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EVIDENCE OF A THIAMINE DEFICIENCY IN SHEEP FED MAIZE SILAGE

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Résumé

MISE EN ÉVIDENCE D'UNE CARENCE EN THIAMINE CHEZ LE MOUTON RECEVANT UN RÉGIME D'ENSILAGE DE MAÏS. — Le présent travail sur moutons correspond à une étude comparée de l'incidence de la consommation d'un régime foin ou ensilage de maïs sur les teneurs en thiamine du contenu du rumen et de certains tissus et organes, l'activité transcétolasique du sang et l'activité fermentaire des microorganismes du rumen.

Les teneurs en thiamine du jus de rumen des animaux soumis à l'ensilage sont environ trois fois plus faibles qu'avec le foin. Le passage d'un régime foin à un régime ensilage de maïs entraîne une diminution importante des teneurs en vitamine B1 du cerveau, des reins, du cœur, du foie, de la paroi du rumen et du gros intestin. Parallèlement, on note une baisse significative de l'activité transcétolasique et de l'effet TPP. Le régime n'a par contre aucun effet significatif sur les teneurs en ammoniac ou en acides gras volatils. Seules les proportions molaires des acides butyrique et isovalérique sont significativement plus élevées pour la ration ensilage de maïs.

Les relations entre les fermentations de type butyrique, l'activité thiaminasique de l'ensilage de maïs et un rôle éventuel des clostridia sont discutées. L'ensemble des résultats montre que le régime ensilage de maïs provoque un état carenciel chronique et accusé en thiamine.

The introduction of maize-silage in winter diets of sheep is often accompanied by the appearance of serious nervous disorders (Van Quackebeke, 1971 ; Espinasse, 1973), resembling those of polioencephalomalacia or necrosis of the cerebral cortex which is characteristic of thiamine inadequacy (Jensen *et al.*, 1956).

It is conventionally accepted that ruminants, owing to syntheses by the ruminal micropopulation, are not dependent on an exogenous intake

of vitamins of group B. Certain authors (Phillipson and Reid, 1957 ; Rerat, 1960) have, however, pointed out that substantial variations occur in the magnitude of these syntheses according to the nature of the diet. The hypothesis that a lack of thiamine (vitamin B1) may arise with such a peculiar food as silage made from the whole maize plant has been proposed (Cottreau, 1972) and takes into consideration the similarity of some disorders engendered by this diet and

those observed in cases of a known lack of vitamin B1 (Petersen and Jensen, 1975).

The present work consists of a comparative study of the effects of the consumption of a hay or maize-silage diet on the intake of thiamine and the thiamine-synthesising and degrading capacity of ruminal microorganisms. It is concerned with the influence of the diet on :

1. the concentrations of thiamine in the rumen contents and the amounts passing through the reticulo-omasal orifice,
2. the thiamine content of different organs and tissues and the transketolase activity of the blood,
3. the metabolic activity (particularly thiaminase production) of the ruminal microorganisms.

Materials and Methods

Experimental animals and apparatus

The experiments were carried out on 22 adult Merino sheep, either fistulated or not, weighing on average 45 kg and kept in individual cages.

Table 1 indicates the allocation of the animals to groups, their feeding protocol, the experimental period associated with each diet and the different criteria employed.

In the first experiment, each fistulated animal received in succession *ad libitum* hay, maize silage (the fermentation characteristics of which are indicated in table 2) and hay. A kinetic study of the postprandial changes in the concentration of thiamine and the fermentation of the rumen content was carried out.

In a second experiment, the amount of hay was reduced so as to equate the ingested vitamin B1 of the hay diet with that of the maize silage fed *ad libitum*.

In a third experiment, animals were fed for three months with both of the experimental rations, then were slaughtered for organ and tissue samples.

Taking and preparations of samples

1. Ruminal contents

At the time of the kinetic study, the animals previously starved for 12 h received a food and

Table 1. - Distribution of animals according to their mode of feeding and different criteria retained for each study

	Fistulated animals				Non-fistulated animals	
	Fed <i>ad libitum</i>			With rationed hay diet for 2 months	Fed for 3 months	
	With hay for 2 months	With maize silage for 3 months	With hay		With maize silage	
Number of animals	4	4	4	4	3	3
<i>Pre-experiment</i>						
habituation to diet	+	+	+	-	+	+
daily intake	+	-	+	-	+	+
<i>Kinetics in vivo</i>						
NH ₃	+	+	+	-	-	-
volatile fatty acids	+	+	+	-	-	-
thiaminase	+	+	+	-	-	-
polyethylene glycol	+	+	+	+	-	-
thiamine	+	+	+	+	-	-
daily intake	+	+	+	+	-	-
<i>Vitamin condition</i>						
blood						
transketolase	+	-	+	-	+	+
thiamine	+	-	+	-	+	+
organs ^a						
thiamine	-	-	-	-	+	+

a : kidneys, lungs, heart, and digestive tract.

water ration for the first two hours. At that point and again 3 h later, a warm solution of polyethylene glycol 4000 (PEG), used as a marker for the liquid phase of the reticulo-rumen to estimate its volume as well as the reticulo-omasal outflow rate, was introduced into the rumen (Hyden, 1961).

The ruminal contents, sampled each hour for 9 h, were strained through six layers of surgical gauze.

On the homogenised filtrate the following analyses were carried out: thiamine, volatile fatty acids (VFA), thiaminase, ammonia and PEG.

2. Blood and organs

The thiamine status of the animals was determined on the one hand from the transketolase activity of the blood of the fasting subjects and, on the other hand, from the thiamine content of certain organs (kidneys, lungs, liver, heart, digestive tract) and the nervous and muscular tissues of the slaughtered animals (table 1).

Analytical methods

The percentages of dry matter, minerals, total nitrogen (Kjeldahl method), cellulose (Weende method) and fats (Soxhlet extractor) in the experimental forages were determined.

The concentrations of thiamine in the ruminal juice and the forages were determined by the fluorimetric method (Freed, 1966 ; Edwin *et al.*, 1975) as modified by Massengo (1977); its concentration in the blood and organs was assayed by the method of Edwin *et al.* (1975).

The transketolase activity of the blood was measured according to the Warnock method (1970). Thiamine pyrophosphate effect was estimated as the percentage of stimulation of transketolase activity by addition of thiamine pyrophosphate in the blood sample.

PEG was determined by the Hyden method (1961). Three criteria were used to determine the metabolic activity of the ruminal microorganisms :

1. Ammoniogenesis: the ammonia level was determined by the gaseous diffusion method of Conway.

2. VFA production: total VFA and the proportions of the individual acids were determined using an Intersmat IGC-120 FB chromatograph with a glass column packed with 25 % N-pentylglycol adipate, 2 % H₂PO₄ on chromosorb W (80-100 mesh) at 140 °C.

3. Thiaminase activity was measured by the radioactive method of Edwin and Jackman (1974).

The Student « t » test was used to test significance of differences among treatment means.

Results

Thiamine percentage of the ruminal contents

1. Comparison between the amount of ingested thiamine on the two diets

The analysis of the experimental feeds (table 3) indicates that the hay was nearly 3-fold richer in thiamine than the maize-silage.

The ingestion of dry matter, and therefore of thiamine, were also significantly greater on average with the hay diet (P<0.01) for the experimental meals distributed every two hours (table 4).

With *ad libitum* feeding, the level of daily consumption of dry matter on hay was not significantly different from that for maize-silage. Consequently, thiamine ingestion with the hay diet was definitely greater than with the maize-silage diet (table 4).

2. Influence of the diet on the percentage of thiamine in the rumen contents

2.1. Effect of the diet

For the two diets, the thiamine concentrations in the ruminal juice did not vary significantly with time during the kinetic study (fig. 1). On the other

Table 2. - Conservation characteristics of maize-silage

	Mean ± standard error
<i>Volatile fatty acids in silage (g/kg dry matter)</i>	
Acetic acid	56.1 ± 5.6
Propionic acid	6.6 ± 1.1
Isobutyric acid	5.6 ± 0.3
Butyric acid	4.4 ± 1.6
Isovaleric acid	5.5 ± 1.1
Valeric acid	6.9 ± 2.3
N-NH ₃ (% NT)	8.0 ± 3.9
pH	3.5 ± 0.9

hand, whatever the time of measurement, these levels were much lower with the silage diet.

2.2. Effect of the experimental period

The progressive substitution of a maize-silage diet for a hay diet involves a considerable and sustained reduction of the thiamine concentration in the ruminal juice. The return to a hay diet was accompanied, on the other hand, by an increase in the thiamine concentration, which levelled out at approximately the initial level (fig. 2).

The regular and depressive effect of the maize-silage diet from the second to the fourth month on the thiamine percentages of the rumen contents (fig. 2), was significant ($P < 0.05$).

3. Influence of the diet on the amounts of thiamine conveyed through the reticulo-omasal orifice

The amounts of thiamine, Q2 and Q5, contained in the rumen 2 and 5 h after the beginning of the meal, were calculated from the measurements of its concentration and the estimate of the corresponding volumes of the rumen contents. The liquid volume of the rumen and the rate of outflow of content which passed through the reticulo-omasal orifice, were determined by using PEG as a marker. If one concedes that there is no thiamine absorption at the rumen level, the comparison of the average (Qd) of the difference (Q2 - Q5) to the average amount of thiamine (Qt) which has left the rumen owing to

Table 3. - Chemical composition of forages

Composition	Diet (mean \pm s.e.)	
	Grass hay	Maize-silage
Ash (%)	8.6 \pm 0.9	5.5 \pm 0.6
N \times 6.25 (%)	6.7 \pm 0.5	8.9 \pm 0.7
Fiber (%)	34.4 \pm 1.0	27.5 \pm 1.7
Fats (%)	1.0 \pm 0.8	2.4 \pm 0.3
NNE (%)	49.3 \pm 1.3	55.7 \pm 1.2
Thiamine (μ g/g, DM)	8.6 \pm 1.0	3.1 \pm 0.4
Dry matter (%)	88.0 \pm 1.2	26.4 \pm 1.6

Table 4. - Forage intake levels and corresponding thiamine ingested

	Diet (mean \pm s.e.) ^a	
	Grass hay	Maize-silage
<i>Pre-experimental period (14 assays)</i>		
Ingested/day (g of dry matter)	1 415.1 \pm 248.8	1 265.7 \pm 80.5
Thiamine ingested/day (mg)	12.1 \pm 0.9 _e	3.9 \pm 0.3
<i>Trials in vivo (13 assays)^b</i>		
Ingested/meal (g of dry matter)	483.3 \pm 50.2 _e	289.5 \pm 59.6 _f
Thiamine ingested/meal (mg)	4.1 \pm 0.4 _e	0.89 \pm 0.26 _f

a : values affected with different letter on the same line are significantly different ($P < 0.05$).

b : 2 hours meal *ad libitum*.

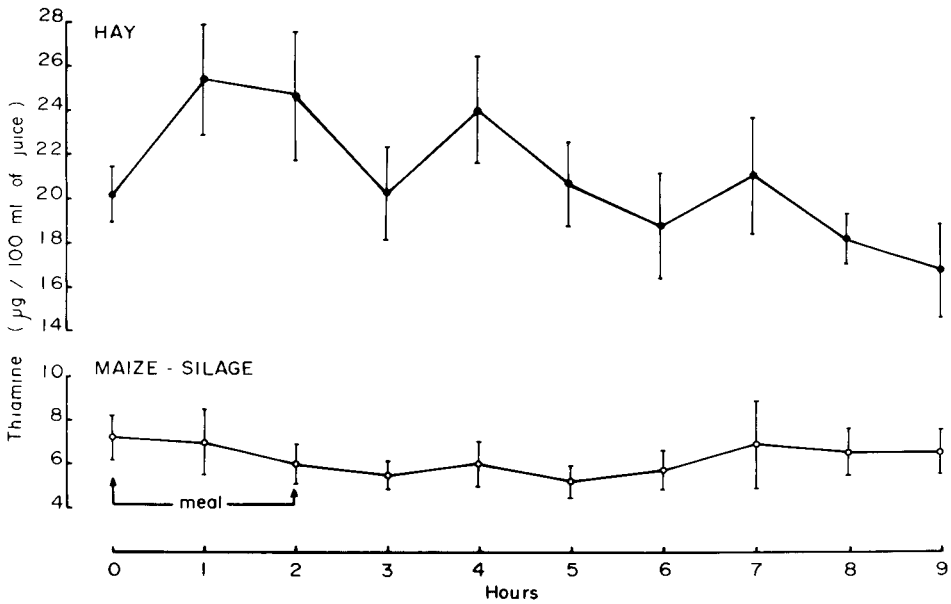


Fig. 1. — Changes in the thiamine content of the rumen (µg/100 ml of strained juice, mean, standard error, n = 13). This evolution is studied during nine post prandial hours *in vivo*, the animals receiving a food and water ration *ad libitum* only during the first two kinetics hours. (n) number of kinetics.

the conveyance, leads one to conclude that :

- a) if $Q_d < Q_t$, thiamine synthesis occurred,
- b) if $Q_d = Q_t$, either equilibrium was reached between breakdown and synthesis, or no synthesis took place ;
- c) if $Q_d > Q_t$, either thiamine breakdown or breakdown exceeded synthesis.

With *ad libitum* feeding, table 5 indicates that with the hay diet thiamine synthesis occurred (Q_t being significantly greater than Q_d). One notes, on the other hand, that very little synthesis resulted (Q_d comparable to Q_t) with the maize-silage diet.

With a rationed diet (ingested hay dry matter being restricted to ensure that exogenous intake of thiamine was equivalent to the highest vitamin level measured on maize-silage with *ad libitum* feeding), the same calculations (table 6) indicate that a state of equilibrium resulted for both diets (Q_d comparable to Q_t). The influx into the rumen of organic matter during the experimental meal

was, on average, 389.6 g for maize-silage and only 151.3 g for hay.

Thiamine concentration in the blood and tissues

1. Transketolase activity and thiamine concentration of whole blood

The ingestion of maize-silage was not only accompanied by a fall in the concentration of thiamine in the rumen juice (fig. 2), but also by a significant drop in the transketolase (TK) activity ($P < 0.01$) and in the thiamine concentration in whole blood ($P < 0.05$) (table 7). The decline in TK activity was accompanied by a fall in the thiamine pyrophosphate stimulation (TPP effect) rather than the rise that might have been expected).

2. Thiamine concentration in the organs

A general decrease was observed in the thiamine concentrations of the organs of sheep fed on maize-silage as compared with the hay

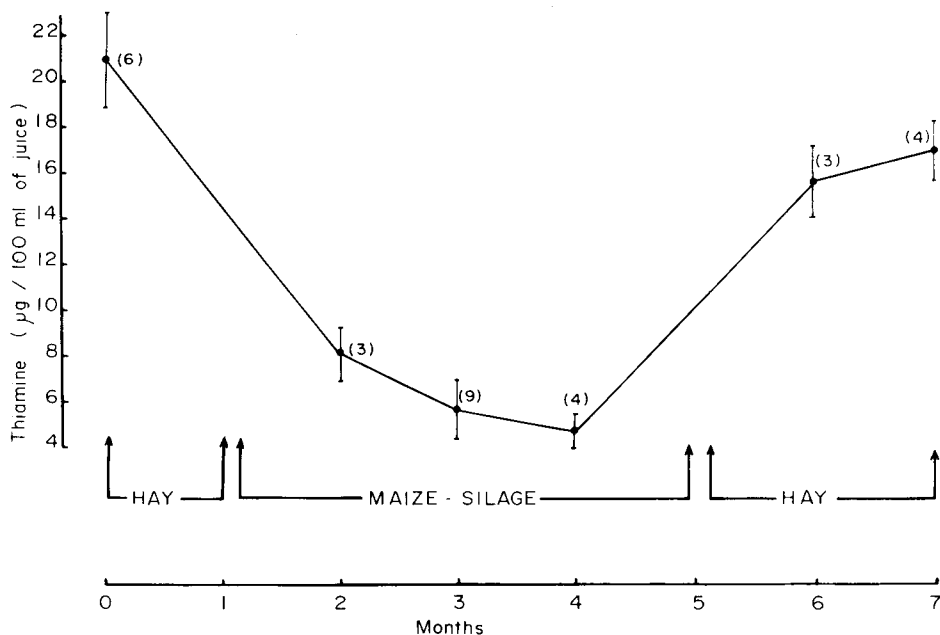


Fig. 2. — Influence of the experimental period on the thiamine content (in $\mu\text{g}/100\text{ ml}$ of juice) of the rumen juice (mean, standard error). One notes a considerable fall in the percentages, following the transfer to a maize-silage from a hay diet and again a rise in the opposite case. (n) number of estimations.

Table 5. - Estimation of the amount of thiamine in the ruminal juice at two and five hours after the meal (*ad libitum* feeding), of the net reduction between these two times and of the outflow of thiamine from the rumen

	Diet (mean \pm s.e.) ^a	
	Grass hay (on seven kinetics)	Maise-silage (on eleven kinetics)
<i>Post-prandial period</i>		
2 hours		
thiamine content ($\mu\text{g}/\text{l}$)	216.76 \pm 21.89	58.66 \pm 16.88
total thiamine (μg)	1 447.98 \pm 167.50	285.45 \pm 120.5
5 hours		
thiamine content ($\mu\text{g}/\text{l}$)	209.86 \pm 12.93	50.16 \pm 17.45
total thiamine (μg)	960.00 \pm 101.80	181.52 \pm 85.75
Net reduction of thiamine between 2 and 5 h (μg)	487.18 \pm 159.23a	103.93 \pm 86.27
Total amount of thiamine having moved between 2 and 5 h (μg)	877.12 \pm 142.88b	162.38 \pm 79.28

a : values affected with different letter in the same column differ significantly ($P < 0.05$).

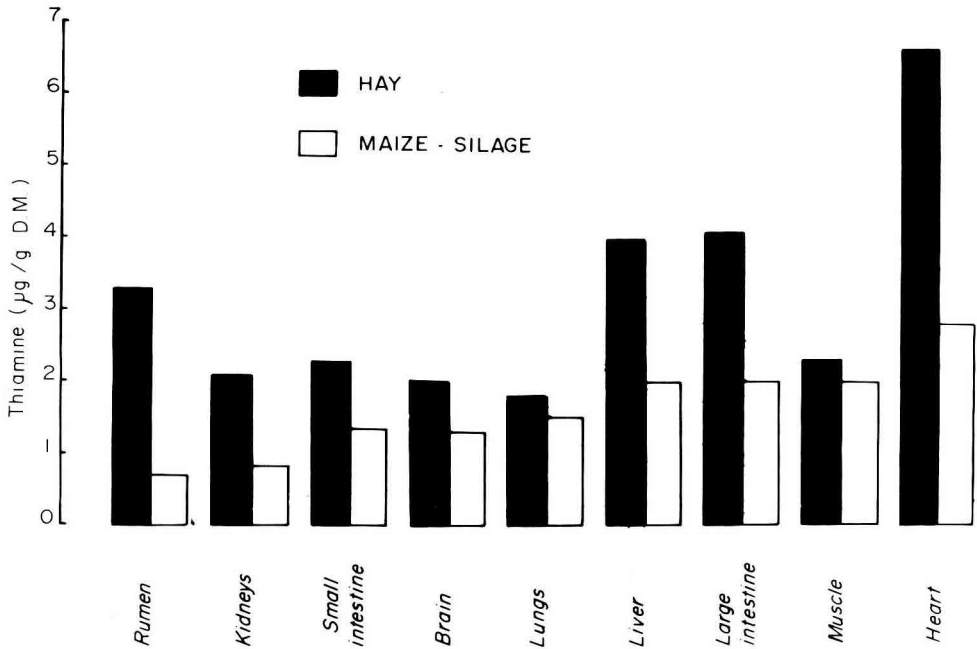


Fig. 3. — Vitamin content of the organs of sheep fed on hay or maize-silage (mean). One notes a general fall in the content with the maize-silage diet which is most pronounced in the cases of the heart, liver, kidney, rumen and large intestine.

Table 6. - Estimation of total quantities of thiamine in the rumen juice at two hours and five hours after the mean (with the exogenous supply of thiamine equivalent for both diets)

and of the amounts with disappeared between these times and which were carried onward by the flow

	Diet (mean ± s.e.)	
	Grass hay (4 kinetics)	Maize-silage (4 kinetics)
<i>Post-prandial period</i>		
2 hours		
thiamine content (µg/l)	189.70 ± 15.70	74.05 ± 13.08
total thiamine (µg)	776.10 ± 62.00	408.20 ± 95.30
5 hours		
thiamine content (µg/l)	168.85 ± 29.34	64.60 ± 4.20
total thiamine (µg)	435.63 ± 75.71	220.29 ± 39.30
Net reduction of thiamine between 2 and 5 h (µg)	345.94 ± 89.29	187.94 ± 134.62
Total amount of thiamine having moved between 2 and 5 h (µg)	392.07 ± 99.67	195.19 ± 43.18

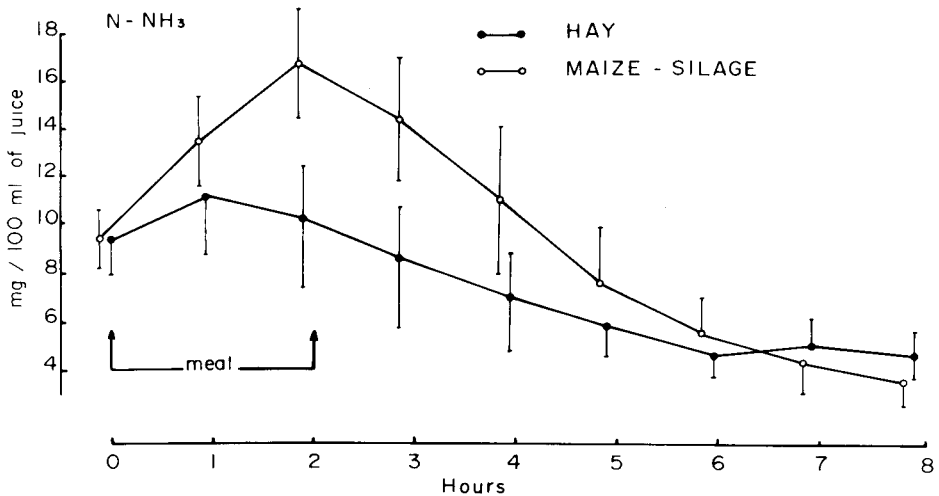


Fig. 4. — Influence of the diet on ammoniogenesis (mean, standard error, $n = 9$). One notes a significantly greater ammoniogenesis ($P < 0.05$) with the maize-silage than with the hay in the first two periods after feeding. $n =$ number of kinetics.

diet (fig. 3). This reduction was particularly pronounced for the heart, the rumen, the large intestine, the liver and the kidneys (these are organs that most actively phosphorylate thiamine).

Fermentation activity of the rumen contents

1. Ammoniogenesis

The concentrations of ammonia in the ruminal juice were significantly greater at times T2 and T3 after the beginning of the meal ($P < 0.05$) for the maize-silage diet (fig. 4); they were comparable for the two diets outside this period.

2. VFA production

The diets did not have a profound effect on the total VFA content of the rumen juice. However, compared with the hay diet, a significant reduction in the percentage of acetic acid occurred on the silage diet, which was compensated by a significant increase in the proportions of isobutyric, isovaleric and valeric acids (table 8).

3. Thiaminase activity of forages and rumen juice

The diets did not have a significant effect on the thiaminase activity of rumen juice (table 9).

However, the results obtained from the forages after maceration revealed a very significant rise in the activity for the maize-silage.

Discussion and Conclusions

Since these analytical results for ruminal thiamine content were obtained from strained juice, the concentrations of thiamine recorded can be attributed to the resultant of intake plus synthesis less degradation and absorption but including only the vitamins present in microbes and fine food particles. This procedure, although neglecting the thiamine involved in the filtration residue (coarse food particles and fixed microorganisms), avoids the disadvantage of the heterogeneity of the total contents which, besides the fluid part, is comprised of the recently ingested food and the residues of previous meals.

From the comparisons made in this study it is obvious that maize-silage has both a short and long-term depressive effect on the thiamine concentration of the rumen contents. This

Table 7. - Transketolase activity and thiamine content of whole blood according to the diet

	Diet (mean ± s.e.) ¹ (10 samples)	
	Grass hay	Maize-silage
Transketolase activity of whole blood (μ M/ribose/ml/h)		
Normal	6.5 ± 1.2a	2.4 ± 0.5b
with thiamine pyrophosphate	8.4 ± 1.5a	2.7 ± 0.4b
thiamine pyrophosphate effect (%)	29.2 ± 0.5a	12.5 ± 0.9b
Thiamine content of whole blood (μ g/100 ml)	6.8 ± 0.7a	4.6 ± 0.7b

1 : values affected with different letter on the same line differ significantly (P <0.05).

Table 8. - Influence of the diet on the molar proportion of each volatile fatty acid, two hours after the beginning of the meal

Volatile fatty acids (percentage of total VFA) (n = 9)	Diet (mean ± s.e.) ¹	
	Grass hay	Maize-silage
Acetic acid	64.17 ± 3.55a	56.65 ± 1.72b
Propionic acid	19.97 ± 3.45	22.00 ± 0.93
Isobutyric acid	1.97 ± 0.54	2.75 ± 0.82
Butyric acid	10.23 ± 1.36a	15.82 ± 1.97b
Isovaleric acid	1.12 ± 0.46a	2.73 ± 0.68b
Valeric acid	1.34 ± 0.43	2.46 ± 0.75

1 : values affected with different letter on the same line differ significantly (P <0.001).

Table 9. - Thiaminase activity of rumen juice and of forages

Thiaminase activity	Diet (mean ± s.e.)	
	Grass hay (9 kinetics)	Maize-silage (17 kinetics)
<i>in forage</i> (μ M of thiazole/min/100 g dry matter)	none	25.5 ± 2.8
<i>in rumen</i> (μ M of thiazole/ml/min) expressed in hours after feeding		
0	1.3 ± 0.2	1.4 ± 0.2
1	1.2 ± 0.6	0.8 ± 0.2
2	1.3 ± 0.7	1.1 ± 0.5
3	1.3 ± 0.3	0.7 ± 0.4
4	1.0 ± 0.2	1.1 ± 0.3
5	1.3 ± 0.4	0.8 ± 0.2
6	1.2 ± 0.3	0.6 ± 0.1
7	0.6 ± 0.2	0.9 ± 0.3
8	1.2 ± 0.4	1.0 ± 0.3
9	1.3 ± 0.5	0.8 ± 0.2

undermines the theory according to which a weak exogenous influx of thiamine would stimulate microbial synthesis (Kon and Porter, 1953), but conforms to the conception of other authors who have pointed out large variations in the percentages of thiamine of the rumen contents dependent upon the size of the exogenous supply of this vitamin (Phillipson and Reid, 1957; Buziassy and Tribe 1960; Rerat, 1960).

The depressive effect of maize-silage is also detectable in the blood by a fall in the blood thiamine concentration and transketolase activity, the latter being one of the three enzymes for which thiamine plays the role of coenzyme in the form of thiamine pyrophosphate (TPP). It is said to be the only one that determines the physiological effectiveness of the vitamin in relation to the real thiamine status of the subjects (Quaghebeur *et al.*, 1974; Mc Candless *et al.*, 1976).

The TPP effect, that is to say, the percentage of stimulation of the transketolase activity obtained after addition of thiamine pyrophosphate in the incubation environment, is normally rendered greater as the thiamine deficiency becomes more acute. However, according to Brin (1962), with the occurrence of a thiamine deficiency there could be not only a loss of coenzyme (TPP), but also of apoenzyme to varying degrees. Our results support this observation, the effect to TPP being weak in animals fed on the maize diet. On the other hand, this stimulation manifested itself in normal proportions (Warnock, 1970) in subjects receiving the hay. Thus one possibility is that the silage diet induced a repressive phenomenon on the biosynthesis of apotransketolase in association with low thiamine concentrations. This question should be investigated and awaits clarification.

At the organ and tissue level, the decrease in the concentration of thiamine was systematic in animals which had received the maize-silage diet. The thiamine concentrations of the hepatic tissue (4 µg/g DM) with a hay diet are close to those reported by Lewis *et al.* (1967) for pruruminant lambs (5 to 7.3 µg/g DM) receiving a conventional diet. Though our results are inconclusive, it seems that the manifestation of the deficiency in the form of cerebral cortex necrosis (CCN) is a final stage that is only reached through extremely low vitamin concentrations. Indeed, neither in our work (1.2 µg/g DM of the brain for animals receiving maize-silage), nor in that of Lewis *et al.* (1967) (0.5 µg/g DM of the brain of subjects receiving a diet deficient in thiamine), was CCN noticed. With maize-silage, it is to be feared, however, that this stage can be rapidly

reached by young animals with very high production performances as was observed by Pierson and Jensen (1975) in feedlot farms. The considerable decrease in the thiamine content for the heart of subjects fed on maize-silage may be linked with the cardiomyopathies sometimes recorded when there is a thiamine deficiency.

Altogether these experimental results show clearly that a maize-silage diet causes a state of thiamine deficiency. The etiology of this deficiency can be looked for a priori in a low intake and in a lack of microbial synthesis on such a food. In this study such a synthesis was only confirmed with the hay diet fed *ad libitum*. The fact of having reduced the supply of organic matter by controlling the hay intake to provide a vitamin intake equivalent to that of the maize-silage ration, appeared to stop thiamine synthesis short. One can suppose in this case that a decrease in microbial activity accounts for the phenomenon.

With the maize-silage diet, neither the influx of organic matter, nor the ammonia concentrations of the rumen capable of ensuring maximum microbial proteosynthesis (Roffler *et al.*, 1976), nor the total VFA content can be considered as limiting factors in comparison with the hay diet. In the case of this diet, then, one can consider two possibilities :

- either the micropopulation synthesizing the thiamine is present but its effect is disturbed by other factors (thiaminases, for example),
- or it is absent and, in this case, a modification of the fermenting species should account for it.

Now, the molar distribution of volatile fatty acids present in the rumen, two hours after the beginning of the meal, for example, revealed significantly greater proportions of butyric acid with the maize-silage diet compared with the hay ration, for which the fermentations tended to be more of the acetic type. The microbial species induced by the two diets were, therefore, different and one can imagine the existence of germs of the genus *Clostridium* with the maize-silage diet. *Clostridia* are sporulating bacteria that can elaborate the thiaminases whose presence was tested in the silage and in the rumen of animals ingesting this type of forage. *Clostridium sporogenes* is, in particular, a known source of thiaminases in the rumen of sheep with cerebral cortex necrosis (Edwin and Jackman, 1973; Shreeve and Edwin, 1974). This led us to measure the thiaminase activity of the ruminal juice and the forages for the two diets. The results obtained from the forages after maceration supported our hypothesis : no thiaminase acti-

activity at all for the hay ; a very great activity for the maize-silage (25.5 μ M of thiazole/min/100 g of DM). On the other hand, the results from the ruminal juice are surprising : large, comparable thiaminase activities, revealing the existence of thiaminases in the ruminal juice with both the diets. This result, if at least unexpected, is not however unique : Loew *et al.* (1970), Edwin and Jackman (1973) and Roberts and Boyd (1974) have reported a notable thiaminase activity in the ruminal juice of clinically normal animals. One can suppose that with a diet like hay, rich in thiamine, either the synthesis and the exogenous influx offset the possible destruction due to thiaminase activity with, as a resultant, a normal vitamin content in the subjects, or the thiaminase activity that we have measured is only *potential* and in reality does not manifest itself in the same way with the two diets, through a lack of cosubstrates on the hay diet. Indeed, the thiaminase

activity (I and II) is linked to the presence of cosubstrates in the reaction environment. Several substances, which range from the simple amino compounds like methylamine and nicotinic acid (Edwin *et al.*, 1968) to veterinary products like anthelmintics and tranquilizers (Roberts and Boyd, 1974), can play this role. On the recommendation of Edwin, we have used on the one hand pyridine as cosubstrate and on the other hand a radioactive thiamine substrate of high specific activity (10 μ Ci/ μ M) in order to detect very low levels of thiaminase activity. Studies in progress are aimed particularly at specifying this influence of cosubstrates present in the environment on the *real* thiaminase activity of the ruminal juices induced by the two diets.

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Summary

Two groups of Merino sheep (with or without rumen fistulae) were used to study the effect of feeding maize-silage (compared to hay) on : thiamine concentrations in rumen fluid, tissues and organs ; blood transketolase activity ; microbial fermentation in the rumen.

After sheep were transferred from a hay diet to maize-silage, thiamine concentrations in ruminal fluid fell to about 25 % of control values on hay. Thiamine levels greatly decreased in kidney, heart, liver, rumen tissue and large intestine. Blood transketolase and TPP effect significantly decreased ($P < 0.01$). Ruminal ammonia nitrogen and total volatile fatty acid (VFA) concentrations were not affected by the two diets. However, both butyric acid and isovaleric acid were significantly higher ($P < 0.001$) in rumen fluid of sheep fed maize-silage. No significant differences were detected between diets for ruminal thiaminase activity regardless of the time of sampling.

Relationships between butyric fermentation, thiaminase activity in maize-silage diet and a possible role of *Clostridium* are discussed. It was concluded that there is a marked thiamine deficiency in sheep fed maize-silage ; the factors involved need further study.

References

- BRIN M., 1962. Effects of thiamine deficiency and of oxythiamine on rat tissue transketolase. *J. Nutr.*, **78**, 179-184.
- BUZIASSY C., TRIBE D.E., 1960. The synthesis of vitamins in the rumen of sheep. I. Effect of diet on the synthesis of thiamine, riboflavin and nicotinic acid. *Aust. J. Agric. Res.*, **11**, 989-1001.
- COTTEREAU P., 1972. Les incidences de l'alimentation à base d'ensilage de maïs sur les productions et la santé des bovins. *Rev. Méd. Vét.*, **123**, 147-167.
- EDWIN E.E., LEWIS G., ALLCROFT R., 1968. Cerebro-cortical necrosis : a hypothesis for the possible role of thiaminases in its pathogenesis. *Vet. Rec.*, **83**, 176-178.
- EDWIN E.E., JACKMAN R., HEBERT N., 1975. An improved procedure for the determination of thiamine. *Analyst*, **100**, 685-695.
- EDWIN E.E., JACKMAN R., 1973. Ruminal thiaminase and tissue thiamine in cerebro-cortical necrosis. *Vet. Rec.*, **92**, 640-641.
- EDWIN E.E., JACKMAN R., 1974. A rapid radioactive method for determination of thiaminase activity and its use in the diagnosis of cerebro-cortical necrosis in sheep and cattle. *J. Sci. Food Agric.*, **25**, 357-368.

- ESPINASSE J., 1973. Pathologie liée à l'alimentation par l'ensilage de maïs chez les ruminants. *Recl Méd. Vét.*, **149**, 1271-1282.
- FREED M., 1966. Thiamine. In : *Methods of vitamin assay*, **6**, p. 123-145, 3rd ed., Interscience, London.
- HYDEN S., 1961. Determination of the amount of fluid in the reticulo-rumen of the sheep and its rate of passage to the omasum. *Kgl. Lantbrukshögsk. Ann.*, **27**, 51-79.
- JENSEN R., GRINER L.A., ADAMS O.R., 1956. Polioencephalomalacia of cattle and sheep. *J. Am. Vet. Med. Assoc.*, **129**, 311-321.
- KON S.K., PORTER J.W.G., 1953. The B1 vitamin content of the rumen of steers given various diets. *Proc. Nutr. Soc.*, **12**, XII.
- LEWIS G., TERLECKI S., MARKSON L.M., ALLCROFT R., 1967. Thiamine status of lambs in relation to cerebro-cortical necrosis. *Proc. Nutr. Soc.*, **26**, 1, XIII.
- LOEW F.M., DUNLOP R.H., CHRISTIAN R.G., 1970. Biochemical aspects of an outbreak of bovine polioencephalomalacia. *Can. Vet. J.*, **11**, 57-61.
- Mc CANDLESS D.W., CURLEY A.D., CASSIDY C.E., 1976. Thiamine deficiency and the pentose phosphate cycle in rats : intracerebral mechanism. *J. Nutr.*, **106**, 1144-1151.
- MASSENGO J., 1977. *Recherches sur la carence en thiamine chez le mouton recevant un régime d'ensilage de maïs*. Thèse de Docteur-Ingénieur, INP Toulouse.
- PHILLIPSON A.T., REID R.S., 1957. Thiamine in the contents of the alimentary tract of sheep. *J. Nutr.*, **11**, 27-41.
- PIERSON R.E., JENSEN R., 1975. Polioencephalomalacia in feedlot lambs. *J. Am. Vet. Med. Assoc.*, **166**, 257-259.
- QUAGHEBEUR D., OYAERT W., MUYLLE E., DE ROOSE P., 1974. Blood thiamine levels and transketolase activities as a diagnostic method for thiamine adequacy in cattle. *Zentralbl. Veterinärmed. A*, **21**, 851-860.
- RERAT A., 1960. *Synthèse et absorption des vitamines B dans le tube digestif du mouton adulte*. Thèse Doct. Sci. Nat., Paris.
- ROBERTS G.W., BOYD J.W., 1974. Cerebro-cortical necrosis in ruminants : occurrence of thiamine in the gut of normal and affected animals and its effect on thiamine status. *J. Comp. Pathol.*, **84**, 365-374.
- ROFFLER R.E., SCHWAB C.G., SATTER L.D., 1976. Relationships between ruminal ammonia and non protein nitrogen utilization by ruminants. III. Influence of intraruminal urea infusion on ruminal ammonia concentration. *J. Dairy Sci.*, **59**, 80-84.
- SHREEV J.E., EDWIN E.E., 1974. Thiaminase producing strains of *Clostridium sporogenes* associated with outbreaks of cerebro-cortical necrosis. *Vet. Rec.*, **94**, 330-338.
- VAN QUACKEBEKE E., 1971. Les problèmes posés par l'utilisation du maïs. *Rev. Pâtre*, **118**, 14-16.
- WARNOCK L.G., 1970. A new approach to erythrocyte transketolase. *J. Nutr.*, **10**, 1057-1062.