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Effects of Hot Melt Processed Nano Iron 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-Fe 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 average body weight 21.50±0.38 kg selected from AICRP pig farm, and allotted into four treatment groups. The treatment groups were T0 (Control), T1 (100mg inorganic iron), T2 (75mg organic iron) and T3 (50mg nano iron). Results of 90day feeding trial showed that there was 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 existed significant difference (P<0.05) from 60th to 90th day among the different treatment groups. The feed intake and digestibility of nutrient was not showing any significant affect except NFE digestibility which was significantly (P<0.05) higher in T3 group. In blood biochemical parameters, serum protein and serum iron was found significantly (P<0.05) higher in 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@50mg per kg of diet improves overall growth performance, nutrients digestibility and hematological profile to the experimental pig to prevent the occurrence of piglet anaemia.

KEYWORDS: Electron Microscopy, HD-K75 pig, Nano-iron, Nutrients digestibility, Organic iron, Serum profiles,

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Iron (Fe), an essential trace element for pigs, is needed for proper blood hemostasis and count of hemoglobin (Hansen, 2009 and Peri et al., 2016). Iron deficiency anemia in suckling piglets is caused by a low supply of this mineral below the daily requirements; it mainly affects newborn animals 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, weanling 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. Sow's milk contains an average of only 1 mg of iron per liter (Brady et al., 1978) which cannot fulfill the iron requirements. As iron is required as a component of hemoglobin in red blood cells which also plays an important role in the body as a constituent of several metabolic enzymes. To avoid piglet anemia, the diet of sows is usually supplementation with inorganic or organic iron which have limitations regarding their bioavailability, and may not meet the mineral requirement. So, nanotechnology is a field of research offering innovative and promising products that, among others, have been recently used to generate nutrients with increased bioavailability. As bioavailability of nano iron is more so could be useful with the objective of this study is to evaluate the effects of dietary nano iron for optimum health and production performance of weaned piglets. In the present study an attempt has been taken to modify the nature of some critical nutrients with an aim to improve its bioavailability and minimize their wastage through excreta. By virtue of their inherent nature of inertness to other interacting nutrients present in GIT, nano-nutrients seems to fit best in precision feeding of livestock. By employing environment benign soft chemistry approach different varieties of dietary nano-shaped iron particles have been synthesized in our lab.

MATERIALS AND METHODS

Laboratory Preparation and Micro-analytical Characterization of synthesized nano-dimensional particles

Nano iron standards are prepared from ferric chloride anhydrous in laboratory under manual control and system and their standardization checked in atomic absorption spectrophotometry (Chatterjee et al., 2007). In order to determine the particle size and potential, 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 nano-particles.

Micro-analytical Characterization of synthesized nano-dimensional particles

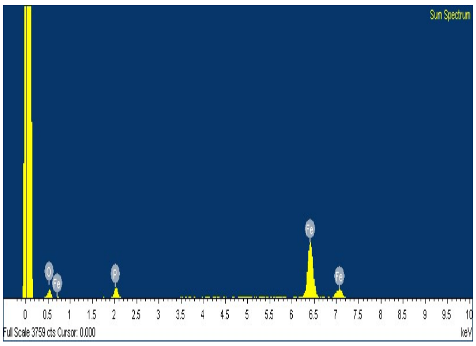

Fig. 1. Depicting the EDx study of FPNP (ferric phosphate nano-particles)

Fig.2. The EDx study of nano-structured $FePO_4$ ensured its elemental composition and purity (bundle shaped appearance)

In the present study an attempt has been taken to modify the nature of some critical nutrients with an aim to improve its bioavailability and minimize their wastage through excreta. By virtue of their inherent nature of inertness to other interacting nutrients present in GIT, nano-nutrients seems to fit best in precision feeding of livestock. By employing environment benign soft chemistry approach different varieties of dietary Nano-shaped iron particles have been synthesized in our lab.

The basal diet was formulated as per the ICAR 2013 recommendation (Table 1) which was fed in the morning (9:00h) by subtracting the equal amount of maize from basal diet. Before housing of the piglets, the floor pens were thoroughly disinfected using fumigants and flame gun. A total of 24 weaned pigs (HDK-75) were randomly allotted with an average initial body weight (BW) of 21.5 ± 0.36 kg on the basis of initial BW 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 its treatment having 6 pigs. The treatment included: control (no iron), inorganic iron (100mg/kg of diet), organic iron (75mg/kg of diet) and nano iron (50mg/kg of diet). Prepared experimental diets (table 1) were fed for 90 days among the group and diets proximate composition mentioned.

Table1. Ingredient and chemical composition of the experimental basal diets (% on air dry basis)

Attributes	% DM basis
Maize	59.0
Wheat bran	13.5
Groundnut cake	11.0
Soyabean meal	15.0
Mineral mixture	1.50
Chemical composition	
OM	92.5
CP	18.6
CF	5.10
EE	3.74
NFE	65.06
Total ash	7.50
Calcium	1.11
Phosphorus	0.23

The body weights were recorded in the first day of this experiment and subsequent fortnightly interval till the end of the experiment. Feed intake was recorded and remaining feed were measured to calculate for 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 place in a digestibility cage. The measured feed is offered and feed residue, feces voided were measured and representative of each sample were dried in hot air oven. The proximate analysis for experimental diets of feed and feces was followed according to (AOAC, 2006).

Blood was collected in start, mid and at end of the trial from each pig from anterior vena cava in the morning (Before watering and feeding) into a vacutainer tube from all the pigs at 0, 30, 60, 90 days of feeding trial. For biochemical analysis blood was collected in a red top clot activator vial, kept slanting with ice pack for 30 minutes and centrifuged it in 1500 rpm for 15 minutes to separate serum for analysis of glucose, total protein, albumin, globulin, A:G, AST, ALT, serum iron and BUN by using commercial kit and by biochemical analyzer. The experimental data were subjected to statistical analysis (SPSS version 20) using a one way analysis of variance described by Snedecor and Cochran (2004). Significance was defined at $P < 0.05$. All the values represent mean ± standard errors of the mean.

RESULTS AND DISCUSSION

Nano iron preparation from ferrous sulphate under laboratory condition examined under FESEM &TEM. The results showed that scanning images of nano iron under field-emission scanning electron microscopy (FESEM) and transmission electron-microscope (TEM) which revealed their structural homology and bundled fashion appearances (Fig.1 and 2). Similarly, Ingole et al. (2010) also reported that by using glucose as a reducing agent, the size of synthesized iron nano particles studied under TEM analysis and results were found in the range of 20-80 nm. In piglet hepatic iron stores are generally low in piglet (Lipinski et al., 2010 and Bessman, 2020).Only limited literature is available on the evidence of oral form of nano iron (Laboratory based) contribution to grower pigs to prevent piglet anaemia. So, in this experiment, the author observed that the final body weight and total gain of the experimental animal significantly ($P<0.05$) affected from 60[th] day onwards (Table 2) among the different treatment groups.

Table 2. Effect of dietary Fe concentration and source on growth performance in grower pigs

Attributes		T1	T2	T3
Initial body weight	8.50± 0.06	8.65±0.07	8.40±0.05	8.65±0.10
Final body weight	29.04b± 0.35	28.08b±0.32	27.37b±0.325	26.13a ±0.430
Total gain (kg)	29.17b±0.004	29.72a±0.003	30.26a±0.001	32.75c±0.004
ADG (g/ day)*	324b±0.36	330a±0.35	336a±0.11	363c±0.41
Total feed intake (kg)	111.4± 3.20	110.8±3.12	110.66±3.13	108.4±2.84
FCR*	3.72a ± 0.16	3.67b±0.10	3.56c±0.19	3.30d±0.12

*T_0, basal diet; T_1, basal diet with 100ppm inorganic iron (w/w %); T_2, basal diet with 75ppm organic iron (w/w%); T_3 basal diet with 50ppm (w/w%) of nano- iron

*Values bearing superscripts a,b,c,d differ significantly ($P<0.01$)

However, significant (P<0.05) difference existed from 60th day onwards to 90th day among the different treatment groups. No influence (P>0.05) of supplementation was manifested seen from the data presented in the 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 (P<0.05) higher in T3 group.

Table 3. Effect of dietary Fe concentration and source on nutrient digestibility in grower pigs

Digestibility co- efficient(%)	T0	T1	T2	T3
DM	69.84±0.431	70.28±1.188	70.91±0.147	72.19±1.165
CP	72.38±0.39	73.04±1.08	73.60±0.13	74.90±1.05
OM	77.78±0.98	77.82±0.47	78.23±0.22	79.84±0.42
CF	28.32±1.02	30.08±2.79	30.12±0.35	31.83±2.85
EE	77.014±0.33	77.17±0.91	78.06±0.06	78.43±0.90
NFE	82.27c±0.25	83.14bc±0.67	84.29ab±0.08	84.96a±0.63
N-retention	70.82 ±1.83	72.99 ±0.57	72.76± 0.28	73.18±1.85
Fe-retention	29.76d±0.63	31.61c±0.16	35.41b±0.13	39.68a±0.12

*Values bearing superscripts a,b,c,d differ significantly (P<0.01)
*T0, basal diet; T1, basal diet with 100ppm inorganic iron (w/w %); T2, basal diet with 75ppm organic iron (w/w%); T3 basal diet with 50ppm (w/w%) of nano- iron

In present study, growth performance due to supplementation of iron was affected significantly from 75th and 90th day of experimental period among which nano fed group showed better growth performance compared with the other treatment groups, which may be due to higher bioavailability of iron under nano form, because nano particles having following novel characteristics, such as, greater specific surface area, higher surface activity, high catalytic efficiency and stronger adsorbing ability which has ability to transport directly to target organs by avoiding fast degradability and improved several health benefits. This was in agreement with Ranjan et al., 2012; Deng et al., 2021 and Lee et al., 2019. However, it differs from Bhuyan et al.(2020), Lee et al. (2019). In case of ADG, there is significantly(P<0.05) higher in 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 4th to 6th fortnight and found better FCR in low doses nano iron with higher iron availability which is supported by Lewis (1999); Kang et al. (2014) and Bhuyan et al.(2020).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.

Table 4. Effect of dietary Fe concentration and source on blood biochemical profiles in grower pigs

Attributes	T0	T1	T2	T3
Serum glucose (mg/dl)	91.4±4.24	92.2±4.24	91.8±4.24	92.3±4.24
Serum protein (g/dl)	6.57±0.17	6.90±0.23	6.93±0.26	7.37±0.30
AST (u/l)	36.49±0.26	36.13±0.31	36.01±0.27	35.98±0.30
ALT (u/l)	43.94±0.32	43.45±0.29	43.45±0.27	43.01±0.30
Serum iron (µg/dl)	130a±4.26	131a±3.92	133b±4.85	135b±4.68
BUN (mg/dl)	14.98±0.23	14.88±0.25	15.19±0.21	15.32±0.21

*T_0, basal diet; T_1, basal diet with 100ppm inorganic iron (w/w %); T_2, basal diet with 75ppm organic iron (w/w%); T_3 basal diet with 50ppm (w/w%) of nano iron

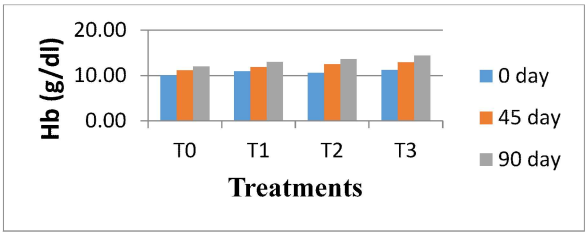

Fig 3. Effect of dietary Fe concentration and source on heamoglobin profiles in grower pigs

However, serum protein and heamoglobin concentration (Fig.3) was significantly higher (P< 0.01) in experimental groups of 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. From table 4, blood biochemical profile, specially the blood glucose level was not affected significantly among different treatment group which was found similar by Bhuyan et al. (2020) where as total serum protein was influence significantly higher in nano iron fed group may be due to enhance protein digestion which increases protein level in blood as mentioned by Matthews et al.(1998); Bhuyan et al.(2020). Serum albumin, globulin and A:G was not affected by inclusions of iron in pig which was found similar in Bhuyan et al. (2020) findings.

Similarly, AST and ALT level was found within normal physiological range 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 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 level in mice treated with 25, 50 and 75 ìg/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 ($FeSO_4$), organic iron@100mg per kg of diet (Methio-chelated) and nano iron@100 and 50mg per kg of diet ($FePO_4$). Significantly higher serum iron in nano iron@50mg/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 Fe nanoparticle (< 100 nm) allows Fe to be absorbed through the intestinal mucus barrier. In addition, this explains the lower serum Fe 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 the table 4 the BUN level of all the treatment groups was found within 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 ($FeSO_4$), organic iron@100mg per kg of diet (Methio-chelated) and nano iron@100 and 50mg per kg of diet ($FePO_4$).

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 ($FeSO_4$) @100mg/kg of diet and organic iron (methio-chelated) @75mg/kg of diet. Inclusions of nano iron @50mg/kg of diet has no adverse effect to the pigs and suggested to the diet of piglet to prevent the occurrence of piglet anemia.

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