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Short note

Vitamin B₁₂ levels in ewe colostrum and milk and in lamb serum

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Summary — Vitamin B₁₂ was measured in samples of normal ovine colostrum and milk and in sera of lambs and ewes taken at various stages of lactation, from lambing up to 16 d. Colostrum obtained within 24 h of lambing contained high concentrations of vitamin B₁₂, but within a few days the levels fell. The lambs received high levels of vitamin B₁₂ from their dams' colostrum but a parallel decrease of vitamin B₁₂ levels in lambs' serum and ewes' milk was subsequently observed. Milk vitamin B₁₂ was analysed by the microbiological method (*Lactobacillus leichmannii*, ATCC 7830) and a radioisotope dilution (RID) technique. Milk vitamin B₁₂ concentrations were greater with the microbiological technique compared with the RID method. The radioassay method has been shown to be as reliable as the microbiological method for measuring vitamin B₁₂ in ovine milk except for very low levels.

vitamin B₁₂ / colostrum / milk / sheep

Résumé — Concentrations en vitamine B₁₂ dans le colostrum et le lait de brebis et dans le sérum des agneaux. Les concentrations en vitamine B₁₂ ont été mesurées dans le colostrum, le lait et le sérum de 10 brebis ainsi que dans le sérum des agneaux depuis le jour de l'agnelage jusqu'à 16 j après la mise bas. Le colostrum obtenu 24 h après l'agnelage contenait une concentration élevée de vitamine B₁₂ mais celle-ci diminuait rapidement. Les agneaux recevaient du colostrum maternel une grande quantité de vitamine B₁₂; celle-ci diminuait parallèlement dans le sérum des agneaux et dans le lait de brebis. Les 2 méthodes utilisées pour doser la vitamine B₁₂ (méthode microbiologique et technique de dilution radioisotopique) ont donné des résultats semblables dans le lait sauf pour des concentrations faibles de vitamine B₁₂.

vitamine B₁₂ / colostrum / lait / mouton

* Correspondence and reprints

INTRODUCTION

Vitamin B₁₂ (cyanocobalamin) is an essential nutrient occurring in milk, which acts as an integral part of various enzymes in the body. Its deficiency affects red-blood-cell maturation, reduces nitrogen retention and limits the concentration in blood and liver. Vitamin B₁₂ deficiency in ruminants is due to insufficient dietary intake of cobalt, which is required by the rumen bacteria for the synthesis of this vitamin. In lambs, until the rumen is functional, the only vitamin B₁₂ supply is their mother's milk. It is believed that the concentration of vitamin B₁₂ in the milk is a reliable index of cobalt sufficiency in ruminants (Annenkov, 1982). However there is little published information about the concentration of vitamin B₁₂ in normal ewe colostrum and milk.

Microbiological assays, using *Lactobacillus leichmanii* or other microorganisms, have long been the most frequently used methods for quantitative determination of vitamin B₁₂ in milk. The development of radioisotope dilution (RID) assays resulted in a trend towards these rapid and less tedious techniques. With human milk, the microbiological methods for assaying vitamin B₁₂ have largely been replaced by radioassay methods (Thomas *et al*, 1979), which were also adapted to veterinary medicine.

A comparative study of vitamin B₁₂ levels has been made in the liver and sera of sheep measured by microbiological and radioassay methods (Millar and Penrose, 1980; Mohammed and Lamand, 1985; Schultz, 1987) but no evaluation has been made of these methods when applied to the analysis of vitamin B₁₂ in ewes' milk.

This paper compares microbiological and radioassay methods to establish whether the RID technique may be used in the rapid determination of vitamin B₁₂ in ewe colostrum and milk. In addition, it deals with the dynamics of vitamin B₁₂ in colostrum,

milk and serum from sheep and in lambs' serum during the first 16 d after lambing.

MATERIALS AND METHODS

Ten Castellana ewes aged 2–6 yr and their 10 lambs were used for this study, with 5 other lambs which did not receive colostrum. Blood, colostrum and milk samples were obtained immediately after lambing, at 24 and 48 h and thereafter at 4, 8 and 16 d, except for the colostrum-deprived lambs, which were only sampled twice, newborn and at 24 h. In addition, milk samples from other ewes were also used during the first month *post partum* for the correlation study.

The jugular vein was used for acquisition of samples. Sera for vitamin B₁₂ determinations were separated within 2 h of collection and were stored at –20°C for no longer than 1 month before analysis.

Samples of colostrum and milk were obtained by manual expression from normal lactating ewes. None of the donors were receiving treatment with vitamin B₁₂ or antibiotics.

The milk was extracted as described by Gregory (1954), to liberate the vitamin from its carrier. The sample, once prepared, was kept at –20°C until its determination.

Microbiological assay was performed as described by Hansen and Hauschildt (1974), using *L. leichmannii* 313 (ATCC–7830). Cyanocobalamin was used as a reference standard in a concentration range of 1–40 pg/ml. Diluted sample or standard was added to 5 ml medium (Difco Vitamin B₁₂ assay medium) and the contents of the tubes were adjusted to 10 ml with distilled water. Before adding the assay organism, the tubes were sterilized by autoclaving. After incubation at 37°C for 24 h, growth of the assay organism was measured as turbidity at 540 nm using a Perkin–Elmer Lambda 5 spectrophotometer. The samples and standards were assayed in triplicate.

The Solid Phase No-Boil DualCount[®] kit, from Diagnostic Products Corporation, was used for vitamin B₁₂ measurement in serum and milk. This was considered as an ideal commercial product for the determination of this vitamin in ovine serum in a comparative study (Schultz, 1987). Purified hog intrinsic factor was employed as the binder for vitamin B₁₂ in this kit. The same sample extracts

were used for the RID method, and the procedures recommended by the manufacturers were used. All samples and standards were assayed in duplicate. Radioactivity due to [⁵⁷Co] was determined with an LKB-1282 Compugamma counter.

Statistics for intra-assay precision were calculated for each of 3 samples from the results of 10 estimates in a duplicate assay. For inter-assay precision, statistics were calculated using milk samples of 3 different concentrations of vitamin B₁₂ and based on 5 estimates.

RESULTS

Vitamin B₁₂ levels in serum, colostrum and milk samples of ewes and serum lambs, taken from lambing day up to 16 d after, determined by both methods are shown in table I. The lambs that received colostrum at 24 h of life registered significantly ($P < 0.001$) higher levels (3.38 ± 0.54 ng/ml) for vitamin B₁₂ than those that had no colostrum intake (1.18 ± 0.48 ng/ml) and were fed on milk substitutes. For newborn lambs, the same levels were 1.05 ± 0.43 ng/ml and 1.01 ± 0.45 ng/ml, respectively.

Comparison of the vitamin B₁₂ levels found using the microbiological and the RID assay kit gave an equation of the regres-

sion line ($y = 0.852x + 2.387$) and a correlation coefficient of 0.75 ($P < 0.001$). Both analytical methods for measuring vitamin B₁₂ in 40 milk samples had a coefficient of variation $< 15\%$.

The intra-assay precision for ovine milk samples was between 4.7 and 9.0% for the microbiological method and 7.6 and 7.8% for RID assay kit. The inter-assay precision ranged between 7.8 and 9.5% for the first method and between 7.4 and 18% for the second (table II).

DISCUSSION

The vitamin B₁₂ in serum from ewes remained above the adequate range for adult sheep (Fisher and MacPherson, 1990) and significant changes associated with lambing or lactation were not detected.

In lambs, colostrum ingestion produced a notable increase of vitamin B₁₂ serum levels (table I), which decreased progressively during the following days, falling even below the level registered on the first day of life, and on day 16 the lowest level was registered. The rise in serum vitamin B₁₂ coincided with the absorption of colostrum vita-

Table I. Colostrum and milk B₁₂ concentrations determined by *L. leichmannii* and RID methods ($n = 8$), and serum vitamin B₁₂ levels in lambs and ewes on the day of parturition (day 0), at 24 h (day 1), 48 h (day 2) and on days 4, 8 and 16.

Day	Colostrum and milk (ng/ml)				Serum (ng/ml)	
	Microbiological method		RID method		Lamb	Ewe
0	214.5 ± 28	(11.5) ^a	205.7 ± 34	(16.5)	1.05 ± 0.43	1.80 ± 0.72
1	67.5 ± 23	(34.6)	54.0 ± 16	(30.1)	3.38 ± 0.54	1.32 ± 0.71
2	39.2 ± 19	(48.3)	38.4 ± 16	(40.5)	2.01 ± 0.63	1.54 ± 0.71
4	28.2 ± 13	(46.9)	27.5 ± 12	(42.7)	1.00 ± 0.30	1.69 ± 0.76
8	11.0 ± 2.0	(18.5)	10.2 ± 2.0	(18.4)	0.52 ± 0.22	1.55 ± 0.68
16	7.2 ± 0.8	(10.7)	6.4 ± 1.0	(20.1)	0.34 ± 0.10	1.36 ± 0.40

^a Mean ± SD (coefficient of variation (%)).

Table II. Milk vitamin B₁₂ concentrations (ng/ml) determined by *L. Leichmannii* and RID methods.

Sample No	Microbiological method		RID method	
	Mean ± SD	CV	Mean ± SD	CV
Intra-assay (n = 10)				
1	11.7 ± 0.6	4.7	10.0 ± 0.8	7.8
2	7.2 ± 0.7	8.6	6.3 ± 0.5	7.6
3	6.0 ± 0.5	9.0	4.5 ± 0.4	7.8
Inter-assay (n = 5)				
4	12.1 ± 0.9	7.8	9.6 ± 0.9	9.4
5	7.4 ± 0.6	8.6	5.6 ± 0.4	7.4
6	2.9 ± 0.3	9.5	1.8 ± 0.4	18.0

CV: coefficient of variation (%).

mins. In the same way, the lambs that received colostrum at 24 h registered significantly ($P < 0.001$) higher levels than those that had no colostrum intake. Serum vitamin B₁₂ concentration in lambs responds rapidly to ingestion and thus serum determination is not a good indicator of deficiency (Millar and Penrose, 1980; Sutherland, 1980).

The vitamin has no functional role in serum and blood, which may simply act as a transport mechanism (McMurray *et al*, 1985) and as a store (Suttle, 1986) of it. According to Halpin and Caple (1982), colostrum provides the essential source of vitamin B₁₂ to the newborn lamb after birth. Under cobalt sufficient conditions, the lambs start to produce their own vitamin B₁₂ from about 1 month of age, when they become ruminants. The vitamin B₁₂ requirement for sheep is about 11 µg daily (Marston, 1970). In preruminants cobalamin is a required nutrient, although the requirement for lambs is not precisely known.

Milk vitamin B₁₂ concentrations registered a notable decrease during the first

24–48 h after lambing, and after the 8th day provides little vitamin B₁₂ to the lambs (table I). Similar dynamic results were observed in human samples; the colostrum obtained within 48 h of delivery contained high concentrations of vitamin B₁₂ by within a few days the levels declined (Thomas *et al*, 1979; Samson and McClelland, 1980). Our results are different in their dynamics from those registered by Wohlt *et al* (1981) for milk from Dorset sheep, in which no significant differences were observed between day 0 (5.5 ng/ml), day 5 (5.1 ng/ml) or day 28 (6.2 ng/ml), but levels of vitamin B₁₂ in raw sheeps' milk were similar, after the 8th day, to those reported by Williams *et al* (1976) (9.8 ng/ml), Scherz and Kloos (1981) (5.1 ng/g), and Scott and Bishop (1986) (6.4 ng/ml).

The method's precision was acceptable (< 10%) except in one case where low levels of vitamin B₁₂ concentrations were found with the RID method (table II). The radio-assay method gave results that correlated well ($P < 0.001$) with those obtained with the *L. leichmannii* method, but this method gave higher values. The major discrepancy between the methods compared was that the microbiological method gave higher results with samples of low vitamin B₁₂ concentration. It has been suggested that deoxyribosides (Gregory, 1954) and thymidine (Gullberg, 1973) may stimulate the growth of the assay organism in the determination of vitamin B₁₂ in human milk. However, in a further study of the ability of these and other metabolites to stimulate the growth of the assay organism, none of these substances stimulated growth (Samson and McClelland, 1980).

We do not know which factor or factors may cause the differences obtained between methods, although the RID assay kit contains a purified hog intrinsic factor to eliminate problems with non-specific binding proteins and should bind only true vitamin B₁₂. Österdahl and Johansson (1988) re-

commended the use of a purified intrinsic factor for measuring true vitamin B₁₂ content in food for the RID assays.

The RID method has been shown to be as reliable as the microbiological method for measuring vitamin B₁₂ in ovine milk except at very low levels. It is faster than the microbiological method and is not subject to problems of sample contamination.

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