EVALUATION OF ESSENTIAL OIL AND INJECTABLE TRACE MINERAL ON BULL GROWTH PERFORMANCE AND FERTILITY

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

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December 2018

Major: Animal Science

EVALUATION OF ESSENTIAL OIL AND INJECTABLE TRACE MINERAL ON BULL GROWTH PERFORMANCE AND FERTILITY

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ACKNOWLEDGEMENTS

Dr. Chase Runyan and Dr. Michael W. Salisbury's continued efforts and contributions to Angelo State University secured the facilities, subjects on trial, contacts and resources needed to complete the objectives of this study. Dr. J. Will Dickison and Mr. Colton Harris completed data collection and analysis of semen and fertility factors. Ranch Manager, Mr. Cody Riddle and other research associates appointed to the Angelo State University Management, Instruction and Research center (MIR) aided in the daily animal husbandry practices to sustain herd health. I truly appreciate the guidance, time and efforts from Dr. Runyan and this committee. Most importantly, I would like to acknowledge my wife, Allison Sullivan for her endless grace, support and encouragement.

ABSTRACT

The objective of this study was to evaluate the effects of an essential oil mixture (EOM) in conjunction with injectable trace mineral (ITM) on growth performance and fertility of growing, yearling bulls. Angus bulls born in the consecutive springs (2016; n=37 and 2017; n=29) were weaned, weighed and stratified by weight and sire across two diet treatment groups (EOM and CONTROL). Additionally, a similar stratification procedure was used, generating injectable trace mineral subsets (ITM-Yes and ITM-No) of bulls, within each diet group. In 2016 (yr 1), d -35 and 2017 (yr 2) d -32, bulls were weaned and exposed to CONTROL diet for total mix ration (TMR) adaptation. At d 0, weight and scrotal measurements were taken as well as dietary treatments applied. Weight and scrotal measures were collected every 28 days, with ITM implemented at d 42 and 70 in yr 1 and d 47 and 75 in yr 2. Final assessment of weight and scrotal measurement, as well as semen quality measures, were concluded on d 98 in yr 1 and d 103 in yr 2. Differences due to ITM as a main effect were observed in the overall weight measures only, as bulls in the ITM-Yes subset exhibited higher overall weight measures ($P = 0.05$), even though no weight differences were observed at d 0 as a result of stratification structure. Differences in post-weaning average daily gain (ADG) and semen motility scores were observed due to the diet × ITM (year) interaction. The ITM-Yes subset bulls of yr 2, achieved a greater post-weaning ADG compared to the ITM-No subset within the EOM diet consuming group ($P < 0.01$). Implementing ITM can improve weight gain and augment semen motility scores while dietary EOM alone has limited impact on growth measures and semen motility.

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INTRODUCTION

The per capita consumption of beef in the United States, during 2016, was more than 56 pounds; since 1965, we have consumed approximately 74 pounds of beef per person in the United States each year (USDA, NASS 2018). In effort to continuously meet the demand of the potentially growing human population, while addressing rising public concern toward animal health and well-being as well as environmental factors, we must relentlessly pursue new knowledge, discovering and implementing new technologies and practices of animal husbandry. The challenge to increase production and improve proficiency is being addressed across all concentrations of study. Nutrient requirements change throughout stages of development and rate of growth is affected by nutrient requirements (NRC, 2000). Genetic potential may only be met or reached with adequate nutrition and husbandry. Cattlemen in every phase or stage of production consider costs-to-gain, with feed expenses often accounting for approximately 65% of the cost required to maintain a breeding herd (Arthur et al., 2004; Van der Westhuizen et al., 2004). Growing public concern combined with costs of production drives the need for new knowledge, discoveries and continued development of technologies to aid cattlemen in their efforts to sustain and improve proficiencies in production. Growing public concern regarding potential health risks and environmental impacts, possibly resulting from excessive use of antibiotics as growth promoters in animal production, led to the Food and Drug Administration (FDA) prescription or veterinary feed directive (VFD) requirement for all antibiotic use in livestock. The announcement of this

intention in 2015, spurred on efforts to identify non-synthetic alternatives for pathogen immunological response and production factors. Of these alternatives, essential oils (EO) have been a primary alternative focus. In ruminant animals, EO as feed additives have shown to improve feed efficiency and control the spread of pathogens (Bampidis et al., 2005). Wallace (2004) identified properties of EO that impede the degradation of protein in the rumen, increasing the amino acid (AA) availability for intestinal uptake. Trace minerals (TM) are required for physiological processes that greatly influence growth and development. Current husbandry practices implemented to supply and sustain TM requirements in beef cattle operations include: reliance on nutritional wisdom of the species, free-choice, granule, block, tubs, boluses, oral drench, inclusion in the total mix ration (TMR) and injection. Trace mineral deficiencies are a result of diet insufficiencies or the reduction of availability due to antagonisms. Adequate status of TM is required to combat deficiencies caused by antagonists (Puls, 1994). By incorporating an injectable trace mineral (ITM), cattlemen may be able to avoid the deficiencies and better meet nutrient requirements during times of greater stress. Further efforts to understand the influence and interactions of technologies as well as mechanisms from supplementation of TM are needed to clarify value. Animal husbandry practices must continue consideration of combining multiple technologies to meet nutrient requirements and improve proficiency for growth and development. The objective of this study was to evaluate the effects of essential oil mixtures (EOM) in conjunction with ITM on growth performance and fertility of growing, yearling bulls.

LITERATURE REVIEW

Essential Oils

Essential oils (EO) are steam volatilized or extracted secondary plant metabolites (Calsamiglia et al., 2007) that have been considered as potential alternatives to growth promoters for animal production (Thakare, 2004). Dudareva and Pichersky (2004) determined the chemical composition of EO depends on or may be modified by the species, the source of plant (geographic location), the harvesting practices, the anatomical structure utilized (root, stem, leaf, flower/ bloom, bark) and method of isolation (extraction by non-aqueous solvent, cold expression, steam distillation, etc.). Terpenoids (mono- and sesqui-) and phenylpropanoids are the primary active metabolites of EO. According to Zwenger and Basu (2008), terpenoids (limonene, thymol, carvacrol, linalool, etc.) are derived from a basic, five carbon structure (C5H8) of an isoprene unit through the mevalonate pathway, and De Cássia da Silveira e Sá et al. (2014) identifies phenylpropanoids (cinnamaldehyde, eugenol, anethole, etc.) as three carbons bound to an aromatic ring of six carbons from phenylalanine synthetized by the shikimate metabolic pathway. According to Ultee et al. (2002), these hydrophobic metabolites prevent normal cell physiology, depleting cell ATP, thus preventing cell growth and ultimately resulting in cell death. Boadi et al. (2004) supports the premise that methane (CH4) losses (via eructation) reduces gross energy (GE) in ruminants, as Bodas et al. (2012), finds EOs could inhibit rumen methanogenesis; therefore, the proper administration of EO could provide mitigation of greenhouse gas effects while improving feed efficiency (Benchaar et al., 2006). McIntosh et al. (2003) supports the use of EO as adequate levels and attachment of specific bacteria introduced to the rumen, may lead to a more efficient utilization of dietary nitrogen, inhibiting the breakdown of AA. Observation of

other technologies, such as monensin and tylosin (Potter et al., 1985), have shown to reduce dry matter intake (DMI), with improved gain-to-feed ratio (G:F) and is similar to that observed by Stock et al. (1995). Other efforts incorporating EOM have shown no differences in DMI between control and treatments (Meyer et al., 2014). Similar observations in the Chaves et al. (2008) study reflect diets incorporating carvacrol and cinnamaldehyde for lambs had no DMI response compared with controls. The Khiaosa-ard and Zebeli (2014) study aimed at investigating the effects of EO and their bioactive compounds (EOBC) on rumen fermentation in vivo, revealed a decreased acetate to propionate ratio, which may favor beef production. Interactions of feedstuffs and EO as feed additives have shown to increase hot carcass weight (HCW), as the Meschiatti et al. (2016) effort compared a coarsely ground corn (CGC) diet including EO to the CGC diet including a monensin product, resulting in subjects exposed to the EO exhibiting greater HCW. Similar efforts of Acedo et al. (2016) concluded EO combined with *a*-amylase increased growth factors as well as DMI and could be a more beneficial alternative to monensin.

Trace Minerals

Primary mineral deficiencies occur when cattle are lacking a particular mineral from direct diet sources; whereas, secondary mineral deficiencies occur when the normal physiological process of breakdown and absorption (metabolism) is inhibited by an alternative mineral, such as: sulfur (S) binding with copper (Cu), preventing metabolic function (Gooneratne et al., 1989). Adequate TM status is required to combat deficiencies caused by antagonists (Puls, 1994). Much effort has been allocated toward TM implementation into diets. The Arthington et al. (2004) effort provides support toward supplementing Cu, Zinc (Zn), and Manganese (Mn), as reproductive performance of young,

growing heifers was increased. The Genther et al. (2014) study, utilizing ingestible TM added to diet, improved subject carcass merit as well as growth of mildly deficient steers. Implementing an ITM bypasses the gastrointestinal tract, improving concentrations in the liver (Pogge et al., 2012) as well as the performance of beef cattle (Richeson and Kegley, 2011). Arthington et al. (2014) supports ITM effects on performance of beef calves in post-wean stages of development. Copper aids in the formation of hemoglobin, bone, melanin and keratin and is an essential component of enzymes that contribute to cellular respiration, cross-linking of connective tissue, central nervous system formation, reproduction and immunity (NRC, 2000). Copper deficiencies cause decreased conception rates, infertility, anestrus, and fetal resorption in murine, porcine, and bovine (Hostetler et al., 2003). Di Costanzo et al. (1986) suggests that Mn highly influences hormone synthesis and the stimulation of cholesterol-steroid synthesis within the ovary. Hormone synthesis requires Mn to convert mevalonic acid to squalene, which stimulates the synthesis of cholesterol (Olson et al., 1999). The need for Mn was further illustrated by Hostetler et al. (2003) as Mn concentrations were situated within the ovary and pituitary glands. Ovarian follicles and corpus lutea (CL) of ewes absorbed a larger amount of radioactively labeled Mn than ovarian or extraovarian reproductive tissues, suggesting the accumulation of Mn within the CL and follicles for the synthesis of hormones (Hidiroglou, 1979). Selenium (Se) enables function of many enzymes and proteins (Allan et al., 1999). Selenium deficiencies often result in reproductive disorders such as retained placentas, infertility, cystic ovaries, metritis, delayed conception, and erratic, weak or silent ovulation periods leading to poor fertilization (Corah and Ives, 1991). Zinc (Zn) influenced overall fat metabolism in steers (Spears and Kegley, 2002) and is implicated in the synthesis and secretion of insulin. The effort of Zezeski et al.

(2016) reflects Zn concentrations in the liver improved acrosome integrity of bull spermatozoa. Price et al. (2016) suggests that TM can improve the rate for sexual maturity in post-weaned Angus bulls. In continued effort to improve production, new understanding is needed to determine and clarify the influence of an adequate supply at appropriate developmental stages of TM to growing, performance cattle.

MATERIALS AND METHODS

These experiments were conducted on the Angelo State University Management, Instruction and Research center (MIR) at dry lots located: 31°31'29.9"N 100°31'31.0"W. All animal handling procedures were conducted in accordance with and approved by the Angelo State University's Institutional Animal Care and Use Committee Protocol # 18-201.

Data was collected from Angus bulls born in the consecutive springs 2016 (Year 1: n =37) and 2017 (Year 2: n=29). The design of this experiment was a 2 x 2 factorial, where subjects were weaned, weighed and stratified by wean weight and sire across two diet treatment groups, an EOM group and an EOM naïve group (CONTROL). Additionally, a similar stratification procedure was used, generating injectable trace mineral (ITM) subsets within each diet group (ITM-Yes and ITM-No). In each year, d -35 in Year 1 (yr 1) and d -32 in Year 2 (yr 2), subjects were weaned and exposed to CONTROL diet for total mix ration (TMR) adaptation. At d 0, weight and scrotal measurements were collected and dietary treatments were applied. Weight and scrotal measures were collected every 28 days, with ITM treatments implemented at d 42 and 70 in yr 1 and d 47 and 75 in yr 2. Final assessment of weight and scrotal measurements, as well as semen quality factors, were collected on d 98 in yr 1 and d 103 in yr 2. Subjects were confined to dry lots, treatment diets provided access to fresh water and hay grazer hybrid (*sorghum almum*) *ab libitum* from weaning through post-study. Total mix ration percentages as well as nutrient composition are reflected in Table 1. Diets are designed to be iso-caloric and iso-nitrogenous, with minimal variation in feed mixing personnel throughout the diet treatment period.

Table 1. Total Mix Ration Percentages and Nutrient Composition

Ingredient	EOM Diet %	CONTROL Diet %
Cracked Corn	30.00	30.00
DDG + solubles	20.00	20.00
Cottonseed Hulls	30.00	30.00
Alfalfa pellets	14.50	14.50
Molasses	4.00	4.00
Mineral Premix[1]	2.50	2.50
Essential Oil Mixture	0.06	0.00
Nutrient, DM		
DM %	89.35	89.35
Crude Protein %	15.55	15.55
NEm Mcal/kg	0.30	0.30
NEg Mcal/kg	0.19	0.19
Crude Fat %	5.44	5.44
ADF	29.19	29.19
NDF	40.30	40.30
Calcium	0.88	0.88
Phosphorus	0.38	0.38

[1]Premix: 17.5-19%Ca, 18.1-20.6%NaCl, 1,075 ppm Mn, 1,780 ppm Zn, 3.95 ppm Se, 89,184 I/kg Vitamin A, 29,728.03 ppm Vitamin D, 493.83 ppm Vitamin E

Growth of subjects was evaluated every 28 days by bull capture and restraint in the Qcatch 8500 (Q series, Arrow Cattle Group, Canada) working chute, assessment of weight was collected, utilizing the Gallagher Weigh Scale W610 model V2. Scrotal circumference (SC), was measured with mechanical scrotal tape (Irwin USA). Low-stress handling techniques were applied in all handling procedures. Average Daily Gain (ADG) was calculated by Year and compiled for analysis. Assessment of weight and scrotal measures were collected in period 1 (d -35 to d 0 in yr1; d -32 to d 0 in yr 2), period 2 (d 0 to d 42 in yr 1; d 0 to d 47 in yr2), period 3 (d 42 to d 70 in yr 1; d 47 to d 75 in yr 2), period 4 (d 70 to d 98 in yr 1; d 75 to d 103 in yr 2), with a combined assessment of periods reflected in post-wean. Table 2 illustrates project days and procedures.

Table 2. Procedure days and associated dates across years

	Yr 1		Yr 2	
Procedure	Day	Date	Day	Date
Wean, sort, collect weights, scrotal measurements	-35	9/28/2016	-32	9/26/2017
collect weights and scrotal measurements; EOM treatment	0	11/2/2016	0	10/27/2017
collect weights and scrotal measurements; ITM treatment	42	12/14/2016	47	12/14/2017
collect weights and scrotal measurements; ITM treatment	70	1/11/2017	75	1/11/2018
collect weights and scrotal measurements semen evaluation	98	2/8/2017	103	2/8/2018

At d 0 in both years, bulls in the EOM groups were transitioned to the dietary EOM treatment, at a diet inclusion rate of 0.004 kgs per head daily as per product label instruction. Bulls in the ITM-Yes subset were administered ITM at a dosage rate of 1 mL per 45.35 kg of body weight, as per product label instruction, immediately following bull body weight collection on d 42 and 70 in yr 1 and d 47 and 75 in yr 2. On d 91 in yr 1 and d 97 in yr 2, an initial semen sample was collected via electro-ejaculator and discarded to verify a common reproduction tract dispel of all bulls. At d 98 in yr 1 and d 103 in yr 2, final semen samples were collected and semen motility scores were assessed using the same trained personnel in both years, using a 5-point scale as described in Table 3. This table illustrates the measure of

motility of semen samples presented in this report. Samples were analyzed by the Quick Check Gold Bull Test, in yr 2 only.

Table 3. Semen grading scores for semen motility parameters

Scale	Grade	Characteristics
5	(+++++) Excellent	More than 80% of the sperm show vigorous motion. Swirls are formed due to the movements of the sperm. The movements are rapid and changing and hard to observe individual sperm samples in undiluted semen.
4	(++++) Very good	About 70-80% of the sperm show vigorous motion which causes waves and eddies but not as vigorous as the excellent grade.
3	(+++)	About 45-70% of the sperm are in motion. Motion is vigorous. Waves and eddies are formed slow across the sample.
2	(++) Fair	30-40% of the spermatozoa are in motion. Movements are vigorous. No waves or eddies present.
1	(+) poor	Little to no mobility found. < 20% of the spermatozoa are in motion. Not progressive and little oscillation.

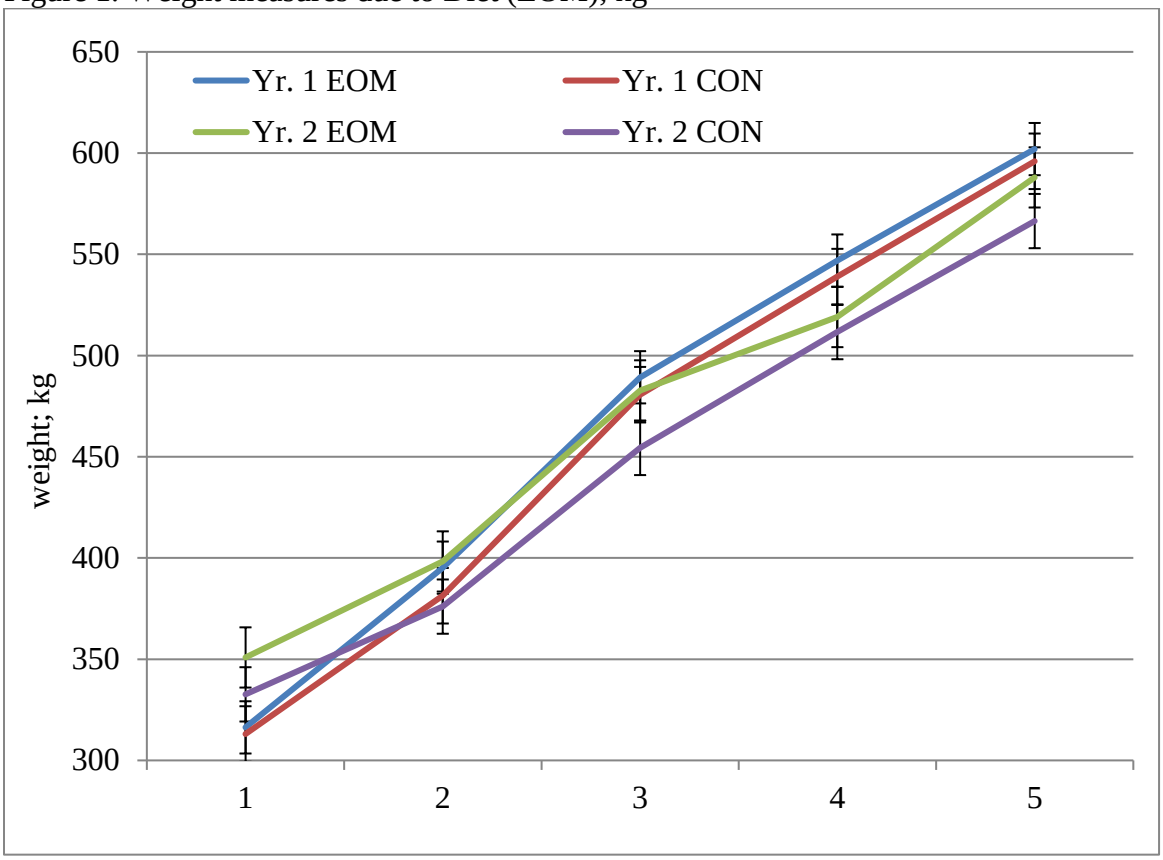
Adapted from Hossain et al. (2012).

Data from both years was compiled and mixed model procedures of SAS (v. 9.2; SAS inst, Inc., Cary, NC) were utilized. Weight measures were analyzed with a model that includes fixed effects of day, diet, ITM, and interactions of diet × age of dam (AOD) nested within year, diet × day (year), diet × ITM (year), diet × ITM × day. These measures were analyzed as repeated measures, utilizing the first order autoregressive covariance structure. All ADG variables and semen quality observations were analyzed with a model using similar fixed effects and only the diet × ITM (year) interaction. These models did not include the repeated measures statements. Differences in the least squares means, using the PDIFF option, were observed and fixed effects, resulting in $P \geq 0.15$ and interactions resulting $P \geq 0.25$ were removed prior to final analysis.

RESULTS AND DISCUSSION

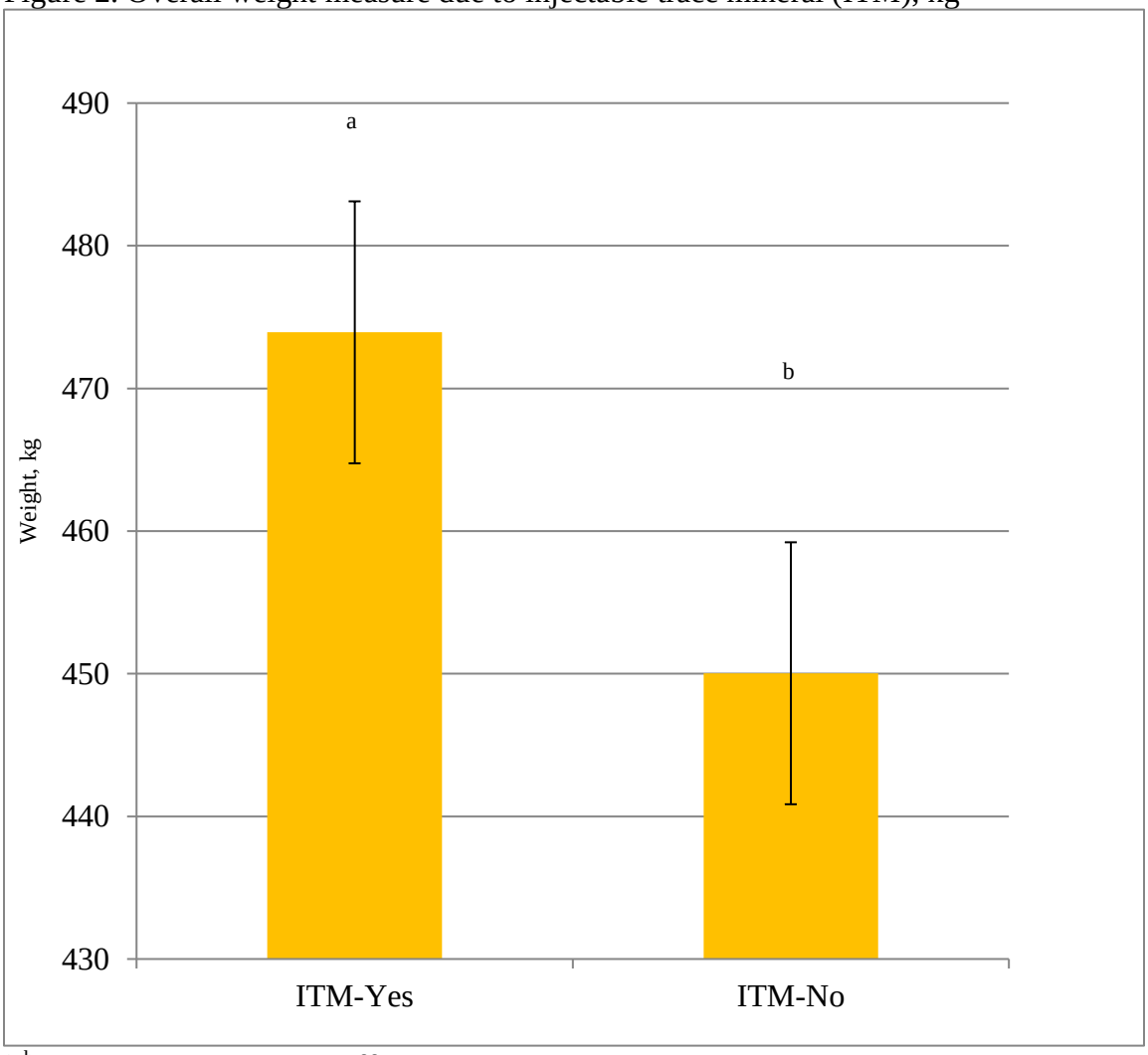
Weight Measures. No significance in overall weight measures were observed due to the main effect of diet alone (Figure 1).

Figure 1. Weight measures due to Diet (EOM), kg



Due to the stratification procedures, no weight differences were observed for any treatment or treatment interactions at d 0 within or between any diet group or ITM subset. Variation was observed in these data due to diet by day (year); however, no significant difference within a single day exists. Differences in overall weight measures were observed due to the main effect of ITM. Subjects in the ITM-Yes subset, exhibited greater overall weight measures ($P = 0.05$) and are illustrated in Figure 2.

Figure 2. Overall weight measure due to injectable trace mineral (ITM), kg



[a, b] superscripts designate differences ($P = 0.05$)

Trace mineral nutrition helps cattle recover more rapidly from stress and is vital for optimum performance of beef cattle (Genther et al., 2014). This data supports ITM can improve weight gain. The difference in the overall weight measure may be better explained by examining the differences in post-wean ADG.

Average Daily Gain. The ADG analysis yielded several sources of variation and resulting *P* – values are presented in Table 4.

Table 4. *P* - values for Average Daily Gain due to main effects by period

	Year	Diet	Sire	AOD	ITM	Diet*ITM (Year)
Period 1	0	0.25	0.02	0.04	0.02	0.66
Period 2	0.07	0.82	0.80	0.56	0.96	0.59
Period 3	0.83	0.04	0.47	0.28	0.24	0.09
Period 4	0.21	0.14	0.40	0.48	0.01	0.35
Post-wean	0.04	0.43	0.40	0.05	0.28	0.04

P - values of weight measurements; filled/ highlighted areas designate differences ($P \leq 0.05$).

Assessment of ADG collected in period 1 (d -35 to d 0 in yr 1; d -32 to d 0 in yr 2) across both years, revealed sire, AOD, and ITM influenced ADG; however, diet was not introduced until day 0 and ITM treatments were initiated on day 42 in yr 1 and day 47 in yr 2, which would have been the last days of period 1. Therefore, the influence of the diet and ITM treatments are not reasonable for these computed differences in ADG for period 1. Diet influenced ADG in period 3; however, the CON subjects had higher ADG (1.91kg/ subject) compared to those exposed to EOM treatment (1.55kg/ subject). Those subjects exposed to ITM treatment in period 4 (d 70 to d 98 in yr 1; d 75 to d 103 in yr 2), reflected less ADG

than the ITM-No subset (2.2kg/ subject) compared to those ITM-yes subjects (1.86kg/ subject).

While the diet and ITM treatments along with the diet by ITM interaction was the primary objective of this study, data suggests that AOD and ITM are important variables for influencing ADG, as differences were observed due to these effects in more periods, more frequently than other main effects; additionally, sire is not of primary concern in these data but included into the final analysis of all periods to avoid confounding result interpretation due to its known source of variation. Even though the genomic impact of EOM and ITM application is beyond the scope of this study, future effort is warranted into these potential effects.

Significance in ADG was observed due to the main effect of age of dam (AOD), within period 1 ($P = 0.01$) and post-wean ($P = 0.05$) observation schedules (Table 5). In observation periods 1 and post-wean, bulls out of three-year-old dams exhibited higher ADG (2.67kg/subject) in period 1 and (2.29kg/subject in post-wean).

Table 5. Least squares mean estimates of Average Daily Gain due to Age of Dam, kg

	AOD 2	SEM	AOD 3	SEM	AOD 4	SEM	AOD 5	SEM	AOD 6+	SEM
Period 1	1.51[a]	±0.25	2.67[b]	±0.27	1.65[a]	±0.24	1.76[a]	±0.28	1.80[a]	±0.13
Period 2	2.31	±0.31	2.32	±0.33	1.64	±0.30	1.64	±0.34	1.76	±0.17
Period 3	1.97	±0.32	1.94	±0.34	1.95	±0.30	1.13	±0.35	1.65	±0.17
Period 4	2.23	±0.25	1.85	±0.27	2.22	±0.23	1.91	±0.27	1.92	±0.13
Post-wean	2.03[x]	±0.14	2.29[y]	±0.14	1.83[x]	±0.13	1.60[x]	±0.15	1.77[x]	±0.07

[a, b] superscripts designate differences ($P \leq 0.05$) within Period 1 ADG
[x, y] superscripts designate differences ($P \leq 0.05$) within Post-wean ADG

Post-wean ADG. Variation of ADG was observed due to the interaction of Diet*ITM (Year), with differences ($P = 0.04$) in the post-wean observation schedule for yr 1 (Table 6).

Table 6. Least squares mean estimates of Average Daily Gain for the Diet*ITM (Year) interaction, kg

	Yr.1: ITM-No				Yr. 1: ITM-Yes			
	EOM	SEM	CON	SEM	EOM	SEM	CON	SEM
Post-wean[a]	2.01	0.14	1.96	0.12	2.16[a]	0.13	2.09	0.13

[a] superscripts designate differences ($P \leq 0.05$)

Post-wean observation schedule results depict those subjects in yr 1, which were exposed to the interaction of treatments (EOM and ITM), exhibited higher ADG (2.16kg/subject).

Significant sources of variation were observed in this data for post-wean ADG due to the Diet*ITM (Year) term only (Table 7). In the yr 2, the ITM-Yes subset bulls achieved a greater post-wean ADG as compared to the ITM-No subset within the EOM diet consuming group (2.53 and 1.02 respectively; $P < 0.01$).

Table 7. Least squares mean estimates of Post-wean ADG of Diet*ITM (Year), kg

	Yr 1				Yr 2			
Diet	ITM-Yes	ITM-No	SEM	*P* =	ITM-Yes	ITM-No	SEM	*P* =
EOM	2.17	2.02	0.15	0.32	[a]2.53	[b]1.02	0.16	< 0.01
Control	2.09	1.96	0.13	0.33	1.67	1.78	0.12	0.48

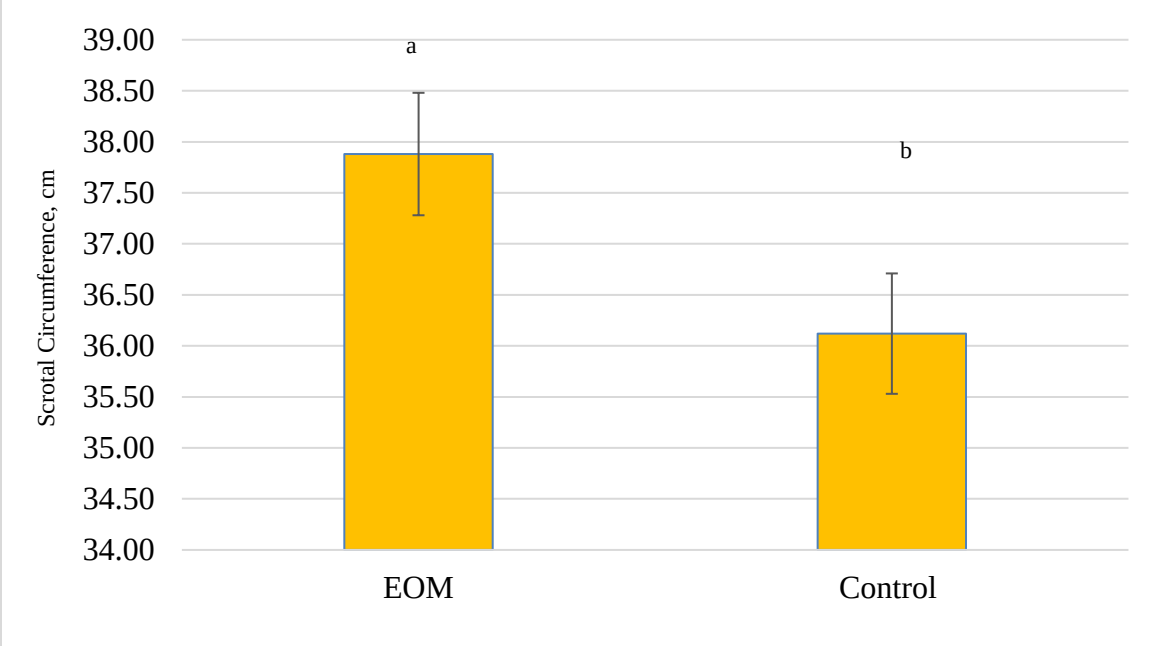
[a, b] superscripts designate differences ($P \leq 0.05$)

The Melo et al. (2016) effort involving Nellore bulls, comparing growth parameters influenced by monensin to EOM, expressed no support toward EOM on growth performance; whereas, the Meschiatti et al. (2014) effort evaluated the interaction of essential oils with *a*-amylase, which improved animal performance. Sherrill et al. (2017) supports stressed or struggling cattle benefit the most from ITM and Stokes et al. (2018) suggest that body

condition score (BCS) may be improved with ITM at certain stages of development. Sire and the interaction of essential oil mixtures (EOM) combined with an ITM can influence ADG. Age of Dam and ITM greatly influences ADG in young, growing bulls. Cattlemen in the cow-calf and purebred phases of production, should consider AOD as a factor for influencing ADG, as well as implementing ITM in their initial processing or herd health plan.

Scrotal Circumference. Results of Rusk et al. (2002), indicate that bulls with larger scrotal circumference (SC) increased yearling body weight (BW) and improved fertility. Gipson et al. (1985) found correlation between SC and the percent live sperm, sperm concentration and motility as well as potential breeding efficiency score. Differences (P = 0.05) in SC due to diet were observed (Figure 3).

Figure 3. Scrotal Circumference due to Diet (EOM), cm



[a, b] superscripts designate differences (P = 0.05)

While these differences were observed at the time of analysis, caution is warranted as to the interpretation of the biological differences this data implies. Most common breeding

soundness exams include a minimum of 30 cm for acceptable SC for yearling age bulls and it is important to note that no bulls in this study would have failed a breeding soundness exam due to inferior SC.

Sperm Concentration. Sperm concentration has been considered a factor of semen quality (Shelke and Dhami, 2001; Belorkar et al. 1988). Prior efforts support sperm concentration positively influences motility (Everett et al., 1978; Mathevon et al., 1998). Sperm concentration varies in bulls (Graffer et al. 1988; Seidel and Foote, 1969; Shelke and Dhami 2001). No significance in sperm concentrations were observed due to any main effect or interaction of main effects in this study.

Semen Motility. Differences in semen motility scores were also observed due to the Diet*ITM (Year) term only, and this data is presented in Table 8.

Table 8. Semen Motility Scores due to Diet*ITM (Year)

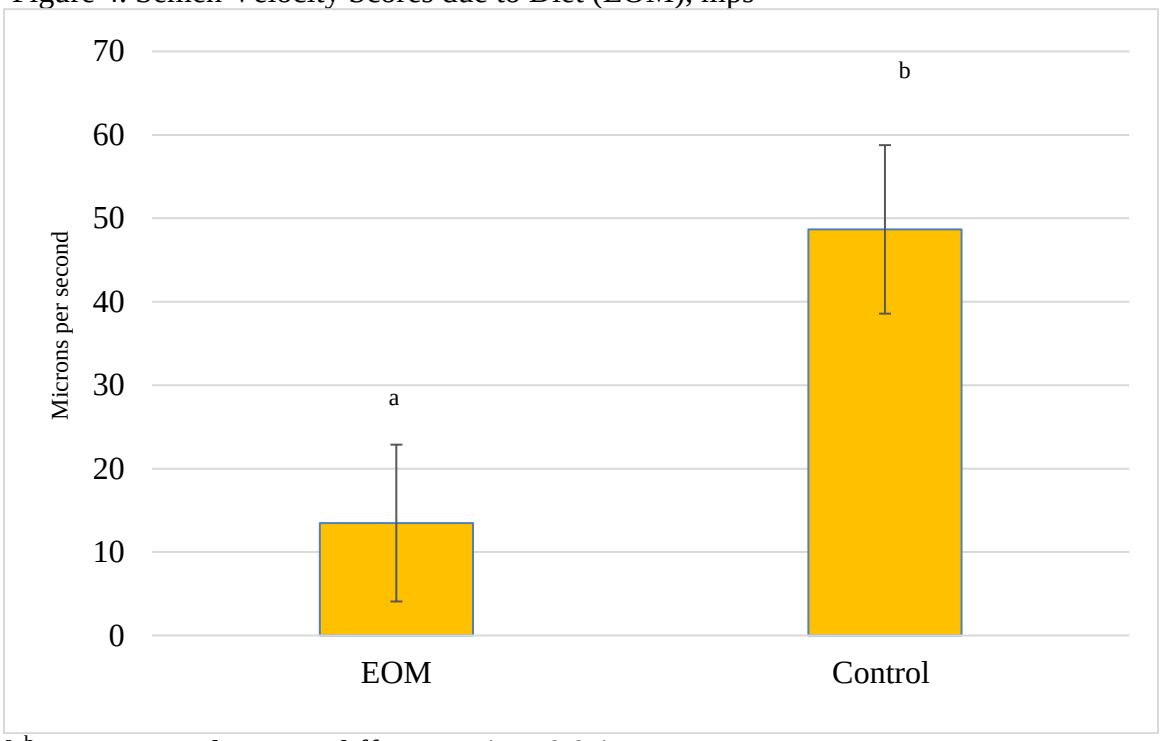
	Yr 1				Yr 2			
Diet	ITM-Yes	ITM-No	SEM	*P* =	ITM-Yes	ITM-No	SEM	*P* =
EOM	3.18	2.79	0.68	0.77	3.73	3.38	0.73	0.64
Control	[a]3.70	[b]2.33	0.61	< 0.01	[x]4.33	[y]2.03	0.69	< 0.01

Least squares means of semen motility scores for Diet × ITM (Year).
[a, b] superscripts designate differences ($P \leq 0.05$) of Yr. 1 ITM effects within respective Diet.
[x, y] superscripts designate differences ($P \leq 0.05$) of Yr. 2 ITM effects within respective Diet.

Significant differences were observed within the CONTROL diet bulls only in both years, as the ITM-Yes subsets in both years had greater least squares means (LSM) estimates than the ITM-No bulls ($P < 0.01$). Alexander (2008) explains that semen samples that have a 30% or greater progressive motility score are declared as acceptable breeders in most breeding soundness exam (BSE) criteria; therefore, all bulls in this data, with motility scores of "2" or greater would pass a standard BSE for motility.

Semen Velocity. As motility is one of the most important factors of fertility, Nagy et al. (2015) concludes the most useful characteristic of semen motility, regarding fertility prediction, is velocity. As shown in Figure 4, subjects in yr 2 CON showed stronger velocity than those exposed to the EOM ($P < 0.05$). This analysis of data may encourage further efforts to determine cytotoxicity of the EOM (calcium carbonate, garlic oil, cinnamaidehyde, silicon dioxide, di-glycerides of fatty acids and mineral oil.

Figure 4. Semen Velocity Scores due to Diet (EOM), mps



[a, b] superscripts designate differences ($P < 0.05$)

While the minimum number of motile sperm required for fertility is different per individual bull (Sullivan and Elliot., 1968), Pace et al. (1981) found fertility increased with increasing numbers of structurally intact and motile sperm. Applied kinematics of the Nagy et al. (2015) effort provides support for strong consideration of velocity toward fertility prediction.

IMPLICATIONS

Continued efforts to implement new knowledge, practice and technology in animal husbandry are essential to meet the demand for beef of a potentially growing and food-conscience population. Animal husbandry practices must continue consideration of combining multiple technologies to meet nutrient requirements and improve proficiency for growth and development. The objective of this effort was to evaluate the effects of EOM in conjunction with ITM on growth performance and fertility of growing, yearling bulls. Essential oils have been a primary focus toward alternative feed additives. While EOM and ITM treatments as well as the interaction of those EOM and ITM treatments were the primary objective of this study, data suggests that AOD and ITM are important variables for influencing ADG. This study suggests that dietary EOM has limited impact on growth measures and semen motility observations; however, when applied in conjunction with ITM, EOM can improve post-weaning ADG. Furthermore, implementing trace mineral homeostasis via ITM can improve weight gain and augment semen motility scores.

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APPENDIX

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

01/20/18

Chase Runyan, Ph.D.
Assistant Professor
Co-Chair IACUC
Department of Agriculture
Angelo State University
ASU Station #10888
San Angelo, TX 76909

Dear Dr. Runyan:

Your proposed project titled, "Evaluation of Cinnagar and injectable trace minerals on bull growth and semen quality parameters" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 1-20-2018, and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 18-201. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Steve Brewer, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee