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COPPER AND ZINC DEFICIENCIES TREATMENT BY INTRAMUSCULAR INJECTIONS IN SHEEP

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Résumé

TRAITEMENT DES CARENCES EN CUIVRE ET EN ZINC PAR INJECTION INTRAMUSCULAIRE CHEZ LE MOUTON. — Dix brebis ont reçu soit une injection de cuivre pulvérulent, soit une injection d'oxyde de zinc par voie intramusculaire, en suspension dans l'huile d'olive. Dans chaque expérience, les résultats des injections ont été comparés avec dix brebis témoins. L'augmentation du cuivre plasmatique et hépatique a été significative. L'augmentation du zinc plasmatique a également été significative. Ces élévations de teneurs plasmatiques ont débuté respectivement vers le 6e jour et le 3e jour après l'injection et se sont poursuivies jusqu'aux 21e et 17e jours. Aucune inflammation violente n'est apparue. Ce traitement semble donc efficace sans provoquer de réaction secondaire néfaste.

Introduction

Trace element salts in solution are generally strongly caustic and produce abscesses in the injection site. Copper per os dosing is efficient for treating deficient animals (Lamand *et al.*, 1969). Blackmon *et al.* (1967) consider this type of treatment adequate for treating zinc deficiencies as well. However trace element should be provided every day for at least ten days and this treatment is time consuming. Therefore we tried to perfect a copper and zinc deficiency treatment allowing the injection of enough trace element, by an easy route (intramuscular) without detrimental inflammation or shock or pain and effective for a period sufficient to limit the treatment to one injection.

Material and methods

Twenty adult Ile de France ewes weighing 45 kg on average were used in each experiment. The animals received as their only diet a pasture hay with a copper content of 4.3 mg/kg D.M. and zinc content of 28 mg/kg D.M. (lower than the deficiency limit in hay ; Lamand *et al.*, 1977).

Heparinized blood was obtained three times a week from each animal. Plasma

were analysed for copper and zinc by atomic absorption spectrophotometry (Bellanger and Lamand, 1975). Ceruloplasmin was determined by the method of Sunderman and Nomoto (1970) and results expressed as optical density units.

Copper injection experiment

After a preexperimental period of 20 days, animals were classed by pairs according to increasing cupremia levels. One ewe of each pair was injected intramuscularly in the neck with 50 mg of copper in the form of fine dust (one micron or more as granulometry *).

Copper was suspended by agitation in 4 ml of purified olive oil *.

The control sheep were injected as treated animals but with oil alone. Experimental animals were slaugtered 21 days post injection and their liver was sampled.

Zinc injection experiment

Animals were paired as already described and the treated ewes received 200 mg of zinc oxide (Prolabo) by intramuscular injection in the neck suspended in 4 ml of purified olive oil *. Control animals received only oil.

Results

No inflammation or any other side effect appeared in any animal as a result of the injections either with copper or zinc.

Copper injections

At the end of the preexperimental period, blood copper levels were variable but remained (during the whole experiment) above 70 µg/100 ml (the deficiency limit usually adopted). The copper injection raised the ewes cupremia by 29 µg/100 ml (Table 1) while the controls cupremia fells by 12 µg/100 ml ($P < 0.01$). Similarly (Table 2) ceruloplasmin was raised in copper injected animals (66.5 O.D. units) and lowered in controls (101 O.D. units) ($P < 0.01$).

Liver copper content was significantly higher ($P < 0.05$) in injected animals than in the controls (Table 3). Cupremia rose significantly on the 6th day post injection (Fig. 1) and was still higher than the controls on the 21st day. Ceruloplasmin is well correlated to plasma copper ($r = 0.832$, 378 D.F.).

Plasma bilirubin level remained normal in all animals during the whole experiment (< 0.4 mg/l).

Zinc injections

Plasma zinc (Table 4) increased in treated animals (by 17.2 µg/100 ml) while it diminished in the controls (by 7 µg/100 ml, $P < 0.001$). A significant increase appeared

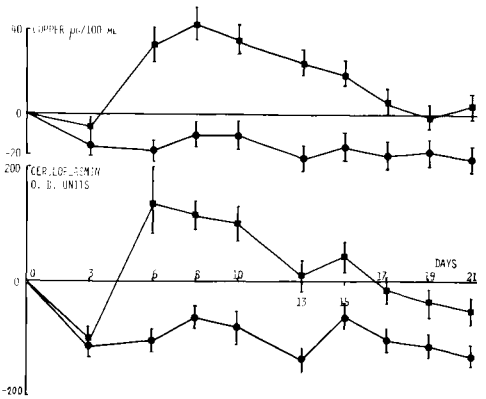

Fig. 1 : Evolution of plasma copper and ceruloplasmin after copper injection compared to mean preexperimental level.
——●—— Controls.
——■—— Treated animals.

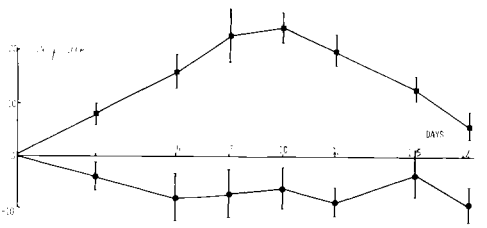

Fig. 2 : Evolution of plasma zinc after zinc injection compared to preexperimental level.
——●—— Controls.
——■—— Treated animals.

* I.N.R.A.-A.N.V.A.R. Patent PROLONTEX ® PROLIGO, Paris.

Table 1 : Comparison of mean plasma copper during preexperimental period and experimental period (after copper injection) [μg/100 ml] in paired ewes.

Pair N°	Injected			Controls		
	Before treatment	After treatment	Difference	Before treatment	After treatment	Difference
1	89	119	30	95	83	— 12
2	104	127	24	103	112	9
3	104	105	1	115	112	— 2
4	116	181	66	118	105	— 13
5	132	182	50	120	105	— 16
6	135	173	38	135	123	— 12
7	135	149	13	142	150	— 9
8	142	144	2	149	136	— 13
9	148	182	34	161	126	— 35
10	169	205	35	168	130	— 38
Mean			29*			— 12*

* Significantly different ($P < 0.001$).

Table 2 : Comparison of mean plasma ceruloplasmin during preexperimental period and experimental period (after copper injection) [O.D. units] in paired ewes.

Pair N°	Injected			Controls		
	Before treatment	After treatment	Difference	Before treatment	After treatment	Difference
1	325	386	61	361	243	— 118
2	369	408	39	424	429	5
3	366	296	— 70	452	370	— 82
4	400	634	234	427	293	— 134
5	482	649	167	435	298	— 137
6	410	465	55	427	353	— 74
7	491	446	— 45	530	551	21
8	544	494	— 50	500	377	— 123
9	514	596	82	573	399	— 174
10	548	650	102	607	410	— 197
Mean			66*			— 101*

* Significantly different ($P < 0.005$).

on the 3rd day post injection and lasted at least until the 17th day when the animals were slaughtered.

Discussion

Many copper complexes have been successfully used (copper glycinate, methionine, EDTA complexes) to prevent or cure copper deficiency in ruminants (Camargo *et al.*, 1962 ; Cunningham, 1959 ; Moule *et al.*, 1962 ; Sutherland *et al.*, 1955 ; Hemingway *et al.*, 1970 ; Ishmael *et al.*, 1970). However these complexes produce more or less inflammatory reactions at the injection site (Ishmael, 1970 ; Hemingway *et al.*, 1970).

Copper oxide has been successfully used as per os dosing in a particulate form (Dewey, 1977) but this type of treatment is less easy to handle than an injection and the copper is still submitted to copper absorption variations from an animal to another one.

We tried subcutaneous injections in the dewlap of 400 mg of copper glycinate in 10 Friesian or Holstein cows. The skin fold thickened from 2 to 10 cm in 4 days and 19 days later the dewlap was still 5 cm thick (Lamand, 1969, unpublished results).

The copper fine dust is an insoluble form which is liberated slowly out of the injection site by a moderate inflammatory process. The zinc oxide behaves similarly.

The observed hypercupremia is maximum on the 6th day (Fig. 1) and as ceruloplasmin evolution parallels plasma copper, the injected copper has probably been metabolized. The liver copper storage increased to normal levels, without attaining 1 000 mg/kg

Table 3 : Comparison of hepatic copper levels at slaughter (μg/g D.M.).

Pair N°	Injected	Controls
1	113	45
2	390	288
3	450	282
4	630	705
5	990	570
6	456	328
7	855	322
8	595	246
9	400	382
10	810	134
Mean	569*	331*

* Significantly different ($P < 0.05$).

Table 4 : Comparison of mean plasma zinc during preexperimental period and experimental period (after zinc injection) [μg/100 ml] in paired ewes.

Pair N°	Injected			Controls		
	Before treatment	After treatment	Difference	Before treatment	After treatment	Difference
1	63	92	29	58	64	6
2	65	75	10	65	65	0
3	67	86	19	67	63	— 4
4	68	87	19	69		
5	71	89	18	69	64	— 5
6	74	84	10	72		
7	77	102	25	76	77	1
8	78	100	22	79	66	— 13
9	80	90	10	83	68	— 15
10	86	96	10	98	72	— 26
Mean			17*			— 7*

* Significantly different ($P < 0.001$).

M.S., the value considered as a toxic limit (Underwood, 1971). No hemolytic crisis occurred since plasma bilirubin level remained normal throughout the experiment. Zinc mobilisation was slower since the maximum plasma zinc was not obtained until the 10th day post injection.

The plasma copper and zinc diminution observed in the control animals indicates that the copper and zinc contents of the hay were deficient in these elements. As already seen (Grace, 1972), in spite of low plasma zinc the ewes remained clinically normal throughout the experiment.

The experiment described shows that the copper or zinc injection is efficient for at least 21 days for copper and 17 days for zinc. Copper has been stored in the liver. As zinc is stored only to a slight extent (Mills *et al.*, 1967) the improvement from the zinc injection lasts so long as there is some zinc left at the injection site.

Clinically the injected animals remained normal without any pain and palpation of the neck of the ewes gave no reaction.

A further experiment in cows showed similar results (Lamand unpublished results) but the best form of copper was copper oxide * and zinc dust * to induce a moderate inflammation and thus the optimum delayed effect. In this experiment cattle showed stronger inflammatory reactions than sheep.

The results described indicate that both powder of metal or oxide of copper or zinc suspended in purified olive oil may be injected intramuscularly without any detrimental effect. This treatment increases the copper or zinc plasma levels and is thus able to cure a deficiency in the considered element. The copper injected is stored and the effect of the injections lasts more than three weeks.

* I.N.R.A.-A.N.V.A.R. Patent. PROLONTEX ® PROLIGO, Paris.

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Summary

In two experiments, compared to 10 control, 10 ewes were injected intramuscularly either with metallic copper dust or zinc oxide, suspended in purified olive oil. Copper injection raised the plasma and hepatic copper of the treated ewes. Similarly, injected zinc oxide produced a rise in plasma zinc. These increases started respectively on the 6th and 3rd day after the injection and lasted till the 21st or 17th day. No detrimental inflammation appeared. This form of treatment is therefore efficient against trace element deficiencies without any side effect.

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