Needle-free injection devices versus regular injection techniques for iron supplementation to piglets.

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Contents:

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1. Abstract

Piglets are born with a small supply of iron; therefore they receive an iron injection when they are three days old. A needle-syringe device is commonly used in swine industry. Over the last years, the interest in newer techniques, like needle- free injection devices, has increased because of risk of disease transmission. There are no studies on the efficiency of needle-free injection with compressed gas. Therefore, the aim of this study is to evaluate the efficiency of needle free injection of iron in newborn piglets. Nine piglets were selected. Randomly 3 piglets were assigned to each of the three groups. In every litter there were three groups. At three days of age, the first group received an iron injection with a syringe- injection device. The second group received an iron injection with a Needle-free device (MS Pulse©). The third group did not receive an iron injection.

The results show that hemoglobin and hematocrit values are similar for syringe injection device group and the needle-free group. This is also seen with Fe concentration in the blood. The body weight of the piglets that did not receive iron was lower compared to the bodyweight of the other piglets.
The present results show that there is no difference between the syringe-injection device and the needle-free device. The piglets that did not receive an iron injection have lower hematological and iron parameters compared to the other piglets. Their body weight was also lower. Not enough iron is available in the environment in the first 26 days, to increase the iron concentration in the blood.
This paper suggests that Needle-free devices are as effective as syringe-injection devices with respect to iron administration in preventing anemia.

2. Introduction

Iron deficiency Anemia is a common disorder in the swine industry. Piglets are born with a small supply of iron and have a rapid growth; they reach four times their birth weight within three weeks. The blood volume and the number of red blood cells also have to increase, the erythroid cells of the bone marrow use the iron in the blood for the synthesis of haemoglobin. (Lipinski, Starzynski et al. 2010) The daily intake of iron should be approximately 15 mg. (Lipinski, Starzynski et al. 2010) The milk of the sow contains 1.22 mg/L of iron, if the sow did not received an iron supplemented diet. (Pond, Veum et al. 1965) Therefore, the iron supply in the body of the piglets will be too low, if the piglets drink 1 L of milk per day. The piglets develop a hypochromic, microcytair anemia. (Goff) Anaemic piglets are characterized by a rough hair coat and pale mucosal membranes. In the standard management at pig farms, the piglets receive an injection of 200 mg of iron when they are three days old. These piglets will not develop anemia. (Goff)

Iron metabolism:

Iron is an important substance in red blood cells. The balance is accomplished by the absorption of iron from the diet, and the loss of iron through waste products. If the animal looses blood it will also lose iron. (Jain 1993) When the piglet does not have enough iron, the amount of red blood cells produced by the bone marrow will be reduced. Moreover, the red blood cells have a lower content of haemoglobin. Red blood cells are the transporters of oxygen in the body. Oxygen binds to haemoglobin, specifically to the iron in the haem-group. (Goff)

There are two forms of iron, the ferrous form (Fe 2+) and the ferric form (Fe 3+). The body receives iron by uptake in the intestinal tract. (Goff) In the environment iron occurs in organic complexes, this is mainly the ferric form. In the stomach hypocloric acid releases the iron from the organic complex and converts it to the ferrous form. The ferrous form can be transported over the cell membrane; uptake of iron takes place in the upper duodenal tract. (Jain 1993)The intestines absorb iron in the ferrous form. (Goff)After uptake, the iron can be transported into the blood, or can be stored in the intestinal cells by binding to ferritin. (Goff)The binding to ferritin is in the ferric form, so the cell converts the ferrous form in the ferric form. The duodenal cell can be lost, because of cell renewal. When this happens, the iron stored in the duodenal cell is lost in the feces. (Jain 1993) Free iron in blood can be excreted by the kidney. To prevent loss of iron, iron binds to transferrin in the blood. (Goff) Red blood cells have transferrin receptors. If the transferrin-iron complex binds to the red blood cell, the transferrin releases the iron. (Goff) Iron can be stored in different ways in the liver, spleen or bone marrow, either with ferritin or with hemosiderin. (Goff)(Jain 1993)

In the early stage of anaemia, the blood smear is normocytic- normochromic, this view is also seen in healthy piglets. In a later stage the anaemia is more promined expressed and the blood cells are microcytic-hypochromic.(Jain 1993) Egali et al. (1999) concluded that piglets are born with anaemia, a low hemoglobin concentration is seen at day one. After birth the red blood cells are still immature. (EGELI, FRAMSTAD 1999)The erythrocytes of fetal blood are larger than the erythrocytes of an adult. The fetal blood values of animals increase during pregnancy. So the red blood cell count, hemoglobin and hematocrit reach the highest concentration with birth. During fetal life the mean corpuscular volume and the mean corpuscular hemoglobin decreases, also after birth they decrease and stabilize after a few months. Fetal hemoglobin will be replaced with hemoglobin A and the size of the erythrocyte will decrease to the adult form. (Jain 1993) MCV is a good indicator for anemia after birth. If the piglets do not receive iron, the cells are microcytic and the MCV value will decrease. Red

blood cell count is not an accurate parameter to diagnose anemia, since an increase in either microcytic or macrocytic cells would also cause the RBC to increase. (EGELI, FRAMSTAD 1999)

Doses control:
The doses given to piglets is a balance between two outcomes: the prevention of anaemia and the risk of iron toxicity. (Lipinski, Starzynski et al. 2010)

The most common method used in swine industry is 200 mg of iron dextran injection intra- muscular. Svoboda et al (2007) compared intra-muscular injection and subcutaneous injection of iron. Both methods were effective to restore iron concentrations in the blood. (Svoboda, Drábek 2007) Calvo and Alleu (1986) injected the piglets with a 100 mg iron injection. Low concentration of hemoglobin, hematocrit and low ferritin concentration were measured suggesting that 100 mg of iron is not enough for the piglets iron metabolism and a second injection is preferred. (Calvo, Allue 1986)

Maes et al (2011) showed that oral administration of iron was as effective as intra- muscular injection of iron. The iron-rich feed was provided three times into two weeks. Each piglet received 10 g of iron-rich feed on each period. The other group received a 1 ml injection of 200 mg iron dextran complex. Higher hemoglobin concentrations were seen with the oral uptake of iron, also the piglets performance and feed intake were similar with both treatments.(Maes, Steyaert et al. 2011) Similar studies were done in the past. Maner et al. (1959) showed that piglets receiving oral iron tablets became anemic. However, it was not sure whether the piglets actually swallowed the tablets. (Maner, Pond et al. 1959)

Iron supplementation is injected intra-muscular in the neck. Iron toxicity is not common, but death occurs quickly in the first 6 hours. (The Merck Veterinary Manuel. 2005) Lipinski et al. (2010) investigated if a split protocol can be used to minimize toxicity effect in the piglet and still have a positive effect on the metabolism of iron. Piglets received 2 injections of iron of 40 mg on the third day and on the tenth day or one dose of 100 mg on the third day. The first injections prevent the decrease of the main haematological parameters, as RBC, haemoglobin and hematocrit. Another advantage of this protocol is the prevention of high Hepc mRNA expression. (Lipinski, Starzynski et al. 2010)

Different receptors in the duodenum cells are responsible for iron transport into the cell. Lipinski et al. (2010) detected two receptors: divalent metal transporter (DMT1) and ferroportin (Fpn). In the first few days of the piglets' life the expression of these proteins is very low. At day four, the expression is detected for the first time. These first few days the enterocytes will be transformed from fetal to adult, which might be an explanation for the low expression of DMT1 and Fpn. So the absorption of iron oral in piglets is not as effective as in adult pigs. (Lipinski, Starzynski et al. 2010) Another explanation for the low uptake in the first two days is the down regulation of Fpn due to hepcidin. Hepcidin is a one of the mechanisms to regulated absorption of iron in the apical duodenum. In this study the Hepc mRNA was high in the first two days of the piglets' life; this is comparable with a 200 days old pig. After day two the Hepc mRNA declined, so theoretically it is possible for the piglet to obtain iron from the environment after day two. The iron concentration increased after iron supplementation, the ferroportin receptor is down regulated. (Lipinski, Starzynski et al. 2010)

Different studies suggested that piglets at an older age maintain their iron because there is enough iron in the environment. This suggests that piglets living in an outdoor production will have enough

iron after birth and will not develop anaemia. Szabo et al. (2002), however, showed that even in outdoor productions iron is a necessary supplement for young piglets. (Szabo, Bilkei 2002)

Needle-free device versus injection-syringe device:
A needle-syringe device is commonly used in the swine industry for vaccination, antibiotics and iron supplementation. It is easily adjustable in different situations and they are inexpensive. In the management of today efficiency, safety and animal stress are factors to take in consideration. Therefore Needle-free injection devices are the newest technology on the market. Needle- free injections can penetrate the skin or the subcutaneous tissue and thus deliver the vaccine into the tissue. (Chase, Daniels et al. 2008)

The tissue depth depends on the force generated by the injector, and ranges from the dermis to the muscle. The force that is needed to penetrate the skin is generated by a compressed gas. A small jet of the vaccine creates a hole in the skin due to erosion, and the vaccine is delivered trans dermally. An advantage for vaccines is the dispersion of the fluid through the tissue. With a needle-syringe device, there will be the formation of a bolus at the tip of the needle. The dispersion of needle-free devices is more dispersed through the tissue. The fluid follows the path of least resistance. (Chase, Daniels et al. 2008)

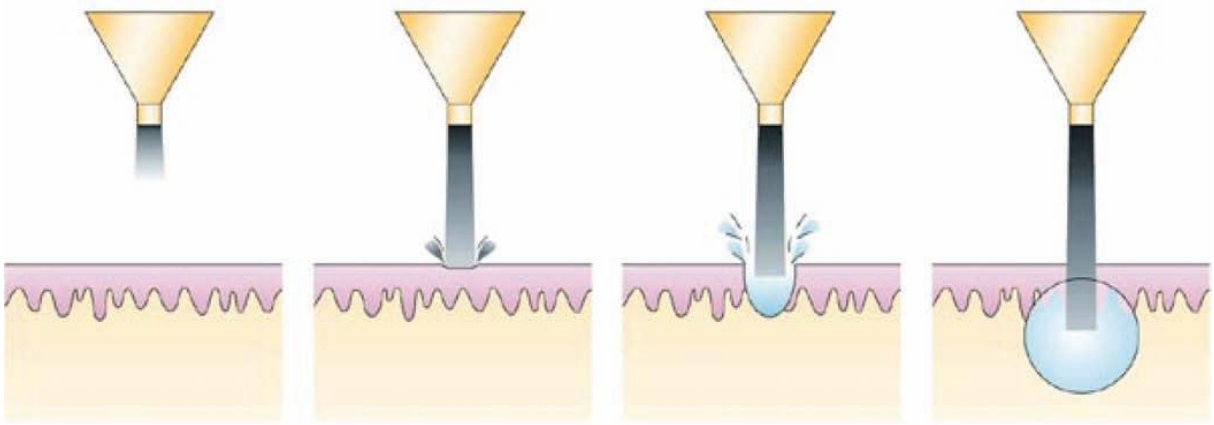


Figure 1: ''Trans dermal injection with a needle-free device. Due to increasing pressure the jet shoots out the nozzle. A hole formation is seen on the skin. The fluid that is accumulated in the hole, slows down the incoming jet. The hole formation is stopped. Distribution of the fluid is dependable of type of needle-free device, thickness of the skin, viscosity of the liquid and pressure.'' (Chase, Daniels et al. 2008)

In Houser et al. (2004) pigs received two vaccinations for *Mycoplasma hyopneumonia* and one for psuedorabiës virus (PRV). Serologic research showed that the serological response of vaccination was higher than the animals without any treatment. The conclusion of this study was that needle-free injections were as efficient as a needle-syringe device. (Houser, Sebranek et al. 2004)

The skin and subcutaneous tissue is the first barrier for pathogens, therefore, a lot of antigen-presenting cells are present. Chase et al. (2008) showed that, when the antigen is delivered to the subcutaneous tissue, many antigen-presenting cells are reached and an advanced immune response is triggered. (Chase, Daniels et al. 2008)

There are also disadvantages of needle-free injection devices. To build the system in the stable is an investment. Also the employees need training how to use the device and to maintain the system. Needle-syringe devices are easily adaptable to different situations. Injection pressure or volume should be adjusted for different pigs, treatments, doses and viscosity. (Chase, Daniels et al. 2008) Another disadvantage for using needle-free devices is that the first stream makes the skin wet. The user will think that the animal has not been properly injected, even though the correct substance has been administrated. (Chase, Daniels et al. 2008)

An injection with a needle can cause abscesses and muscle damage, this is undesirable in the meat industry. A bigger risk is the breakage of needles. When the needles are continuously used in different animals, the probability of bending and breaking gets higher. Needle tips and fragments can be detected in the meat with a metal detector. A lot of needle fragments are missed with a metal detector, so this is not a guaranty for needle free meat. There are different reasons why the needles are hard to detect. The needle exists from two or more different metals and the size is small. Also the orientation from the needle in the meat is an important factor if the needle can be detected. Needle-free injection devices would be safer for the consumption of meat. (Houser, Sebranek et al. 2004) Needle-stick injuries are common by swine veterinarians, the risk is very high because the syringe can contain infectious agents or vaccines, sedatives etc. (Chase, Daniels et al. 2008, Weese, Faires 2009)

When the surface of the skin is contaminated with bacteria the risk of translocation is higher. If bacteria penetrate the skin, there is a higher risk of abscesses formation. Ray et al. (2010) studied whether E. coli translocation decreased when a needle-free injection device is used compared to injection syringe device. The surface of the muscle was sprayed with E-coli, only the top layer was inoculated. Then the muscles received a needle-free injection or a syringe- injection, at different depts. The E-coli counts were higher for needle-free injection than for needle-syringe device, in all the different depts. (Ray, Dikeman et al. 2010) Nicolas et al. (1989) thought that if *Arcanobacterium pyogenes* was present on the surface of the pig an injection site, abscess formation would occur more with needle-free devices than needle-syringe injection. More abscesses were present after using the needle-free device. (Nicholas M. Kiefer, George R. Neumann 1989)
Houser et al. (2004) concluded that there was no difference seen in either group for muscle damage also no abscesses were found in needle-free and needle-syringe device groups. (Houser, Sebranek et al. 2004)

For the different methods of injection there are benefits and disadvantages. The aim of this study is to evaluate the efficiency of needle free injection of iron in newborn piglets. Therefore different parameters are taken into consideration. The piglets that do not receive iron develop a microcytic-hypochromic anemia and have a lower body weight. In this study the outcomes of the two different injection methods are compared and the hypothesis is that a needle free injection device is as efficient a syringe-injection devices with respect to iron administration in preventing anemia.

3. Materials and Methods

Animals:
72 piglets were used from the research farm, the Tolakker in Utrecht. From 8 litters, 9 piglets were selected and randomized in three different treatment groups with 3 piglets for each treatment. 24 piglets were given an injection with iron using a syringe-device. 24 piglets were given a needle-free injection of iron and 24 piglets didn't receive an iron injection. All piglets were housed in a box, with a half slatted plastic floor and half concrete floor. At three days of age the tails were clipped and the piglets received ear tags. 7 days after birth the piglets were given acess to solid food. Piglets could be transferred to another sow if they were weak. Weaning took place at an age of 26 days. After weaning 2 or 3 litters were mixed and kept in a pen with half concrete floor and half slatted plastic floor. Drink nipples and solid food were available ad libitum. Cage enrichment was available in the pens.

Experiment Design:
1 ml iron injection is given when the piglets are three days old. Iron was administrated to the piglets in the form of Iron Dextran (200 mg/ml). (Gleptosil from Alstoe Limited). A MSPulse© needle-free injector was used in the needle-free group. The syringe-injection device group received the injection intramuscular into the neck.

Sampling and Analyzing:
Blood samples were taken, before the first iron injection at 3 days of age. More blood samples were drawn at 14, 26 and 40 days of age. Blood samples were drawn from the jugular vein. At three days of age the piglets are small; therefore a 21 G needle is used, with a syringe. The other days a vacuum system was used with a 21G needle. 2 ml of blood is collected in Heparin and Serum tubes.
All the piglets were weighted before the blood sample was taken.

Blood analysis:
The blood was analyzed on the following parameters:
- hemoglobin,
- hematocrit,
- mean corpuscular volume
- mean corpuscular hematocrit
- mean corpuscular hematocrit concentration
- serum iron
- total iron binding capacity
- iron saturation

The morphology of the erythrocyte was analyzed at day 26 and day 40. A blood smear was made and the erythrocytes were visualized through a microscope, 400x projection. A droplet of blood is placed on a slide, with another slide, the blood is dispersed. Then the slide has to dry. After 15 minutes the slide can be colored. The procedure for coloring is: 30 seconds in fixationsolution, 4 seconds in hemacolor red and 6 seconds in hemacolor blue. If the slide is dried the erythrocytes can be visualized through a microscope, 400x projection.

Statistics:
All the data were imported in SPSS 20. A mixed model was used to compare the different treatment

groups. Iron (Fe) is the dependent factor. Different factors were imported in the model, the model that is used is time + treatment + weight + treatment * time. The random factors were litter and piglet (litter). The mean value and standard deviation was used for the graphs.

4. Results

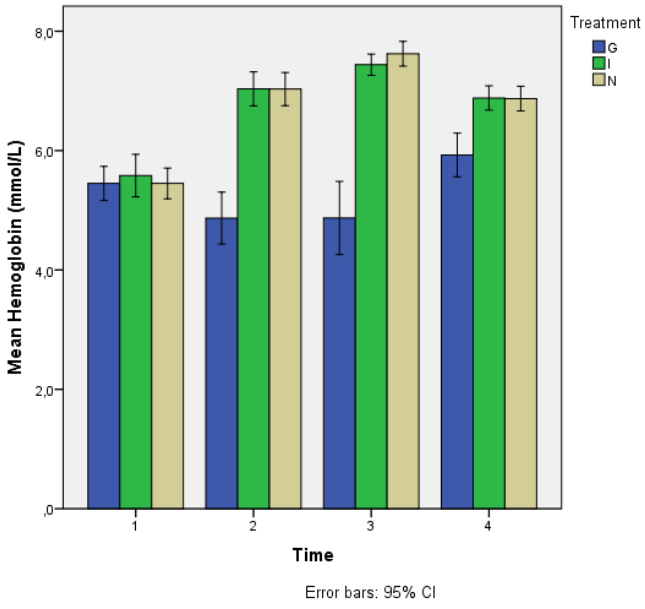
The outcomes for weight, hemoglobin, hematocrit, MCV and MCH at the different points in time of the three treatment groups are given in figure 2 and 3,.

Supplementation of iron increase hematological status of the piglets

The outcomes for weight, hemoglobin, hematocrit, MCV and MCH at the different points in time of the three treatment groups are given in figure 2 and 3. After iron supplementation the hemoglobin, hematocrit, MCV and MCH increased. The group without iron supplementation showed on day 14 (time 2) lower hemoglobin (figure 2, graph A) and hematocrit (figure 2, graph B) than on day 3(time 1).

Hematocrit (graph B) and hemoglobin (graph A) increased after day 3 (time 1) for both treatment groups. A decrease is seen after 26 day for both iron treatment groups for hematocrit and hemoglobin. The hemoglobin, hematocrit, MCV and MCH were similar for needle-free device and syringe-needle device. (figure 2)

A:



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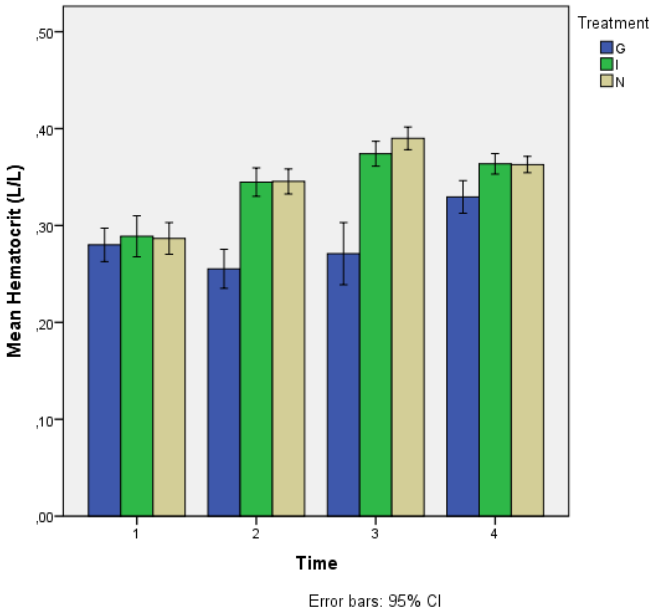

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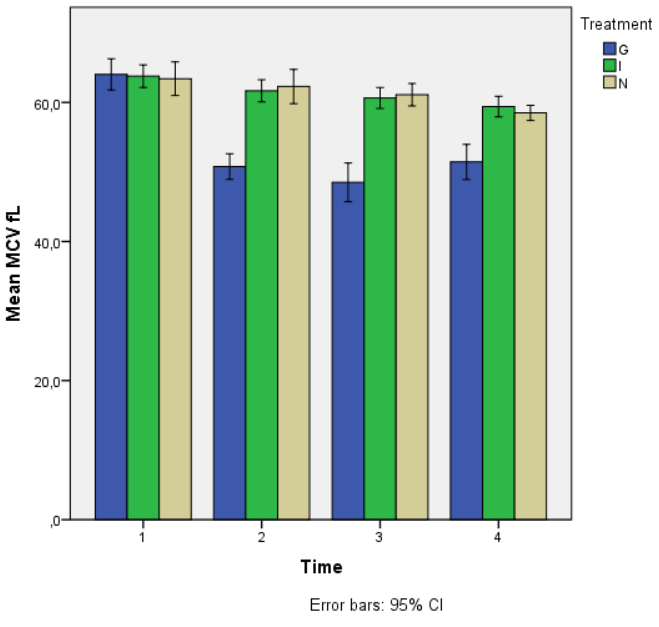

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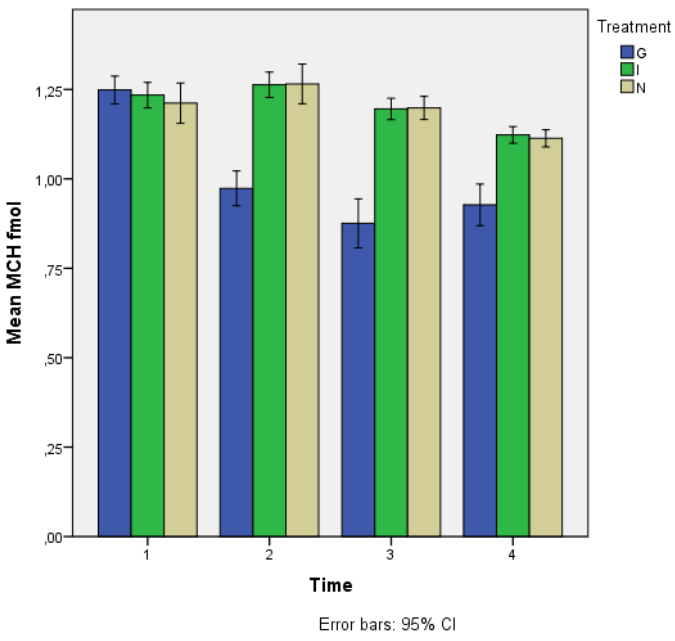


Figure 2: A. Mean Hemoglobin concentration, B. Mean hematocrit concentration, C. Mean corpuscular volume. D. Mean corpuscular hemoglobin, was determined for each group at each time point. (G. no iron, I. syringe-injection, N. needle-free injection)

The mean weight of the piglets increased in time for all the treatment groups. (figure 3)

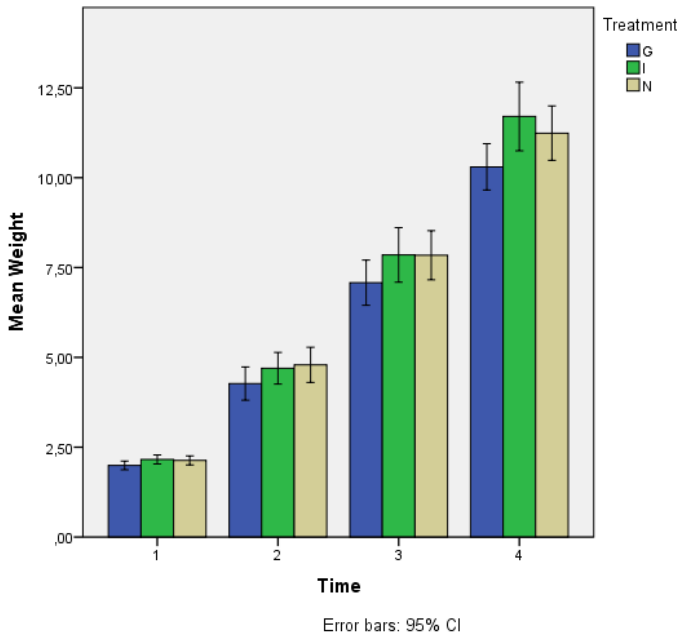
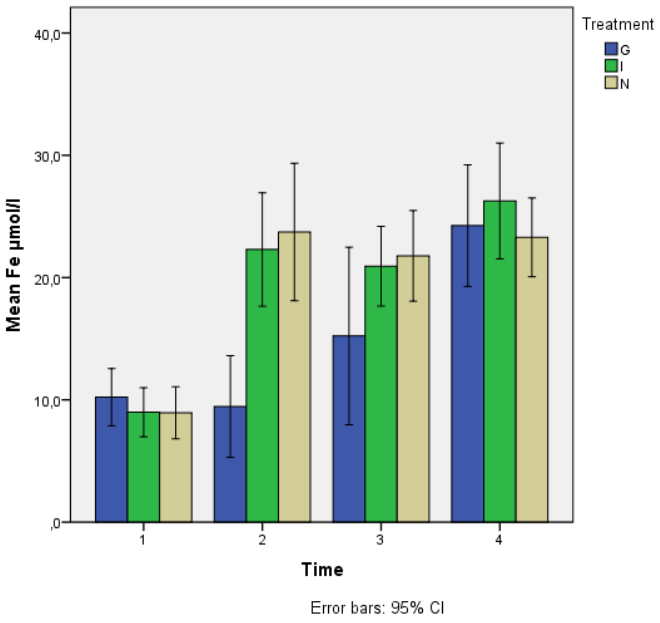


Figure 3: Weight of the piglets measured in kg, was determined for each group at each time point. (G. no iron, I. syringe-injection, N. needle-free injection)

Comparing the outcome of the different treatments:

Fe concentration increased in time for needle-free group and the injection syringe device. (Figure 4) The Fe concentration (figure 4, graph A) for the no iron group decreased between time 1 and 2, an increase is seen after time 2. A decrease of the Fe binding capacity (figure 4, graph B) was seen on time 2 for all three groups. The no iron group had an increase in Fe binding capacity (figure 4, graph B) at time 3. Fe saturation coefficients (figure 4, graph C) showed an increase for needle- free as injection syringe group.

A.



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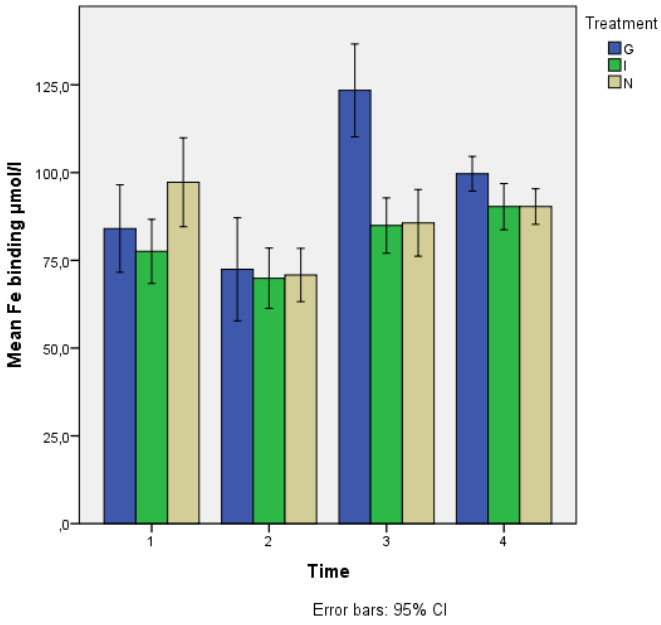

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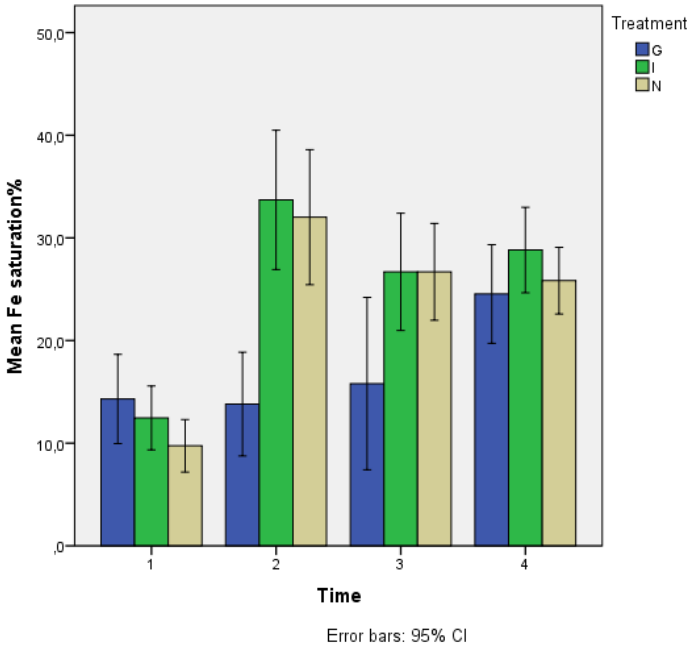

Figure 4: Iron parameters of control, needle-free and syringe group. A. Fe concentration. B. Fe binding capacity. C. Fe saturation coefficient. (G. no iron, I. syringe-injection, N. needle-free injection)

A mixed model is used to statistic evaluate the outcome.

The mixed model was: time + treatment + weight + time * treatment. The data lead to a ratio, the reference was injection with needle-syringe device on time 1; 3 days of age.

In Table 1, Time 2,3 and 4 is compared to time 1. The estimate values were increasing in time, the piglets have a higher Fe concentration on time 2, 3 and 4 compared to time 1.

Table 1: Estimate, Significant and Confidence interval for time 1,2,3 and 4.

Parameter	Estimate	Significant	95% Confidence Interval	
Age in days:			Lower Bound	Upper Bound
Time 1: 3 days	0	-	-	-
Time 2: 14 days	2.7	0.000	1.95	3.74
Time 3: 26 days	3.2	0.000	2.10	4.85
Time 4: 40 days	4.8	0.000	2.66	8.67

All the treatments were compared to the reference; treatment injection, at the different times. So treatment; no iron on time 1 was compared to treatment injection on time 1. The outcome is a ratio, where treatment injection with needle-syringe device is 1, compared to the estimate of needle-free injection and no iron treatment.

Table 2: Estimate, Significant and Confidence interval for time 1,2,3 and 4. The different treatments no iron and needle-free are compared to syringe-injection device.

Parameter	Estimate	Significant	95% Confidence Interval	
Treatment *Age in days :			Lower Bound	Upper Bound
Treatment; no iron * time 1: 3 days	1.08	0.579	0.74	1.40
Treatment; no iron *time 2: 14 days	0.34	0.000	0.25	0.48
Treatment; no iron * time 3: 26 days	0.41	0.000	0.32	0.60
Treatment; no iron *time 4: 40 days	0.80	0.170	0.58	1.11
Treatment Needle-free * time 1; 3 days	0.93	0.631	0.68	1.26
Treatment Needle-free * time 2: 14 days	1.02	0.905	0.74	1.40
Treatment Needle-free * time 3: 26 days	1.00	0.997	0.73	1.38
Treatment Needle-free *time 4: 40 days	0.86	0.320	0.63	1.16

For treatment no iron, at time 1, 2 and 3, the coefficient is smaller than 1. Therefore the mean Fe is lower for treatment no iron at time 2, 3 and 4 compared to the reference at the same time. The estimate increase in time, the ratio between the reference and no iron gets smaller.

For treatment 2 the Estimate coefficient is around 1. This means that the Fe concentration of the reference and needle-free injection are comparable at any given time.

Erythrocyte morphology:

Erythrocyte morphology was analyzed on polychromasia and anisocytose. (Figure 5) In figure 5 the polychromasia and anisocytose of the different treatment groups are given for time 3 and 4.

A: **B:**

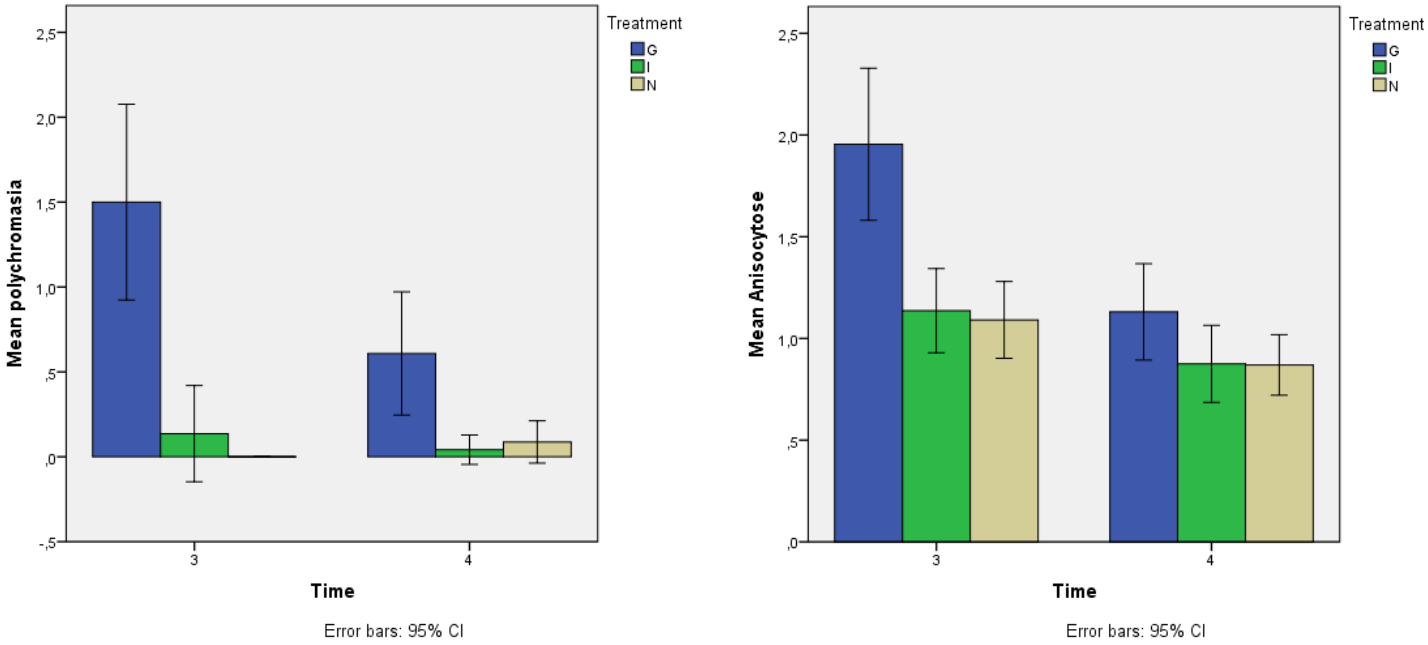

Figure 5: Polychromasia(A) and anisocytose(B) of the three groups, given for time 3 and 4.

The no iron group has a higher frequency of polychromasia and anisocytose.

5. Discussion

The goal of this study is to compare two methods of iron supplementation in piglets. Supplementation of iron using a needle- free injection device gives the same result with respect to mean Fe concentration as using an injection syringe device as seen in figure 2 and 4. The ratio between needle-free and injection device is 1:1, therefore the Fe concentration is the same for both treatment groups. The group that did not receive an iron injection had a lower ratio compared to the injection device. The Fe concentration for this group is lower, the ratio increased in time (Table 2), the difference between the groups gets smaller over time.

In figure 2 the hematological parameters, hemoglobin, hematocrit, MCV and MCH are displayed in bar charts. An increase is seen for hemoglobin and hematocrit for both iron groups, till day 26, at day 40 a slight decrease. The estimate is for day 14 and 26 almost 1, the Fe concentration is equal for both treatments. The difference between needle-free injection and syringe injection is not significant on day 14 and 26. At day 40 there is a lower ratio, the Fe concentration of Needle-free device is lower than injection device. This is also seen in graph A, figure 4.

The no iron group has lower hemoglobin, hematocrit, MCV and MCH. Iron is important for erythropoiesis. This is disturbed if the piglets don't receive iron and the piglet developed microcytair, hypochrome erythrocytes. (Goff) There was a significant difference for the no iron group on day 14 and 26 in comparison with the syringe injection device. Also in figure 4, graph A it can be seen that the iron metabolism was low in the no iron group. The mean concentration on day 14 is lower than on day 3. After day 14, the mean Fe increases, as seen in graph A, but the dispersion is high on day 26 for the no iron group. There are still piglets with low concentration of iron, but there are also piglets that have similar values as the piglets that received an iron injection. On the 40th day the piglets have similar iron values in comparison with the other two groups.
At day 14 the Fe bindings capacity decrease is slim. The iron that is received, is used for growth. The Fe concentration in the blood increases on day 14 for the injection group and for the needle-free group, the binding capacity decreases, iron saturation coefficient is increasing for both groups. The no iron group has a low Fe concentration and a low Fe saturation coefficient. A high binding capacity is seen at 26 days. At day 40 the Fe saturation and bindings capacity coefficient does not differ a lot from the other two groups.

After 7 days solid food was offered to the piglets. These piglets were held with their siblings that received iron. The piglets get older and the normal behavior of rooting increases. The iron that is available in the environment increases in time, since the piglets that receive iron, also lose iron with the feces. It is still uncertain how significant this factor is for iron metabolism in the piglet. The piglets not receiving an iron injection are able to increase their iron concentration in the blood, due to uptake in the environment and solid food. After weaning different groups of piglets are in the same pen. The iron concentration in the environment should be higher. The iron concentration of the no iron group should increase, as is seen in figure 4.

Piglets grow fast in the first few weeks. This is seen in figure 3. On day 3 the mean weight is 2 kg. There is no difference in body weight between the injection-syringe group and needle-free group. The no iron group has a lower mean weight at all the measure points than the injection group and the needle-syringe group. (figure 3) After 7 days solid food was available in the pens. All the piglets receive the same diet. After weaning, the no iron group should increase their body weight to the

same levels as the injection-syringe group. As seen in figure 4, the iron concentration is almost similar for all groups and it was expected that the body weight would not differ anymore. In this report it is shown that the difference in weight between those groups is still increasing at 40 days. Piglets that did not receive iron at birth still have a disadvantage after weaning. Iron and food is available, but to be able to gain the same weight 2 weeks after weaning, iron is essential for the piglets at birth.

Polychromosia and Anisocytose were mostly seen in the no iron group. A scale of 1,2 and 3 was given to show the difference between high percentage and a low percentage of anisocytose and polychromosia. A high percentage was a 3, a low percentage a 1. Anisocystose is seen in all blood smears at day 26. Young blood cells have a bigger volume than older erythrocytes. This is seen in all young animals. At 40 days of age not all piglets show anisocystose. (figure 5, graph B) The no iron group showed a lot of variety in erythrocyte size. Color difference was evaluated with the same scale of anisocystose. Piglets that receive iron did not show polychromosia. The no iron group did show polychromosia. In the no iron group at 26 days microcytair and hypochromic erythrocytes were also detected. This method is an interpretation. In this data anisocytose and polychromosia are seen in the no iron group, and is a sign of iron anemia.

In summary, the result show that needle-free injection device gives the same hematological and iron parameters as injection-syringe device. Also the advantage to give piglets an iron injection three days after birth an iron injection is reviewed and a higher weight is seen in the iron treatment groups. Needle-free injection device is as effective as an injection-syringe device.

6. Literature

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