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BRIEF COMMUNICATION

The effect of blood sampling site on measures of stress in dairy cattle

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INTRODUCTION

Stress has a negative impact on livestock production. Several physiological indicators currently exist to identify when animals are experiencing stress. Recently it has been suggested that the site of blood sampling may influence clinical pathologic parameters in rodents (Nasir *et al*., 1996). Because of the difficulty interpreting multiple physiological measures of stress and their high variation within animals we conducted a study to 1) determine the effect of blood sampling site on physiological measures of stress in dairy cows and 2) identify new quantifiable indicators of stress.

MATERIALS AND METHODS

Twenty Holstein cows (in lactation) were used. Blood samples (10 ml) were collected from the jugular and tail vein on the first day of the study. All cows had normal clinical profiles on the first day of the study. Ten cows were then removed from feed and 60 hours later access to water was removed (stress group, S). Ten cows were allowed continuous ad-lib access to feed and water (control group, C). A second blood sample was collected 72 hours after the first sample. Whole blood and plasma samples were analyzed for clinical parameters (glucose, plasma urea nitrogen, creatinine, phosphorus, calcium, sodium, potassium, chloride, total CO_2, anion gap, total plasma protein, albumin, globulin, albumin/globulin ratio, AST, alkaline phosphatase, total bilirubin, magnesium and ionized magnesium) as well as cortisol, acute phase proteins (haptoglobin and alpha-1 acid glycoprotein) and total white blood cell counts. The experimental design was a completely random design with a 2 x 2 factorial arrangement of treatments (blood sampling site and stress treatment). Data were analyzed using GLM of SAS for repeated measures analysis of variance. There were no significant treatment effects due to blood sampling site so data were pooled and reanalyzed. The final model included treatment (stress vs control), day (1 vs 4) and the treatment by day interaction. Treatment effects were tested with cow within treatment as the error term. The least significant difference test was used to separate means following a significant overall F-test.

RESULTS AND DISCUSSION

There was no blood sampling site effect for any of the physiological parameters measured ($P > .1$). Blood sampling in dairy cattle is most commonly performed at the jugular vein or the tail vein. With reference to the parameters studied, equivocal results were found regardless of the blood-sampling site and indicates that meaningful comparison of results from different blood sampling sites, for these physiological parameters, is appropriate. Stress measures for control and nutritionally stressed dairy cows on day 1 and 4 are shown in Table 1. Nutritional stress is a model of what cattle might face in a natural environment. Validation of the nutritional stress model is indicated by the increase in packed cell volume and total white blood cell counts and a decrease in plasma glucose on day 4 in the stress group. Plasma cortisol increased on day 4 for both groups indicating stress related to the blood sampling procedure itself.

Of particular interest were the changes in haptoglobin, plasma magnesium, ionized magnesium and ratios of these variables following stress. While haptoglobin (an acute phase protein) is generally thought to be a response to tissue injury and infection (Kosmas *et al*., 1997) in this study physical (nutritional) and psychological stress may be reflected in the increase in haptoglobin. Psychological stress has been shown to alter acute phase protein concentrations in humans (Singh *et al*. 1991; Maes *et al*., 1997). Psychological components of this stress model would be frustration due to lack of access to feed while other cows in the same barn had access to feed. Such psychological stress can activate the hypothalamic-pituitary-adrenal (HPA) axis. Corticotrophin releasing hormone, part of the HPA, has been shown to be a mediator of the acute phase response (Hagan *et al*. 1993). Plasma cortisol concentrations in this study increased in both treatment groups and while it is likely that stress due to the sampling procedure increased cortisol concentrations in both groups, our results in the nutritionally-stressed cows is similar to that found by Ward *et al*. (1992).

Changes in haptoglobin and magnesium concentrations may also have been altered due to dehydration (as indicated by the PCV results) or alterations in metabolism resulting from nutritional stress. In general, dairy cows have relatively low magnesium reserves and this most likely played a role in reduced magnesium levels observed in nutritionally stressed cows on day 4. Under conditions of nutrient imbalance in ruminants deamination of amino acids in the liver and formation of urea increase. In this study we did not see an increase in plasma urea nitrogen nor did we see a change in plasma pH. Total bilirubin did, however, increase following stress indicating changes in liver metabolism.

TABLE 1: Least square means for stress measures in control and nutritionally ñstressed dairy cows.

	Treatment					
	Stress		Control			
Variable	Day 0	Day 4	Day 0	Day 4	SEM	P Value
Packed Cell Volume, %	35.8[a]	42.7[b]	33.6[c]	36.6[a]	.44	.0001
Haptoglobin, µg/ml	10.2[a]	172.5[b]	0[a]	0[a]	12.4	.0001
Total White Blood Cells/mm^3	22,137[a]	37,305[b]	22,214[a]	22,187[a]	1,622	.0001
Glucose, mg/dl	62.5[a]	48.2[b]	57.1[c]	63.7[a]	1.69	.0001
Phosphorus, mg/dl	5.9[a]	8.9[b]	5.2[cd]	5.8[d]	.24	.0001
Calcium, mg/dl	8.7[a]	7.8[b]	8.3[a]	9.2[c]	.15	.0001
Anion Gap, mmol/l	14.0[a]	19.7[b]	13.4[a]	14.3[a]	.43	.0001
Total Protein, g/dl	7.0[a]	8.0[b]	6.5[c]	6.9[a]	.15	.0510
Total Bilirubin, mg/dl	.29[a]	.74[b]	.27[a]	.29[a]	.02	.0001
Albumin, g/dl	2.7[ab]	3.2[c]	2.6[a]	2.9[b]	.06	.0420
AST, IU/l	80.4[a]	100.1[b]	77.0[a]	77.3[a]	2.3	.0001
Plasma Magnesium, mg/dl	2.0[a]	1.6[b]	2.1[a]	2.1[a]	.05	.0002
Ionized Magnesium, mmol/l	.43[a]	.39[b]	.45[ac]	.48[c]	.09	.0008
Ratio 1[1]	.21[a]	.25[b]	.21[a]	.22[a]	.07	.0017
Ratio 2[2]	.82[a]	.79[b]	.82[a]	.82[a]	.003	.0018
α1-acid glycoprotien, (g/ml	277.2	289.5	301.0	298.2	7.54	.3240
Cortisol, ng/ml	9.79	22.43	8.31	25.01	2.48	.4166
pH, mmol/l	7.9	7.7	7.9	7.7	.01	.4438
Plasma Urea Nitrogen, mg/dl	10.8	11.6	10.1	10.5	.31	.6193

[abcd] Means within rows with the same superscript do not differ significantly ($P < .05$).

[1] Ratio 1 = Ionized Magnesium/Total Magnesium [Total Magnesium = Plasma Magnesium + Ionized Magnesium].

[2] Ratio 2 = (Total Magnesium – Ionized Magnesium)/Total Magnesium.

Our results indicate that we have not yet separated physiological from psychological stress responses nor do we understand how these two responses interact. Additional research is required using other models of stress to elucidate these responses. Mechanisms driving physiological and/or psychological changes in parameters indicative of the animals stress response are also lacking.

CONCLUSIONS

These results suggest that the site of blood sample collection does not influence the ability to detect changes in physiological stress measures and that haptoglobin and magnesium profiles may be useful physiological and possibly psychological indicators of stress in dairy cattle.

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