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## Menthol Supplementation Has Minimal Effects on Blood Components from Holstein Steers

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### Introduction

Menthol is a naturally occurring compound classified as an essential oil that gives plants of the *Mentha* species their characteristic minty aroma and flavor. Menthol is used as a cooling compound in products ranging from common cold medications to pesticides and has been found to have a wide range of biological activities in different systems within the body. More recently, menthol and other essential oils have been identified as potential alternatives to feed antibiotics and growth promotants. Menthol has been observed to directly affect  $\kappa$ -opioid receptors. Kappa opioid receptors are located in the central nervous system, with a high density found in the hypothalamus. Menthol has been hypothesized to be capable of binding to  $\kappa$ -opioid receptors in the hypothalamus, stimulating neuropeptides involved in the production and release of growth hormone and leading to increased insulin-like growth factor-1 concentration, thus increasing growth. The purpose of this experiment was to evaluate changes in blood metabolites when menthol was incorporated into the diets of steers.

### Experimental Procedures

Holstein steers ( $n = 52$ ; body weight 1,262 lb) were blocked by initial body weight and sorted, within block, to four treatment groups for a 30-day experiment. Steers were housed in individual feeding stalls equipped with fence-line feed bunks and automatic water fountains. The pens were partially covered and grouped into 3 barns, with 20 animals in the first 2 barns and 12 animals in the third barn. Treatments consisted of menthol added at 0%, 0.003%, 0.03%, or 0.3% of diet dry matter (Table 1). Feeding was staggered by 1 hour for each barn.

On day 1 of the experiment, blood samples were obtained via jugular venipuncture at 0, 6, 12, 18, and 24 hours after feeding. Treatment administration began on day 2, and blood samples were again drawn at 0, 6, 12, 18, and 24 hours after feeding. The blood sampling schedule was repeated on days 9, 16, 23, and 30. Body weights were measured on day 1 and 30. Plasma and serum were stored in 5-mL plastic tubes and subsequently analyzed for plasma metabolites of menthol, and serum was used for analysis of insulin-like growth factor 1 (IGF-1) concentration. Feed intakes were monitored daily, and

unconsumed feed was removed from the bunk, weighed, and dried at weekly intervals or as needed to determine actual dry matter intake.

## Results and Discussion

Dry matter intake did not differ ( $P = 0.25$ ) among treatments (Table 2). Average daily gain and feed efficiency were unaffected ( $P > 0.05$ ; Table 2) when menthol was included in the diet. Decreased performance, indicated by poor average daily gains and dry matter intakes, may be attributed to frequent handling of the animals.

The main purpose of this experiment was to assess components in blood related to growth. We observed a menthol  $\times$  time within day interaction ( $P < 0.01$ ; Figure 1) for IGF-1 concentration; however, we concluded that 0.003% menthol had no effect on IGF-1 concentration, but 0.03% and 0.3% menthol treatments negatively affected IGF-1 concentrations initially. Metabolites of menthol in plasma were measured, and we observed no menthol  $\times$  hour interaction ( $P = 0.71$ ; Figure 2), but we observed an effect of menthol ( $P = 0.05$ ) and an effect of time ( $P < 0.01$ ). Menthol metabolites were found in plasma of at least one steer fed 0% menthol. The reason for this is unknown, but it is conceivable that some menthol was inhaled by control steers because of the volatility of menthol and because steers were housed in close proximity to each other. In addition, the half-life of menthol metabolites in bovine plasma is not known; it may be metabolized and excreted rather quickly, which would explain why cattle receiving menthol had no metabolites of menthol in plasma at some sampling times.

## Implications

Addition of menthol to diets of cattle had little or no impact on growth parameters measured in this trial.

## Acknowledgments

The authors would like to thank Steven Hu from Pfizer Animal Health for his assistance in quantifying menthol and its derivatives in plasma.

**Table 1. Diet composition, dry matter basis**

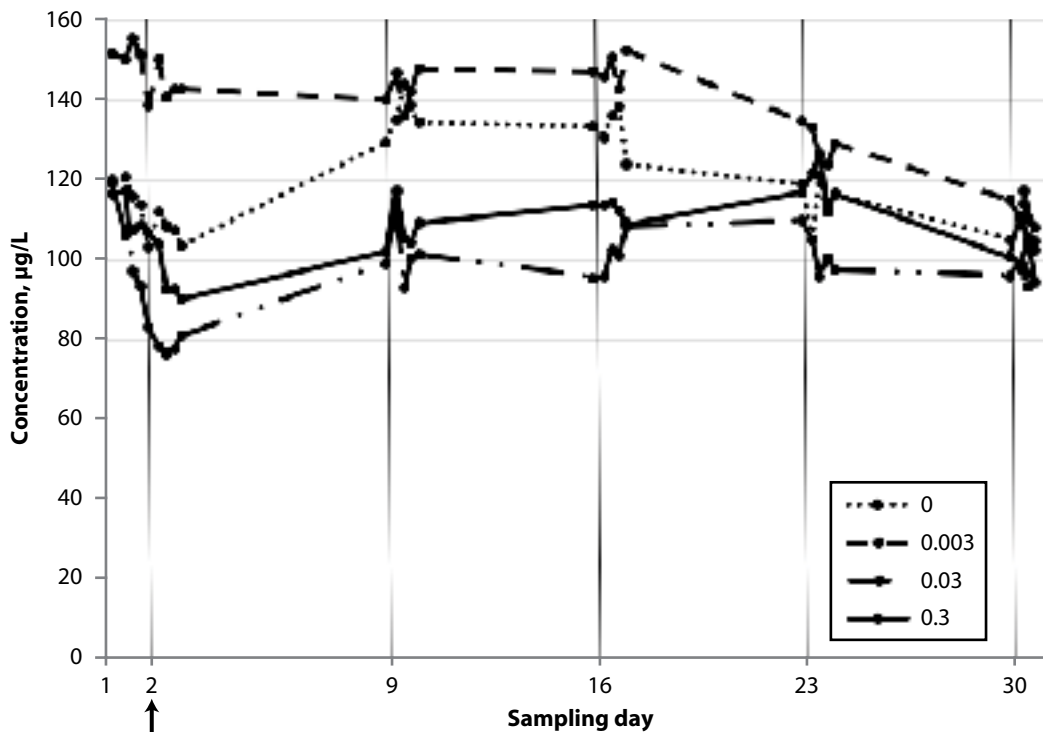
Ingredients, %	Menthol, % diet dry matter			
	0	0.003	0.03	0.3
Steam-flaked corn	50.39	50.39	50.34	49.89
Wet corn gluten feed	33.640	33.637	33.660	33.840
Corn silage	12.00	12.00	12.00	12.00
Feed additive premix <sup>1</sup>	2.16	2.16	2.16	2.16
Vitamin/mineral premix <sup>2</sup>	0.07	0.07	0.07	0.07
Limestone	1.44	1.44	1.44	1.44
Salt	0.30	0.30	0.30	0.30
Menthol	-	0.003	0.03	0.3
Calculated composition, %				
Crude protein	14.00	14.00	14.00	14.00
Calcium	0.62	0.62	0.62	0.62
Phosphorus	0.51	0.51	0.51	0.51
Potassium	0.70	0.70	0.70	0.70
Neutral detergent fiber	20.08	20.08	20.08	20.10

<sup>1</sup> Formulated to provide 300 mg/d Rumensin and 90 mg/day Tylan (Elanco Animal Health, Greenfield, IN) in a ground corn carrier.

<sup>2</sup> Formulated to provide 1,000 IU/lb vitamin A; 10 IU/lb vitamin E; 10 ppm added copper; 60 ppm added zinc; 60 ppm added manganese; 0.5 ppm added iodine; 0.25 ppm added selenium; and 0.15 ppm added cobalt.

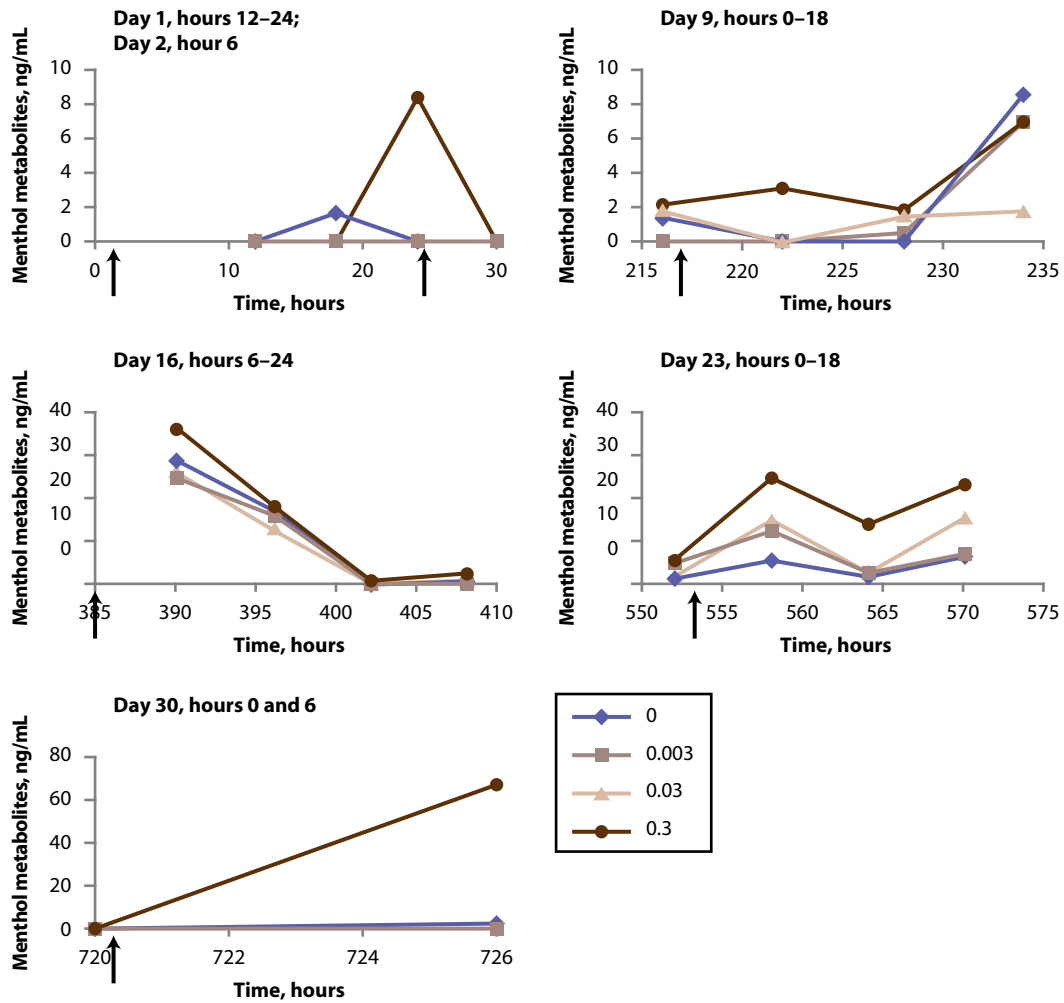
**Table 2. Effect of menthol on feedlot performance of Holstein steers fed for 30 days**

Item	Menthol, % diet dry matter				SEM	P-value
	0	0.003	0.03	0.3		
Average daily gain, lb/day	1.22	1.20	1.12	1.06	0.37	0.98
Dry matter intake, lb/day	16.55	16.53	17.19	15.46	0.79	0.25
Feed:gain	14.93	17.85	16.95	15.38	5.27	0.97



**Figure 1. Effects of menthol on insulin-like growth factor 1 concentrations from Holstein steers on days 1, 2, 9, 16, 23, and 30 and at hours 0, 6, 12, 18, and 24 post-feeding.**

Treatments consisted of 0, 0.003, 0.03, and 0.3% diet dry matter menthol. Arrow indicates beginning of treatment administration. Menthol  $\times$  time within day interaction,  $P < 0.01$ ; effect of menthol,  $P = 0.26$ ; effect of time within day,  $P < 0.01$ ; SEM = 16.2.



**Figure 2. Concentration of menthol metabolites in plasma obtained from Holstein steers.** Diets were formulated to contain 0, 0.003, 0.03, and 0.3% diet dry matter menthol. Arrow indicates feeding relative to time of blood collection. Menthol  $\times$  hour interaction,  $P = 0.71$ ; effect of menthol,  $P = 0.05$ ; effect of hour,  $P < 0.01$ ; SEM = 17.29.