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Blood and urinary metabolites in cattle differing in susceptibility to bloat

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ABSTRACT

Blood samples from 159 animals of known susceptiblity to bloat were analysed for a range of proteins, minerals, acid base status, hormones and enzymes. Urine samples from the cows and heifers were analysed for minerals and osmolality. Each animal was sampled once only in a fed state. An increase in bloat breeding value of one unit (increasing susceptibility) was significantly associated ($P<0.05$) with a decrease in liveweight of 28 (±6) kg and increases in blood of 0.26 (±0.07) U/l pepsinogen, 0.33 (±0.14) mmol/l urea, 29.5 (±11.3) nmol/l Se, 4.9 (±1.2) mmol/l red blood cell K, 0.5 (±0.2) g/l beta globulin, and 13.9 (±6.1) pmol/l gastrin. The data suggest there may be some physiological or metabolic differences between high and low susceptible animals either arising during the selection and breeding programme or which contribute to the susceptibility mechanism.

Keywords: bloat susceptibility; genetics; metabolites; cattle; blood profile.

INTRODUCTION

The Friesian-Jersey crossbred animals in the high (HS) and low (LS) bloat susceptible herds at Ruakura are the result of 20 years of divergent selection and breeding based on bloat behaviour under challenge (Morris *et al.*, 1991). In addition to the difference in bloat behaviour there is some evidence that selection for and against susceptibility has been associated with differences between HS and LS animals in anatomical and digesta characteristics. LS cows were heavier than HS cows, had heavier heart and kidney weights after adjustment for liveweight, and under some conditions the weight of digesta in the rumen was lower and the percentage dry matter higher in LS than HS animals (Carruthers & Morris, 1988; Carruthers *et al.*, 1988; Morris & Carruthers, 1991). The physiological significance of the differences or the extent to which differences such as organ weights contribute to the mechanism of susceptibility has not been determined. Differences may have resulted from, rather than contributed to, the bloat responses of the two groups. Current research on the bloat herds is focussed on identification of a genetic marker for susceptibility (Wilkins & Morris, 1992). In the history of the bloat herds there has not been any general screening of metabolites or hormones which might indicate that the herds differ in ways other than susceptibility to bloat. This paper reports the results of metabolic profiles determined on the blood and urine of HS and LS animals present in the herds in autumn 1992.

EXPERIMENTAL DETAILS

Animals

A total of 159 animals were sampled in March 1992, including 134 pure-line animals and 25 crosses between HS and LS lines (Table 1). Eleven of the cows were not lactating.

TABLE 1: Numbers of animals sampled

Age/Sex	HS	LS	Crosses
Cows	38	25	15
18 month heifers	12	7	3
6 month heifers	19	6	2
18 month bulls	2	2	0
6 month bulls	14	9	5
Total	85	49	25

Animals were grazed together within age and sex groups and the groups were sampled over several days, as follows: 18 month old heifers and non-lactating cows; 20 cows and 4 bulls; two groups of 24 cows; 6 month old calves (male and female calves sampled on the same day but 1 hour apart in sampling time). Each group of cows sampled was balanced for HS and LS animals.

A breeding value (BV) for bloat was calculated for each animal using a best linear unbiased prediction based on the bloat behaviour of the animal and its relatives. Bloat scores under challenge were obtained for each animal at 6 months of age, using a visual scoring system ranging from 0 (no bloat) to 4 (severe bloat). The BVs are centred around zero and are positive for HS animals and negative for LS animals.

Sampling

On each sampling day the animals were sampled over a 1 to 2 hour period. Variation among animals in feeding pattern prior to sampling was minimised by grazing hungry animals for a defined period prior to sampling. The lactating cows were offered fresh pasture for 1 hour from 0600 to 0700h, prior to milking, and sampling started at 0900h. Non-lactating animals were restricted in feed during the night preceding sampling, offered fresh pasture from 0530 to 0630 h, removed from the paddock and sampling started at 0830h.

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Animals were restrained in a neck crush and blood was sampled into vacutainers from the jugular vein. Urine was sampled, after perineal stimulation, from cows and 18 month old heifers just prior to the sampling of blood.

During the sampling period the cows were drenched with zinc oxide on three days each week. Two of the groups of cows were sampled on a day intermediate between zinc dosing days and the third was sampled on a dosing day but before the zinc was given.

Measurements

Serum or plasma:

All animals: total protein (g/l), albumin (g/l), alpha 1-, alpha 2-, beta-, and gamma-globulins (g/l), vitamin B12 (pmol/l), beta hydroxybutyrate (mmol/l), glucose (mmol/l), pepsinogen (U/l at 37°C), urea (mmol/l), thyroxin (nmol/l), gamma glutamyltransferase (U/l at 30°C), glutamate dehydrogenase (U/l at 30°C), CO_2 (mmol/l), calcium (mmol/l), copper (mmol/l), potassium (mmol/l), magnesium (mmol/l), sodium (mmol/l), phosphate (mmol/l), zinc (mmol/l), chloride (mmol/l), creatinine (mmol/l).

16 HS and 16 LS cows only: renin activity (ng/ml/hour), insulin-like growth factor-1 (ng/ml), gastrin (pmol/l).

19 HS and 13 LS only: cortisol (ACTH test, see below).

Blood:

All animals: selenium (nmol/l), red blood cell potassium (mmol/l), haematocrit (%), mean corpuscular volume (fl), white blood cells (10^9/l).

16 HS and 16 LS cows only: hydrogen ion (nmol/l), pH, pCO_2 (mm Hg), pO_2 (mm Hg), bicarbonate (mmol/l), base excess (mmol/l).

Urine:

Cows and 18 month old heifers: potassium (mmol/l), sodium (mmol/l), phosphate (mmol/l), chloride (mmol/l), pH, osmolality (mosmol/l), creatinine (mmol/l).

Organs:

14 HS and 9 LS cows at slaughter: adrenal gland size, expressed per unit live weight at slaughter.

ACTH test (Verkerk *et al.*, 1994):

On a day separate to when other sampling was carried out, plasma cortisol was determined in 19 HS and 13 LS cows on blood sampled immediately before and at 50 and 120 minutes after intravenous injection by bolus of 0.01 mgACTH/100 kg (Synacthen, CIBA-Geigy). The area under the curve after correction for the value at 0 minutes was used to assess each animal's adrenal cortex responsiveness to ACTH.

Concentrations of minerals in the urine were expressed as a percent of the urine creatinine values, and the fractional excretion rate of each mineral was calculated as the ratio (urine:serum) of the creatinine-adjusted values (both blood and urine) for each mineral.

Liveweights were recorded on separate days for each age/sex group but within two weeks from measurement days.

Statistical analysis

All animals were included in regression analyses of metabolite concentrations on bloat breeding value, fitting separate straight lines for each age and sex group. Percent Friesian of each animal (all animals were Friesian-Jersey cross) was included as a covariate. Means and standard errors of the difference between means (SED) from analyses of data for pure-line animals are also presented for some variables.

RESULTS

Bloat BVs and percent Friesian for pure-line HS and LS animals are shown in Table 2. Percent Friesian was higher for HS than LS animals. At the time of sampling the productions (kg/cow/week) by pure-line HS and LS lactating cows, respectively, were 49.7 and 50.1 kg milk (SED 4.3, P>0.1), 2.30 and 2.65 kg fat (SED 0.18, P<0.1), 1.93 and 1.99 kg protein (SED 0.15, P>0.1), and 2.32 and 2.32 kg lactose (SED 0.22, P>0.1).

TABLE 2: Bloat breeding values and percentage Friesian for cows, 6 and 18 month heifers (F6mth, F18mth), and 6 and 18 month males (M6mth, M18mth)

	Bloat BV			% Friesian		
Group	HS	LS	SED	HS	LS	SED
Cows	0.64	-0.41	0.04***	43.6	40.4	1.7+
F18mth	0.72	-0.47	0.07***	41.4	37.1	1.9*
F6mth	0.79	-0.38	0.11***	41.8	34.7	2.5**
M18mth	0.98	-0.45	0.10*	41.5	33.0	2.5
M6mth	0.74	-0.42	0.09***	45.0	36.3	1.8***

The regression coefficients for each variable against bloat BV, with all animals included and including percent Friesian in the model, for those variables with significant slopes on breeding value are shown in Tables 3 and 4. Table 3 shows overall coefficients for those variables with no significant BV-age interactions. Mean values for pure-line HS cows are given as reference points. An increase in bloat breeding value of one unit (increasing susceptibility) was significantly associated with a decrease in liveweight of 28 kg and increases in blood of 0.26 U/l pepsinogen, 0.33 mmol/l urea, 29.5 nmol/l Se, 4.9 mmol/l red blood cell K, 0.5 g/l beta globulin, and 13.9 pmol/l gastrin. Liveweight increased as percent Friesian increased but the other variables were not associated with % Friesian. Although coefficients were significant the variation among animals was large. For example, the data for pepsinogen is shown in Figure 1. The 0.26 U/l increase in pepsinogen per unit increase in breeding value was an 8% increase relative to the range in pepsinogen values and a 16% increase relative to the mean pepsinogen value.

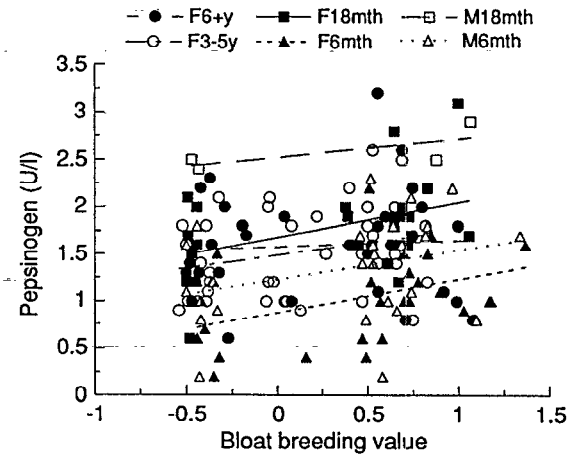
Table 4 shows regression coefficients for variables showing a significant BV-age interaction. Individual years of age were considered for cows but 3, 4 and 5 year old cows did not differ and their data were combined and compared with data for cows aged 6 years and older. Non-lactating cows were considered separately from lactating cows but their data are not presented as there were few animals. Within cows aged six years and older an increase of one unit in breeding value was associated with decreases of 3.9 g/l in total protein in blood and 0.14 mmol/l in magnesium status and an in-

TABLE 3: Mean values for HS cows and overall slopes and standard errors (± SE) for significant regressions of variables on bloat breeding value (BV) and percent Friesian (%F) for variables with no significant BV-age interactions.

Variable	Mean HS cows	BV	%F
Liveweight (kg)	371	-27.6*** (±5.5)	0.94* (±.39)
Pepsinogen (U/l)	1.67	0.26*** (±0.07)	-0.003 (±0.005)
Urea (mmmol/l)	6.95	0.33* (±0.14)	-0.004 (±0.009)
Selenium (nmol/l)	387	29.5* (±11.3)	-1.04 (±0.77)
rbc K (mmol/l)[a]	33.9	4.9*** (±1.2)	-0.16 (±0.08)
ß-globulin (g/l)	12.0	0.5* (±0.2)	-0.02 (±0.01)
Gastrin (pmol/l)	79.8	13.9* (±6.1)	-0.08 (±.51)

[a] red blood cell potassium

FIGURE 1: Blood pepsinogen values and regression lines for 6 and 18 month heifers (F6mth, F18mth), cows aged 3-5 and 6 years and older (F3-5y, F6+y), and 6 and 18 month males (M6mth, M18mth) of known bloat breeding value.



crease of 3.4 mmol/l in CO_2 content of blood. Within this group the ages ranged from 6 to 13 years in both susceptibility groups and the age distributions were similar. Also similar were the levels of production in HS compared to LS cows aged 6 years and older. For the other variables shown in Table 4 there was a significant association with breeding value within at least one of the age/sex groups sampled.

Variables not given in Tables 3 and 4 were found to be not significantly associated with BV for bloat and their data are not shown.

A 10% increase in percentage Friesian was associated (P<0.05) with increases (±SE) of 2.1 (±0.8) mmol/l serum creatinine, 0.9 (±0.4) g/l total protein and 0.7 (±0.2) µmol/l Cu and decreases of 0.03 (±0.01) mmol/l Mg, 0.7 (±0.3) % haematocrit and 0.7 (±0.3) fl mean corpuscular volume.

DISCUSSION

Six metabolites and liveweight were significantly associated with bloat breeding value across all age and sex groups. Another nine variables were associated with breeding value for at least one age/sex group. Further variables may also be associated with breeding value but this was not apparent under the single-sampling regime. The difference in liveweight observed between HS and LS animals reflects differences evident throughout the breeding of the HS and LS herds (Morris *et al.*, 1991). Although liveweight and % Friesian were associated (Table 3), the difference between HS and LS animals was not due to % Friesian as HS animals were lighter than LS animals but contained a higher % Friesian.

When analysing large numbers of variables, 5% of the analyses would be expected to be significant at P<0.05 by chance, therefore some of the associations may be spurious. Usefulness of a single sample is limited; at best, the results may indicate which physiological processes might be investigated further in order to obtain more information about factors contributing to the mechanism of susceptibility to bloat, or that might be useful as phenotypic markers of

TABLE 4: Regression slopes and standard errors (± SE) of variables on bloat breeding value (BV) where there were BV-age/sex interactions, for 6 and 18 month heifers (F6mth, F18mth), cows aged 3-5 and 6 years and older (F3-5y, F6+y), and 6 and 18 month males (M6mth, M18mth).

	Age/sex group					
Variable	F6mth	F18mth	F3-5y	F6+y	M6mth	M18mth
ß-hydroxy Butyrate	0.03	-0.04	0.03	0.03	0.13***	0.03
	(±0.03)	(±0.03)	(±0.03)	(±0.03)	(±0.03)	(±0.06)
Creatinine	-1.4	-4.1	-0.8	2.3	0.7	12.6*
	(±2.7)	(±2.8)	(±2.6)	(±2.6)	(±2.5)	(±5.2)
a2-globulin	0.7	2.5***	-0.75	-0.5	-0.04	-0.4
	(±0.5)	(±0.6)	(±0.5)	(±0.5)	(±0.5)	(±1.0)
Total Globulin	3.3*	5.9**	-1.0	-3.1	0.6	-1.7
	(±1.7)	(±1.7)	(±1.6)	(±1.6)	(±1.6)	(±3.2)
Total Protein	2.5	2.8	-1.0	-3.9**	-0.5	1.0
	(±1.5)	(±1.5)	(±1.4)	(±1.4)	(±1.4)	(±2.8)
CO_2	-0.03	-0.79	-1.14	3.37***	-0.66	-1.35
	(±0.86)	(±0.90)	(±0.84)	(±0.82)	(±0.81)	(±1.64)
Mg	0.05	0.01	-0.06	-0.14***	0.04	0.01
	(±0.04)	(±0.04)	(±0.04)	(±0.04)	(±0.04)	(±0.07)
White Blood Cells	1.6*	-1.3*	0.1	-0.5	0.2	-1.2
	(±0.6)	(±0.7)	(±0.6)	(±0.6)	(±0.6)	(±1.2)
Urinary Creatinine		-1097*	270	-74		
		(±449)	(±421)	(±409)		

susceptibility. A single sampling regime for Band 4 protein in saliva did not distinguish HS and LS animals (Carruthers & Morris, 1993), although previous studies on animals from the same herd had indicated susceptibility groups may differ in Band 4 concentration (Clarke *et al.*, 1974).

Some of the variables which were associated with breeding value have been significantly associated with production traits in sheep and cattle. Selection lines for fleeceweight in sheep (McCutcheon *et al.*, 1987) and milk production in cattle (Tilakaratne *et al.*, 1980) differed in plasma urea concentrations by 0.80 mmol/l and 0.92 mmol/l, respectively, compared to the difference of 0.33 mmol/l per unit change in bloat breeding value in the present study. In some studies the difference between lines has been consistent across a range in feeding conditions (Tilakaratne *et al.*, 1980) but in others specific feeding conditions were required (McCutcheon *et al.*, 1987) or differences were apparent at one age but not another (Sejrsen *et al.*, 1984).

Some breeds of sheep exhibited two phenotypically distinct groups for red blood cell potassium concentration (Evans, 1954). Animals with low blood potassium drank less and excreted lower urine volumes than those with high potassium (Evans, 1957). The threefold difference between high and low lines of sheep was considerably greater than that observed between HS and LS animals. If the lower potassium concentrations observed in LS animals were associated with differences in water metabolism, then water intakes by LS animals would have been expected to be lower than those of HS animals. However, drinking-water intakes by LS cows were observed to be either similar to, or higher than, those of HS cows in a series of trials (Carruthers *et al.*, 1988), suggesting that, in contrast to the findings in sheep, the differences in red blood cell potassium between HS and LS animals were not associated with differences in water metabolism.

Pepsinogen content of the blood can reflect leakage of pepsinogen from the abomasum to blood from an ulcer or gut damage due to parasites, or possibly reflect the level of cell activity in the abomasum. Values for HS and LS animals were within normal ranges so high levels of parasites were not indicated. However, urea, ß-globulin and gastrin in blood may also increase as a result of abomasal damage due to parasites. An investigation of parasite numbers or damage due to parasites in HS and LS animals may be warranted.

The results did not support previous claims that bloat-prone animals have higher blood phosphorus levels than non-bloating animals (Brown *et al.*, 1960).

Animal factors contributing to the mechanism of susceptibility to bloat have not been fully defined; various physiological processes have been suggested to be involved but are without supporting data. Most of these focus on rumen function or on inputs to the rumen such as saliva, and it is likely that more than one factor is involved. In this study, several variables may have been associated with abomasal characteristics or resistance to parasites of HS and LS animals. Selection for and against susceptibility to bloat may have resulted in two groups of animals which differ in characteristics additional to but not related to bloat. However, the variation among animals in all of the variables measured suggested that they would not be useful as predictors of breeding value, unless further studies established that HS and LS animals responded differently in response to some treatment challenge.

ACKNOWLEDGEMENTS

The following assistance is acknowledged: RS Ellison and GK Hoggard (Ruakura Animal Health Laboratory), H Simpson (Massey University, gastrin analysis) and J Speed (Waikato Hospital, renin analysis) for laboratory analyses and HV Henderson (AgResearch Ruakura) for statistical analysis.

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