Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Performance response of neonatal calves to milk enriched with organic iron

Melika Hamedi, Abdolmansour Tahmasbi and Abbasali Nasserian*

Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran

*Corresponding author,
E-mail address:
tahmasebi@ferdowsi.um.ac.ir

Received: 01 Jun 2022,
Accepted: 17 Aug 2022,
Published online: 05 Dec 2022,

ORCID
Melika Hamedi
0000-0001-5117-8560
Abdolmansour Tahmasbi
0000-0001-5979-9783
Abas-Ali Naserian
0000-0003-1179-6262

Abstract The current study aimed to investigate the effects of different sources of organic iron (native product in comparison to foreign ones) in the milk on plasma mineral concentration (Fe, Cu, Ca, P, and Zn), body weight, starter intake, feed conversion ratio (FCR), health condition, body measurements, and fecal score. Female calves (n=36) were randomly allocated to 3 groups (12 calves per treatment) and fed control milk (no iron supplement) (C), a low dose of iron (LF) consisting of 400 mg of Fe supplementation with the purity of 10% iron (native) and high dose of iron (HF) which had 200 mg of Fe supplementation with 20% purity (foreign). All animals were kept in individual pens and had ad libitum access to starter feed and water during the experimental period. From the first day of age to weaning (56 days), starter intake was measured daily. Body weight, skeletal measurements, fecal score, and health parameters were monitored weekly until weaning age. Calves that received the iron supplements had higher plasma Fe concentration, average daily gain (ADG) and starter intake, in contrast, they had low Cu, Ca, and P content and FCR than the control group. Supplementing milk with iron resulted in improved health status. Results showed that native Fe had the same impacts as the foreign product. There were no notable effects of treatment on skeletal parameters, fecal score, heart rate, respiratory rate, and rectal temperature. According to this experiment, applying iron supplements in diets requires taking into account the iron supply via other sources such as milk and or solid feeds. According to this experiment, although calves' health and performance improved regardless of iron sources, the amount of iron supplied through other sources such as milk or solid feed should also be considered, when using iron supplements in the diet.

Keywords: calf health, iron supplement, neonatal calves, performance

Introduction

The early life of calves is the critical state for their productive period, which can conceivably play an incredible role in their performance, health condition, and future milk production (Lopes et al., 2021). Neonatal calves do not have a functional rumen, it seems that they are more similar to monogastric animals that must pick up their required nourishing ingredient from milk (Xiao et al., 2021). In the early stages of life, calves suffer from a lack of immune systems. Therefore, this condition makes them face vital challenges, mainly the risk of morbidity and death (Wu et al., 2021).

One of the most significant negative aspects of milk feeding is the incidence of gastrointestinal diseases, such as diarrhea (Qadeer et al., 2021). On the other hand, milk cannot provide all nutrients required by the calf's immune system, especially iron. Whole milk is a weak source of iron and its iron concentration is in the range of 0.3 to 0.6 mg/kg (Allan et al., 2020), which means that young calves rear on milk are at risk of anemia.

Iron plays critical roles in skeletal growth, hematopoiesis (hemoglobin and myoglobin), enzymatic activity (Wysocka et al., 2020), immunity, and antioxidant defenses (cytochrome oxidase, peroxidase, and catalase) in the body, therefore it is considered as an essential trace element (Harvey. 2008). Accordingly, the great challenge of infancy is adequate iron supply, which leads to iron deficiency if not achieved. Anemia caused by iron deficiency has been reported in dairy calves (Joerling and Doll, 2019). Suckling calves, the only bovine group, are generally at risk of iron deficiency and to which supplemental iron needs to be provided. Iron deficiency anemia is a more common problem for veal calves but not in young bulls that are fed a formulated diet (Bami et al., 2008). The iron requirement of animals varies that depends on their age, sex, and physiological stages. Young animals are more sensitive to iron deficiency in the early stages of life, but neonatal have some iron stores in their bodies. Based on the National Research Council recommendations (NRC, 2001), a six-week-old calf with 0.9 kg dry matter (DM) intake/day requires 100 mg of iron/kg DM. Whole milk alone cannot meet the calf's needs for iron. Although there are different sources of iron, like hay and cereals, due to the industrial rearing of calves and the lack of access to soil-contaminated feed, they cannot meet their requirements from feed sources (Ramin et al., 2014). This problem explains the necessity of iron supplementation in diets.

In neonatal calves, iron supplementation has improved growth rate and disease resistance (Atyabi et al., 2006). Mohri et al. (2010) indicated that a remarkable increase in total weight gain and mean daily weight gain without any notable effect on red blood cell (RBC) parameters in calves that received the iron supplement. Some experiments reported an increased hemoglobin concentration and performance before pre-weaning periods in calves that received iron supplementation orally or by injection (Völker and Rotermund, 2000). Bami et al. (2008) stated that parenteral iron supplementation enhanced weight gain in one-month age dairy calves. On the other hand, increased iron concentration can have toxic effects on animal health, intestinal cells, permeability, and absorption of other elements. In most dairy farms, added iron supplements to the diet are non-organic sources, which can undergo oxidation, react with other substances and increase insolubility (Podder et al., 2021).

There are some inhibitors which play as a antagonism role of iron absorption (phytates, polyphenols, calcium, and protein), which can reduce the bioavailability of iron in the body. Since amino acid chelated iron is the main form of iron-absorbed by the body, so in comparison to inorganic iron, which should transform to chelate salts; organic one can be absorbed directly, without competing with other minerals (Yu et al., 2000). Therefore, in animal nutrition, to avoid deficiency and increase the bioavailability of the element, most research focuses on using iron chelate or organic iron-containing compounds. The purpose of the present study was to evaluate the impact of two sources of protein complex iron (with different purity of iron) on growth parameters, health scores, and some other minerals in neonatal calves during the milk feeding period.

Materials and methods

This study was conducted at the dairy research center (Faculty of Agriculture, Ferdowsi University of Mashhad) in Mashhad, Iran. All procedures and animal handling were approved by the Agricultural Animal Care and Use Committee of the Ferdowsi University of Mashhad, which were done based on the Iranian Council of Animal Care (1995).

Animals and experimental design

Thirty-six Holstein Friesian heifer calves were weighed immediately after birth, isolated from their dams, and kept in individual pens (mean birth weight 40.11 ± 1.99 kg). They were fed by dam's colostrum for the first three days of life, and then from the fourth day up to 56 days of life; they received four L of herd milk two times a day (7 AM and 7 PM). The calves were randomly allocated to 3 treatment groups (12 calves per treatment): 1) control group (C) was fed a control diet (without iron supplement), 2) a low dose of iron (LF) consisting of 400 mg of Fe supplementation with the purity of 10% (native product), and 3) high dose of iron (HF) which contained 200 mg of Fe supplementation with 20% purity (iron-Glycinate, BSAF, Germany). Our hypothesis was to supply the same scale of pure iron from two different iron supplements. We wanted to examine whether the native product (LF) was comparable to the foreign one (HF) in calf performance. We tried to compensate for the low purity of the native product by increasing the amount of this Fe source: therefore, the calves received the same amount of iron but from two different origins.

All animals had *ad libitum* access to the starter feed (20% of barley, 40% of corn, 29% of soybean meal, 4% of sugar beet pulp, 4.85% of wheat bran, 0.5% of calcium carbonate, 0.7% of dicalcium phosphate, and 1.0% of vitamin and mineral mix on a dry matter basis) and freshwater during the experiment. To stimulate pre-stomach development, solid feed, composed of starter and alfalfa hay; was offered to all calves. The starter refusals were weighed every morning to obtain the daily starter intake (SI). Samples of the starter and alfalfa hay and feed residual of each calf were collected and analyzed. Calves were weighed weekly before the morning milk feeding. Body weight (BW) and body measurements including the heart girth (circumference of the belly before feeding), body length (distance between the points of shoulder and rump), withers height (distance from the base of the front feet to withers), hip height (distance from the base of the rear feet to hook bones) and hip-width (distance between the points of hook bones) were measured at 56 days of age.

Blood sampling and analysis

Blood was collected from the jugular vein into an evacuated tube containing K2 EDTA on day 56 of age for mineral determination (Becton Dickenson, Rutherford, -

NJ). Blood samples were centrifuged at 1,200 × g for 15 min at 4°C, and plasma samples were transferred into microtubes and stored at −80°C until further analysis (Thavasu et al., 1992). Iron plasma concentrations were analyzed by spectrophotometer method using commercial kits (Pars Azmon, Iran).

Animal health condition monitoring

The calves were monitored daily for clinical symptoms such as dehydration, vitality, and general health. Health conditions (fecal scores and respiratory rate) were recorded on the last day of the experiment (56 days of age). Feces were scored for consistency, color, and fluidity based on the method described by Larson et al. (1977) on a scale of 4 (1 = normal, 2 = soft, 3= runny feces, and 4 = watery with splatters). If calves had a fecal score of 2 or higher, they were treated before morning feeding with electrolyte solution (5.5–7.0% sodium, 1.65% potassium; solution (2 kg/calf).

Heart rate (HR) values were obtained with a stethoscope placed on the left chest wall, respiratory rate (RR) (breaths/min) was recorded by counting the movements of the abdominal muscles on the flanks during respiration, and rectal temperature (RT) was measured before morning feeding at the end of the experiment, following the procedures of Feitosa and Gonçalves (2014). All these measurements were done by one person to eliminate human errors, and data were recorded when the calves were standing in the cages. Starter and hay were periodically sampled after drying by oven at 55°C, and they were ground through 1-mm mesh for determination of the DM, ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to AOAC (2002) as follows 89.76% DM, 19.32% CP, 17.23% NDF, 8.12% ADF, 3.20% EE, and 5.80% ash on a DM basis.

Statistical analysis

The data were analyzed using the PROC GLM (SAS, 2008) in a completely randomized design, with the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is the variable, μ is the mean of value, T_i is the effect of treatment, and ε_{ij} is the effect of experimental error.

The Tukey's test was applied for the comparison of means (P<0.05).

Results

The changes in final BW, average daily gain (ADG), SI, and feed conversion ratio (FCR) from birth to 56 days are shown in Table 1. Final BW was significantly affected by organic iron supplementation, HF and LF had higher BW than the control (P<0.01). SI was higher in calves that received a high dose of iron supplement compared to control (P<0.01). ADG was significantly affected by using the iron supplements. Among the three groups, HF had the highest ADG (P<0.01). Supplementing organic iron decreased significantly FCR, calves that received HF had the lowest FCR (P<0.01).

The LF and HF calves had higher plasma Fe concentrations and lower plasma Cu, Ca, and P concentrations compared to the control (P<0.01 Table 2).

The results of the respiratory rate, heart rate, rectal temperature, and fecal score are presented in Table 3. The highest respiratory rate was recorded in the first week of age, decreasing gradually to 38.3 per min at 56 days of age. There were no significant differences among groups in heart rate during the study. Rectal temperature, fecal score, heart rate (Table 3), and skeletal growth (including withers height, body length, heart girth, hip-width, and hip height) were not significantly affected by the sources of iron supplement (Table 4).

Table 1. BW, ADG, SI, FCR in calves that were fed with iron supplemented milk

Item	Treatment			SEM	P-Value
	Control[1]	Low Fe[2]	High Fe[3]		
Final body weight(kg)	67.37^{b}	69.34^{a}	69.85^{a}	0.506	0.010
Average daily gain (g/d)					
Day 1-56	475.9^{c}	498.8^{b}	539.5^{a}	4.306	0.000
Starter intake (kg/d)					
Day 1-56	0.53^{b}	0.54^{ba}	0.56^{a}	0.004	0.003
Feed conversion ratio (FCR)					
Day 1-56	2.31^{a}	2.22^{b}	2.08^{c}	0.022	0.000

[1]Calves did not receive any Fe supplementation,
[2]Calves that received 400 mg of Fe supplementation which contained 10% pure Fe,
[3]Calves that received 200 mg of Fe supplementation which contained 20% pure Fe
[a,...,c]Within row, means with common letters are not different (P>0.05).

Discussion

The obtained results of the current study approved that iron supplements improved BW and ADG, which were the consequence of increased SI, while Zhang et al. (20-16) stated that high iron content in the rat's diet resulted in a decrease in BW and ADG. In contrast with our results, Bostedt et al. (2000) found that iron supplementation had not any significant effects on the weight gain of neonatal calves. Similar to our results, Bami et al. (2008) observed an increase in ADG in receiving iron calves. An increase in ADG during the first weeks of calf's life can improve first lactation yield and mammary tissue (Allan et al., 2020).

Including lactoferrin (iron-transport protein) in the diet of neonatal calves increased per-weaning weight gain and heart girth gain (Joslin et al., 2002). Heinrichs et al. (2017) expressed that BW significantly correlated to heart girth, and they stated that a heart girth is a good tool in determining the BW of pre-weaned calves in commercial farms. Regarding this point that measuring heart girth with a meter is a practical alternative tool for determining a calf's weight instead of the weight scale, it should be considered that there is a tendency to overestimate the BW of animals that are less than 150 kg in this method, that may result in management and nutritional problems (Sherwin et al., 2021). Although no significant difference in heart girth was found among treatments in the current experiment, a tendency to increase was observed in iron-supplemented groups. Additionally, using visual estimates for predicting BW can be affected by breed, age, physiological states, and also nutrition, as we observed the significant increase in BW of calves and the non-significant increase in skeletal growth.

In the experiment of Lindt and Blum (1994), calves that were fed with milk replacer which contained iron (10 mg Fe/kg of milk replacer) developed marked anemia and reduced growth performance than those fed with milk replacer with 50 mg Fe/kg. They concluded that this amount of Fe would seem physiologically appropriate for calves. Since the calves of the current experiment received 40 mg of iron in their consuming milk, a tendency to increase in SI was obtained over the entire experiment for calves that received HF from birth to weaning age. Based on the obtained results, iron is probably the reason for greater SI, which led to improved performance. Since iron deficiency decreases feed intake, ADG, and growth (Ceppi and Blum., 1994), supplying iron to calves can improve SI and growth (Radostits et al., 2000). As one of the complications of iron deficiency anemia is the decline in appetite (Ghrayeb et al., 2020), it is supposed that the proper amount of iron in the diet is needed for normal appetite and glucose utilization (Ceppi et al., 1994). In agreement with the current study, the notable growth response to applying iron in calves, in those fed by hay and starter along with milk, was observed (Mohri et al., 2006; Bami et al., 2008). The proper growth in the neonatal period of calf's life, is critical for whole-of-life performance (Diaz et al., 2001). During the neonatal phase, growth occurs mainly in the bone and muscle, and feed efficiency is higher in this stage rather than in an adult (Nejad et al., 2013). In the present study, FCR is statistically affected by iron supplements during the first eight weeks of age. Our results were in agreement with McFarlane et al. (1988), who mentioned that low iron content in diet negatively affected weight gain, FCR, and calf's health. However, in another study, adding iron supplementation to the diet of veal calves had no statistically impact on BW, FCR, and body size (Cui et al., 2016). Although in the current study, control calves did not suffer from anemia, the lower BW, ADG, and SI in them indicated the iron content of the diet (milk and starter) was not sufficient to meet the calf's requirement for normal growth and performance.

Table 2. Plasma Fe, Cu, Ca, P, and Zn concentrations in calves that were with by iron supplemented milk

Item (mg/kg)	Treatment			SEM	P-Value
	Control[1]	Low Fe[2]	High Fe[3]		
Fe	1.531[b]	1.699[a]	1.764[a]	0.480	0.005
Cu	0.833[a]	0.712[b]	0.707[b]	0.012	0.000
Ca	117.8[a]	115.8[b]	114.5[b]	0.507	0.000
P	62.83[a]	60.83[b]	60.58[b]	0.438	0.002
Zn	1.177	1.171	1.169	0.020	0.965

[1]Calves did not receive any Fe supplementation
[2]Calves that received 400 mg of Fe supplementation which contained 10% pure Fe
[3]Calves that received 200 mg of Fe supplementation which contained 20% pure Fe
[a, b] Within row, means with common letters are not different (P>0.05).

There are variations in the blood Fe concentrations in young calves, and it can cover wide ranges from 55.84 μg/dL to 167.53 μg/dL (Kupczyński et al., 2017). On the other hand, the mechanism of iron absorption is not complete in early life, and this may affect the concentration of iron. During the first week of life, blood iron concentration dropped (Rajabian et al., 2017), which confirms the necessity of iron supplementation. Iron is an essential trace element related to growth and is critical for various metabolism activity such as enzyme co-factors. According to the current study, both organic iron supplements had the same statistical effect on the plasma Fe concentration. It is needed to mention that the first sign of iron deficiency is the reduction of blood Fe concentration, which is rare in calves reared by milk replacer due to the excellent iron content (Joerling and Doll, 2019). According to Yu et al. (2000), using organic iron (iron from an amino acid complex) led to an increase in pig's serum iron, which can affect animal growth and fight diseases and infections, that observed in piglets (Li et al., 2018). In line with our findings, applying organic iron to lambs resulted in high iron levels (Asadi et al., 2022). As iron is a key nutrient that is involved in health and immunity, the proper levels of it are needed for optimal immune responses (Mohus et al., 2018), it is the reason for the high prevalence of infection in iron deficient veal calves (Gygax et al., 1993).

According to the antagonistic relationship of iron with other minerals, applying organic iron to milk resulted in a reduction of plasma Cu, Ca, and P concentrations. Trace minerals are in an antagonistic or synergistic relationship. Some minerals such as zinc, calcium, copper, and chromium are in an antagonistic relationship with iron, so an abundance of them lead to problem in iron absorption (Angelova et al. 2014), in severe condition, they result in iron deficiency. In other aspects, some minerals are in synergistic relation with iron which can be a helpful tool for diagnosing the reason for an iron deficiency. The deficiency of minerals that are in synergistic relation with iron, especially those that are participated in iron metabolism, results in iron deficiency (Angelova et al. 2014). Copper acts as a co-factor of active enzymes in superoxide dismutation, electron transport, and iron oxidation (de Romaña et al., 2011). It was shown that high consumption of iron had a preventing impact on Cu uptaking (Humphries et al., 1983). The excess iron as ferrous sulfate and ferric citrate in the lamb's diet resulted in a decline of plasma Cu while having no impact on Ca, P, and Mg (Sefdeen, 2017). Cu supplementation can negatively impact serum Fe (Van den Top, 2005). As divalent metal transporter1 (DMT1) is a mediator which transports both Fe and Cu, that builds a competitive condition between Fe and Cu in transport and bioavailability (Arredondo and Núñez, 2005). Applying iron to the lamb's diet increased plasma Fe concentration and decreased the Cu significantly, but it had no impact on Zn, Ca, and P (Ali Arabi et al., 2018). A similar study reported that ferrous carbonate in lamb's diet decreased plasma Cu statistically and did not affect P and Zn concentration (Prabowo et al., 1988). Applying high iron levels to the lamb's diet (more than 600 ppm) led to a reduction in P absorption (Mejia Haro et al., 2009). Calcium has a reverse impact on iron absorption, Ca consumption generally prohibits iron uptaking (Lönnerdal, 2010). Like to Ca (Walczyk et al., 2014), phosphates are famous inhibitors of iron absorption (Nakao et al., 2015).

Applying inorganic iron has economic reasons, particularly iron dextran and iron sulfate. However, recent studies are focusing on using organic forms, which can easily be absorbed by high bioavailability, which is started in monogastric animals, and approved that using an organic form of iron is more efficient than inorganic type (Kupczyński et al., 2017) due to their readily absorption.

Newborn calves are susceptible to respiratory and gastrointestinal disorders when fed milk (Ring et al., 2018). Iron deficiency in calves can lead to more risk of infection in the respiratory and gastrointestinal tract (Franciosi et al., 2018). The results confirmed that probably the experimental calves did not suffer any iron deficiency during the trial; therefore, respiratory or gastrointestinal diseases were not reported. The normal range for heart rate is 100-140 beats per minute (Mee, 2008), so our finding is considered on a standard scale. The HR of calf declined from 115.50±2.94 to 102±0.75 bpm at birth. This reduction continued up to 30 days of life (Piccione et al., 2010). The reason for high HR in calves was the low systolic volume in newborn calves, which means that the heart needs to pump blood at a higher systolic rate to the cardiovascular system. As calf's systolic volume is low at birth; thus, for the inability to change the cardiac output, the systolic volume should be hardboard by an increase in heart rate. Algers et al. (2006) expressed that iron-deficient calves had exhibited several adaptations, including elevated heart rhythm and urinary noradrenaline altering reactivity of the hypothalamic-pituitary-adrenal (HPA) axis. The non-significant differences between treatments indicated that calves did not suffer any iron deficiency, even in the control group.

In neonatal calves, metabolically regulating body temperature is incomplete (NRC, 2001). The environmental stress can affect metabolic and physiological conditions and changes (Klopp et al., 2021). In calves, the physiological range of rectal temperature is 38.1 to 39.2 °C, so obtained data from these studies were considered close to the normal range. Burfeind et al. (2010) stated that the sources of error for RT mainly are due to procedure, device, and depth of insertion into the rectum and defecation, they stated that the error could amount to as much as ± 0.5°C, and this is a part of or data explanation.

Table 3. Respiratory rate, heart rate, rectal temperature and fecal score in calves that were fed with iron supplemented milk

Item	Treatment			SEM	P-Value
	Control[1]	Low Fe[2]	High Fe[3]		
Respiratory rate	38..65	38.06	38.13	0.238	0.18
Heart rate	102.10	101.80	102.80	0.960	0.78
Rectal temperature	38.02	38.16	38.22	0.754	0.20
Fecal score[4]	1.42	1.40	1.38	0.025	0.52

[1]Calves did not receive any Fe supplementation
[2]Calves that received 400 mg of Fe supplementation which contained 10% pure Fe
[3]Calves that received 200 mg of Fe supplementation which contained 20% pure Fe
[4]a classification scale of fecal score was described as follow (1 = normal, 2 = viscous feces, 3= runny feces, and 4 = runny feces with splatters)
Within row, means with common letters are not different (P>0.05)

Iron metabolism in the body is complicated. Hurrell and Egli (2010) claimed that there is no physiological mechanism for iron excretion. Most iron requirements (90%) are supplied by endogenous sources, especially from the breakdown of circulating red cells. Digestive diseases are more common in neonatal calves, which can lead to a reduction of the immune system and death in calves, as diarrhea (Rell et al., 2020, Marcato et al., 2021). Consequently, it can affect ADG (Machado and Ballou, 2022) and carcass characteristics (Tautenhahn et al., 2020). To prevent the adverse deathful effects of diarrhea, scoring the feces is a helpful and wise method. The color of feces and its consistency indicated the health situation of the calf's digestive tract. Fecal scoring based on color and consistency is the most helpful and accurate way to evaluate animal's health (Larson et al., 1977). Graham et al. (2018) expressed that immediate identification and rehydration of affected calves with diarrhea is critical before the calves develop clinical signs of dehydration. By getting old, there is a tendency to improve the feces consistency; however, calves receiving iron supplements had harder feces than the control group. Neuberger et al. (2016) reported that iron deficiency is one of the noninfectious factors implicated in the development of calf diarrhea. Based on this conclusion and our observation (not identified diarrhea in all treatments), it can be postulated that experimental calves did not have iron deficiency.

Table 4. Body measurements in calves that were fed with iron supplemented milk

Item (cm)	Treatment			SEM	P-Value
	Control[1]	Low Fe[2]	High Fe[3]		
Withers height	86.78	87.47	87.45	1.14	0.885
Body length	76.91	78.16	78.23	0.933	0.536
Heart girth	93.87	95.48	94.08	1.17	0.933
Hip width	21.66	21.72	21.77	0.524	0.988
Hip height	89.13	89.32	89.38	0.536	0.945

[1]Calves did not receive any Fe supplementation
[2]Calves that received 400 mg of Fe supplementation which contained 10% pure Fe
[3]Calves that received 200 mg of Fe supplementation which contained 20% pure Fe

There was a tendency to increase skeletal growth by aging. That may be attributed to greater solid feed intake, metabolically and physiologically, which indicates better digestive tract performance in these group animals. Although body weight gain and skeletal parameters growth were affected by feed intake, Marcondes and Silva (2021) expressed that during the neonatal period, the body size is mainly affected by genetic potential, nutrition, and management policy. Despite the significant starter consumption and ADG, it was expected that skeletal body size would also be affected by treatments. The results obtained from this experiment are consistent with other reports (Ferreira et al., 2013, Santos et al., 2015). The trial error may be responsible for non-significant changes. Additionally, when a calf has access to limited amounts of milk, it has a low growth rate in comparison to a calf that has ad libitum access to milk (van Niekerk et al., 2021). In the present study, they fed only 4 L milk per day, and this may be explained by the reason for the differences.

In the current study, anemia was not observed in control calves and none of the calves did not suffer from infections or diseases. As the decrease in plasma Fe can have an impact on immune response and calf's performance, obtained results of our study indicated that applying iron, whether from homemade or foreign commercial brand products, at a dose of 40 mg/ day, improved growth, performance, and health in neonatal calves. According to our hypothesis, which was the investigation of the bioavailability of organic iron from, two different sources with different purity, in most determined items two iron supplementation had the same effect. Just in calves that received high purity of iron, FCR was practically lower than two other groups.

Conclusion

Our data indicated that iron supplements with different sources increased plasma Fe concentration, ADG, SI, and decreased FCR compared to the control group. Two different organic iron supplementation with different purity (foreign and native products) had a similar impact on the calf's general health condition and feed intake, which indicated the same function of native Fe in comparison to foreign products. The obtained data of the control group have shown that the iron content in milk satisfied the basic iron requirements, so increasing iron via supplementation improved animal performance. Further trials should be focused on high iron concentrations in the diet of calves and measuring iron-related parameters in the blood, such as hemoglobin and in the liver, as well as the hepcidin-ferroportin axis and cytotoxicity. It is recommended for future study regarding iron metabolism in pre-ruminate animals, the functional difference in iron absorption, and the effect of this element in the body on genome and proteome assessment.

Acknowledgments

We want to thank the staff of the Dairy Research Center of the Ferdowsi University of Mashhad for their help and cooperation during this study.

Statement of Ethics

All procedures and animal handling were approved by the Agricultural Animal Care and Use Committee of the Ferdowsi University of Mashhad, Iran (Approval no: 326/458/2018).

Conflict of Interests

No potential conflict of interest was reported by the author(s).

Funding

The current study was supported by a grant from the Ferdowsi University of Mashhad, Iran (grant number: 3/45769).

References

Algers, B., Broom, D., Canali, E., Hartung, J., Smulders, F., van Reenen, C., Veissier, I., 2006. The risk of poor welfare in intensive calf farming systems: an update of the Scientific Veterinary Committee Report on the Welfare of Calves. *European Food Safety Authority Journal* 366, 1-36.

Ali Arabi, H., Zand, N., Bahari, A., Hajivaliei, M., Zaboli, K., 2018. Effect of iron source on performance, some minerals, thyroid hormones and blood metabolites of Mehraban male lambs. *Journal of Animal Science Research* 28, 77-92. (in persian)

Allan, J., Plate, P., Van Winden, S., 2020. The effect of iron dextran injection on daily weight gain and haemoglobin values in whole milk fed calves. *Animals* 10, 853-863.

Angelova, M.G., Petkova-Marinova, T.V., Pogorielov, M.V., Loboda, A.N., Nedkova-Kolarova, V.N. and Bozhinova, A.N., 2014. Trace element status (iron, zinc, copper, chromium, cobalt, and nickel) in iron-deficiency anaemia of children under 3 years. *Anemia* 2014, 718089-718096.

AOAC., 2002. Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

Arredondo, M., Núñez, M.T., 2005. Iron and copper metabolism. *Molecular Aspects of Medicine* 26, 313-327.

Asadi, M., Toghdory, A., Hatami, M., Ghassemi Nejad, J., 2022. Milk supplemented with organic iron improves performance, blood hematology, iron metabolism parameters, biochemical and immunological parameters in suckling dalagh lambs. *Animals* 12, 510-521.

Atyabi, N., Gharagozloo, F., Nassiri, S., 2006. The necessity of iron supplementation for normal development of commercially reared suckling calves. *Comparative Clinical Pathology* 15, 165-168.

Bami, M.H., Mohri, M., Seifi, H.A., Tabatabaee, A.A., 2008. Effects of parenteral supply of iron and copper on hematology, weight gain, and health in neonatal dairy calves. *Veterinary Research Communications* 32, 553-561.

Bostedt, H., Hospes, R., Wehrend, A., Schramel, P., 2000. Effects of the parenteral administration of iron preparations on the iron supply status during the early development period of calves. *Tierärztliche Umschau* 55, 305-315.

Burfeind, O., Von Keyserlingk, M., Weary, D., Veira, D., Heuwieser, W., 2010. Repeatability of measures of rectal temperature in dairy cows. *Journal of Dairy Science* 93, 624-627.

Ceppi, A., Mullis, P.E., Eggenberger, E. and Blum, J.W., 1994. Growth hormone concentration and disappearance rate, insulin-like growth factors I and II and insulin levels in iron-deficient veal calves. *Annals of Nutrition and Metabolism* 38, 281-286.

Ceppi, A. and Blum, J.W., 1994. Effects of growth hormone on growth performance, haematology, metabolites and hormones in iron-deficient veal calves. *Journal of Veterinary Medicine Series A* 41, 443-458.

Cui, K., Tu, Y., Wang, Y.C., Zhang, N.F., Ma, T. and Diao, Q.Y., 2016. Effects of a limited period of iron supplementation on the growth performance and meat colour of dairy bull calves for veal production. *Animal Production Science* 57, 778-784.

de Romaña, D.L., Olivares, M., Uauy, R., Araya, M., 2011. Risks and benefits of copper in light of new insights of copper homeostasis. *Journal of Trace Elements in Medicine and Biology* 25, 3-13.

Diaz, M.C., Van Amburgh, M.E., Smith, J.M., Kelsey, J.M. and Hutten, E.L., 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. *Journal of Dairy Science* 84, 830-842.

Feitosa, F., Gonçalves, F., 2014. Semiology of the respiratory system of large animals. In: Feitosa, F.L.F. (Eds.), Veterinary Semiology urinary incontinence: the art of diagnosis. 3rd ed. Roca, Sao Paulo, Brasil, pp. 313-331.

Ferreira, L., Bittar, C., Silva, J., Soares, M., Oltramari, C., Nápoles, G., Paula, M., 2013. Performance and plasma metabolites of dairy calves fed a milk replacer or colostrum silage. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 65, 1357-1366.

Franciosi, C., Rocha, T.G., Fagliari, J.J., 2018. Hematological and biochemical parameters of neonatal Holstein calves supplemented with iron. *Pesquisa Veterinaria Brasileira* 38, 234-243.

Ghrayeb, H., Elias, M., Nashashibi, J., Youssef, A., Manal, M., Mahagna, L., Refaat, M., Schwartz, N. and Elias, A., 2020. Appetite and ghrelin levels in iron deficiency anemia and the effect of parenteral iron therapy: A longitudinal study. *PloS One* 15, 0234209-0234222.

Graham, A., Renaud, D., Duffield, T., Kelton, D., 2018. Calf cleanliness does not predict diarrhea upon arrival at a veal calf facility. *Journal of Dairy Science* 101, 3363-3366.

Gygax, M., Hirni, H., Wahlen, R.Z., Lazary, S. and Blum, J.W., 1993. Immune functions of veal calves fed low amounts of iron. *Journal of Veterinary Medicine Series A* 40, 345-358.

Harvey, J.W., 2008. Iron metabolism and its disorders. *Clinical Biochemistry of Domestic Animals* 6, 259-285.

Heinrichs, A., Heinrichs, B., Jones, C., Erickson, P.S., Kalscheur, K., Nennich, T., Heins, B.J., Cardoso, F., 2017. Verifying Holstein heifer heart girth to body weight prediction equations. *Journal of Dairy Science* 100, 8451-8454.

Humphries, W., Phillippo, M., Young, B., Bremner, I., 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. *British Journal of Nutrition* 49, 77-86.

Hurrell, R., Egli, I., 2010. Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition* 91, 1461S-1467S.

Joerling, J., Doll, K., 2019. Monitoring of iron deficiency in calves by determination of serum ferritin in comparison with serum iron: A preliminary study. *Open Veterinary Journal* 9, 177-184.

Joslin, R., Erickson, P.S., Santoro, H., Whitehouse, N.L., Schwab, C.G., Rejman, J., 2002. Lactoferrin supplementation to dairy calves. *Journal of Dairy Science* 85, 1237-1242.

Klopp, R.N., Yoon, I., Eicher, S., Boerman, J.P., 2021. Effects of feeding Saccharomyces cerevisiae fermentation products on the health of Holstein dairy calves following a lipopolysaccharide challenge. *Journal of Dairy Science 105*, 1469-1479.

Kupczyński, R., Bednarski, M., Śpitalniak, K., Pogoda-Sewerniak, K., 2017. Effects of protein-iron complex concentrate supplementation on iron metabolism, oxidative and immune status in preweaning calves. *International Journal of Molecular Sciences* 18, 1501-1512.

Larson, L., Owen, F., Albright, J., Appleman, R., Lamb, R., Muller, L., 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. *Journal of Dairy Science* 60, 989-991.

Li, Y., Yang, W., Dong, D., Jiang, S., Yang, Z., Wang, Y., 2018. Effect of different sources and levels of iron in the diet of sows on iron status in neonatal pigs. *Animal Nutrition* 4, 197-202.

Lindt, F., Blum, J., 1994. Occurrence of iron deficiency in growing cattle. *Journal of Veterinary Medicine Series A* 41, 237-246.

Lönnerdal, B., 2010. Calcium and iron absorption—mechanisms and public health relevance. *International Journal for Vitamin and Nutrition Research* 80, 293-299.

Lopes, R.B., Bernal-Córdoba, C., Fausak, E., Silva-del-Río, N., 2021. Effect of prebiotics on growth and health of dairy calves: A protocol for a systematic review and meta-analysis. *PloS One* 16, e0253379-e0253387.

Machado, V.S., Ballou, M.A., 2022. Overview of common practices in calf raising facilities. *Translational Animal Science* 6, 234-245.

Marcato, F., van den Brand, H., Kemp, B., Engel, B., Schnabel, S., Hoorweg, F., Wolthuis-Fillerup, M., van Reenen, K., 2021. Effects of transport age and calf and maternal characteristics on health and performance of veal calves. *Journal of Dairy Science* 105, 1452-1468.

Marcondes, M.I., Silva, A.L., 2021. Determination of energy and protein requirements of preweaned dairy calves: A multistudy approach. *Journal of Dairy Science* 104, 11553-11566.

McFarlane, J.M., Morris, G.L., Curtis, S.E., Simon, J. and McGlone, J.J., 1988. Some indicators of welfare of crated veal calves on three dietary iron regimens. *Journal of Animal Science* 66, 317-325.

Mee J.F, 2008. Managing the calf at calving time. Proceedings of the 41st Annual Conference, American Association of Bovine Practitioners, North Carolina, USA, 228-230.

Mejia Haro, I., Brink, R.D., Mejia Haro, J., 2009. Effects of inclusion of different levels of iron in lamb diets on apparent absorption and retention of phosphorus. *Journal of Animal and Veterinary Advances* 8, 19-22.

Mohri, M., Sarrafzadeh, F. and Seifi, H.A., 2006. Effects of oral iron supplementation on haematocrit, live weight gain and health in neonatal dairy calves. *Iranian Journal of Veterinary Research* 7, 34-37.

Mohri, M., Poorsina, S., Sedaghat, R., 2010. Effects of parenteral supply of iron on RBC parameters, performance, and health in neonatal dairy calves. *Biological Trace Element Research* 136, 33-39.

Mohus, R.M., Paulsen, J., Gustad, L., Askim, A., Mehl, A., DeWan, A.T., Afset, J.E., Asvold, B.O., Solligard, E. and Damas, J.K., 2018. Association of iron status with the risk of bloodstream infections: results from the prospective population-based HUNT Study in Norway. *Intensive Care Medicine* 44, 1276-1283.

Nakao, M., Yamamoto, H., Nakahashi, O., Ikeda, S., Abe, K., Masuda, M., Ishiguro, M., Iwano, M., Takeda,

E. and Taketani, Y., 2015. Dietary phosphate supplementation delays the onset of iron deficiency anemia and affects iron status in rats. *Nutrition Research* 35, 1016-1024.

Nejad, J.G., Hosseindoust, A., Shoae, A., Ghorbani, B., Lee, B.H., Oskoueian, E., Hajilari, D., Amouzmehr, A., Lohakare, J.D. and Sung, K.I., 2013. Effects of feeding levels of starter on weaning age, performance, nutrient digestibility and health parameters in Holstein dairy calves. *Asian-Australasian Journal of Animal Sciences* 26, 827-830.

Neuberger, A., Okebe, J., Yahav, D., Paul, M., 2016. Oral iron supplements for children in malaria-endemic areas. *The Cochrane Database of Systematic Reviews* 2, CD006589-CD006718.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th rev. National Academy Press, Washington DC, USA.

Piccione, G., Casella, S., Pennisi, P., Giannetto, C., Costa, A., Caola, G., 2010. Monitoring of physiological and blood parameters during perinatal and neonatal period in calves. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 62, 1-12.

Podder, R., Glahn, R.P., Vandenberg, A., 2021. Iron-and zinc-fortified lentil (Lens culinaris Medik.) demonstrate enhanced and stable iron bioavailability after storage. *Frontiers in Nutrition* 7, 614812-614823.

Prabowo, A., Spears, J., Goode, L., 1988. Effects of dietary iron on performance and mineral utilization in lambs fed a forage-based diet. *Journal of Animal Science* 66, 2028-2035.

Qadeer, M.K., Bhatti, S.A., Nawaz, H., Khan, M.S., 2021. Effect of milk or milk replacer offered at varying levels on growth performance of Friesian veal calves. *Tropical Animal Health and Production* 53, 1-8.

Radostits, O.M., Mayhew, I.G. and Houston, D.M., 2000. Veterinary clinical examination and diagnosis. WB Saunders, London, UK.

Rajabian, F., Mohri, M., Heidarpour, M., 2017. Relationships between oxidative stress, haematology and iron profile in anaemic and non-anaemic calves. *Veterinary Record* 181, 265-265.

Ramin, A., Asri-Rezaei, S., Paya, K., Eftekhari, Z., Jelodary, M., Akbari, H., Ramin, S., 2014. Evaluation of anemia in calves up to 4 months of age in Holstein dairy herds. *Veteriner Fakültesi Dergisi (Istanbul)* 40, 1-6.

Rell, J., Wunsch, N., Home, R., Kaske, M., Walkenhorst, M., Vaarst, M., 2020. Stakeholders' perceptions of the challenges to improving calf health and reducing antimicrobial use in Swiss veal production. *Preventive Veterinary Medicine* 179, 104970-104980.

Ring, S., McCarthy, J., Kelleher, M., Doherty, M., Berry, D., 2018. Risk factors associated with animal mortality in pasture-based, seasonal-calving dairy and beef herds. *Journal of Animal Science* 96, 35-55.

Santos, F., De Paula, M., Lezier, D., Silva, J., Santos, G., Bittar, C., 2015. Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. *Animal* 9, 958-965.

SAS, 2008. SAS User's Guide: Statistics. Version 9.2. SAS Institute Inc., Cary, North Carolina. USA.

Sefdeen, S., 2017. Effect of Dietary Iron on Copper Metabolism of Sheep. Ph.D. Thesis, Harper Adams University, United Kingdom.

Sherwin, V., Hyde, R., Green, M., Remnant, J., Payne, E., Down, P., 2021. Accuracy of heart girth tapes in the estimation of weights of pre-weaned calves. *Veterinary Record Open* 8, e16-24.

Tautenhahn, A., Merle, R., Müller, K., 2020. Factors associated with calf mortality and poor growth of dairy heifer calves in northeast Germany. *Preventive Veterinary Medicine* 184, 105154-105164.

Thavasu, P.W., Longhurst, S., Joel, S.P., Slevin, M.L. Balkwill, F.R., 1992. Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions. *Journal of Immunological Methods* 153, 115-124.

Van den Top, A.M., 2005. Reviews on the Mineral Provision in Ruminants (VIII): Iron Metabolism and Requirements in Ruminants. CVB Documentation Report Nr. 40, Centraal Veevoederbureau: Lelystad, Netherland.

Van Niekerk, J., Fischer-Tlustos, A., Wilms, J., Hare, K., Welboren, A., Lopez, A., Yohe, T., Cangiano, L., Leal, L., Steele, M., 2021. ADSA foundation scholar award: New frontiers in calf and heifer nutrition from conception to puberty. *Journal of Dairy Science* 104, 8341-8362.

Völker, H., Rotermund, L., 2000. Possibilities of oral iron supplementation for maintaining health status in calves. *Deutsche Tierarztliche Wochenschrift* 107, 16-22.

Walczyk, T., Muthayya, S., Wegmüller, R., Thankachan, P., Sierksma, A., Frenken, L. G., Thomas, T., Kurpad, A., Hurrell, R. F., 2014. Inhibition of iron absorption by calcium is modest in an iron-fortified, casein- and whey-based drink in Indian children and is easily compensated for by addition of ascorbic acid. *The Journal of Nutrition* 144, 1703–1709.

Wu, S., Li, X., Chen, X., Zhu, Y., Yao, J., 2021. Optimizing the growth and immune system of dairy calves by subdividing the pre-weaning period and providing different milk volumes for each stage. *Animal Nutrition* 7, 1296-1302.

Wysocka, D., Snarska, A., Sobiech, P., 2020. Iron in cattle health. *Journal of Elementology* 25, 3.

Xiao, J., Chen, T., Alugongo, G.M., Khan, M.Z., Li, T., Ma, J., Liu, S., Wang, W., Wang, Y., Li, S., 2021. Effect of the length of oat Hay on growth performance, health status, behavior parameters and rumen fermentation of Holstein female calves. *Metabolites* 11, 890-906.

Yu, B., Huang, W.J. and Chiou, P.W.S., 2000. Bioavailability of iron from amino acid complex in weanling pigs. *Animal Feed Science and Technology* 86, 39-52.

Zhang, Y., Wang, Z., Li, X., Wang, L., Yin, M., Wang, L., Chen, N., Fan, C., Song, H., 2016. Dietary iron oxide nanoparticles delay aging and ameliorate neurodegeneration in drosophila. *Advanced Materials* 28, 1387-1393.