



# Influence of a Supplemental Blend of Essential Oils Plus 25-hydroxy-vitamin-D3 on Feedlot Cattle Performance during the Early-growing Phase under Conditions of High-ambient Temperature

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## ABSTRACT

**Background:** During the first stage of fattening, performance of cattle is more affected by high ambient load. A strategy to mitigate the negative effects of high ambient temperatures on energy efficiency is by monensin supplementation. However, the present concern about the use of antibiotics as feed additives has led to search for safe alternatives. Due to its nature, essential oils and supplementary vitamin D3 represent a potential substitute to monensin in cattle subjected to high environmental heat load. For this reason, The objective of this study was to compare supplemental monensin vs the novel combination essential oils plus vitamin D3 on growth performance and dietary NE of feedlot bulls exposed to elevated ambient temperature during the initial 84-d on feed.

**Methods:** Ninety crossbreed young bulls (228.0±7.1 kg initial weight) were used in 84-d trial to evaluate a blend of essential oils plus 25-hydroxy-Vit-D3 as a feed additive to alleviate the harmful effects of the high-ambient temperature on feedlot cattle performance during the early-growing phase. Dietary treatments (9 replicates/treatment) were supplemented with: 1) 24 mg of sodium monensin/kg diet DM (MON), or with 2) 119.12 mg/kg diet DM of a combination of standardized mixture of essential oils (119 mg) plus 0.12 mg of 25-hydroxy-vitamin-D3 (EO+HyD). Average THI was 82.7±3.2.

**Result:** There were no treatment effects on day-to-day fluctuations in DMI. However, EO+HyD tended to increase DMI (4.3%, P=0.06). Supplemental EO+HyD increased daily weight gain (8.3%, P<0.01) and gain-to-fed ratio (4.0%, P=0.03). Supplemental EO+HyD tended to increase estimated dietary net energy (2.5%, P=0.07) and observed-to-expected dietary NE ratio (3.0%, P=0.07). This effect can be attributed to a 7% reduction in the maintenance requirement. The combination of EO+HyD may be a valuable tool to optimize growth-performance and feed efficiency of cattle under conditions of high ambient heat load.

**Key words:** Cattle, Dietary energy, Essential oils, High-ambient temperature, Monensin, Performance, Vitamin D<sub>3</sub>.

## INTRODUCTION

The ionophore monensin sodium (MON) is widely used feed additive (20 to 30 mg/ kg diet) in many countries for enhancement of feedlot cattle gain efficiency. Increased gain efficiency has been attributed to antimicrobial effects on ruminal fermentation favouring increased ruminal molar proportions of propionate and decreased methane energy loss and to a decreased risk of subclinical acidosis consequent to daily intake fluctuations (Barreras *et al.*, 2013; Azzaz *et al.*, 2015; Marques and Cooke, 2021). This latter effect may be especially pertinent for cattle exposed to high ambient load (HAT), as fluctuations in feed intake tend to be greater under those conditions (NASEM, 2016). Barreras *et al.* (2013) observed that in cattle finished under HAT conditions (THI = 79), supplemental MON decreased daily feed intake fluctuation (1.7 vs 4.5%) and increased gain efficiency (5%). The improvement in gain efficiency was attributed to a 10% reduction in estimated maintenance requirement. However, MON supplementation tends to

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decrease DMI (3 to 8%) that may lead to decreased ADG (Duffield *et al.*, 2012). Furthermore, due to the potential risk for development of antibiotic resistance, there is a global trend toward banning the use of supplemental antibiotics (WHO, 2017). Ionophore supplementation is currently prohibited within the European Union (European Commission, 2003; Directive 1831/2003/CEE) and this trend is expanding to various countries around the world. Essential oils (EO; such thymol, eugenol, vanillin, guaiac and limonene) have been investigated as an alternative to conventional antibiotics such as MON. Like the ionophores, EO have antibiotic-like characteristics affecting ruminal fermentation and nutrient absorption (Drouillard, 2018) and growth performance of feedlot lambs (Arteaga-Wences *et al.*, 2021) and cattle (Meschiatti *et al.*, 2019). In recent reports, 25-hydroxy-vitamin-D3 (HyD) supplemented at 0.1 mg/kg of diet increased ADG (Gouvea *et al.*, 2019), carcass weight (Carvalho and Perdigao, 2019) and dressing percentage (Acedo *et al.*, 2019). Researchers attributed these effects to enhanced protein accretion. Osei-Amposha *et al.* (2019) observed that HAT negatively affects muscle protein accretion rate. The objective of this study was to compare the conventional use of supplemental MON vs the novel combination EO+HyD on intake variation, growth performance and dietary NE of feedlot bulls exposed to elevated ambient temperature during the initial 84-d on feed.

## MATERIALS AND METHODS

All animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care, the experimental protocol was approved by the Universidad Autonoma de Sinaloa Animal Use and Care Committee (Protocol #11062021).

### Weather measurement and THI estimation

Climatic variables (ambient temperature and relative humidity) were obtained every hour from on-site weather equipment (2 equipment; Thermohygrometer AVALY, Mod. DTH880, Mofeg S.A., Zapopan, Jalisco) throughout the experimental period. The temperature humidity index was calculated using the following formula:

$THI = 0.81 \times T + RH (T - 14.40) + 46.40$  (Habeeb *et al.*, 2018).

### Animal processing, housing and feeding

Ninety young bulls (approximately 10 mo age;  $228.0 \pm 7.1$  kg initial shrunk weight, approximately 50% Zebu breeding with the remainder represented by continental and British breeds in various proportions) were used to evaluate the treatments effects on characteristics of growth-performance and dietary energetic. The trial was conducted at the Feedlot facilities located in Guasave, Sinaloa, México ( $25^{\circ}33' N$  and  $108^{\circ}25' W$ ). The site is about 50 m above sea level and has a dry climate. On arrival into the feedlot (approximately 3 weeks before initiation of the experiment), cattle were vaccinated for bovine rhinotracheitis and parainfluenza 3 (TSV-27, Pfizer Animal Health, México), clostridials (Fortress

7, Pfizer Animal Health, Mexico) and *Pasteurella haemolytica* (One Shot, Pfizer Animal Health, México) and treated against parasites (CYDECTIN® NF, Pfizer Animal Health, México; Trodax, Merial, México). Cattle were injected with 500,000 IU vitamin A, 75,000 IU vitamin D<sub>3</sub> and 50 IU vitamin E (Synt-ADE, Zoetis) and were implanted with 40 mg of trenbolone acetate and 8 mg of estradiol 17 $\beta$  (Revalor G, MSD Salud Animal Mexico, Santiago Tianguistenco, México) and individually weighed. Because feed and water were not withdrawn before weighing, weights were reduced (pencil shrink) by 4% to account for digestive tract fill (NRC, 2000). Cattle were blocked by weight into 2 weight groupings and randomly allocated within weight grouping 2 treatments (9 pens/treatment, 5 bulls/pen). Pens were 5 $\times$ 12 m with 19 m<sup>2</sup> of shade and were equipped with automatic waterers and fence-line feed bunks (2.37 m in length). Experimental phase lasted 84-d. The basal diet composition is given in Table 1. Dietary treatments consisted in the basal diet supplemented as follows: 1) 24 mg of sodium monensin/kg diet DM (MON; Rumensin 90®, Elanco Animal Health, Indianapolis, IN), or 2) 119.12 mg/kg diet DM of a combination of standardized mixture of essential oils (119 mg) plus 0.12 mg of 25-hydroxy-vitamin-D<sub>3</sub> (EO+HyD). Sources of EO and HyD were commercial standardized products, CRINA Ruminants® and HY3.D® (DSM Nutritional Products, Basel, Switzerland). Diets were prepared at weekly intervals. Daily feed allowances to each pen were adjusted to allow minimal (< 5%) feed refusals. The amounts of feed offered and feed residual were weighed daily. Cattle were provided fresh feed twice daily at 0800 and 1400 hours in a 40:60 proportion (as fed basis). Feed bunks were visually assessed between 0700 and 0730 hours each morning, feed residuals were collected and weighed for determination of feed intake. Adjustments to daily feed offerings were made at the afternoon feeding.

### Laboratory analyses

Feed and residual feed samples were collected daily and stored at 4°C. Samples were composited weekly for dry matter determination (oven drying at 105°C until no further weight loss; method 930.15; AOAC, 2000).

### Calculations

The estimations of expected DMI and dietary net energy were performed based on measures of initial and final shrunk body weight (SBW). Average daily gain (ADG) was computed by subtracting the initial SBW from the final SBW and dividing the result by the number of days on feed (84 d). The gain efficiency was computed by dividing ADG by the daily DMI. One approach for evaluation of the efficiency of dietary energy utilization in growth-performance trials is the ratio of observed-to-expected DMI and observed-to-expected dietary NE. Based on diet NE concentration and measures of growth performance, there is an expected energy intake. This estimation of expected DMI is performed based on observed ADG, average SBW and NE values of the diet (Table 1): expected DMI, kg/d =  $(EM/NE_m) + (EG/NE_g)$ , where EM (energy required for maintenance, Mcal/d) = EM =

$0.077W^{0.75}$ , EG (energy required for gain, Mcal/d) =  $ADG^{1.097} \times 0.0557W^{0.75}$  (NRC, 1984) and  $NE_m$  and  $NE_g$  (Mcal/kg) are corresponding NE values based on the ingredient composition (NASEM, 2016) of the experimental diet (Table 1). The observed dietary net energy was calculated using EM and EG values and DMI observed during experiment by means of the quadratic formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c}$$

Where

$x = NE_m$ , Mcal/kg,  $a = -0.877DMI$ ,  $b = 0.877EM + 0.41DMI + EG$ ,  $c = -0.41EM$  and  $NE_g = 0.877NE_m - 0.41$  and observed dietary  $NE_g$  was estimated from observed dietary  $NE_m$  as follows:  $0.877NE_m - 0.41$  (Zinn *et al.*, 2008).

### Statistical analyses

Treatment effects on growth performance and dietary energetics were analysed as a randomized complete block design (MIXED procedure SAS, 2007), with pen as the experimental unit. The fixed effect consisted of treatment and block as the random component. For comparing DM intake pattern, equality of mean effects and homogeneity between variances (CV1 vs CV2) were tested using Brown and Forsythe's variation of Levene's test. In all cases, least squares mean and standard error are reported and contrasts are considered significant when the P value  $\leq 0.05$  and tendencies are identified when the P value  $> 0.05$  and  $\leq 0.10$ .

## RESULTS AND DISCUSSION

The experiment was conducted during summer season. Average of THI was  $82.7 \pm 3.2$  with a minimum and maximum of 75.1 and 90.2, respectively (Table 2). Daily maximal THI exceeded 80 "danger or "emergency" range (Habeeb *et al.*, 2018) for every day (average of 5.3 h daily above THI 80) of the 84-d study. Based on THI coding, bulls were exposed, on a daily basis, to conditions of stressful ambient heat load (Silanikove, 2000).

Daily intake of additive treatments averaged 167 mg/d for MON, 863 mg/d for EO and 0.87 mg/d for HyD, respectively. The daily dose of MON (0.590 mg/kg LW) was within the recommended dose to for increased gain efficiency (Duffield *et al.*, 2012). The daily dosage of EO (3 mg/kg LW) and HyD (0.003 mg/kg LW) were within the range of levels previously shown to enhance growth performance or carcass (Acedo *et al.*, 2019; Carvalho and Perdigao, 2019; Toseti *et al.*, 2020).

Effects of treatments on DMI, growth performance and dietary energetics are shown in Table 3. Compared with MON, EO+HyD tended to increase DMI (4.3%;  $P=0.06$ ). The observed DMI observed for MON and EO+HyD treatments were -7.6 and -4.3% lower than expected based on dietary energy concentration and on average SBW (Table 1 and 3) for cattle under favorable environmental conditions (NASEM,

2016). Mader *et al.* (1999) observed an average decrease in DMI of 14% when cattle were exposed to high ambient temperature (average THI=79.1) for short period times (17-d). The slight reduction in DMI observed in the present trial reflects a "long term" adaptation to the persistent ambient heat load. It is expected that day-to-day fluctuations in DMI will be greater in cattle fed exposed to high ambient heat load (NRC, 2000). Although variations in DMI of up to 15% do not seem to appreciably affect ADG and gain efficiency (da Silva *et al.*, 2018), fluctuations of greater than 15% may lower gain efficiency (Zinn, 1994). In warm climates the risk of higher intake variations become greater. A perceived advantage of supplemental MON is reduced variation in DMI (Galyean and Rivera, 2003), that can be particularly advantageous during period of extreme ambient conditions

**Table 1:** Diet formulations and feeding program during early-growing phase (1-84d).

Item	Experimental diets	
	MON	CRINA+HyD
<b>Ingredient composition, % DM basis</b>		
Corn stover	20.00	20.00
Steam-flaked corn	55.00	55.00
Tallow	2.50	2.50
Soybean meal	10.00	10.00
Cane molasses	9.00	9.00
Agromix-SP <sup>a</sup>	2.50	2.50
DSM-dilution 1 <sup>b</sup>	1.00	-
DSM-dilution 2 <sup>c</sup>	-	1.00
<b>Nutrient composition, % DM basis<sup>d</sup></b>		
Crude protein	13.22	13.22
Neutral detergent fiber	22.74	22.74
Calcium	0.84	0.84
Phosphorous	0.31	0.31
<b>Calculated NE, Mcal/kg</b>		
Maintenance	1.99	1.99
Gain	1.34	1.34

<sup>a</sup>Agromix SP contained: CP, 53.0%, Calcium, 13.6%; Phosphorous, 0.40%; Magnesium, 1.0%; Potassium, 0.71%; NaCl, 15%; Co, 5.59 ppm; Fe, 2759 ppm; Zn, 2913 ppm; Cu, 20 ppm; Mn, 1674 ppm; vitamin A, 225 IU/g; vitamin E, 1.26 IU/g.

<sup>b</sup>Premix-DSM dilution contained (by 10 kg): Calcium 20.9% g; Phosphorus, 3.9%; Vit A, 40,000 KUI; Vit D3, 50,000 KUI, Vit E, 1.87 ppm, biotin, lactonúcleo industrial (trace mineral) 50 g/kg, and 24 g Monensin.

<sup>c</sup>Premix-DSM dilution contained (by 10 kg): Calcium 20.9% g; Phosphorus, 3.9%; Vit A, 40,000 KUI; Vit D3, 50,000 KUI, Vit E, 1.87 ppm, biotin, lactonúcleo industrial (trace mineral) 50 g/kg, and the combination of 119 g EO plus 0.12 g HyD/kg diet. (CRINA and HyD<sub>3</sub>; DSM Nutritional Products, Basel, Switzerland, Nutritional Products, Basel, Switzerland).

<sup>d</sup>Nutrient composition and net energy values are based diet formulation and tabular values for individual feed ingredients (NASEM, 2016).

**Table 2:** Ambient temperature (Ta), mean relative humidity (RH) and mean calculated temperature-humidity index (THI)<sup>a</sup> registered during the phase 1 of the experiment.

Week	Mean T <sub>a</sub> , °C	Min T <sub>a</sub> , °C	Max T <sub>a</sub> , °C	Mean RH, %	Min RH, %	Max RH, %	Mean THI	Min THI	Max THI
1	36.75±1.3	31.44±1.1	42.06±1.5	38.57±4.1	23.93±1.1	53.21±7.1	86.01±2.0	76.93±1.2	95.09±2.8
2	36.96±1.6	32.06±1.9	41.86±1.3	38.00±7.1	20.21±0.6	55.79±4.5	86.84±3.6	78.03±2.5	95.66±4.7
3	31.83±2.9	26.31±2.1	37.35±3.7	40.32±2.7	29.18±2.8	51.46±3.8	80.06±3.7	72.36±2.3	87.76±5.0
4	34.08±2.9	28.79±1.9	39.36±2.8	23.21±1.7	20.43±0.9	26.00±2.6	78.90±2.2	73.04±1.7	84.76±2.6
5	32.00±3.1	26.75±2.6	37.26±3.8	29.14±4.5	24.57±1.8	33.71±7.9	80.39±3.3	77.39±3.7	86.39±2.9
6	30.56±2.4	26.35±1.3	34.77±1.1	52.84±3.6	45.25±2.4	60.43±2.7	76.26±1.6	66.43±1.3	86.09±1.9
7	30.93±2.7	26.35±2.3	35.51±3.0	77.23±2.7	62.93±1.8	91.54±3.8	86.20±3.6	77.89±1.3	94.52±3.2
8	30.89±1.8	26.70±1.7	35.09±1.9	75.14±3.7	62.43±3.4	87.86±5.3	85.60±3.4	78.24±3.5	92.96±3.4
9	29.85±0.6	24.64±1.1	35.07±0.5	71.43±3.2	56.46±2.0	86.39±4.7	83.68±1.9	74.68±1.5	83.68±1.9
10	29.26±0.7	25.23±0.8	33.29±0.7	72.80±1.5	61.04±1.8	84.57±1.7	82.44±0.7	75.54±0.8	89.34±0.9
11	30.07±1.6	25.51±1.4	34.63±1.7	73.96±2.5	59.50±1.4	88.43±4.2	84.35±2.5	76.40±2.3	92.31±2.8
12	27.97±1.7	23.66±2.0	32.27±1.3	68.91±4.9	56.04±4.2	81.79±5.5	79.97±2.8	72.76±3.1	87.17±2.5
Mean 1-84 d	31.76±2.8	26.98±3.1	36.54±2.6	55.13±3.0	43.50±2.7	66.76±4.8	82.67±3.2	75.12±2.5	90.22±2.4

<sup>a</sup> THI = 0.81 × ambient temperature + [(relative humidity/100) × (ambient temperature- 14.4)] + 46.4. THI code (Normal THI < 74; Alert >74-79; Danger 79-84 and Emergency > 84).

**Table 3:** Effect of supplemental monensin (MON) or blended oils combined with vitamin D<sub>3</sub> (EO+HyD) on growth performance in growing phase of feedlot cattle.

Item	Additives <sup>a</sup>		SEM	P value
	MON	EO+HyD		
Days on feed	84	84		
Pen replicates	9	9		
Live weight, kg <sup>b</sup>				
Initial	228.05	228.04	2.42	0.88
Final	339.43	349.50	3.25	0.04
Daily gain, kg/d	1.326	1.446	0.029	<0.01
Dry matter intake, kg/d	6.946	7.256	0.10	0.06
DMI variation, CV%	3.24	6.74	2.29	0.39
Gain to feed ratio	0.191	0.199	0.003	0.03
Dietary net energy, Mcal/kg				
Maintenance	1.91	1.95	0.017	0.07
Gain	1.26	1.30	0.015	0.07
Observed-to-expected dietary NE				
Maintenance	0.95	0.98	0.009	0.07
Gain	0.94	0.97	0.011	0.07
Observed-to-expected DMI	1.054	1.025	0.010	0.07

<sup>a</sup> MON=Monensin 24 mg/kg diet DM (Rumensin 90®, Elanco Animal Health, Indianapolis, IN); EO+HyD = standardized source of a mixture of essential oils 119 mg/kg diet DM (CRINA; DSM Nutritional Products, Basel, Switzerland) plus 0.12 mg/kg diet DM of 25-hydroxy-vitamin-D<sub>3</sub> (HyD<sub>3</sub>; DSM Nutritional Products, Basel, Switzerland)

<sup>b</sup> Initial shrunk and final weight is the full live weight reduced 4% to adjustment for gastrointestinal fill.

when risk of intake variation is expected to be highest. Barreras *et al.* (2013) observed that in feedlot heifers finished under high ambient heat load (THI=79.2), the variation in energy intake was 2.5 times lower in ionophore-supplemented heifers (CV= 1.7%) than for heifers fed a non-supplemented control diet (CV = 4.5%).

Compared with MON, EO+HyD increased (8.3%, P<0.01) ADG. This enhancement in ADG may reflect, in part, a potentiating effect of HyD on net protein retention and

hence, lean tissue growth (Carvalho and Perdigo, 2019; Martins *et al.*, 2020). Toseti *et al.* (2020) observed that in Nellore bulls supplemented with EO (CRINA Ruminants®) plus exogenous α-amylase, ADG was 8.5% greater than observed in bulls fed MON. The increased ADG in their study was largely due to a 5% increase in DMI. Thus, they did not observe a treatment effect on gain efficiency. However, in the present study, EO+HyD also increased gain efficiency (4.0%; P=0.03). Thus, the difference in ADG between MON



and EO+HyD was not solely a reflection of differences in DMI. Based on growth performance measures, supplemental EO+HyD tended to increase (2.5%,  $P=0.07$ ) dietary net energy and the observed-to-expected dietary NE ratio (3%,  $P=0.07$ ) compared with MON. An alternative approach for expressing the treatments effects on energetics in the present trial is by assumption that the changes in efficiency of energy retention is affected solely by the environmental effects on the maintenance coefficient (MQ). Accordingly, the MQ for the present study can be estimated as follows:

$$MQ = \frac{(NE_m \times [DMI - \{EG/NE_g\}])}{SBW^{0.75}}$$

where  $NE_m$  and  $NE_g$  corresponds to the NE values of the diet based on tabular values (Table 1; NASEM, 2016) and EG = energy requirement for weight gain (Mcal/d). Thus, given the NE values for the basal diet are consistent with expected based on tabular values, the corresponding MQ for bulls fed MON was 0.084 Mcal/  $W^{0.75}$ , while the MQ for bulls fed EO+HyD was 0.079 Mcal/  $W^{0.75}$ . Accordingly, the increases of MQ above specified standard (0.077 Mcal/  $SBW^{0.75}$ ; NASEM, 2016) was 8.3% for MON and 2.5% for EO+HyD. This estimated increase in MQ for bull MON is in good agreement (0.99) with the expected increase based on standard heat load equation NRC (2000). Based on energetic efficiency, the apparent mitigating effect of EO+HyD on cattle response to ambient heat load could be partially explained by certain metabolic adjustments in adapted cattle. In heat stressed dairy cattle, supplemental EO lowered the frequency of high rectal temperature and increased blood oxygenation (Brito da Silva et al., 2020). More research is needed to elucidate the potential physiological effects of this novel combination of EO and HyD in cattle growing under high ambient load.

## CONCLUSION

The combination of a blend of essential oils plus 25-hydroxy-vitamin-D<sub>3</sub> (EO+HyD) may help optimize growth-performance and feed efficiency of feedlot cattle exposed to high ambient heat load. Under the experimental conditions in which this trial was carried out, the benefits (based on growth performance and energetic efficiency) of supplemental EO+HyD were superior to that of supplemental MON.

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## Conflict of interest

The authors declare no conflict of interest.

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