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NOTE

EFFECTS OF YEAST ON NITRATE TOXICITY IN DAIRY STEERS

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ABSTRACT

Twelve Holstein steers were fed diets consisting of 1) corn silage and concentrate with yeast, 2) corn silage and concentrate without yeast, 3) alfalfa silage and concentrate with yeast and 4) alfalfa silage and concentrate without yeast to determine the effects of live yeast culture on nitrate toxicity. All steers were dosed with nitrate on a daily basis during the experimental period. Blood samples were analyzed for pH, partial pressure of carbon dioxide and oxygen, packed cell volume, hemoglobin and methemoglobin. Methemoglobin level increased ($P<0.01$) with time after initiation of nitrate feeding for all treatments. Results indicated that live yeast culture at 0.1% of the diet dry matter did not significantly reduce nitrate toxicity in steers.

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اثرافزودن مخمر به جیره گوساله های اخته هولشتین برای خنثی کردن مسمومیت نیتراتی

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به ترتیب دانشجوی سابق دکترای علوم دامی (اکنون استاد یار گروه علوم دامی دانشکده کشاورزی دانشگاه صنعتی اصفهان، اصفهان، ایران) و استادان بخش علوم دامی دانشگاه کنتاکی لگزینتون، کنتاکی، آمریکا.

چکیده

به دوازده گوساله نر اخته هولشتین یکی از چهارجیره زیر داده شد: ۱- سیلوی ذرت و کنسانتره با مخمر، ۲- سیلوی ذرت و کنسانتره بدون مخمر، ۳- سیلوی یونجه و کنسانتره با مخمر و ۴- سیلوی یونجه و کنسانتره بدون مخمر. هدف از انجام این آزمایش بررسی اثر تغذیه مکمل مخمر به این گوساله ها برای خنثی کردن مسمومیت نیتراتی بود. هرروز صبح ، در طول مدت آزمایش به گاوها ۵۵۰ میلی گرم نیترات به ازاء هر کیلوگرم وزن بدن همراه با کنسانتره داده شد. نمونه های خون برای pH , Pco_2, Po_2, PCV، هموگلوبین و مت هموگلوبین آنالیز شد. مقدار

هموگلوبین خون همه گاوها در طول آزمایش زیاد شد ($P<0.01$). تفـاوت هـای معنـی داری در تیمارهای مختلف در ساعات ها و روزهای مختلف دیده شد ولی این تفاوت ها بخـاطر رژیـم هـای غذا یی و نه به دلیل افزودن مخمر بود. این آزمایش نشان داد که افزودن مخمر به جیره گوساله های اخته هولشتین اثر چندانی برخنثی کردن مسمومیت نیتراتی نداشت.

INTRODUCTION

Nitrate toxicity is a significant metabolic problem in livestock Accumulation of high nitrite as the result of high nitrate intake, and abnormally lower conversion of nitrite to ammonia in the rumen is the main factor for developing nitrate toxicity (1). Sublethal nitrate consumption (0.5 to 1.0%) has been shown to cause abortion, lower milk production, loss of body weight or reduced growth, and produce vitamin A deficiency (4, 11). However, other studies have shown that feeding forages containing up to 2.3% nitrate did not cause abortion, lower milk production or reduced growth (3).

Live yeast culture is a feed additive that is very palatable and has been used to increase feed consumption (10). There are field reports indicating beneficial effects of feeding live yeast culture in reducing incidence of nitrate intoxication (9). Therefore, the objective of this study was to determine if live yeast culture added to livestock feedstuff, could reduce the severity of nitrate toxicity.

MATERIALS AND METHODS

Twelve Holstein steers weighing 120-225 kg were housed in University of Kentucky Coldstream farm's metabolism barn and were randomly assigned to a 2x2 factorial arrangement of treatments with three replications. The experiment consisted of a 14 day preliminary period and a 12 day challenge period. Total mixed rations (TMR) were fed to steers twice a day and orts were weighed each morning before feeding. Treatment diets consisted of 1) corn silage and concentrate (soybean meal to corn ratio 50:50) with dietary yeast (YC, Yea-sacc, Alltech, Inc., 3031 Catnip Hill Pike, Nicholasville, KY, U.S.A.), inclusion, 2) corn silage and concentrate without dietary yeast inclusion, 3) alfalfa silage and concentrate (corn only) with dietary yeast inclusion and 4) alfalfa silage and concentrate without dietary yeast inclusion. Silage to concentrate ratio was 2:1 on a dry matter basis. Every morning during the challenge period all steers were dosed with nitrate in the form of KNO_3 in TMR. Since dosages of 400 mg of nitrate kg^{-1} body weight have been reported to produce significant increases in methemoglobin (MetHb) concentration in ruminants (7), 550 mg kg^{-1} body weight was selected to produce reasonable test of yeast culture effect. According to Alltech, Inc., recommendation, yeast was added to concentrate to make up a 0.1% yeast concentration in TMR. Composition of supplement and forages are shown in Table 1.

Jugular blood samples were collected into vacutainer tubes at 0, 2, and 4 h post feeding on day 1, and 4 h post feeding on days 2 (28 h), 3 (52 h), 5 (100 h), 8 (172 h), and 12 (268 h). Blood samples were analyzed for pH, partial pressure of carbon dioxide (P_{CO_2}), partial pressure of oxygen (P_{O_2}), packed cell volume (PCV), hemoglobin (Hb, mg dL^{-1}), and MetHb (mg dL^{-1}). Blood MetHb was analyzed within 2 h after collection using a modified Evelyn and Malloy procedure (6). This procedure determines MetHb as % of

Table 1. Concentration of ingredients in the experimental diets (dry matter basis).

	Diets			
	Corn silage		Alfalfa silage	
Items	with yeast (0.1%)	without yeast	with yeast (0.1%)	without yeast
Corn silage	66.7	66.8	-	-
Alfalfa silage	-	-	66.7	66.8
Corn	16.3	16.3	32.6	32.6
Soybean meal	16.3	16.3	-	-
Dicalcium Phosphate	0.3	0.3	0.3	0.3
salt	0.3	0.3	0.3	0.3

of total Hb. Blood Hb was analyzed using Sigma kit procedure (catalog number 525). Another additional sample was collected for blood gas analyses, placed on crushed ice and analyzed within 1 h for blood pH, P_{CO_2} and P_{O_2} by blood gas analyzer (Model 2132-05; Instrumentation Laboratories, Inc. Lexington, KY).

The general linear model (GLM) procedure of SAS (8) was used for all statistical analyses and least square means analysis was used for data analysis. Data on blood composition were analyzed with repeated measurement in time. Treatments were compared by using least significant difference (LSD).

RESULTS AND DISCUSSION

Close visual observation and frequent heart rate measurements of steers during the experimental period indicated no significant apparent health problem in steers in any treatment group. The average dry matter intake ranged between 7.5 and 12.5 kg $d.^{-1}$

Nitrate (Fig.1) increased the level of MetHb ($P<0.01$) with time in the blood indicating the toxic effect on the animals. However, this conversion of Hb to MetHb was only 18% at 172 h post feeding and none of the steers showed any clinical signs of nitrate toxicity. This supports a previous study (1) in which clinical signs of nitrate toxicity appeared only when more than 40% of total Hb was converted to MetHb.

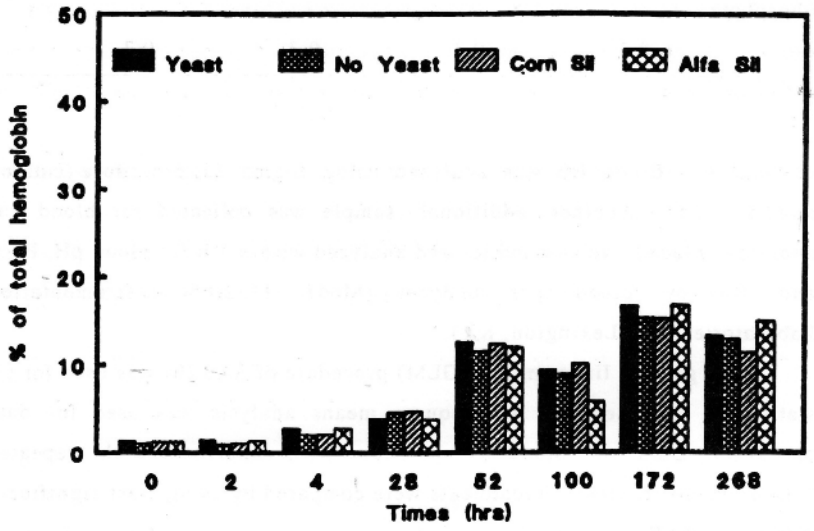

Fig. 1. Concentration of blood methemoglobin as a percent of total hemoglobin of steers fed 550 mg nitrate per kg BW d^{-1}.

Overall means of blood pH, P_{CO_2}, P_{O_2}, PCV, Hb, and MetHb are shown in Table 2. These overall means were not altered significantly by adding live yeast culture. However, some effects of feeding regime were observed in overall means. In this study animals receiving yeast tended ($P<0.06$) to have a lower blood P_{CO_2} content than those with no yeast. Overall mean of P_{CO_2} for steers fed alfalfa silage was higher ($P<0.1$) than steers fed corn silage. The higher cationic effect of alfalfa silage when compared with corn silage is the probable reason for the higher level of P_{CO_2} in the blood of steers fed alfalfa silage. Steers fed diet 1 showed lower P_{CO_2} ($P<0.06$) than those on treatment 4. Steers fed yeast tended to have higher blood MetHb concentration. This trend was also observed by Horn and Lusy (5).

Changes in blood pH at selected times of post feeding are shown in Fig. 2. During the first 28 h post treatment, pH values of blood increased for all treatments. This observation was probably due to dietary nitrate inclusion. Van't Klosser *et al.* (11) showed that intra-venous nitrite infusion

Table 2. Overall least square mean of blood pH, partial carbon dioxide tension (P_{CO_2}mm Hg), partial oxygen tension (P_{O_2}, mm Hg), packed cell volume (PCV), hemoglobin (Hb, mg d L^{-1}) and methemoglobin (MetH b, mg d L^{-1}) of steers fed four different diets.

Treatments		pH	P_{CO_2}	P_{O_2}	PCV	Hb	MetHb
1-Corn	silage†	7.347	58.5^{a}	22.0	33.32	12.78^{c}	7.7
2-Corn	silage	7.435	49.9^{ab}	21.9	31.45	11.71^{b}	6.9
3-Alfalfa	silage†	7.440	50.6^{ab}	20.8	32.37	12.96^{ac}	7.8
4-Alfalfa	silage	7.380	52.8^{b}	21.3	32.39	12.71^{abc}	7.6

† Treatment groups fed mixed diets with yeast.

a,b Means with different superscript within same column differ ($P<0.06$).

b,c Means with different superscript within same column differ ($P<0.1$).

in pregnant cows resulted in a nonsignificant increase in maternal blood pH. However, the fetal blood pH decreased slightly. Cohn *et al.* suggested that the lower blood pH of fetuses was possibly due to production of lactic acid by anaerobic glycolysis during a hypoxic situation.

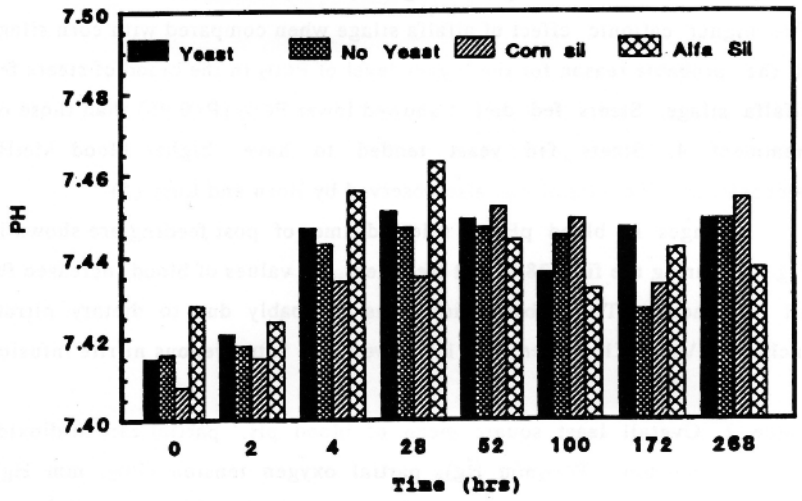

Fig. 2. Blood pH of steers fed 550 mg nitrate per kg BW d^{-1}

The change of Po_2 during post feeding times did not show any clear effect of yeast in any treatment. However, other studies (11) have shown that maternal and fetal blood Po_2 levels decreased significantly ($P<0.05$) after nitrite infusion in maternal blood and the lowest fetal Po_2 coincided in time with the highest concentration of fetal MetHb.

Blood PCV changes did not show any consistent treatment pattern across post feeding time. However, other studies (12) have shown higher PCV in sheep that were infused ruminally with higher levels of sodium nitrate.

Overall Hb means in cows fed diet 2 was lower than cows fed diet 1 ($P<0.1$). Steers supplemented with yeast tended to have higher Hb concentrations. Total blood Hb increased after the first day of nitrate feeding This observation is in agreement with Yoon *et al.* (12) who reported a higher total blood Hb content as a result of higher sodium nitrate infusion in the rumen of sheep. The increased blood Hb was possibly due to the increased conversion of Hb to MetHb after feeding nitrate.

CONCLUSION

Dietary inclusion of yeast at 0.1% of DM had no significant effect in reducing nitrate toxicity in Holstein steers. However, whether higher levels of dietary yeast culture addition may reduce nitrate toxicity remains to be investigated.

LITERATURE CITED

1. Buck, W.B., G.D. Osweiler and G.A. Van Gelder. 1976. Clinical and Diagnostic Veterinary Toxicology, 2nd ed. Kendall/Hunt, Dubuque, Iowa, U.S.A. pp.109-115.
2. Cohn, H.E., G.J. Piasecki and B.T. Jackson. 1980. The effect of fetal heart rate on cardiovascular function, during hypoxemia. Amer. J. Obstet. Gynecol. 138:1190-1197.
3. Crawford, R.F. 1960. Some effects of nitrates in forage on ruminant animals. Ph.D. thesis, Mann Library, Cornell University, Ithaca, NY,U.S.A. pp. 1-156.

4. Hale, W.H., F. Hubbert and R.E. Tylor. 1961. The effect of concentrate level and nitrate addition on hepatic vitamin A stores and performance of fattening steers. J. Anim. Sci..20:934-943.
5. Horn, G.W. and K.S. Lusy. 1984. Preliminary study on the effect of yeast culture supplementation on nitrate/nitrite induced methemoglobinemia in lamb and steers. Vet. Hum. Toxicol. 26:309-313.
6. Leahy, T. and R. Smith. 1960. Notes on methemoglobin determination. Clin. Chem. 6:148-152;
7. Lewis, D. 1951. The metabolism of nitrate and nitrite in the sheep. Biochem. J. 48:175-180.
8. SAS. 1985. SAS User's Guide: Basics. SAS Inst., Inc. Cary, NC, U.S.A.
9. Stone, C.W. 1976. Yeast Culture Research Brief. Dimond V Mills. Inc., Cedar Rapids, IA, U.S.A. pp. 1-3.
10. Streeter, C.L., G.W. Horn and J.E. McClung. 1981. Yeast culture in a free-choice mineral supplement for stoker cattle grazing wheat pasture. Oklahoma Agric. Exp. Sta. Res. Rep. Mp. 108:101-105.
11. Van't Klooster, A.Th., M.A.M. Taverne, A. Malestein and E. M. Akkersdijk.. 1990. The pathogenesis of abortion in acute nitrite toxicity of pregnant dairy cows. Theriogenology 33:1075-1089.
12. Yoon, C., J.M. Park and W.B. Chun. 1988. Effect of nitrate administration on rumen fluid and blood composition of sheep. Korean J. Anim. Sci. 30:233-239.