

Research Article

CONTROL OF IRON DEFICIENCY ANAEMIA IN PIGLETS THROUGH 2-7-10-15 MODULE OF ORAL IRON SUPPLEMENTATION

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Received 02 June 2022, revised 24 July 2022

ABSTRACT: Iron deficiency anemia is the leading cause of piglet mortality. It happens due to low iron stores in piglets at birth, increasing body weight with the high demand for hemoglobin carrying red blood cells, presence of a very low amount of iron in sow's milk, and immature mechanism of iron absorption in piglets. Iron supplementation is the only way to control it. The present study investigated the efficacy of oral iron supplementation in two different doses @ 30 mg/kg body weight and 150 mg/kg body weight on suckling piglet performance, control of iron deficiency anemia, and blood as well as organ iron status. The iron supplementation was given on the 2nd, 7th, 10th, and 15 th- day post birth. Oral iron supplementation to piglets improved growth parameters, hemoglobin level, serum Fe and serum ferritin levels, and organ (liver and spleen) Fe levels. Moreover, at weaning, hemoglobin levels of supplemented piglets were normal whereas the un-supplemented piglets were suffering from iron deficiency anemia. Therefore, oral iron supplementation @ of 30 mg/kg body weight on 2-7-10-15 days post-birth may be recommended for control of iron deficiency anemia and improvement of iron status in piglets.

Key words: Iron deficiency anemia, Andaman local pig, Oral iron supplementation, Iron status.

INTRODUCTION

Iron, an important micronutrient, plays vital roles in several cellular processes such as transportation of oxygen, electron transfer reactions, energy metabolism, gene regulation, regulation of cell growth, and development of immune functions (Beard 2001, Ganz and Nemeth 2015). Unlike other microminerals, body iron homeostasis is regulated through iron absorption as mammals lack a mechanism for the excretion of excess iron (Hallberg and Hulthén 2000). In piglets, insufficient iron absorption leads to iron deficiency which results in reduced circulation of red blood cells and ultimately anemia (Kim *et al.* 2018). It occurs due to low level of iron stores at birth, presence of limited iron in sow milk, immature mechanism of iron absorption in piglets, and rapid growth rate of new-born piglets requiring a large amount of hemoglobin carrying red blood cells (Kegley *et al.* 2002, Antonides *et al.* 2015). The condition of iron deficiency anemia (IDA) is more severe in piglets reared in confinement as they don't have access to soil and have to depend on sow

colostrum and milk which also cannot meet the iron requirement of piglets (Kolb 1963, Antonides *et al.* 2015).

Iron deficiency anemia is the most common nutritional deficiency disorder in pre-weaning piglets and all breeds of pigs are susceptible to developing the disease if not treated properly (Collard 2009, Lipiński *et al.* 2013). The negative impact of IDA in piglets is well studied (Venn *et al.* 1947, Svoboda *et al.* 2004, Szudzik *et al.* 2018). It results in a reduced growth rate, high incidence of diarrhea and pale and emaciated conditions in piglets (Larkin and Hannan 1985, Peters and Mahan 2008). The piglets suffering from IDA lose body condition rapidly and have thin-walled hearts and edema in the lungs, muscles, and connective tissues (Knight and Dilger 2018, Perri *et al.* 2016). If IDA is not treated in time, the piglets may experience rapid breathing and sudden death. Piglets experience this condition during nursing, weaning, and post-weaning periods (Perri *et al.* 2016). Supplementation of iron is the only effective way to control IDA in piglets. Iron may be administered either parenterally or orally.

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Nowadays, it's a common practice to give an intramuscular injection of iron dextran to day 3-6 old piglets (Svoboda and Drabek 2005, Svoboda *et al.* 2017). Sometimes an injection of iron can cause stress, and increase the chances of infection and iron overdosing in animals (Roberts 1998, Loh *et al.* 2001, Dong *et al.* 2020). Sometimes, parenteral iron administration can show conflicting results despite an injection of 200 mg of iron to the piglets after farrowing (Perri *et al.* 2017). Therefore, oral supplementation of iron is sometimes preferred to be safe without any chance of infection and overdosing and easy to be administered to the piglets as either paste or can be mixed with drinking water. Hansen (1998) and Loh *et al.* (2001) reported that iron supplementation with drinking water was able to combat anemia and higher Hb levels as compared to non-supplemented animals were observed (Hansen 1998, Loh *et al.* 2001). However, in oral iron supplementation, the dose and timing of iron supplementation are very important to control IDA in piglets. Moreover, a single dose may not be sufficient to avoid iron deficiency anemia in piglets (Chen *et al.* 2019). On the other hand, overdosing is also harmful; a single dose of iron (200 mg generally practiced) either administered parent rally or orally may cause iron toxicity due to iron overload and can cause sciatica nerve paralysis (Holmgren 1996, Hansen 1998, Miller and Ullrey 1997). In the present study, we evaluated the effect of two iron supplementation doses (30 mg/kg body weight and 150 mg/kg body weight) which were supplemented orally on the 2nd, 7th, 10th, and 15th-day post birth. The effect of iron supplementation on growth parameters, hemoglobin level, serum, liver, and spleen iron level, and serum ferritin level was evaluated.

MATERIALS AND METHODS

Ethical approval

Experiments were conducted humanely under the relevant national and institutional guidelines. The protocol of the current experiment was approved by the Institute Animal Ethics Committee (IAEC).

Experimental animals and design

The experiment was conducted at the institute pig farm of ICAR-Central Inland Agricultural Research Institute, Andaman and Nicobar Islands, India (11.6060° N, 92.7058° E) on Andaman local pig, indigenous pig germplasm of Andaman and Nicobar Islands. A total of 60 day-old piglets (Initial body weight: 1.34 ± 0.23 kg) of the Andaman local pig breed from ten farrowings were selected and participated in a 28-day study (up to weaning). The piglets were allowed to stay with their

respective mothers (sows) throughout the experimental period. On day 0 (at birth), the piglets were randomly divided into three groups (Fig. 1) with 20 piglets in each group (10 male and 10 female). There were 1 male and 1 female piglet per treatment for each sow. The Control group didn't receive any iron supplementation whereas the two treatment groups received ferrous sulfate supplementation at a dose rate of 30 mg/kg body weight (T1) and 150 mg/kg body weight (T2) respectively. Ferrous sulfate supplementation was given on the 2nd, 7th, 10th, and 15th day of birth. During that period, sows were allowed to nurse their piglets and standard managerial practices were followed.

Growth performance

The body weight of individual piglets was recorded on days 0, 7, 14, 21, and 28 after birth. Average daily gain (ADG) was calculated for individual piglets by subtracting initial body weight from final body weight divided by the number of days each piglet was in the study.

Blood sample collection

Blood samples were drawn on days 0, 7, 14, 21, and 28 by jugular venipuncture using a 22-gauge needle. After collection, blood samples were divided into two parts, one part was kept in a vacutainer coated with heparin (Hebei Xinle Sci & Tech Co., Ltd. Hebei Province, China) for hematological analysis and the other part was transferred to a vacutainer containing clot activator (J. K. Diagnostics, Rajkot, India) for serum separation. For serum separation, the blood samples were centrifuged at $500 \times g$ for 5 mins at 25 °C and the separated serum samples were stored in cryovials at -80°C for further analysis.

Hemoglobin estimation using Sahli's Haemoglobinometer

20 µl of fresh whole blood was taken in Sahli's graduated hemoglobin tube and mixed with N/10 hydrochloric acid solution to convert hemoglobin to brown color acid hematin. This was then diluted with distilled water till the brown color matched with the brown glass standard. The hemoglobin value (g/dl) was read directly from the scale.

Serum iron content and total iron binding capacity (TIBC)

Serum iron and TIBC were estimated using a commercially available kit (Iron/TIBC kit, Coral Clinical systems, Goa, India). Ferrozine/MgCO₃ method was employed for the estimation. The wavelength used for both tests was 570 nm.

Measurement of serum ferritin concentration

Serum ferritin was measured by sandwich enzyme-linked immune-sorbent assay technology using a commercial kit (Porcine Ferritin ELISA Kit, Life Technologies, Delhi, India) as per the protocol recommended by the manufacturer.

Measurement of iron and copper content in liver and spleen

At the end of the experiment, 3 piglets from each group were sacrificed and liver and spleen samples were collected for estimation of iron and copper content. The samples were collected aseptically, washed in 1X Phosphate Buffered Saline (PBS), and then kept immediately in sterile plastic centrifuge tubes (Genaxy Scientific Pvt. Ltd., Delhi, India) in an ice box. Organ Fe and Cu concentration was determined by using a flame atomic absorption spectrometer (AAS4141, Electronics Corporation of India Limited, Hyderabad, India). Liver and spleen samples were dried in a hot air oven for 24 h and fresh and dry weights were recorded. Dried samples were taken in a crucible and the crucible containing the dried sample was then kept in a muffle furnace at 500 °C for 2 h. The crucible was cooled and its weight was recorded carefully. The increase in the weight of the crucible gives the weight of acid soluble ash. 10 ml dilute HCl (1:1) was added to the crucible containing ash, covered with a watch glass, and digested for 20-30 mins in the water bath. Watch glass was removed and the contents in the crucible were rinsed with 5 % HCl solution. The contents were filtered through What man filter paper No. 42 into a 50 ml volumetric flask. The contents were mixed properly and preserved for the estimation of Fe and Cu.

Statistical analysis

The data were presented as mean \pm standard deviation. Data were analyzed using GraphPad Prism software (<http://www.graphpad.com>). The statistical difference among groups was determined by one-way analysis of variance (ANOVA), $p < 0.05$ was considered statically significant.

RESULTS AND DISCUSSION

Growth performance

The results of growth performance like body weight and average daily gain (ADG) are presented in Table 1. No significant difference in body weight of piglets was observed among the three groups up to day 14 of age. But, at 21 and 28 days of age, the piglets in the supplemented groups (T1 and T2) recorded significantly higher body weight compared to control piglets. There

was no significant difference in body weight between the two supplemented groups (T1 and T2) at all the time points of the study. Regarding ADG, the three groups did not show any significant difference for 0-14 days. On the other hand, supplemented groups (T1 and T2) showed significantly higher ADG as compared to the control group for days 14-28 and overall ADG (days 0-28).

In the present study, supplemented groups (T1 and T2) recorded significantly higher body weights compared to control piglets on days 21 and 28 post birth and significantly higher ADGs as compared to the control group for days 14-28 and overall ADG (day 0-28) (Table 1). The results of our study are in agreement with Murphy *et al* (1997) who reported a positive correlation between Hb level and body weight gain in the piglets. It was noted in an earlier study in piglets that higher body weight was not related to higher Hb levels, instead had higher iron requirements making them more prone to IDA (Perri *et al.* 2016). In our study, piglets that received iron dosages showed improved body weight and daily gain than the group which didn't receive any iron dose. Some of the iron doses which were earlier reported to be necessary to maintain adequate Hb concentrations and body weight gain are 25 mg/kg (Braude *et al.* 1962) and 35-40 mg/kg of body weight (Egeli and Framstad 1998). The improved growth performance observed in our study is supported by many studies (Bhattarai and Nielsen 2015a; Szudzik *et al.* 2018).

Table 1. Growth parameters (body weight and average daily gain) of control and supplemented piglets.

Days	Control	T1	T2
Body weight (kg)			
0D	1.36 \pm 0.11	1.32 \pm 0.14	1.34 \pm 0.12
7D	2.11 \pm 0.21	2.13 \pm 0.19	2.22 \pm 0.19
14D	3.18 \pm 0.15	3.24 \pm 0.18	3.28 \pm 0.15
21D	4.16 \pm 0.25 ^A	4.39 \pm 0.11 ^B	4.48 \pm 0.12 ^B
28D	5.07 \pm 0.19 ^A	5.47 \pm 0.13 ^B	5.61 \pm 0.16 ^B
ADG (kg)			
0-14D	0.130 \pm 0.015	0.137 \pm 0.018	0.139 \pm 0.011
14-28D	0.135 \pm 0.014 ^A	0.159 \pm 0.018 ^B	0.166 \pm 0.010 ^B
0-28D	0.132 \pm 0.013 ^A	0.148 \pm 0.014 ^B	0.153 \pm 0.011 ^B

(Data are presented as mean \pm standard deviation. ^{A,B}Different superscripts in the same row indicate significant difference. Control group didn't receive any iron supplementation whereas T1 and T2 received FeSO₄ @ 30 mg/kg body weight and 150 mg/kg body weight respectively on day 2nd, 7th, 10th and 15th post birth.)

Control of iron deficiency anaemia in piglets through 2-7-10-15 module of oral iron supplementation

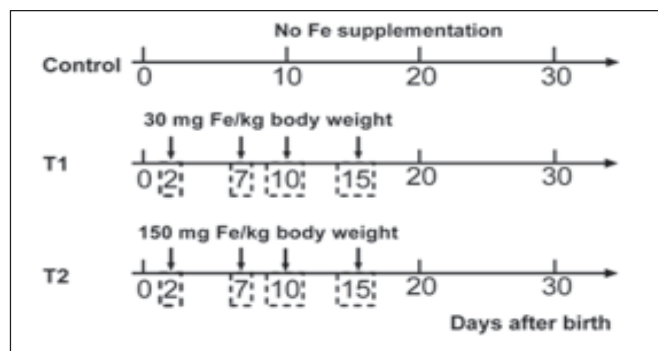


Fig. 1. Schematic diagram of experimental groups. (Numbers within boxes indicate the days on which iron supplementation was given to the piglets).

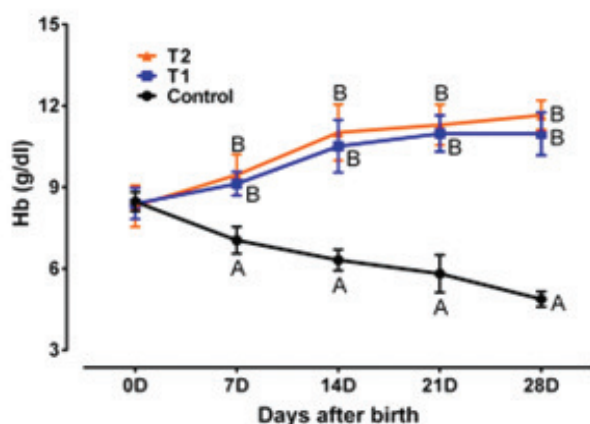


Fig. 2. Haemoglobin concentration of control and supplemented piglets. (Data are presented as mean \pm standard deviation. ^{A,B}Values with different superscripts in a particular time point indicate significant difference among the groups. Control group didn't receive any iron supplementation whereas T1 and T2 received FeSO₄ @ 30 mg/kg body weight and 150 mg/kg body weight respectively on day 2nd, 7th, 10th and 15th post birth).

Haemoglobin concentration

Hb (g/dl) levels (Fig. 2) of piglets of all groups were almost similar on zero-day. But from 7-day post birth onwards, the Hb concentrations of supplemented groups (T1 and T2) were significantly higher than the control group. On the other hand, the two supplemented groups did not show any significant difference in Hb concentration in any of the time points of measurement. On day 28 post birth, the Hb concentration of the control group was 4.88 ± 0.28 g/dl which indicated that the piglets were suffering from severe iron deficient anemia. The values (g/dl) of Hb concentration of T1 and T2 were normal (>0.9 g/dl), 10.97 ± 0.79 , and 11.66 ± 0.56 respectively at day 28 post birth.

Iron status and anemia in piglets are generally evaluated based on Hb level as 80-90 % of iron is utilized for the

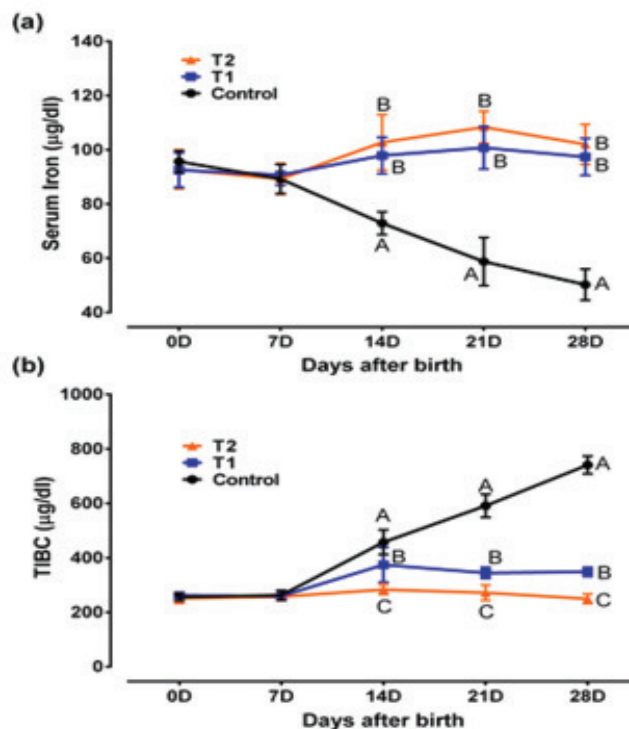


Fig. 3. Serum iron level (a) and total iron binding capacity (b) of control and supplemented piglets. (Data are presented as mean \pm standard deviation. ^{A,B,C}Values with different superscripts in a particular time point indicate significant difference among the groups. Control group didn't receive any iron supplementation whereas T1 and T2 received FeSO₄ @ 30 mg/kg body weight and 150 mg/kg body weight respectively on day 2nd, 7th, 10th and 15th post birth).

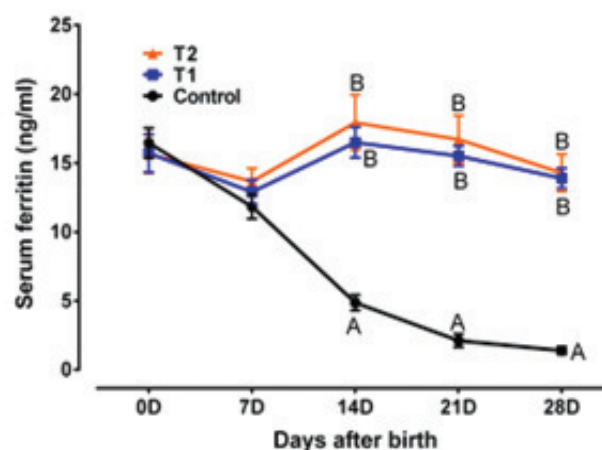


Fig. 4. Serum Ferritin level of control and supplemented piglets. (Data are presented as mean \pm standard deviation. ^{A,B}Values with different superscripts in a particular time point indicate significant difference among the groups. Control group didn't receive any iron supplementation whereas T1 and T2 received FeSO₄ @ 30 mg/kg body weight and 150 mg/kg body weight respectively on day 2nd, 7th, 10th and 15th post birth).

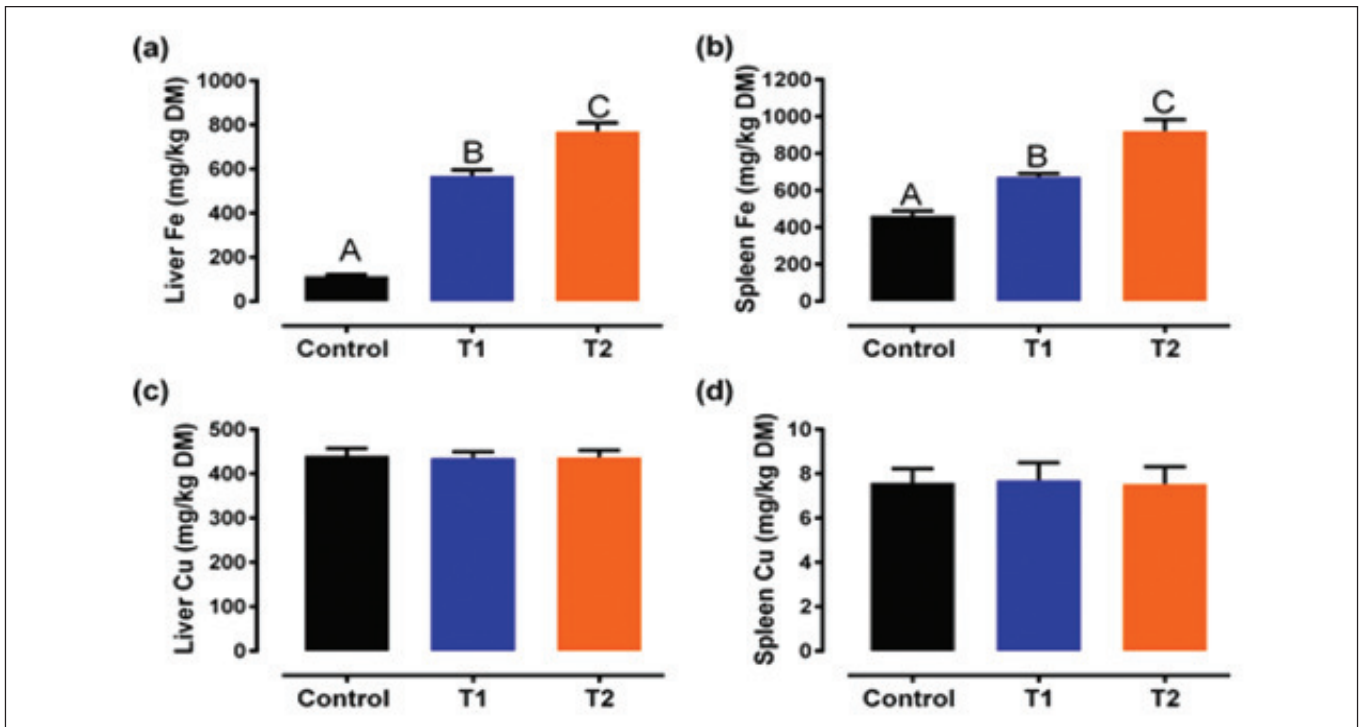


Fig. 5. Organ iron and copper of control and supplemented piglets. [(a) Liver iron, (b) Spleen iron, (c) Liver copper and (d) Spleen copper. Data are presented as mean \pm standard deviation. ^{A,B,C}Values with different superscripts indicate significant difference. Control group didn't receive any iron supplementation whereas T1 and T2 received FeSO_4 @ 30 mg/kg body weight and 150 mg/kg body weight respectively on day 2nd, 7th, 10th and 15th post birth].

formation of Hb (Perri *et al.* 2016). Different researchers suggested different levels of Hb for the diagnosis of anemia. The threshold value for Hb count is 8.0 g/dl and below this indicates anemia (NRC 1979, Perri *et al.* 2016). As per Wei *et al.* (2005), an Hb level below 6 g/dl is anemic whereas more than 10 g/dl is normal. On the other hand, Svoboda and Drabek (2005) suggest that a Hb level below 8 g/dl is anemic. As per Bhattarai and Nielsen (2015b), an Hb level below 9 g/dl is anemic. In the present study, control piglets at weaning developed anaemia as the average Hb value was 4.88 ± 0.28 g/dl (Fig. 2) On the other hand, the values (g/dl) of Hb concentration of T1 and T2 were normal (>0.9 g/dl), 10.97 ± 0.79 and 11.66 ± 0.56 respectively at weaning. Due to the lower Hb in a control group, the group lagged in body weight, serum iron levels, liver and spleen iron content, and serum ferritin. The groups which received iron supplementation showed more than double Hb concentration which is reflected in their body weight, serum iron, organ iron, and ferritin.

Serum iron content, total iron binding capacity (TIBC), and serum ferritin level

No significant difference in serum iron level (Fig. 3a) was observed among the groups on day 0 and day 7 post

birth. Significantly higher values of serum iron in both supplemented groups than control group were observed on day 14, day 21 and day 28 post birth whereas the two supplemented groups did not show any variation on those days.

Total iron binding capacity (Fig. 3b) of the control group was significantly higher than the two supplemented groups on day 14, day 21, and day 28 post birth whereas no significant difference among the groups was observed on day 0 and day 7 post birth. Moreover, T2 showed significantly lower values of TIBC than T1 on day 14, day 21, and day 28 post-birth.

Control and supplemented groups (T1 and T2) didn't show any significant variation in serum ferritin concentration on day 0 and day 7 post birth. On the other hand, serum ferritin levels in T1 and T2 were significantly higher than in a control group from day 14 onwards, whereas no significant difference between T1 and T2 was observed (Fig. 4).

Extracellular iron, serum iron, and TIBC are all important indicators for assessing the iron status in piglets. There is growing evidence that serum ferritin is the most sensitive marker for both animal and human iron reserves (Furugouri *et al.* 1983, Theil *et al.* 2007). TIBC, serum

iron, and ferritin are believed to be interrelated and are reliable indices to understand the development of anemia. In the present study, it was found that serum iron levels and ferritin were significantly lower in the control group as compared to supplemented groups (Fig. 3 and Fig. 4). From this we can understand that iron supplementation is necessary for piglets to maintain an iron level and only sow's milk is not sufficient. To better understand the relation between Hb, serum iron, ferritin, and TIBC, it can be concluded that groups that were given iron supplementation had metabolically digested the iron and that was reflected in their hematological indices. The results of our study were also supported by a former study by Smith *et al* (1984).

Liver and spleen iron and copper content

Iron levels in the liver (Fig. 5a) and spleen (Fig. 5b) were significantly higher in supplemented groups (T1 and T2) as compared to the control group. Between the supplemented groups, T2 recorded significantly higher levels of liver iron and spleen iron than T1. No significant variation in the liver (Fig. 5c) and spleen copper (Fig. 5d) concentration was observed among the three groups.

A positive correlation between organ (liver and spleen) iron concentration and diet iron level has been reported (Abbaspour *et al.* 2014). Significantly higher iron storage in the liver in the spleen in supplemented groups indicated higher absorption of iron from a feed. The results of the study are consistent with the findings of Li *et al* (2018) and Chen *et al* (2019).

It is well accepted that the supplementation of ferrous sulphate preparations is the standard treatment for iron deficiency due to its good bioavailability and efficacy (Santiago 2012). In the present study, however, an absorption rate of ferrous sulfate was not investigated. In a classical study by Marsh *et al* (1943), it was found that following supplementation of ferrous sulfate for several weeks, the absorption rate was around 50 %. Moreover, retention of large amounts of iron did not influence the hemoglobin values. In the present study also, no significant difference in hemoglobin levels between animals supplemented with the low and high doses of ferrous sulfate was observed. Other several studies in humans (Jacobs *et al.* 1984, Saha *et al.* 2007, Yasa *et al.* 2011) and others (Geisser and Muller 1984, Jacobs 1987, Sharp and Srai 2007) investigated the effects of ferrous salts supplementation on iron status and it was found that absorption was low and several dietary factors influenced absorption of non-heme iron. Most of the studies were undertaken in humans or the rodent model. The absorption rate, toxicity-related study, and molecular mechanism of

non-heme iron supplementation in the porcine model should be investigated in the future in detail.

CONCLUSION

Considering the results from the study, it can be concluded that both the groups of iron supplementation showed higher Hb, body weight gain, and higher serum iron and ferritin compared to the un-supplemented group. Moreover, iron should be supplemented in more than one dose at different intervals such as days 2, 7, 10, and 15 with proper monitoring of Hb level. If only one dose of iron is administered, there are chances that in the 3 and 4th-week post birth the piglet can have a depletion of iron reserves in the body and starts to show slow growth and weakness. In conclusion, supplementation of iron at the rate of 30 mg/kg body weight on 2-7-10-15 day post birth improved growth performance, improved serum as well as stored iron and organ iron status.

ACKNOWLEDGEMENTS

The authors are thankful to NABARD, Port Blair for providing the fund to carry out the experimental work. The authors are also thankful to the Directorate of Agriculture, Andaman and Nicobar Administration, Haddo, Port Blair for providing facilities to conduct serum and liver iron estimation using an Atomic absorption spectrometer.

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Control of iron deficiency anaemia in piglets through 2-7-10-15 module of oral iron supplementation

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***Cite this article as:** Sawhney S, Bhattacharya D, Ponraj P, Sunder J, Sujatha T, Mondal S, Chakurkar EB, De AK (2022) Control of iron deficiency anaemia in piglets through 2-7-10-15 module of oral iron supplementation. *Explor Anim Med Res* 12(2): 187-194. DOI: 10.52635/eamr/12.2.187-194.