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KALE ANAEMIA IN RUMINANTS

II. OBSERVATIONS ON KALE-FED SHEEP*)

By

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Some effects of feeding locally grown marrow-stem kale to cattle have been reported by *Grant et al.* (1968). The study covered haemoglobin levels, packed cell volume, in vitro erythrocyte uptake of triiodothyronine and for blood serum the levels of calcium, phosphorus, magnesium, GOT and OCT activity, total protein and the protein fractions. Among the cows fed large amounts of kale (60 kg per day) there was a significant drop in haemoglobin levels and the packed cell volume. There were no other changes which could be ascribed to the kale.

As a further step in assessing the effects of heavy kale feeding to ruminants, the haemoglobin level, packed cell volume, erythrocyte count, and Price-Jones curve were followed in sheep fed kale grown in another area of the country.

MATERIALS AND METHODS

Twenty male Lantras lambs, five months old and originating from 1 flock were taken from pasture and stabled on September 24th. Ten lambs served as controls and were fed hay and crushed mixed grain with free access to straw throughout the experiment. The other group of 10 lambs was placed directly on a ration of 1.5 kg marrow-stem kale per animal per day with free access to straw. After 13 days the amount of kale was increased to 2.5 kg and after 31 days kale was offered ad lib. and straw was removed from the pens. During this part of the experiment daily con-

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sumption was 3 to 4 kg per lamb. After 45 days of kale feeding, supplies of kale were exhausted and the kale group was fed the same ration as the control group until the experiment was concluded after 76 days.

The kale was cut and trucked twice weekly from 2 farms in the vicinity of Stockholm and fed whole to the lambs.

Blood samples from each animal in both groups were taken twice weekly. Haemoglobin levels, packed cell volume (PCV) and erythrocyte counts were made on heparinized samples (*Paulson & Åberg* 1965). Price-Jones curves to determine the range of erythrocyte diameters were made with the aid of an automatic counter (Celloscope 102) for the same 2 animals in each group throughout the experiment. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values for PCV, Hb and erythrocyte counts.

For Heinz body counts 0.1 ml fresh venous blood was mixed with 0.2 ml 0.1 M sodium citrate solution in an Ellerman tube and centrifuged for 5 min. The supernatant was discarded, 0.2 ml of freshly filtered 0.5 % methyl violet solution was added to the sediment, the tube was shaken well and then allowed to stand for 5 min. After re-centrifugation for 5 min. the supernatant was again discarded and thin smears made of the concentrated blood cells. The counts were made with the aid of an ocular with a hair-line pointer; the slides were moved at random and all the cells in the different fields lying along the pointer were counted. Two hundred erythrocytes were counted, and the percentage containing Heinz bodies was calculated.

The results were evaluated statistically (t-test) and with conventional symbols.

RESULTS

There were no clinical signs which could be attributed to the experimental procedure. Both groups remained apparently healthy, although the growth rates for both groups were not particularly good.

The values obtained for Hb levels, erythrocyte counts, PCV and Heinz body counts throughout the experiment are illustrated in Figs. 1, 2 and 3, and for MCV and MCHC in Table 1. The t-values and the degree of significance for the differences are also indicated for each sampling occasion. The amount of kale fed has also been indicated for reference.

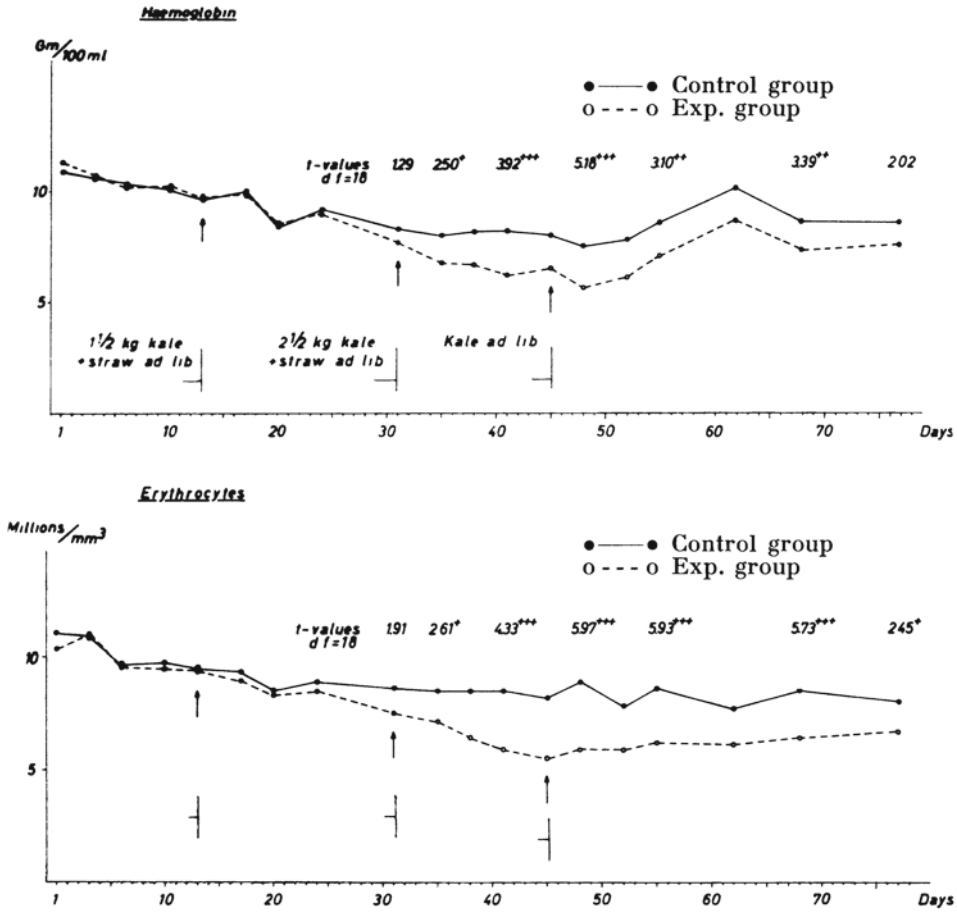

Figure 1. Haemoglobin levels and RBC counts. From day 1 the kale lambs received 1.5 kg each per day, from day 13 2.5 kg each per day, and from day 31 kale ad lib. Return to control ration after day 45.

For both the control group and the kale group, the first part of the experiment, i.e. the change-over from pasture to stable feeding, was associated with a drop in erythrocyte counts, Hb and PCV.

From about day 24 onwards, the values for the control group became more stable, while those for the kale group continued to decline. Statistically significant differences, however, could be demonstrated on day 35 ($P < 0.05$) and day 42 ($P < 0.001$), i.e. during the period when kale was offered ad lib.

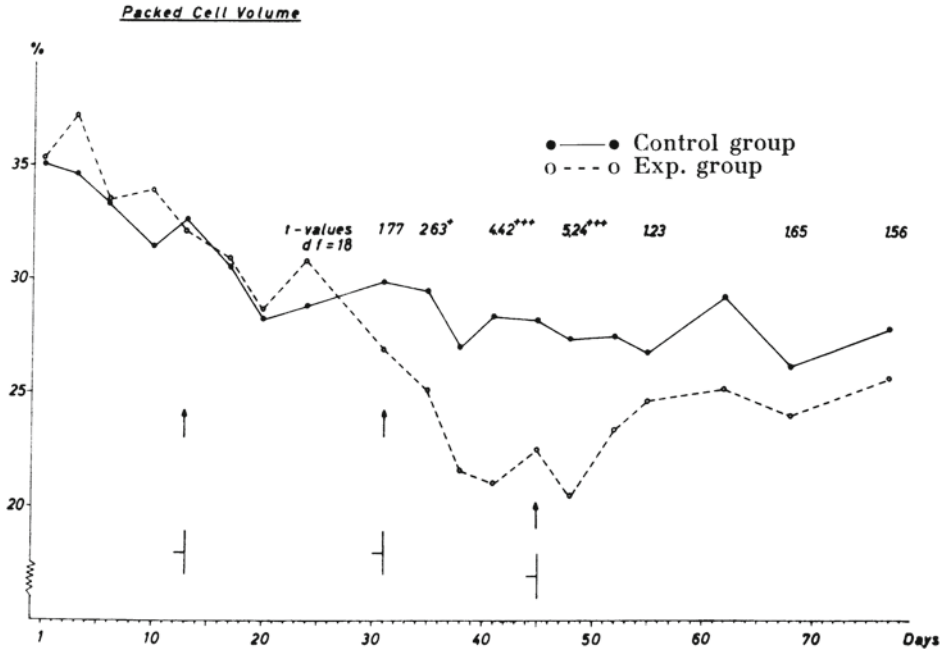

Figure 2. Packed cell volume. From day 1 the kale lambs received 1.5 kg each per day, from day 13 2.5 kg each per day, and from day 31 kale ad lib. Return to control ration after day 45.

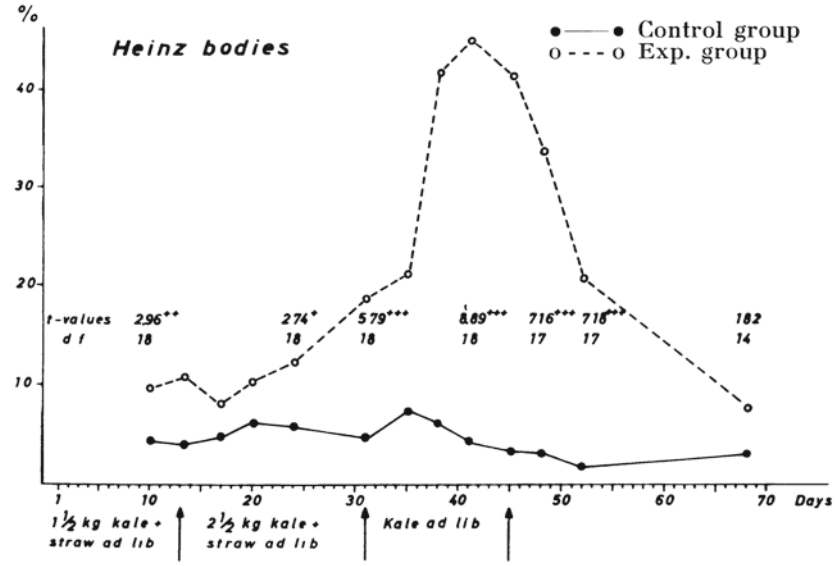

Figure 3. Heinz bodies. From day 1 the kale lambs received 1.5 kg each per day, from day 13 2.5 kg each per day, and from day 31 kale ad lib. Return to control ration after day 45.

Table 1. Mean corpuscular haemoglobin content and volume.

Day	Haemoglobin content				t value
	control		kale-fed		
	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$	
1	10	1.00 ± 0.06	8	1.10 ± 0.10	2.62*
17	10	1.08 ± 0.07	10	1.12 ± 0.09	1.06
24	10	1.05 ± 0.10	10	1.07 ± 0.09	< 1
35	10	0.95 ± 0.09	10	0.96 ± 0.07	< 1
41	10	0.97 ± 0.09	10	1.06 ± 0.16	1.56
48	10	0.85 ± 0.08	10	0.95 ± 0.09	2.65*
52	10	1.02 ± 0.13	10	1.06 ± 0.11	< 1
55	10	1.00 ± 0.07	10	1.15 ± 0.09	4.23***
68	10	1.02 ± 0.05	10	1.16 ± 0.06	5.61***
77	8	1.07 ± 0.09	9	1.13 ± 0.09	1.35

Day	Volume				t-value
	control		kale-fed		
	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$	
1	10	3.20 ± 0.17	8	3.44 ± 0.34	1.94
17	10	3.29 ± 0.25	10	3.51 ± 0.28	1.83
24	10	3.26 ± 0.38	10	3.64 ± 0.27	2.55*
35	10	3.49 ± 0.53	10	3.59 ± 0.37	< 1
41	10	3.36 ± 0.25	10	3.58 ± 0.49	1.25
48	10	3.11 ± 0.29	10	3.52 ± 0.44	2.47*
52	10	3.60 ± 0.43	10	4.08 ± 0.54	2.20*
55	10	3.13 ± 0.28	10	4.04 ± 0.67	3.98***
68	10	3.08 ± 0.15	10	3.77 ± 0.48	4.35***
77	8	3.59 ± 0.39	9	3.83 ± 0.25	1.52

From day 1 the kale lambs received 1.5 kg each per day, from day 13 2.5 kg each per day, and from day 31 kale ad lib. Return to control ration after day 45.

After the end of the kale-feeding period, the Hb and RBC slowly rose, but were still significantly lower for the kale group on day 67, some 3 weeks after transfer to the same ration as the control group.

The Price-Jones curves for the 2 lambs in both groups underwent pronounced but in principle similar changes during the course of the experiment. The initial curves had a single distinct peak between the 4.5 and 5.0 μ intervals but as the experiment progressed, the curves became irregular but in general low and broad-based without distinct peaks.

The Heinz body counts are illustrated in Fig. 3. By 10 days there was a significant difference ($P < 0.01$) between the kale and the control groups. During the period of kale feeding ad lib. the counts were very high, but declined markedly by 7 days after kale period was finished. The difference was still significant ($P < 0.001$) at this point. The smears made between day 52 and day 68 were technically unsatisfactory. On day 68, i.e. 23 days after the end of the kale feeding period, there was no significant difference between the groups.

Even during the period of heavy kale feeding usually 1 Heinz body per erythrocyte, occasionally 2, but never more, were observed.

Pronounced anisocytosis and to a lesser degree poikilocytosis were noticed in the smears from both groups, a change also reflected in the Price-Jones curves.

DISCUSSION

The change from grazing to stable feeding was associated with a drop in the Hb levels and erythrocyte counts for the control group as well as the kale group. In the control cattle in the previous experiment, however, the Hb levels rose after stabling and the total serum protein level declined. For the present, these observations have to be taken simply as a sign that the change in environment has haematological — as well as other — effects upon ruminants without being able to pinpoint either a common pattern or more specific causes. There was a moderate degree of parasitism among both groups of lambs (from the same flock and the same pasture) with the usual species represented but not Haemonchus or Monezia.

The group of lambs fed kale developed statistically significantly lower Hb levels and erythrocyte counts than the control lambs from day 31, when kale was offered ad lib. The Heinz body counts (from day 10 onwards) were significantly higher for the kale-fed animals and increased precipitously during the ad lib. period.

The statistically significant differences in the blood values for the control and the kale groups permit the term "kale anaemia" even although the Hb levels and erythrocyte counts did not fall to extremely low values.

Taken together with the results for cattle reported previously, it appears that marrow-stem kale from farms in different areas

of this country and in different years has the property of depressing Hb levels and erythrocyte counts in ruminants. This agrees with *Rosenberger*'s observations (1939, 1943, 1950) cited in a previous report (*Grant et al.* 1968).

Concerning the amount and duration of kale feeding necessary to induce a drop in Hb levels in ruminants it appears that the lambs tolerated 1.5 kg kale and probably also the 2.5 kg per day. The highly significant ($P < 0.001$) changes were first noted in the samples taken some 10 days after kale was fed ad lib. (3—4 kg per day). The significantly higher Heinz body counts which were observed only 10 days after the beginning of the experiment and at the beginning of the heavy kale feeding period, however, suggest that the erythrocytes were already in an abnormal state during the periods when smaller amounts of kale were fed. The high Heinz body counts persisted at least a week and probably somewhat longer in the kale group after return to the control ration.

The course of kale anaemia was followed by blood samples twice weekly. At these short intervals there was no evidence that the decline in Hb levels or erythrocyte counts was intermittent. PCV values followed the same continuous downward trend to the end of kale feeding period.

The question whether or not the drop in Hb levels, erythrocyte counts and PCV values would have continued or levelled off could not be answered in this experiment. Supplies of kale were exhausted after only 14 days of the heavy kale feeding period, and only a week after significant changes were detected in the Hb levels, erythrocyte counts and PCV values.

After the end of the kale feeding experiment, these values began to rise again although the Hb levels and erythrocyte counts were still significantly lower some 3 weeks after the change-over from kale to the control ration.

The MCV and MCHC values for the kale group were also significantly increased in samples taken 10 and 22 days after the end of the kale feeding period, a macrocytic hyperchromic shift of "recovery" compatible with a low, broad-based Price-Jones curve. That the control group also acquired a squat Price-Jones curve undoubtedly reflects the haematological changes in this group accompanying the change-over from pasture to stable feeding, although these were clearly of a much lesser magnitude than the changes induced by kale feeding.

Comparison with previous descriptions of naturally-occurring and kale-induced anaemia in ruminants can be only general, since all the previous reports lack statistical analysis of their results and have usually been based on only single or a few animals (see survey in our previous paper). On the whole, the haematological pattern displayed by our animals corresponded with that described for cattle and sheep (see particularly *Penny et al.* 1961, 1964 and *Clegg & Evans* 1962).

One difference concerns the temporal relation between Heinz body formation and a drop in Hb and erythrocyte values. Our kale sheep had a significantly high Heinz body count long before significant drops in Hb or erythrocyte levels. In the cattle of *Penny et al.* (1964), high Heinz body counts coincided with the Hb and erythrocyte decrease. With reservation for possible true species difference our results for sheep suggest that Heinz body formation is a more subtle manifestation than the changes in the Hb and erythrocyte values which are governed by the amount of kale consumed in relation to the regenerative capacity of the bone marrow.

Another noteworthy difference between our results and those cited is the apparent effect of taking our animals off pasture on the Hb levels, erythrocyte counts and ultimately the Price-Jones curves. We could also consistently demonstrate Heinz bodies in the erythrocytes of the control sheep exposed to the same abrupt change in environment and diet.

The differences in kinetics and temporal relationships between our results and those of others, particularly of *Penny et al.* (1961, 1964), can also be taken as illustrating the need to apply to kale anaemia the more refined techniques now available for studies of erythrocyte and haemoglobin metabolism.

From a practical viewpoint, what has been demonstrated in this and in our previous experiment is that the feeding of kale grown in this country is statistically associated with a drop in Hb levels and erythrocyte counts and that even moderate amounts of kale induce erythrocyte abnormalities (Heinz bodies), before these values are depressed. Furthermore we found no evidence for local, yearly or major individual differences in the effects of kale feeding. An abrupt change-over from pasture to stable feeding in itself had noticeable haematological effects on both cattle and sheep.

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SUMMARY

The haematological effects (Hb, RBC, PCV, Price-Jones curve, MCV, MCHC and Heinz body counts) of feeding lambs on marrow-stem kale were followed and the results evaluated statistically.

The lambs were taken directly from pasture and stabled for the experiment. The control group (10 lambs) were fed hay and crushed grain.

The kale-fed group (10 lambs) received 1.5 kg each per day (13 days), then 2.5 kg per day (18 days) and then free access (14 days) before being placed on the control ration.

A statistically significant drop in Hb levels and erythrocyte counts were noted about a week after the ad lib. feeding of kale was begun. This decline was long proceeded by a significant rise in Heinz body counts. Withdrawal of kale was followed by a macrocytic hyperchromatic recovery pattern.

The abrupt change from grazing to stable feeding in itself was apparently associated with a drop in Hb levels, packed cell volumes and erythrocyte counts. Even the control sheep developed Heinz bodies in their erythrocytes although significantly fewer than the kale-fed animals.

ZUSAMMENFASSUNG

Anämie beim Schaf nach Markstammkohlfütterung.

Bei Fütterung mit Markstammkohl von Lämmern sind die hämatologischen Veränderungen (Hämoglobin, Hämatokrit, Anzahl der Erythrozyten, Price-Jones-Kurven, Volumen und Hämoglobingehalt der Erythrozyten und Heinz-Körperchen) untersucht und statistisch analysiert worden.

Die Lämmer wurden direkt von der Weide eingestallt, als der Versuch begann. Die Kontrollgruppe (10 Tiere) wurden mit Heu und Schrot ausfüttert. Die Versuchsgruppe (10 Tiere) bekam 1,5 kg Markstammkohl täglich pro Tier in 13 Tagen und 2,5 kg in 18 Tagen, darauf freien Zugang in 14 Tagen. Danach bekamen sie dasselbe Fütter wie die Kontrollgruppe.

Ungefähr eine Woche, nachdem die Tiere freien Zugang zum Markstammkohl gehabt hatten, wurde eine statistisch signifikante Senkung des Hämoglobinwertes und der Anzahl Erythrozyten gefunden. Dieser Senkung ging eine signifikante Steigerung der Anzahl Heinz-Körperchen voraus. Bei beendeter Markstammkohlfütterung fand man ein makrozytär hyperchromes Heilungsbild. Der schnelle Übergang vom Weidegang zur Stallfütterung scheint eine Senkung des Hämoglobingehaltes und der Erythrozytenanzahl zu verursachen. Auch in der Kontrollgruppe wurden Heinz-Körperchen in den Erythrozyten gesehen, aber die Anzahl war signifikant geringer als die in der Versuchsgruppe.

SAMMANFATTNING

Anämi vid utfodring med fodermärgkål åt får.

I samband med utfodring med fodermärgkål till lamm har de hämatologiska förändringarna (Hb, antal röda blodkroppar, hämatokrit, Price-Jones kurvor, blodkroppsvolym, blodkropparnas hämoglobinhalt och Heinz kroppar) studerats och bearbetats statistiskt.

Lammen togs direkt från betet och installades till försöket. Kontrollgruppen (10 djur) utfodrades med hö och gröpe. Försöksgruppen (10 djur) fick 1,5 kg fodermärgkål per djur och dag i 13 dagar och 2,5 kg i 18 dagar samt fri tillgång i 14 dagar. Därefter fick de samma utfodring som kontrollgruppen.

En statistiskt signifikant sänkning av Hb-värdet och antalet röda blodkroppar sågs ungefär en vecka efter att djuren fått fri tillgång till kålen. Denna sänkning hade föregåtts av en signifikant stegring av antalet Heinz kroppar. När kålutfodringen avslutats inträdde en makrocytär, hyperkrom avläkningsbild.

Den hastiga övergången från bete till stallutfodring tycks orsaka en sänkning av Hb, hämatokrit och antal röda blodkroppar. Även hos kontrollgruppen förekom Heinz kroppar i röda blodkropparna men signifikant färre än i försöksgruppen.

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