EFFECT OF BOVINE LACTOFERRIN ON UTILIZATION OF ORALLY ADMINISTERED IRON IN SUCKLING PIGLETS

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Abstract

Piglets, on days 3 and 10 of age, were given orally 100 mg of Fe^{2+} as iron fumarate and 34.5 mg of Fe^{3+} from iron-saturated lactoferrin (group L) or 134.5 mg of Fe^{2+} as iron fumarate (group F). Positive control group received 200 mg of Fe^{3+} dextran i.m. on day 3 and negative control group was treated with Fe^{3+} dextran on day 21. Haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and iron concentration in blood plasma were significantly lower in group L compared to group F. The replacement of the part of the iron dose by iron from iron-saturated lactoferrin had negative effect on iron status of piglets.

Key words: piglets, haemoglobin, anaemia, iron, dextran, lactoferrin.

Material and Methods

The sows and their litters were kept in farrowing units on plastic floor until weaning. The farm had 400 Large White x Landrace breed sows. All the piglets were weaned at the age of 28 d. Prestarter was offered ad libitum to all piglets from days 7-35 (Seltek, Tekro s.r.o. Praha, iron content 238 mg/kg). The piglets were individually tattooed with a number in the ear. The piglets were divided into 4 groups (split litters). There were two experimental groups and two control groups.

Group L. The piglets (n=22) were given orally 100 mg of Fe^{2+} as iron fumarate and 34.5 mg of Fe^{3+} from iron-saturated lactoferrin on day 3 of life. The same dose was repeated on day 10. A 23% saturated bovine lactoferrin (Favea. s.r.o.) was used in the study.

Group F. On day 3 of life, the piglets (n=22) were given orally 134.5 mg of Fe^{2+} as iron fumarate. The same dose was repeated on day 10.

Group D (positive control group). The piglets (n=22) were given orally 134.5 mg of Fe^{3+} as iron fumarate on day 3 of life. The same dose was repeated on day 10.

Group A (negative control group). The piglets (n=21) were not given any iron preparation till
the 3 weeks of age. At this age, the piglets were injected i.m. with 200 mg of Fe$^{3+}$ in the form of iron dextran.

**Sampling and analyses.** Blood (2 ml) was collected from the *vena cava cranialis* on days 7, 14, 21, 28, and 35 of age. EDTA (ethylenediaminetetraacetic acid) was used as anticoagulant for the haematological examination. Heparin was used as anticoagulant for the determination of iron concentration in blood plasma.

Haematological examination included: haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The indices were determined by haematological analyzer Celtac Alfa (Nihon Kohden).

Iron concentration in blood plasma (Fe) was determined photometrically measuring iron complex with ferrozine.

The piglets were weighed (BW) at birth (day 1) and on days 7, 14, 21, 28, and 35 of age.

**Statistical analyses.** The results were evaluated statistically by analyses of variance (ANOVA). Values with $P < 0.05$ (*) and $P < 0.01$ (**) expressed significant difference between groups L and F.

**Results**

The results are presented as mean values and standard deviations of each index in Figs. 1-8.

**Differences of the examined indices among groups.**

- **Day 7.** Iron concentration in blood plasma in group F was higher than in group L ($P < 0.01$).
  - In group A, Hb ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$), MCH ($P < 0.05$) and Fe ($P < 0.01$) were found to be lower compared to groups D, L, and F.

- **Day 14.** Hb ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$), MCH ($P < 0.01$), and Fe ($P < 0.01$) in group L were significantly lower compared to group F. Hb ($P < 0.01$), PCV ($P < 0.01$), RBC ($P < 0.05$), MCV ($P < 0.01$), MCH ($P < 0.01$), Fe ($P < 0.01$) and BW ($P < 0.01$) in group A were lower than in groups L, F, and D.

- **Day 21.** Hb ($P < 0.01$), PCV ($P < 0.01$), MCV ($P < 0.01$) and MCH ($P < 0.01$) in group L were significantly lower than in group F. The same indices and Fe of groups L and F were found to be significantly lower compared to group D ($P < 0.01$). In group A, Hb, PCV, RBC, MCV, MCH, Fe, and BW were lower than in group D ($P < 0.01$).

- **Day 28.** Differences between groups were similar to those found at the age of 21d. In group L, Hb ($P < 0.01$), PCV ($P < 0.05$), MCV ($P < 0.01$) and MCH ($P < 0.01$) were lower than in group F. These indices of groups L and F were also significantly lower than in group D ($P < 0.01$). BW in group D was significantly higher compared to groups L, F and A ($P < 0.01$). Fe in groups L and F was significantly lower ($P < 0.01$) compared to groups D and A.

- **Day 35.** No differences between groups L and F were found at the age of 35d. BW in groups L, F, and A remained significantly lower ($P < 0.01$) than in group D. Fe in groups L and F was significantly lower ($P < 0.01$) compared to groups D and A.

**Dynamics of the examined indices within groups**

- **Group L.** From day 7 Hb, PCV, MCV, MCH and Fe were decreasing and reached minimum values on day 28 ($P < 0.01$). One week after weaning at the age of 35 d no further decrease in the indices was noted and there was a significant increase in Fe content ($P < 0.01$).

- **Group F.** The values of the examined indices were similar to those of group L, i.e. decreasing tendency of Hb, PCV, MCV, MCH, and Fe ($P < 0.01$) till the age of 28 d and a significant increase in Fe concentration ($P < 0.01$) one week thereafter.

- **Group D.** Between days 7 and 14, Hb, PCV, MCV, MCH, and RBC ($P < 0.01$) increased significantly. From day 14 till the end of the trial, only non-significant oscillations of haematological indices were noted. Fe showed decreasing tendency from day 7 to day 28.

- **Group A.** Between days 7 and 21, Hb ($P < 0.01$), PCV ($P < 0.05$), MCV ($P < 0.01$), MCH ($P < 0.01$), and MCHC ($P < 0.01$) decreased significantly. One week after iron dextran application at the age of 21d, there was an increase in Hb, PCV, MCV, MCH, MCHC, RBC, and Fe values (all, $P < 0.01$).

**Discussion**

In both experimental groups (groups L and F), the decreasing tendency of haematological indices and iron concentration in blood plasma indicates that iron reserves started to be depleted. Haemoglobin concentration in the groups were significantly lower compared to intramuscularly iron supplemented piglets (group D). At the age of 28 d mean values of haemoglobin concentration in experimental groups were lower than 80 g/l. The anaemic limit, i.e. the point when anaemia gives rise to clinical symptoms of disease is set by most authors at a haemoglobin concentration lower than 80 g/l (8, 22). We conclude that iron supplementation in both orally supplemented groups were inadequate under conditions of this trial.

One week after weaning no further decrease in haematological indices were found and there was a significant increase in iron concentration in blood plasma. We suggest that this was due to the fact that piglets started to receive iron from prestarter.

The decrease in the examined indices was more profound in the group of piglets which were given lactoferrin (group L). In group L Hb, PCV, MCV, MCH, and iron concentration in blood plasma were found to be significantly lower compared to group supplemented only with iron fumarate (group F). This indicates that iron status in the group that was given lactoferrin was lower.
Fig. 1. Haemoglobin concentration.

Fig. 2. Packed cell volume.

Fig. 3. Red blood cell count.

Fig. 4. MCV.

Fig. 5. MCH.

Fig. 6. MCHC.

Fig. 7. Fe concentration in blood plasma.

Fig. 8. Body weight.
Our idea of using lactoferrin in combination with oral preparation came from the following facts:

1) Bivalent iron (Fe²⁺) should be used for oral iron preparations. However, bivalent iron is not very stable in solution and is easily oxidized to the insoluble trivalent form.

2) Lactoferrin binds iron in its trivalent form. Nagasako et al. (18) discovered that lactoferrin can bind iron at sites other than its chelate-binding sites, thereby stabilizing iron in solution.

3) Fransson et al. (7) found, that lactoferrin-bound iron is absorbed and incorporated into red blood cells to the same extent as ferrous sulphate (bivalent iron) despite the fact that lactoferrin-bound iron is in the trivalent form.

It can be therefore suggested that efficiency of iron preparations could be increased by utilization of otherwise insoluble trivalent form of iron in the presence of lactoferrin.

The existence of technology for large scale isolation of lactoferrin from cow milk offers the possibility of the utilization of bovine lactoferrin in industrial production of oral iron preparations.

The exact mechanism of absorption of iron from iron-saturated lactoferrin remains still unclear. Dual isotope studies on human intestinal cells in culture showed that both lactoferrin and iron are taken up by enterocytes (17). Kawakami showed that both lactoferrin and iron are taken up by enterocytes (17). Kawakami et al. (13) suggested that iron from iron-saturated lactoferrin is significantly absorbed across the intestinal mucosa by an alternative mechanism to that used for the transport of soluble iron salts. It has been hypothesized that lactoferrin receptor in the intestinal brush-border membrane is likely to be responsible for the absorption of lactoferrin-bound iron. Studies on lactoferrin binding to brush-border membrane preparations from mice (10) and human infants (12) support this hypothesis. Gislason et al. (9) have documented in piglets a specific lactoferrin receptor on the brush-border membrane of the small intestine. They also found that human and bovine lactoferrin did not bind to the receptor.

The results reported in this study indicate that iron from bovine lactoferrin could not be utilized because of the degree of species specificity. Therefore, the replacement of the part of the iron dose by iron from iron-saturated lactoferrin had negative effect on piglets iron status.

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**References**


