Metabolic profiles of non-lactating, pregnant heifers during transitioning from kale to swedes in Southland, New Zealand

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Abstract

During winter 2014, a study was conducted in non-lactating pregnant dairy heifers to monitor changes in liver enzymes during a six-week period following a diet change from kale to swedes. Blood samples were collected from 20 cows on Days 0, 4, 7, 14, 28, and 42 after transitioning. Changes were recorded for most blood metabolites, in response to the change from kale to swedes, indicating potential liver inflammation or hepatic cell stress damage. On days four and seven, 6 and 8% of animals had lower than normal concentrations of AST, respectively. Elevated concentrations of GGT and GLDH, relative to Day 0, were recorded on Day 7 and 14 in individual cows. NEFA concentrations peaked on Day 4, possibly indicating a decrease in feed intake during the initial transitioning process. Up to 40 percent of the herd exhibited metabolic changes during the transition period. Although the concentration of some metabolites were above the normal range for individual animals between days 4 and 14, they had decreased to within the normal range by day 28, indicating that cows had become adapted to the new diet. Results suggest that, even with good practice, individual animals within a herd are at risk of liver stress when transitioning onto swedes.

Keywords: Cattle; liver; kale; swedes; winter

Introduction

Brassica crops (in particular rape, kale, turnips and swedes) and fodder beet are an essential source of winter supplementary feed on farms in Southland and South Otago, New Zealand (Nichol et al. 2003; Dalley 2010). Their reliable fast-growing characteristics mean that high-quality readily digestible feed can be produced within a wide range of climatic conditions to fill winter feed deficits (Dalley & Geddes 2012). Swedes, in particular, have been fed to dairy cows in these southern regions for over 50 years. However, it has been well documented that several health problems are associated with the ingestion of brassica crops (Nichol et al. 2003; Collett & Matthews 2014).

It is well established that the Brassica genus is rich in sulphur-containing amino acids and other sulphur-containing secondary plant compounds such as glucosinolates (GSLs). These substances, often with bitter and unpalatable tastes, have evolved as protective mechanisms against disease, insect, and herbivore attack (Bekaert et al. 2012; Cheeke 1995). Concentrations of GSLs generally increase as the plant reaches maturity, so flower-heads and seeds tend to have the highest concentrations within the plant.

Many of the substances are 'pre-toxins', meaning that they can break down into substances that are toxic to animals when the plant tissue is damaged. When plant cells are crushed, e.g., when chewed, the plant's myrosinase enzyme system is activated to hydrolyse the sulphur-containing compounds (Bones & Rossiter 1996). The diversity of these substances and their break-down products results in a spectrum of brassica-associated diseases, which present with a wide range of clinical signs and symptoms (photosensitivity, red water, and goitre) and generally result in poor growth rates in young stock.

The primary toxic effects of GSL derivatives are two-fold: 1) interference with thyroid function because of exposure to the thiocyanate, isothiocyanate, and oxazolidine-2-thione derivatives and 2) liver (bile duct hyperplasia and liver necrosis) and kidney dysfunction because of exposure to the nitrile and epithionitrile derivatives (Collett et al. 2014).

An outbreak of swede toxicity was reported in Southland, New Zealand during June to September 2014 (Dalley et al. 2016). Extensive work was undertaken to determine the cause of this outbreak, and although not fully resolved, it was suggested that it was possibly due to increased glucosinolate concentrations in the plants (Collett et al. 2014; Dalley et al. 2016). The outbreak highlighted the lack of information regarding the health of animals during the transition onto crop. As such, a study was conducted in dry, pregnant dairy heifers during the winter of 2015, to monitor changes in liver enzymes and other metabolic parameters during a six-week period following the diet transition from kale to swedes.

Materials and methods

The Southland Demonstration Farm (SDF), Wallacetown, Southland (46.3298° S, 168.2903° E), was enrolled in the study. The winter diet of rising two-year-old animals on the property, involved a switch from kale to swedes in mid-winter (15th July).

Animal selection

At the baseline blood collection, prior to the diet change, 30 rising 2-year-old Friesian and Friesian x Jersey animals were randomly selected by the farm manager, from a mob of approximately 210. Twenty of these became the 'monitor group' of animals that were sampled at each time point during the study and the remaining 10 were included in the 'random group'.

Animals included in this random selection varied at each sampling. This random group were included to determine whether monitoring of random animals would identify the same level of health risk as that indicated by the monitor animals in which repeated sampling was occurring. At each subsequent sampling, blood samples were collected from the 20 monitor animals and 10 animals randomly selected by the vet. As animals calved they were removed from the trial.

Animal management and crop allocation

For six weeks prior to the commencement of the transitioning, the animals were grazed as a single mob on kale (10 kg dry matter (DM)/cow/day) and supplemented with pasture baleage (4.4 kg DM/cow/day). The swede crop allocation increased gradually from 4.8 to 7.7 kg DM/cow/day during the initial two and a half weeks following the diet change. Pasture baleage and hay were provided as supplements (Table 1) at the same time the animals were offered a new break of crop once the animals were fully transitioned.

Baseline blood samples were collected on the day of transport from the grazier, prior to trucking to the SDF (Day 0). On arrival at the SDF, the animals commenced their diet transitioning onto swedes (Table 1). Blood samples were collected on days 4, 7, and 14 of the transition period and then fortnightly until 42 days after swede introduction. Blood samples from cows that calved within seven days of sampling were excluded from statistical analysis, due to normal metabolic changes associated with parturition.

Table 1 Daily crop and supplement allocation to rising two-year-old dairy heifers during diet transitioning from kale to swedes during mid-winter.

Blood sample day	Crop type	Crop allocation (kg DM/cow)	Pasture baleage allocation (kg DM/cow)	Pasture hay allocation (kg DM/cow)
0	kale	10	4.4	0
	swedes	4.9	5.6	2.8
	swedes	4.8	4.5	1.5
	swedes	5.0	4.7	1.6
4	swedes	5.1	4.8	1.6
	swedes	5.1	4.9	1.6
	swedes	5.3	6.7	0
7	swedes	5.5	6.9	0
	swedes	5.7	7.1	0
	swedes	5.9	7.5	0
	swedes	6.3	7.9	0
	swedes	6.6	6.3	0
	swedes	6.7	6.4	0
	swedes	7.1	6.7	0
14	swedes	7.7	7.3	0

Blood sampling

Blood samples were collected, at approximately 11 am, from the coccygeal vein or artery, using 10ml serum Vacuette tubes (Greiner Bio-one, Kremsmunster, Austria). Blood samples were kept on ice prior to transportation to Gribbles Invermay for analysis. Blood samples were allowed to clot and then centrifuged at 3000 × *g* for the extraction of serum. Serum was analysed for AST, bilirubin, BHBA, GGT, GLDH, NEFA, and TP (Table 2) on a Roche/Hitachi Modular system using the manufacturers method from the Roche Diagnostics catalogue for AST, GDH, GGT, total protein and bilirubin; the manufacturers Wako method for NEFA C and the method of McMurray et al. (1984) for BHBA (Table 2).

Statistical analysis

A total of 73 cows (20 monitor and 52 randomly selected cows) were sampled during the monitoring period. Monitor cows were sampled up to six times and random cows one or two times. Four cows had missing calving dates or were empty and were excluded from the analysis. Blood samples collected from animals closer than eight days before calving and any time after calving were excluded from the analysis. As a result, some cows originally designated monitor cows became random cows due to only having one or two sample results and data are not presented for the 42 day samples as there were insufficient samples remaining after cows within eight days of calving were removed. Analysis was therefore completed on a total of 44 cows with a total of 86 valid samples (13 monitor and 31 randomly selected cows).

To determine the normal concentration range for each metabolite in the sample population the pooled standard deviation (SD) was calculated as the √MSE of an ANOVA model with cow and day included as fixed effects. The lower and upper 95% confidence limits for each day were then calculated as

$$\text{Lower limit}_{\text{Day=t}} = \text{Mean}_{\text{Day=t}} - 2 * \text{SD}$$

$$\text{Upper limit}_{\text{Day=t}} = \text{Mean}_{\text{Day=t}} + 2 * \text{SD}$$

Table 2 Summary of the normal concentration ranges and disease indictors of blood metabolites measured in a group of rising two-year-old dairy heifers during diet transition from kale to swedes during mid-winter.

	Metabolite	Name	Normal Range
Liver damage indicators	AST	Aspartate Aminotransferase	58-193 IU/l
	GGT	Gamma glutamyl transferase	6-37 IU/l
	GLDH	Glutamate dehydrogenase	0-59 IU/l
	Bilirubin	Bilirubin	0-13 µmol/l
Energy balance indicators	BHBA	Beta-hydroxybutyrate	0-1.0 mmol/l
	NEFA	Non-esterified fatty acids	0.4-0.9 mmol/l
Protein balance indicators	TP	Total Protein	60-90 g/l

Table 3 Mean, upper and lower 95% confidence interval (CI) concentrations of blood metabolites from a monitor group of rising two-year-old dairy heifers during diet transition from kale to swedes during mid-winter.

Parameter	Limit	Day 0	Day 4	Day 7	Day 14	Day 28
AST	Lower 95% CI	6.4	12.0	31.9	9.1	5.7
IU/l	Mean	69.1	74.7	94.6	71.8	68.3
(58-193)	Upper 95% CI	131.8	137.4	157.3	134.4	131.0
Bilirubin	Lower 95% CI	ND	7.3	0.3	0.0	0.0
µmol/l	Mean	ND	10.5	3.6	1.1	1.3
(0-13)	Upper 95% CI	ND	13.8	6.7	4.4	4.6
BHBA	Lower 95% CI	0.25	0.47	0.37	0.49	0.58
mmol/l	Mean	0.43	0.65	0.55	0.67	0.75
(0-1.0)	Upper 95% CI	0.61	0.82	0.72	0.84	0.93
GGT	Lower 95% CI	0.14	2.9	2.1	6.1	0.0
IU/l	Mean	13.6	16.4	15.6	19.5	12.7
(6-37)	Upper 95% CI	27.0	29.8	29.0	33.0	26.1
GLDH	Lower 95% CI	0.0	0.0	0.0	0.0	0.0
IU/l	Mean	7.7	6.5	24.1	26.1	6.7
(0-59)	Upper 95% CI	56.8	55.6	73.2	75.2	55.8
NEFA	Lower 95% CI	0.20	1.05	0.00	0.00	0.00
mmol/l	Mean	0.63	1.48	0.42	0.18	0.20
(0-0.9)	Upper 95% CI	1.06	1.91	0.85	0.60	0.63
TP	Lower 95% CI	63.9	61.6	59.8	63.2	62.0
g/l	Mean	66.8	64.5	62.8	66.1	65.0
(60-90)	Upper 95% CI	69.8	67.5	65.7	69.1	68.0

Results

Monitor cows

Based on normal concentration ranges, as supplied by the laboratory for each metabolite, changes in metabolic profiles were observed during the diet transition onto swedes (Table 3).

Herd average concentrations of GGT and GLDH were within the normal range on Day 0, 7, and 28. However, on Days 7, 14 and 28 between 10 and 30% of the monitor cows were above the normal range for both metabolites. Blood samples were not analysed for bilirubin on Day 0, however Day 4 concentrations were higher than at the other time points and 35% of the monitor animals had concentrations above the normal range. The concentration of AST was below the normal range in 6 and 8% of monitor cows at Day 4 and 7, respectively. For all other time points, the monitor cows were within the normal range.

On Day 4, 100% of the monitor cows had NEFA concentrations exceeding 0.9 mmol/l, however, by Day 7 all animals had returned to within the normal range. Concentrations for BHBA were within the normal range for the monitor cows at every time point.

Concentrations of TP were lower than the normal range in 10% of monitor cows at Day 0 and Day 4, 15% of animals on Day 7 and 20% on Day 14.

Randomly selected cows.

Data from the randomly selected cows demonstrated that, on the most part, the range in metabolite results for these cows were similar to those of the monitor cows (data not presented), however, for all metabolites, some samples were outside the range provided by the monitor cows. For AST, GLDH, and NEFA, less than 10% of random cow samples were outside the range. This percentage increased up to 15% of samples for BHBA and GGT, 25% for bilirubin, and 60% for total protein. With the exception of total protein the majority of random cow samples outside the range were above the 95% confidence interval (CI). For total protein, 71% of random cow samples were lower than the 95% CI of the monitor cows.

Discussion

Concentrations of the enzymes GGT, GLDH, and AST in serum, as well as serum bilirubin, are used to assess whether liver inflammation is present (Table 2). These enzymes leak into circulation when inflammation occurs. Increased levels of GGT are specific for damage to tissues associated with the liver and bile ducts, while GLDH is a sensitive marker for hepatocellular injury (Collett et al. 2015). The enzyme AST, likewise, may reflect liver damage, but can also arise from other sources.

The diet-transitioning process adopted in the SDF heifer herd was considered good practice in that the animals were held on the initial swede allowance of approximately 5 kg DM/cow/day for seven days before increasing up to their full allowance of 8.7 kg over the next seven days. Hay

was also offered with the pasture baleage for the first five days of the transition period and supplements comprised 59% of the diet DM.

No clinical health issues were observed during the monitoring period despite some animals experiencing concentrations of blood metabolites 1.5 to 10 times greater than the normal range, indicating possible toxic effects and potential liver damage in some animals because of the diet change. Anti-nutritional factors, e.g., glucosinolates released from the swedes during ingestion, are one potential cause of this increase in liver enzyme concentration (Collett et al. 2015). The relatively short-lived duration of greater GGT and GDH concentrations (duration of days to weeks) may reflect a relatively short duration of exposure to swede-associated toxins compared with chronic exposure to sporidesmin observed with facial eczema (Towers & Stratton 1978). Elevated GGT and GLDH concentrations have also been reported in cows grazing turnips (Collett 2014) and have been linked to feeding fresh rape (*Brassica napus L.*) in a Canadian study of 34 herds that investigated nutritional risk factors for liver damage (Barnouin & Paccard 1988). The delayed increase in GGT is consistent with a lag period of 10-14 days before GGT activity increases, due to the inducible nature of its enzymatic activity (Smith & Gravett 1986).

Clearance times for liver enzymes in serum are generally in the order of several weeks following the removal of the challenge or adaptation of the animal to the secondary plant compound (Towers & Stratton 1978). Sources of variation in a group of cows sampled at a single point in time include both the severity of a toxic incident, and the time since this incidence occurred. The baseline blood results in the current study exclude previous (more severe) damage as the cause of the liver disease and indicates that the changes observed were the result of mild damage occurring during the transition process. The differences among animals observed within this mob could indicate individual animal differences in their rate of transitioning onto the swede crop or consumption of diets containing different proportions of swedes.

The negative energy balance observed early in the transition process, as indicated by high NEFA concentrations at Day 4, indicate that the animals were actively mobilising adipose tissue, i.e., in negative energy balance at the time of sampling. Possible explanations for this result are incorrect feed DM allocation or poor utilisation of the swedes in the initial feeding period due to these animals being naive to grazing bulb crops or difficulty eating the swede bulb. Despite 100% of the animals having NEFA concentrations above the normal range and a 50% increase in average BHBA concentration between Day 0 and Day 4, BHBA concentrations of all monitor cow samples were below 1 mmol/l indicating the animals were not ketotic (Oetzel 2007).

The monitoring of dry dairy heifers during the transition from kale to swedes indicated that a percentage of the herd exhibited metabolic changes. Although, the concentrations of some metabolites were above the normal range between Days 4 and 14, they had decreased to within normal range by Day 28, indicating that cows had become adapted to the swede diet. Although blood metabolite concentration increases were recorded for a proportion of animals in the current study, the levels recorded for GGT and GLDH were considerably lower than those measured during the 2014 swede toxicity outbreak, as reported by Collett et al. (2015). Collett et al. (2015) monitored blood metabolite levels in mixed-aged cows on two farms in Southland where cows were exhibiting signs of swede toxicity (photosensitisation and/or periparturient metabolic disease) following a diet change from fodder beet or kale to swedes during late winter. Elevated GGT and GLDH concentrations were observed in 42.5% and 40% of animals, respectively. The results support the view that the disease was due to bile duct damage, since GGT is secreted at higher levels from the bile duct linings when this occurs. Collett et al. (2015) also reported an apparent trend for animals on the farms having blood parameters suggesting subclinical disease, a factor also identified in the current study. Knowledge regarding the wider implications of brassica liver disease is still sparse and further research is required.

For some indicators of metabolic health in the current study (AST, GLDH, and NEFA), less than 10% of samples from randomly selected animals fell outside the predicted range, however, for others, e.g., bilirubin and total protein, between 25 and 60%, respectively, were outside the range of the monitor group. For total protein 71% of the outlier samples fell below the 95% CI of the monitor cows, however the reasons for this are unclear. For all other metabolites the random samples outside the predicted range were above the 95% CI but were still within the range of the high samples recorded from the monitor cows.

While both sampling methods resulted in similar conclusions regarding animal health risk at each sampling time, farmers wishing to assess the metabolic status of their animals during a diet change to brassicas or between crops should select animals for repeat sampling. Repeat sampling provides the opportunity to track changes over time for individuals and thus give a better indication of changing disease risk than a random selection of animals at each time point.

Conclusion

Results indicate that even in herds where good diet transition protocols are in place for feed transitioning, a proportion of the herd may experience some liver dysfunction or other metabolic disease. Monitoring blood metabolites in a select group of animals during this period can identify the risk of metabolic health dysfunction, however, lack of information on the impact of such metabolic changes and management factors that cause the challenge currently limits the usefulness of collecting such data.

Acknowledgements

The authors acknowledge Barry Bethune and the farm team at the Southland Demonstration farm for management of the animals during the study, the vet technicians at VetSouth for collection of the samples and Barbara Kuhn-Sherlock for the statistical analysis of the results.

References

Barnouin J, Paccard P 1988. Nutritional risk factors for liver damage in French dairy herds. Facteurs de risque nutritionnels de la pathologie hepatique dans les troupeaux bovins laitiers en France. Canadian Veterinary Journal 29(11): 915-920.

Bekaert M, Edger PP, Hudson CM, Pires JC, Conant GC 2012. Metabolic and evolutionary costs of herbivory defense: Systems biology of glucosinolate synthesis. New Phytologist 196(2): 596-605.

Bones AM, Rossiter JT 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. Physiologia Plantarum 97: 194-208.

Cheeke PR 1998. Natural Toxicants in feeds, forages and poisonous plants. Interstate Publishers Inc, Danville, Illinois. 2nd ed. 479p. ISBN 0-8134-3128-X.

Collett MG 2014. Bile duct lesions associated with turnip (Brassica rapa) photosensitization compared with those due to sporidesmin toxicosis in dairy cows. Veterinary Pathology 51(5): 986-991.

Collett MG, Matthews ZM 2014. Photosensitivity in cattle grazing Brassica crops. International Journal of Poisonous Plant Research 3(1): 6-21.

Collett MG, Stegelmeier BL, Tapper BA 2014. Could nitrile derivatives of turnip (Brassica rapa) glucosinolates be hepato- or cholangiotoxic in cattle? Journal of Agriculture and Food Chemistry 62(30): 7370-7375.

Collett MG, Westwood C, Gill J 2015. Clinical biochemistry and histopathology of brassica liver disease. Proceedings of the Society of Dairy Cattle Veterinarians Annual Conference 2015. Pp. 255-268.

Dalley DE, Geddes T 2012. Pasture growth and quality on Southland and Otago dairy farms. Proceedings of the New Zealand Grassland Association 74: 237-241.

Dalley DE 2010. Achieving wintering targets – critical success factors for different wintering systems in Southland. Proceedings of the South Island Dairy Event. The Caxton Press, Christchurch. Pp. 224-242.

Dalley DE, Verkerk G, Kyte R, McBeth C, Petch S, Kuhn-Sherlock B, Leach C, Irwin A, Harding N, Morley C, Ryan T 2016. Final report: Swede associated toxicity in dairy cattle during winter 2014. An overview of activities supported by DairyNZ. 84 p. http://www.dairynz.co.nz/farm/adverse-events/southland-swedes/ [accessed 9 August 2016]

McMurray CH, Blanchflower WJ, Rice DA 1984. Automated kinetic method for D-3-hydroxybutyrate in plasma or serum. Clinical Chemistry 30: 421–425.

Nichol W, Westwood C, Dumbleton A, Amyes J 2003. Brassica wintering for dairy cows: overcoming the challenges. Proceedings of the South Island Dairy Event pp. 154-172. The Caxton Press, Christchurch.

Oetzel GR 2007. Herd-level ketosis – diagnosis and risk factors. Proceedings of the Preconference Seminar of the American Association of Bovine Practitioners 40th Annual Conference. 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting pp. 67-91. Vancouver, BC, Canada.

Smith BL, Gravett IM 1986. Uniformity of response of identical twin cattle to sporidesmin intoxication. New Zealand Veterinary Journal 34: 217-219.

Towers NR, Stratton GC 1978. Serum gamma-glutamyltransferase as a measure of sporidesmin-induced liver damage in sheep. New Zealand Veterinary Journal 26: 109-112.