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Blood metabolites near calving in twin-pregnant and single-pregnant cows

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ABSTRACT

Nineteen cows, diagnosed 30 to 50 days after insemination as conceiving to a double ovulation and ranging in age from 2 to 8 years, were compared for possible signs of pregnancy toxæmia with a sample group of 18 single-ovulating single-pregnant controls, balanced for age. Blood samples were obtained on four occasions, between 2 and 10 weeks before the date when the first cow was due to calve. Blood samples were analysed for ferroxidase, albumin (A), globulin, total protein, the ratio of albumin to globulin, magnesium, beta-hydroxybutyrate (BOH), gamma-glutamyltransferase and serum or whole blood selenium (Se). There were significant differences between single-pregnant and twin-pregnant cows in the concentrations of A, BOH and whole blood Se. Least-squares means were as follows: A, 33.7 and 32.3 g/l; BOH, 0.46 and 1.62 mmol/l; and whole blood Se 887 and 737 nmol/l. The BOH data in particular were useful in identifying cows possibly requiring extra veterinary supervision.

Keywords Cattle, double ovulation, twin calving, blood metabolites, beta-hydroxybutyrate, repeatability, pregnancy toxæmia.

INTRODUCTION

A beef cattle resource was established by Ruakura in 1982/83 to breed for a high twin calving rate. The screening of 69 foundation cows for this project, purchased with a history of at least two sets of twins each, was described by Morris (1991), with the foundation cows and their female descendants constituting the twin selection herd. Morris (1991) reported that the foundation cows produced a 10.3% twin calving rate (18 sets/174 calvings) during the remainder of their lifetimes; the lifetime performance of their daughters is still being recorded.

In an attempt to increase twin calf survival, and hence the number of potential replacement calves from dams of high breeding value, a study was undertaken to measure blood metabolites near calving in double-ovulating cows and in single-ovulating controls. Of particular interest was beta-hydroxybutyrate (BOH). In cattle, ketone bodies result from mobilisation and oxidation of triglycerides from body fat reserves. If this process becomes excessive due to an imbalance between the energy demands of pregnancy/lactation and dietary intake, then BOH levels rise considerably and clinical signs of ketosis may develop. Some clinical experiences with New Zealand cattle have been reported recently by Vermunt (1987).

MATERIALS AND METHODS

Herd Description

The foundation herd and their descendants were of mixed dairy and beef breeds and crosses as described by Morris (1991). They were managed at Tokanui Station, Te Awamutu as single-suckling or twin-suckling cows.

The part of the herd under study here was all mated by artificial insemination (AI) over a 6-week period from the beginning of November. Ovulation and pregnancy records were obtained by one veterinarian (A.M. Day), using ovarian and uterine palpation *per rectum*, when cows were 30 to 50 days post AI. To achieve this the herd was visited for palpation about every 2 weeks from mid-December until the end of January. Cows were examined only once, at the first visit when at least 30 days post AI,

unless they were found to have produced a double ovulation (in which case they were re-examined at later visits). Each cow was also recorded as single or twin pregnant.

After the calves were weaned in early January, the cows were generally run in groups of 60 to 100 each until July. Yearling heifers and sometimes 2-year-olds, however, were drafted off and managed separately. Groups were rotated around all paddocks, except during April when the area for cattle was restricted. In July, groups were reconstituted according to expected calving date, and cows identified as double ovulators were reallocated into one separate group.

Blood Sampling : 1990 Calvings

A preliminary study of metabolic differences was carried out in the winter of 1990. Thirty-eight cows (age range 2 to 7 years plus one 10-year-old) were blood sampled on July 2 (5 to 11 weeks before their expected dates of calving). Eighteen of the cows had conceived to a double ovulation (Table 1); of these, 8 were thought to be twin-pregnant, 3 others were possibly twin-pregnant and 7 were thought to be single-pregnant. These 18 were drafted from the main herd to a separate management group on July 2, remaining separate until after the last cow calved, and they (alone) were blood sampled again on July 30.

Analyses of serum from both blood samples of the double-ovulating cows included albumin (A), globulin (G), total protein (A+G), magnesium (Mg), BOH and, by calculation, the ratio of albumin to globulin (A/G). The BOH data showed one cow (the 10-year-old) with elevated values of 2.5 and 5.3 mmol/l on July 2 and 30, respectively. She was subsequently found to display the typical clinical symptoms of pregnancy toxæmia, and was successfully treated with daily oral doses of Ketol (Biomac Laboratories Ltd, Auckland) and with dextrose by intravenous injection.

Five cows in the group of 18 calved twins, including the 10-year-old. Results are given in Table 1, showing details of calving outcome. Although it was not possible to be definite, the lives of the twin calves and their 10-year-old mother were probably saved by veterinary intervention; the calves and their mother reached weaning satisfactorily.

Analyses of the two blood samples per cow from the 13 single calvers and the 5 twin calvers showed that, with the small numbers of records available, BOH was the only metabolite significantly related to calving status (means being respectively 0.44 and 1.35 mmol/l, a difference of 0.91 ± 0.41 mmol/l, $P < 0.05$). One other of the 5 cows calving twins also had elevated BOH values (1.5 mmol/l), but her twins were born alive without pre-calving intervention.

For winter 1991, a fuller study was planned, in order to monitor time trends in BOH and other metabolites in both single- and double-ovulating cows, and beginning one month earlier in the season (i.e. early June).

TABLE 1 The reproductive profile of cows used in the 1990 and 1991 studies on the effect of pregnancy status on blood composition.

Calving year	Ovulation record	Putative preg. status	Total pregnant	Calving outcome	
				single	twins
1990	single	single	20	20	-
	twin	twin	8	4	4
		?twin	3	3	-
		single	7	6	1
1991	single	single	18	18	-
	twin	twin	9	5 ^a	4
		?twin	2	1	1
		single	8	8	-

^a One aborted at 6 months of pregnancy

^b Includes one cow apparently pregnant with identical twins

Blood Sampling and Calving Management : 1991

In 1991, the experiment consisted of a total of 19 cows (2 to 8 years of age) which had conceived to a double ovulation, or in one case conceiving to a single ovulation but with an identical twin pregnancy, alongside 18 single-pregnant controls, balanced for age. They were identified and blood sampled on June 5 (day 1). They remained grazing with the rest of the herd for another 5 weeks, during which time further blood samples were obtained on June 24 (day 2) and July 10 (day 3). The whole herd was supplemented daily with MgO applied to hay from July 1 (approximately 35 g/cow/d). The 19 double ovulators were drafted from their herd-mates on July 10 and were managed from then as a separate, preferentially-fed group until the end of calving. A fourth blood sample was obtained from the 19 double ovulators only, on July 31, day 4 (10 to 33 days before the actual calving dates of August 10 to September 2 in this group). The range of actual calving dates in the single ovulator group was from July 28 to September 15.

From July 10, cows in the double ovulator group were supplemented with 2 kg/head/d of a proprietary 'dairy ration', containing 15% protein (minimum). Supplementation was continued until the end of calving, and (for the earlier calvers) into early lactation. As a result of our preliminary findings with elevated BOH in 1990, it was decided to treat affected cows in 1991 with Ketol.

The subsequent calving outcome is shown in Table 1, with 4 out of 9 cows considered to be twin pregnant actually calving twins, along with one of the two cows possibly twin pregnant. One other cow, designated twin pregnant, aborted in June at 6 months of pregnancy, but only one foetus was found.

Blood Parameters : 1991

Serum from the sample on day 1 was analysed for nine metabolites: ferroxidase (Fx), A, G, A+G, A/G, Mg, BOH, gamma-glutamyltransferase (GGT) and selenium (Se). Two vacutainer tubes per cow were obtained on each of days 2, 3 and 4; the first eight traits were analysed from serum, whilst whole blood was used for Se. Results from Se on day 2 were subsequently discarded because the sampling day coincided with the administration of Se to all animals by injection.

Data Analyses

Results for each blood trait in 1991 were analysed using the Genstat (1988) computer programme, fitting fixed effects for actual pregnancy status (twin, $n=5$; single, $n=32$), sampling day (1 to 4), age of cow (3 groups: 2 and 3 years, 4 to 6 years, 7 and 8 years), with and without all possible interaction terms. As no interaction was significant, these terms were not considered further. A restricted maximum likelihood (REML) model (Patterson and Thompson 1971) was also used, fitting the three main effects above as fixed terms, with 'animal' as a random effect. This provided the opportunity to estimate the between-day repeatability for animals. As these blood metabolites were being tested as possible indicators of nutritional or physiological difficulties during pregnancy, some analyses were also carried out where records from the cow which aborted were added to those of the twin calving cows, making 6 animals compared with the remaining 31.

For traits where the effect of twin status was significant, cows with different ovulation/pregnancy status were further subdivided for analysis into three groups, namely twin calvers ($n=5$), other double ovulators ($n=14$), and single ovulators ($n=18$).

Analyses of variance were used to investigate fixed effects on cow weight and birth weights of their calves, in relation to the twin pregnancies and twin births. For cow weight taken on June 5, 1991 ($n=248$ records), fixed effects were fitted for age of cow, coded breed, and pregnancy status (238 single-pregnant, 6 twin-pregnant and 4 cows subsequently found to be non-pregnant). Although there were 6 twin calvers within the whole herd, one of these was not part of the blood sampling study. For birth weight (with 237 singles, 12 twins and 1 aborted and not weighed), fixed effects were fitted for age of dam, coded breed, birth type, sex of calf, perinatal survival (238 alive and 11 dead at birth) and date of birth as a covariate. Corresponding analyses were done on 1990 cow weights (July 2) and calf birth weights as well.

RESULTS

Blood Parameters : 1991

Table 2 shows the least squares means for each blood parameter, classified by day. Standard deviations are also given for each trait. Four of these parameters showed significant differences among days, namely Fx, A/G, Mg and BOH; Fx did not appear to show any time trend, whereas there was an upward trend in A/G and Mg with time. Part of the trend for Mg concentration was due to the daily MgO applications to hay, which began on July 1.

Defining animals with a BOH value of 1.7 mmol/l or over as seriously elevated (i.e. approximately 2 SD above the mean), one twin-pregnant 2-year-old (cow A: 1.8 mmol/l) was identified on sample day 1 and treated with Ketol. Three other twin-pregnant cows showed elevated BOH levels by sample day 3.

and were treated from July 11 (along with continuing treatment for cow A). The fifth twin-pregnant cow showed a BOH level of 1.5 mmol/l on sample day 3, was not treated, and had a normal value of 0.4 mmol/l on sample day 4. Significantly, only the 5 cows which calved twins showed any BOH values greater than 1.1 mmol/l.

Not shown in Table 2 were the effects of age of cow; these were significant for G, A+G, A/G and Mg. Globulin and A+G concentrations increased with age, and consequently A/G declined with age (with values of 0.90 for 2- to 3-year-olds, 0.86 for 4- to 6-year-olds and 0.76 for cows of 7 years or more, s.e.d. 0.04). Corresponding Mg concentrations declined with age from 0.82 to 0.74 to 0.64 mmol/l respectively (s.e.d. 0.04 mmol/l).

Table 2 also gives the animal repeatability estimates among sample days. Almost half of the traits were highly repeatable, with values of at least 0.80, and the remainder showed moderate repeatability except for BOH. Given the sudden increases in BOH in twin-calving cows, and the attempts to treat these cows, the lower repeatability was not surprising.

Table 3 shows the effects of actual pregnancy status, for traits where status was significant. Percentage differences due to twin- versus single-pregnancy status were small for A, but very large and significant for BOH and whole blood Se concentration. Also shown are the analyses where ovulation and calving status were subdivided into three groups. A/G was also included here because it was significant in this analysis, although not for single versus twin pregnancies. The double ovulator/single calving

group were intermediate for A and A/G, but were quite similar to the single-calving cows for BOH and Se.

A further set of analyses including the cow which aborted with the twin calvers did not change the estimated difference between twin and single calvers much, except for BOH, where the value for the extra cow was not elevated.

Body Weights

For cow weight, the effect of pregnancy status (twin versus single) was not significant in 1990 or 1991. For calf birth weight, however, the effect of birth type was 7.8 ± 1.2 kg ($P < 0.001$), with singles averaging 33.1 kg and twins 25.3 kg. There was also a difference of 2.4 ± 1.3 kg ($P < 0.10$) between the weights of calves surviving (30.4 kg) and those of calves dying at birth (28.0 kg). The corresponding analysis of birth weight for calves born in 1990 showed a difference of 7.0 ± 1.7 kg for birth type and 0.5 ± 1.5 kg for calves alive versus dead at birth.

No twin-calving cows died during calving. Two twin-born calves died perinatally (one each from two twin sets). In each case the surviving calf was of similar birth weight to the one that died.

DISCUSSION

Blood Parameters : 1991

Although trends over sample days were not significant for A and G, there was a tendency for A to increase and G to decrease

TABLE 2 Means for each sample day, with phenotypic standard deviations and repeatabilities (r) for each trait, as measured in 37 cows on four occasions in late pregnancy

Trait	Units	Jun 5 Day 1	Jun 24 Day 2	Jul 10 Day 3	Jul 31 Day 4	Sig ^a	SD	r (x100)
ferroxidase ^b	U/l	23.7	21.9	25.9	25.0	***	4.5	61 ± 8
albumin (A)	g/l	32.4	33.5	32.9	33.0	+	1.8	71 ± 7
globulin (G)	g/l	41.3	40.3	39.4	38.4	+	3.8	83 ± 4
total protein (A+G)	g/l	73.6	73.6	72.2	71.4	NS	3.9	80 ± 5
ratio (A/G)		0.79	0.84	0.85	0.87	*	0.11	85 ± 4
Mg	mmol/l	0.67	0.75	0.73	0.81	*	0.11	64 ± 8
beta-hydroxybutyrate	mmol/l	0.76	1.13	0.97	0.92	*	0.48	24 ± 12
gamma-glutamyl-transferase (GGT) ^b	U/l	10.6	10.5	-	-	NS	2.2	66 ± 10
Se - serum	nmol/l	302	-	-	-	-	31	-
- whole blood	nmol/l	-	-	835	800	+	188	92 ± 4

^a Significance tests were for differences among days 1 to 3 (i.e. where all animals were sampled), except for GGT (days 1 and 2) and whole blood Se (days 3 and 4, double ovulators only).

^b Analyses of ferroxidase were at 37°C and GGT at 30°C.

TABLE 3 Effect of calving status or ovulation status on selected blood parameters

Parameter ^a	Calving status			Ovulation/calving status			Average s.e.d. ^b
	Single (S)	Twin (T)	Diff. (S-T)	Single ovulator	Calved twins	Other double ovulators	
Number of animals	32	5		18	5	14	
Albumin	33.7	32.3	1.4+	34.0	32.2	33.4	0.7*
A/G	0.87	0.83	0.04 NS	0.90	0.80	0.87	0.05*
BOH	0.46	1.62	-1.16***	0.44	1.43	0.49	0.13 ***
Whole blood Se	887	737	150*	873	711	928	70**

^a Units: albumin, g/l; BOH, mmol/l; Se, nmol/l

^b Average standard error of differences between pairs

with pregnancy stage. Taking the ratio of A/G resulted in a significant increasing trend over days. Of the other traits significantly affected by sample day (Fx, Mg and BOH), the last two seemed useful for monitoring cows. Because Mg concentration was also associated with age (as also reported by Feyter *et al.*, 1986), the more clear-cut indicator was BOH. Because of a low repeatability over time, BOH would need to be monitored frequently, to provide useful information of assistance in avoiding pregnancy toxemia. In the one case of abortion observed, there was no associated elevation of BOH. Although unlikely to affect the ranking of animals, diurnal variation in BOH has been reported, related to time of feeding (Coggins and Field, 1976), which should be accounted for in future studies.

With hindsight, serum Se was preferable to whole blood Se (Thompson *et al.*, 1991), because of the time lag required to incorporate Se into red blood cells. However, the 8% lower value of serum Se in twin- than single-calving cows (data only available on day 1) was reasonably consistent with the values from whole blood Se on days 3 and 4.

In a related study of metabolites at Whatawhata (W.H. McMillan, personal communication) twin- and single-pregnant recipient cows from an embryo-transplant project were compared prior to calving in 1991. Six out of 10 twin-pregnant cows had BOH concentrations above 1.1 mmol/l, compared with only 1 out of 11 single-pregnant cows with elevated BOH. The differences in BOH concentration due to pregnancy status were significant on 2 of the 4 sampling days between 27 May and 8 August. There was also a significant effect of pregnancy status on 1 of 4 sampling days for whole blood selenium concentration (a 19% lower level in twin-pregnant cows, compared with a difference of 17% overall in our data in Table 3).

Body Weights

A difference of 7.0 to 7.8 kg in birth weight was found between single- and twin-born calves in this study. By contrast, with cows under drylot conditions in Nebraska, USA, Gregory *et al.*, (1990) found a larger difference of 10.5 kg between the two groups. The singles in Nebraska, however, averaged 44.8 kg, and the percentage difference was the same in the two experiments (23.4% in Nebraska and 22.0% at Tokanui). de Rose and Wilton (1991) found differences in Canada of 7.4 kg (16.9%) in birth weight.

We found no significant differences in cow weight between single- and twin-pregnant cows nor in birth weight classified by calf survival, although there were small numbers of twins.

In a Californian study of recipient cows carrying twin or single calves (Koong *et al.*, 1982), it was found in the last

trimester that single-pregnant cows gained weight (allowing for estimated conceptus weight) whereas twin-pregnant cows lost weight. Although data on cow weight change over the critical period were not available in our study, the rationale behind preferentially feeding our twin-pregnant cows was to attempt to allow for assumed differences in energy requirements. Further work is underway at Whatawhata to find out exactly how much is required to supply any such deficiencies under pastoral conditions (W.H. McMillan, personal communication).

CONCLUSIONS

Given the size of differences in metabolite concentrations between single- and twin-pregnant cows, it seemed that BOH was the most useful indicator of pregnancy status and/or of possible pregnancy toxemia requiring intervention.

ACKNOWLEDGEMENTS

We wish to thank the staff at Tokanui Station for care and management of the stock, Messrs A.P. Hurford and B.R. Southey for analyses of data and Mrs J.M. O'Neill for assistance with the blood samples.

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