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Anaemia in Housed Lambs: Effects of Oral Iron on Clinical Pathology and Performance

By *S. Vatn*[1] and *T. Framstad*[2]

[1]Norwegian School of Veterinary Science, Department of Sheep and Goat Research, Sandnes, and [2]Borregaard Industries, Ltd, Sarpsborg, Norway.

Vatn S, Framstad T: Anaemia in housed lambs: effects of oral iron on clinical pathology and performance. Acta vet. scand. 2000, 41, 273-281. – An experiment including 39 pairs of housed twin lambs was performed to evaluate the effect of an oral iron supplement (Fe-MAX® Starter) on clinical pathology, growth rates and disease occurrence. Significant differences between the iron supplemented group (Fe-group) and the controls were seen, for varying periods of time, for all red blood cell and iron parameters examined. In spite of this, 25% of the iron supplemented lambs had haemoglobin values below 80 g/L 4 weeks after treatment, whereas 33% of the controls had corresponding values 3 weeks after treatment, indicating that one single iron dose was insufficient to prevent iron deficiency anaemia. No significant positive effect on live weights was seen. However, the Fe-group had a poorer daily weight gain during the first week after dosing ($p<0.01$), but a better daily gain during the second ($p<0.01$) and third weeks. No effects were seen on disease occurrence. An additional trial with iron dextran injections to 5 untreated 21 days old lambs with varying haemoglobin values, revealed a rapid increase in the red cell distribution in anaemic lambs, with production of macrocytic, mainly normochromic erythrocytes. The new erythrocyte population was visible on the cytogram after 2 to 3 days and on the histogram after 5 days.

Ovine; haematology; blood; Technicon H*1.

Introduction

Housed young lambs have little access to dietary iron, as their main source is «contaminant iron» on the ewe's udder (*Hinds* 1999), and iron deficiency anaemia has been demonstrated in such lambs (*Ullrey et al.* 1965, *Øveråas et al.* 1988, *Green et al.* 1993). In Norway, lambs are normally confined during their first 2 to 4 weeks of life, until spring pastures are adequate in May-June. Housing also seems to be increasingly common in other countries (*Green et al.* 1993). Both daily oral iron supplement to artificially reared lambs (*Dilov* 1983), and intramuscular iron dextran injections to naturally reared lambs have been shown to prevent the decrease in haematological values seen in untreated lambs (*Carlson et al.* 1961, *Tait & Dubeski* 1979, *Bassett et al.* 1995, *Green et al.* 1997).

In a recent study, iron dextran injections had a preventive effect on the development of abomasal bloat, as well as a significant effect on weight gain, red blood cell and iron parameters in lambs (*Vatn & Torsteinbø* 2000). The present study was conducted to evaluate the effects of a single oral dose of amino acid chelated iron on live weight gains, red blood cell and iron parameters, and general disease occurrence. Red cell distribution width (RDW) has previously been found to be a more sensitive indicator of erythropoesis than mean cell volume (MCV) in piglets and kittens (*Weiser & Kociba* 1983, *Egeli & Framstad* 1998). To evaluate whether this was the case in lambs, the haematological effects of iron dextran injections in five 21-day

-old lambs with different haemoglobin (HGB) values were examined, with special emphasis on haemograms, MCV and RDW.

Materials and methods

Oral iron supplementation

Thirty-nine pairs of naturally reared twins of the Rygja and Dala breeds were included in the trial. All data from 2 pairs of twins were excluded from the beginning of the trial due to disease (see below). The lambs were all offspring of ewes and rams of haemoglobin AA type, were born in April, and belonged to the research flock of the Department of Sheep and Goat Research, The Norwegian School of Veterinary Science. They were kept on slatted floors (concrete), where hydrated lime was spread daily, until turnout on pasture at 4-5 weeks of age. All ewes were vaccinated against clostridial diseases (Ovivac, Hoechst Animal Health, Walton, UK) prior to lambing, and the lambs received coccidiostatica (Baycox®, Bayer AG, Leverkusen, Germany) 7 days after turnout on spring pastures and anthelminthica (Valbazen®, Pfizer AS, LLN, Belgium) at monthly intervals during summer grazing.

The lambs were given iron supplementation at the age of 36 h to 5 days (average 3 days). One of each twins received an oral dose of 330 mg iron (Fe-group) (6 ml Fe-MAX® Starter, 55 mg/ml, Borregaard Industries Ltd, Sarpsborg, Norway), and the twin mates received 6 ml 0.9% NaCl (controls). Alternately, the heaviest and the lightest twin were given iron and placebo, and an equal distribution of the sexes were sought. Lambs were weighed at birth, at the day of treatment and once weekly until weaning, 18 weeks later. The male to female ratio was 20:17 (Fe-group) and 19:18 (controls), and mean birth weight was 5.3 kg in both groups.

Twenty-five of the twin pairs were blood sampled 7 times, at treatment (0 weeks) and at 1, 2, 3, 4, 8, and 18 weeks after treatment (1 to 18 weeks). Blood was collected from the jugular vein using K3-EDTA (3ml) and lithium heparin (5ml) evacuated sampling tubes (Venoject®, Terumo Europe N.V., Leuven, Belgium). Blood containing EDTA was analysed automatically on a Technicon H*1® (H*1) (Bayer Instrument Corp. Tarrytown, N.Y., USA), using reference controls (Para Tech®, Streck laboratories, Inc., Omaha, Nebraska, USA). Software designed for murine blood (Technicon* Systems Multispecies Software 3.0:Miles, Inc., Diagnostic division, Tarrytown, N.Y., USA) was used as no specific program for sheep had been developed. The H*1 performs complete red and white blood cell (RBC and WBC) counts, measures mean cell volume (MCV), red cell distribution (RDW), haemoglobin width (HGB), haemoglobin distribution width (HDW) and the so called cellular haemoglobin concentration mean (CHCM). It calculates the haematocrit (HCT), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), and as a quality control CHCM and MCHC are compared. When the difference between the 2 values exceeded 60 g/L, HCT measured by the microhaematocrit method (HCT-Man) was used to replace the HCT obtained from the H*1, as this was erroneously low. Additionally, a manual MCHC was calculated as HGB x 100 / HCT-Man.

The H*1 differentiates between platelets and RBC based on size and refractive index, but very small RBC (<10 fL) are incorrectly counted as platelets, indicated by an asterisk on the platelet count. To correct this, the following formula has proven useful; new RBC Count = Reported RBC Count x ((R Count + Small RBC) / R Count) (personal communication, 1998, Dugaillez and Zelmanovic, Bayer, Tarrytown, N.Y., USA). The «R Count» is the number of cells counted by the H*1 and is listed in the Research 1 screen together with «Small

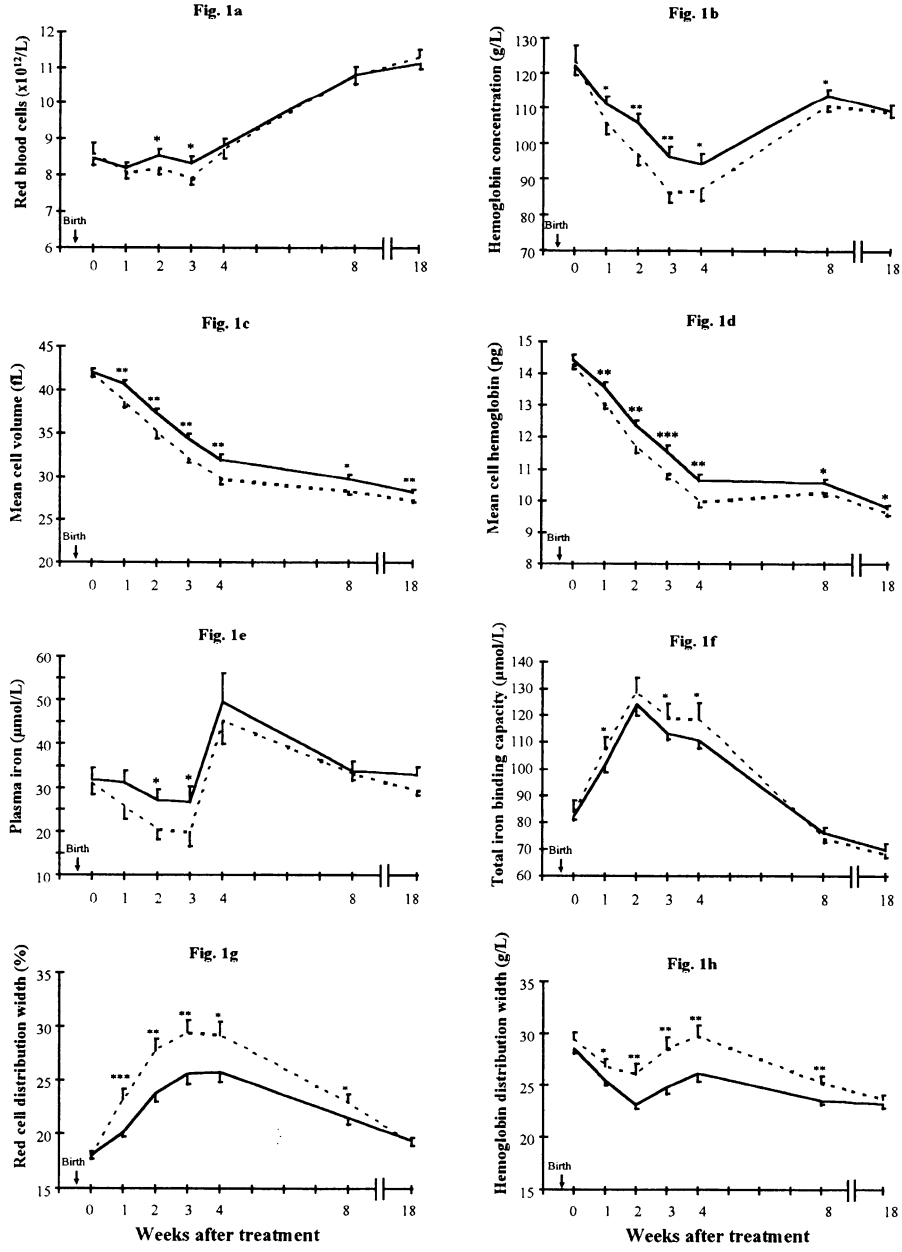

Figure 1. Mean (SEM) haematology and iron values in the iron treated (Fe) group (—) and the controls (- - -) at different times (weeks) after treatment. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ between groups. Number of animals: 0-4 weeks: $n = 48$, 8 weeks: $n = 44$, and at 18 weeks: $n = 42$.

RBC». As these small erythrocytes were not included in the estimation of the MCV and MCH, these were also recalculated. A thorough evaluation of the H*1 for analysis of lamb blood, will be published elsewhere (*Vatn et al.* 2000).

Plasma iron (P-Fe) and total iron binding capacity (P-TIBC) were determined on a Technicon RA® 1000 (Ferrimat-Kit and TIBC additive, bioMérieux, Lyon, France) using controls (Seronorm™ Nycomed Pharma AS, Oslo, Norway). Percent iron saturation (Fe-saturation) was calculated from the former.

Blood films were made to examine the lambs for infection with *Eperytrozoon ovis* at the day of treatment and 2, 4 and 8 weeks later. One lamb was heavily infected with *E. ovis* on the day of treatment and all data from both twins were excluded form the trial. The weights of an additional pair of twins, where the ewe had skin lesions on the udder, were excluded from the beginning of the trial. The weights of other lambs experiencing disease, and their litter mates, were excluded from the day of disease occurrence, provided they lost, or stopped gaining weight. Two pairs of twins were excluded due to mastitis in the ewe.

Statistics were performed using a one-sided paired student t-test, except for the results obtained at the day of treatment, where a two-sided test was used.

Iron dextran injections

Five untreated lambs from the research flock, with HGB values of 55, 76, 80, 81 and 100 g/L at 21 days of age, were injected with 300 mg iron dextran (2.5 ml Idofer®, Boehringer Ingelheim, Agrovet A/S, Hellerup, Denmark) s.c. in the inguinal area. Blood was collected (5 ml EDTA) and analysed with the H*1 at 2, 15, and 21 days of age, and 2, 3, 5 and 8 days post injection. Except for the anaemia, they were clinically healthy and free of *E. ovis, Eimera* spp. and other common gastrointestinal parasites.

Results

Oral iron supplementation

Clinical pathology. The effects of oral iron supplementation on the RBC and iron parameters are shown in Fig. 1. RBC counts and P-Fe values (Figs. 1a and 1e) were significantly higher in the Fe-group than in the controls at 2 and 3 weeks after treatment. For HGB (Fig. 1b) the difference was seen from 1 to 8 weeks, and for MCV and MCH (Figs. 1c and 1d) from 1 week and through the whole sampling period. Significantly lower values were found in the Fe-group for P-TIBC (Fig. 1f) at 1, 3, and 4 weeks, and for RDW and HDW (Figs. 1 g and 1h) from 1 to 8 weeks.

A significant ($p<0.01$) decrease in RBC numbers was found in the control lambs from the

Table 1. Percentage of lambs, in the control and iron treated (Fe) groups, with varying haemoglobin concentrations (HGB g/L) at different times after treatment.

	Time (weeks) after treatment									
	0		2		3		4		8	
HGB	Control	Fe	Control	Fe	Control	Fe	Control	Fe	Control	Fe
≥100	88	96	46	71	9	49	21	41	92	100
90-100	8	4	29	21	29	21	25	21	8	0
80-90	0	0	8	4	29	17	33	13	0	0
<80*	4	0	17	4	33	13	21	25	0	0

Age at treatment (0 weeks) was between 36 h and 5 days. Total number of lambs at each sampling was 48, except at 8 weeks, when 44 lambs were sampled. *HGB < 80 g/l is regarded as an anaemia in this study.

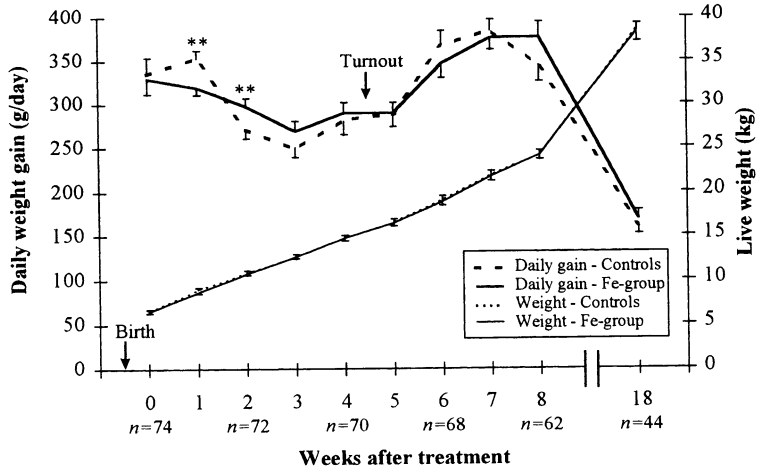

Figure 2. Mean (SEM) daily growth rates (g) and live weights (kg) in the Fe-group and controls at different times (weeks) after treatment. **: $p<0.01$ between groups.

time of placebo treatment to 3 weeks, after which RBC counts increased in both groups (Fig. 1a). The turn of the curve representing RBC counts occurred concomitant to the marked increase in P-Fe values (Fig. 1e). P-TIBC showed a significant increase in values in both groups during the first 2 weeks after treatment. Fe-saturation followed the same curve as P-Fe until 4 weeks after treatment, but in the succeeding period (4 to 18 weeks), both P-Fe and P-TIBC decreased, resulting in a stable Fe-saturation at about 45% (data not shown). The HCT curves (data not shown) closely resembled the HGB curves for both groups. At treatment, the mean HCT in the controls and the Fe-group was 36.3 and 35.7%, respectively, and the lowest mean value (25.6%) was found in the controls at 3 weeks. At this sampling, the Fe-group had a mean HCT of 28.7%, and the difference was significant ($p<0.01$). At 18 weeks both groups had a mean HCT of 31%.

Both groups experienced a significant decrease in MCV and MCH (Figs. 1c and 1d) during the study period, whereas RDW increased significantly from treatment to 3 weeks in both groups.

A large proportion of the lambs had HGB values below 80 g/L at 3 and 4 weeks (Table 1), the lowest value was 53 g/L in a control lamb at 4 weeks. When examining the RBC histogram, 2 erythrocyte populations could be detected, with the microcytic population to the left, and the old population of larger erythrocytes to the right. Only the most anaemic lamb displayed an additional small macrocytic population at 4 weeks. No double peak, only a slight or moderate extension to the left, was seen on the HGB histograms of the anaemic lambs.

The mean values of CHCM (measured by the H*1), and the manually calculated MCHC, were lower in the controls compared to the Fe-group, until 4 weeks after treatment, whereas MCHC calculated by the H*1 revealed higher mean values in the controls (data not shown). However, for all 3 methods of evaluating this parameter, the values for both groups reached their lowest levels at 2 to 4 weeks after treatment.

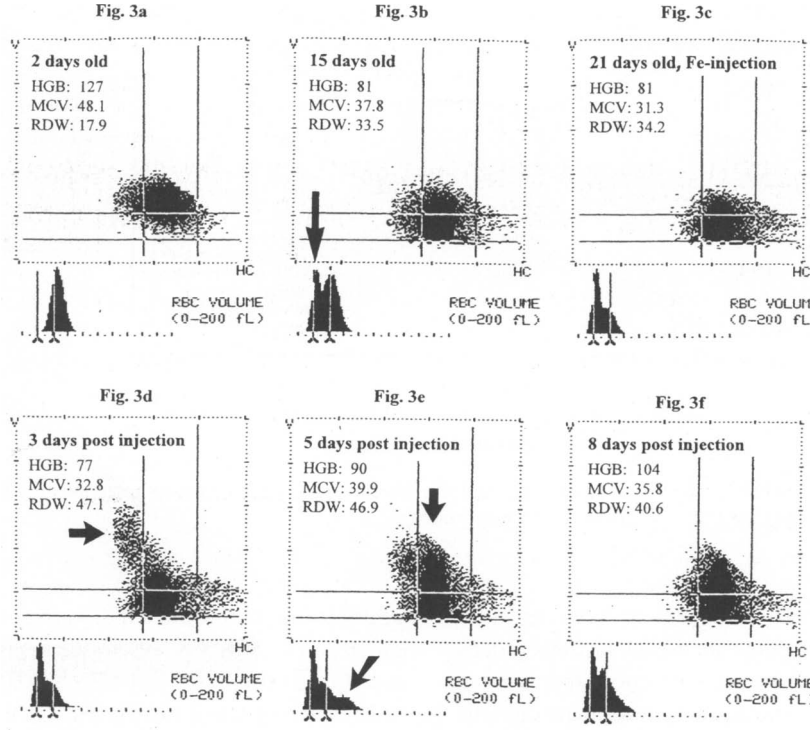

Figure 3. RBC histograms and cytograms in a lamb before and after iron dextran injection. On the cytogram V = volume (vertical axis) and HC = hemoglobin concentration (horizontal axis). On the histogram the vertical bars are set to illustrate the separation between micro-, normo-, and macrocytic red blood cells from left to right, respectively, in adult sheep. The development of a new microcytic population at 15 days of age was easily distinguishable on the histogram (Fig. 3b-bottom, arrow). The first hypochromic macrocytes were first detected in the cytogram (Fig. 3d-top, arrow). Five days post injection the new population was clearly visible on the histogram (Fig. 3e-bottom, arrow), but at this stage the majority of the new RBC produced were normochromic (Fig. 3e-top, arrow).

The mean WBC counts were between 5.5 and 6.5 $x10^9$/L at treatment and showed an increase in both groups from 4 weeks on, to between 8.0 and 9.5 $x10^9$/L at 18 weeks (data not shown).

Growth rates and disease. The difference in daily growth rates and live weights are shown in Fig. 2. The iron treated lambs gained less weight (34 grams per day, $p<0.01$) during the first week after oral iron supplementation, but the second and third week these lambs gained on average 27 ($p<0.01$) and 20 grams more, respectively, than the controls each day. The average total weight gains during the indoor feeding period (from treatment to 4 weeks) was 8.1 and 8.2 kg for the controls and the Fe-group, respectively. No significant difference was found in the live weights between groups, at any time.

Iron treatment had no effect on total disease occurrence until slaughter in the autumn, with

diseases such as interdigital abscesses, diarrhoea, and tick born fever occurring in both groups.

Iron dextran injections

The 4 anaemic lambs (HGB 55-81 g/L) responded to iron dextran injections by producing macrocytic, mainly normochromic RBC. The new RBC population was visible on the cytogram after 3 days and on the histogram after 5 days (Fig. 3). A marked increase was seen in RDW within 3 days in 4 of the 5 lambs, MCV within 5 days, but HDW remained constant. In the most anaemic of these 4 lambs RDW increased already after 2 days (from 32% to 42%). The fifth lamb displayed an increase in HGB from 100 g/L before injection to 115 g/L eight days later, but with no additional increase in RDW and MCV.

Discussion

Significant differences were found between the iron treated (Fe) group and controls for all red blood cell and iron values studied. The effects of oral iron supplementation within the first 5 days of life were seen after one week, and for MCV and MCH it lasted throughout the whole study period (18 weeks). However, a marked transient drop in red blood cell parameters was seen both in the Fe-group and in controls, with HGB values below 80 g/L in more than 25% of all lambs at 3 to 4 weeks after treatment.

In contrast, oral administration of iron glycerophosphate gave good results when constantly added to the milk replacer in artificially reared lambs, whereas in calves the effect of oral treatment was inferior to the intramuscular iron dextran administration (*Dilov* 1983). In naturally reared lambs though, daily administration of oral iron supplement would not be practical.

As almost half the lambs (49%) in the Fe-group had HGB values above 100 g/L at 3 weeks, as opposed to 9% of the controls, these lambs had obviously gained from the iron supplement. On the other hand, 25% the lambs in the Fe-group were anaemic at 4 weeks despite iron supplement, indicating considerable individual differences within the Fe-group. The oral supplement used in our study (Fe-MAX® Starter) contained amino acid chelated iron, developed for use in piglets, and according to the producer, its absorption over the gut should not be age dependent. Possibly, insufficient closure of the oesophageal-reticular grove in some lambs reduced the uptake of the iron supplement. Furthermore, hydrated lime, spread daily on the concrete slats, may have been partly ingested by the lambs, via the ewe's udder, forming a non-absorbable calcium-iron complex. In weaned fattening pigs, a conditioned iron deficiency was caused by adding calcium carbonate to the diet (*Radostits et al.* 1994). Except for one lamb, dribbling of iron solution after dosing was not registered.

Strict hygiene, as applied in our research flock, is, moreover, predisposing for the development of iron deficiency anaemia in housed lambs younger than one month (*Hinds* 1999), possibly explaining the high prevalence of anaemia seen in the controls of the present study. Interestingly, lambs reared outdoors had significantly higher red blood cell parameters at 3 weeks of age than housed lambs (*Green et al.* 1993).

Several studies have proven iron dextran injections to be efficient in preventing the marked decline in red blood cell parameters and Fe-saturation seen in control lambs (*Carlson et al.* 1961, *Bassett et al.* 1995, *Green et al.* 1997, *Vatn & Torsteinbø* 2000).

In iron deficient kittens, erythrocyte volume distribution was found to be a more sensitive parameter for detecting disturbances of RBC size than MCV (*Weiser & Kociba* 1983), and similar findings have been reported in piglets (*Egeli & Framstad* 1998). This was also seen in our study, where RDW increased from below 20

to 25%-35% during the microcytic anaemia in the iron deficient young lambs, and to above 45% subsequent to the iron dextran injection (Fig. 3). No additional increase was seen in the anaemic lambs of the oral trial, except for one very anaemic lamb, despite a rise in Fe-saturation, P-Fe, and RBC counts from 3 to 4 weeks. This age corresponds to the time when lambs start digesting considerable amounts of solid food.

The response in MCV to the iron dextran injections, appeared later than the changes in RDW. Moreover, the natural variation in MCV, from >40 fL in newborn lambs to <30 fL at 18 weeks, makes it a parameter of limited use, unless serial samples are available. As the medium life span of erythrocytes in young lambs is reported to be 46 days (*Jain* 1993a), MCV becomes a more useful parameter in older lambs. Increased MCV values were demonstrated during haemolytic anaemia in three months old *E. ovis* infected lambs (*Øverås* 1969).

Microcytosis precedes the hypochromasia in iron deficiency anaemia (*Jain* 1993b), and in the present study the anaemia demonstrated was microcytic and only moderately hypochromic. The MCHC calculated by the H*1, presented erroneously higher mean values in the controls, and unexpectedly high values were also found in anaemic piglets compared to normal piglets (*Egeli et al.* 1998). The CHCM, measured by the H*1 on all but the smallest but potentially hypochromic cells, agreed better to the manually calculated MCHC (data not shown). For this parameter, the lowest values were found in the control lambs, corresponding to earlier findings in lambs (*Green et al.* 1997). It is unclear why iron treatment seemed to have a significantly negative effect on the daily weight gain during the first week after dosing. Experiments with the same iron supplement to piglets, revealed no such effect (*Framstad* 1998). Our study demonstrated a positive effect of iron supplementation 2 and 3 weeks after dosing, but the total weight gain during the first 5 weeks did not differ significantly between the groups. As experiments with milk replacer containing iron glycerophosphate gave weight gains exceeding those achieved by iron dextran injections (*Dilov* 1983), it seems likely that the dosing in our study was not optimal. Other studies on the effect of iron dextran injections on live weights are contradictory, as significant effect were seen by some (*Carlson et al.* 1961, *Tait & Dubeski* 1979, *Green et al.* 1997), but not by others (*Øverås et al.* 1988, *Bassett et al.* 1995). In a 2 year study including 5 different Norwegian sheep flocks, iron dextran injections within the first week of life resulted in improved total weights at slaughter, but also revealed variations both between farms and seasons (*Vatn & Torsteinbø* 2000).

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Sammendrag

Anemi hos unge lam: effekt av en enkelt dose jern gitt oralt.

Et forsøk med peroral jerndosering til lam som ble holdt innendørs ble utført for å vurdere effekten på hematologi, tilvekst og sjukdomsforekomst. Studien inkluderte 39 tvillingpar, der ett lam fikk aminosyrechelatert-jern (Fe-MAX® Starter) og det andre fikk placebo. Signifikante forskjeller ble registrert i alle undersøkte parametre for hematologi og jern, over varierende tidsintervall. Til tross for dosering med jern hadde 25% av disse lamma hemoglobin verdier under 80 g/L, mens tilsvarende andel av kontrollene var 33%. Dette indikerte at dosen var utilstrekkelig. En signifikant ($p<0,01$) dårligere daglig tilvekst ble sett i den jernbehandla gruppa første uka etter dosering, mens denne gruppa viste økt daglig tilvekst andre ($p<0,01$) og tredje uka etter dosering. Det var ingen signifikante effekter på levende vekter eller sjukdomsforekomst. Et tilleggsforsøk med jerninjeksjoner til fem 21 dager gamle lam med forskjellige hemoglobinverdier, viste en rask økning i erytrocyttsprednings-kurven (RDW). Produksjon av nye makrocyttære, røde blodlegemer var synlig på cytogrammet etter 2 til 3 dager og på histogrammet etter 5 dager.

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Reprints may be obtained from: S. Vatn, Norwegian Meat Research Centre, Pb 396 Økern, 0513 Oslo, Norway. E-mail: synnove.vatn@fagkjott.no, tel: +47 22 09 22 44, fax: +47 22 22 00 16.