0.05$ ) of supplementation was manifested from the data presented in Table 3 in terms of the digestibility co-efficient of various nutrients (DM, CP, OM, CF, and EE) except nitrogen-free extract digestibility which is significantly higher in the T3-group ( $P < 0.05$ ) among the different experimental groups.

**Table 3:** Effect of dietary iron concentration and source on nutrient digestibility in grower pigs

Digestibility co-efficient(%)	Treatments				P value
	T <sub>0</sub> (control)	T <sub>1</sub> (Inorganic100mg/kg of diet)	T <sub>2</sub> (Organic75mg/kg of diet)	T <sub>3</sub> (nano50 mg/kg of diet)	
DM	69.84±0.431	70.28±1.188	70.91±0.147	72.19±1.165	0.311
CP	72.38 ±0.39	73.04 ±1.08	73.60 ±0.13	74.90 ±1.05	0.213
OM	77.78 ±0.98	77.82 ±0.47	78.23 ±0.22	79.84 ±0.42	0.123
CF	28.32±1.02	30.08±2.79	30.12±0.35	31.83±2.85	0.704
EE	77.014±0.33	77.170±0.91	78.064±0.06	78.434±0.90	0.413
NFE	82.27 <sup>c</sup> ±0.25	83.14 <sup>bc</sup> ±0.67	84.29 <sup>ab</sup> ±0.08	84.96 <sup>a</sup> ±0.63	0.018
N-retention	70.82 ± 1.83	72.99 ± 0.57	72.76 ± 0.28	73.18 ± 1.85	0.598
Fe-retention	29.76 <sup>d</sup> ±0.63	31.61 <sup>c</sup> ±0.16	35.41 <sup>b</sup> ±0.13	39.68 <sup>a</sup> ±0.12	<0.01

\*T<sub>0</sub>, basal diet; T<sub>1</sub>, basal diet with 100 ppm inorganic iron (w/w %); T<sub>2</sub>, basal diet with 75 ppm organic iron (w/w%); T<sub>3</sub> basal diet with 50 ppm (w/w%) of nano- iron

### Blood Biochemical Profile

The effects of dietary Fe concentration and source on serum profiles are presented in Table 4. In phase 1, there were no significant differences in blood glucose, serum protein, BUN level, AST, and ALT value. However, serum protein and hemoglobin concentration (fig.4) were significantly higher ( $P < 0.01$ ) in experimental pigs. There was a significant ( $p < 0.05$ ) difference in serum iron (table 4) between pigs fed the organic iron and nano iron-fed diets.

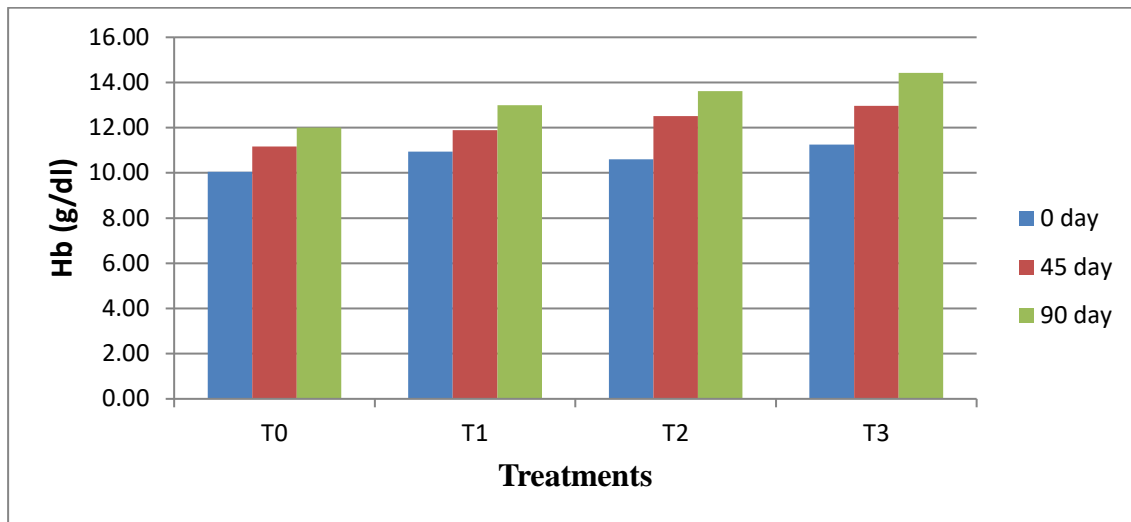
**Table 4:** Effect of dietary iron concentration and source on blood biochemical profiles in grower pigs

Attributes	T <sub>0</sub> (control)	T <sub>1</sub> (Inorganic100 mg/kg of diet)	T <sub>2</sub> (Organic75 mg/kg of diet)	T <sub>3</sub> (nano50 mg/kg of diet)	P value
Serum glucose (mg/dl)	91.40±4.243	92.19±4.243	91.77±4.243	92.28±4.243	0.21
Serum protein (g/dl)	6.57±0.17	6.90 ±0.23	6.93 ±0.26	7.37 ±0.30	<0.01
AST (u/l)	36.49±0.26	36.13±0.31	36.01±0.27	35.98±0.30	0.58
ALT (u/l)	43.94 ±0.32	43.45 ±0.29	43.45 ±0.27	43.01 ±0.30	0.27
Serum iron (µg/dl)	130.55±4.26	131.24±3.92	133.31a±4.85	135.07±4.68	<0.01
BUN (mg/dl)	14.98±0.23	14.88±0.25	15.19±0.21	15.32±0.21	0.17

\*T<sub>0</sub>, basal diet; T<sub>1</sub>, basal diet with 100 ppm inorganic iron (w/w %); T<sub>2</sub>, basal diet with 75 ppm organic iron (w/w%); T<sub>3</sub> basal diet with 50 ppm (w/w %) of nano iron

### Hematological Parameter

The effects of dietary Fe concentration and source on serum profiles are presented in Table 5. In phase 1, there were no significant differences in the percentage of PCV and platelet value and there was a significant ( $p < 0.05$ ) difference in serum RBC value between pigs fed the organic iron and nano iron-fed diets. However, no significant difference ( $P < 0.05$ ) was found in terms of monocyte, lymphocyte, and granulocyte value in the serum among the experimental group, and data are presented in table 5.



**Fig 4.** Effect of dietary iron concentration and source on hemoglobin profiles in grower pigs

**Table 5:** Effect of dietary Fe concentration and source on hematological profiles in grower pigs

Attributes	T <sub>0</sub> (Control)	T <sub>1</sub> (Inorganic 100mg/kg of diet)	T <sub>2</sub> (Organic 75 mg/kg of diet)	T <sub>3</sub> (nano 50 mg/kg of diet)	P-value
PCV (%)	34.93 ±0.41	34.83 ±0.44	35.96 ±0.53	35.96 ±0.53	0.00
Platelet (m/mm <sup>3</sup> )	321.71±0.61	321.93±0.64	322.02±0.52	324.68±1.08	0.11
RBC (m/mm <sup>3</sup> )	5.95±0.06	6.10±0.10	6.13±0.10	6.22±0.10	<0.01
WBC (m/mm <sup>3</sup> )	15.65±0.13	15.43±0.12	15.46±0.17	15.80±0.17	0.49
Lymphocyte count (%)	44.09±0.40	44.80±0.54	44.61±0.72	44.76±0.39	0.43
Monocyte count (%)	3.80±0.039	4.03±0.040	3.79±0.053	4.04±0.064	0.13
Granulocyte count (%)	34.40±0.493	35.24±0.399	34.57±0.509	35.58±0.492	0.82

\*T<sub>0</sub>, basal diet; T<sub>1</sub>, basal diet with 100 ppm inorganic iron (w/w %); T<sub>2</sub>, basal diet with 75 ppm organic iron (w/w%); T<sub>3</sub> basal diet with 50 ppm (w/w%) of nano iron

## Discussion

In the present study, an attempt has been made to modify the nature of some critical nutrients to improve their bioavailability and minimize their wastage through excreta. By their inherent nature of inertness to other interacting nutrients present in GIT, nano-nutrients seem to fit best in the precision feeding of livestock. By employing an environment benign soft chemistry approach different varieties of dietary nano-shaped iron particles have been synthesized in our lab. So, the synthesized dietary nano-minerals have been characterized micro-analytically. The transmission electron microscope (TEM) analysis demonstrated that the particles are predominantly spherical in shape. The average diameter of FeNPs was around 40-60nm (fig.2). “Ingole *et al.*, (2010)” also reported that by using glucose as a reducing agent, the size of iron nanoparticles synthesized, as determined by TEM, was in the range of 20-80 nm. Growth performance, due to the supplementation of iron was affected significantly from the 75<sup>th</sup> and 90<sup>th</sup> day of the experimental period. Among all the groups' nano fed group showed better growth performance compared with the other treatment groups, which may be due to the higher bioavailability of iron under nano form, because nano-particles have the following novel characteristics, such as greater specific surface area, higher surface activity, high catalytic efficiency, and stronger adsorbing ability which also can transport directly to target organs by avoiding fast degradability and improved several health benefits. These results would be confirmed by “(Ranjan *et al.*, 2012; Deng *et al.*, 2021 and Lee *et al.*, 2019)” and not confirmed by Bhuyan *et al.*, (2020) Lee *et al.*, (2019). While in terms of average daily gains (ADG), there is significantly higher in the current experiment (table 2) which is supported by “Bhuyan *et al.*, (2020); Feng *et al.*, (2009), and Lewis *et al.*,(1996)”. Further, FCR was significantly affected from the 4<sup>th</sup> to 6<sup>th</sup> fortnight and found better FCR in low doses of nano iron with higher iron

availability which is supported by “Lewis, (1999); Kang *et al.*, (2014), and Bhuyan *et al.*,(2020)”. From Table 4, the blood biochemical profile, especially the blood glucose level was not affected significantly among different treatment groups which were found similar by Bhuyan *et al.*, (2020) whereas total serum protein was influence significantly higher in nano fed group may be due to inclusions of nano iron that enhance protein digestion which increases protein level in blood as mentioned by “Matthews *et al.*,(1998) and Bhuyan *et al.*, (2020)”. Serum albumin, globulin, and A: G were not affected by the inclusion of iron in pigs which was found similar to Bhuyan *et al.*, (2020) findings. Similarly, AST and ALT level was found within normal physiological ranges which means there was no adverse effect in the liver of pigs by inclusions of nano iron 50mg/kg of diet which is similar in the case of other forms of iron. The present study finding is in good agreement with the findings of Parivar *et al.*, (2018) who reported that there is no significant alteration in AST and ALT levels in mice treated with 25, 50, and 75 µg/mg doses of iron nanoparticles similarly, Bhuyan *et al.*, (2020) also found no significant difference ( $P < 0.05$ ) in AST and ALT level among the different treatment groups by inclusions of inorganic iron 100mg per kg of diet ( $\text{FeSO}_4$ ), organic iron 100mg per kg of diet (Methio-chelated) and nano iron 100 and 50mg per kg of diet ( $\text{FePO}_4$ ). Significantly higher serum iron in nano iron 50 mg/kg of diet fed group may be due to higher bioavailability of nano iron in blood circulation. Kachuee *et al.*, (2019) also mention that particle size is a key parameter to improve absorption efficiency. The smaller size of iron nanoparticles ( $< 100$  nm) allows iron to be absorbed through the intestinal mucus barrier. In addition, this explains the lower serum iron in inorganic iron 100mg/kg of diet. The present study finding is in good agreement with the following findings “(Bhuyan *et al.*, 2020; Leeson *et al.*, 2003; Bruerton, 2005; Lipinski *et al.*, 2010; Li *et al.*, 2018; Yu *et al.*, 2000 and Elshemy, 2018). From table 4 the BUN level of all the treatment groups was found within a normal physiological range which indicates optimal utilization of amino acids (Dukes, 1996) and the present finding is in good agreement with Bhuyan *et al.*, (2020) who also found that BUN level was not significantly affected by inclusion of inorganic iron 100mg per kg of diet ( $\text{FeSO}_4$ ), organic iron 100mg per kg of diet (Methio-chelated) and nano iron 100 and 50mg per kg of diet ( $\text{FePO}_4$ ). Under hematological parameter, there was a significantly ( $P < 0.5$ ) higher PCV in nano fed group may be influenced due to its marked small size that increased the bioavailability and absorption of iron oxide nanoparticles (Wei *et al.*, 2005; Yu *et al.*, 2000; Rincker *et al.*, 2004 and Bhuyan *et al.* 2020). Elshemy *et al.*, (2018) also reported a significant increase in hematocrit value when compared to  $\text{FeSO}_4$  in male albino rats fed iron oxide nano-particles (IONPs) @0.4 mg/kg body weight. In contrast, Lee *et al.*, (2019) reported no significant difference in PCV among different treatment groups supplemented with inorganic iron (100 ppm Fe as  $\text{FeSO}_4$ ), HME-Fe levels (50, 75, and 100 ppm nano-Fe), and organic iron (100 ppm Fe as iron methionine). In the present investigation among treatment groups nano-fed group showed the higher RBC level may be due to high iron in blood that may reflect enhanced erythropoiesis and result in high RBC value. Ajay K. Singh. (2018) also mentioned that iron is a necessary ingredient that is used by erythroid precursor cells for effective erythropoiesis (Hashem *et al.*, 2018; Wang *et al.*, 2013 Li *et al.*, 2018). WBC count was not affected significantly which is in agreement with Lee *et al.*, (2019) and Bhuyan *et al.*, (2020). The platelet count was not influenced by inclusions of different sources of iron viz- nano iron ( $\text{FePO}_4$ ), organic iron (Methio-chelated), and inorganic iron ( $\text{FeSO}_4$ ) which is in good agreement with the findings of Bhuyan *et al.*, (2020).

## Conclusion

The present investigation can be concluded that supplementation of nano iron 50mg/kg of diet in grower pigs has better advantages in growth performance, nutrient utilization, and blood biochemical profiles in grower pigs when compared with other treatment groups fed with inorganic iron ( $\text{FeSO}_4$ ) 100mg/kg of diet and organic iron (methio-chelated) 75mg/kg of diet. Inclusions of nano iron 50mg/kg of diet have no adverse effect on the pigs and are suggested to the diet of the piglet to prevent the occurrence of piglet anemia.

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## Contribution by Authors

Equal contribution. All authors declared that ‘written informed’ consent was obtained from the approved parties for the publication of this article and accompanying images.

## Conflict of Interests

There is no conflict of interest.

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