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SCIENTIFIC NOTE

Impact of presence of zeolite in diets for lambs supplemented with zilpaterol hydrochloride: Growth performance and dietary energetics

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ABSTRACT

Zeolite (ZEO) and zilpaterol hydrochloride (ZH) are feed additives commonly included at the same time in lamb’s diets, but there is no information regarding the potential antagonism between both on growth performance and dietary energy, for this reason, 48 Pelibuey × Katahdin male intact lambs (36.2 ± 4.4 kg initial live weight) were assigned to the following treatments: 1) Basal diet without ZEO and ZH (CON); 2) inclusion of 3% ZEO replacing cracked corn (*Zea mays* L.) and soybean meal (*Glycine max*. (L.) Merr.) in the CON diet (ZEO), 3) CON diet supplemented with 6 mg ZH kg^{-1} diet (ZH), and 4) ZEO diet supplemented with 6 mg ZH kg^{-1} diet (ZEO+ZH). The experimental period was 34 d. There were no treatment interactions ($P \geq 0.21$). The ZEO inclusion diluted the diet net energy (NE) by 2.9%, but compared to CON, DM intake (DMI), average daily weight gain (ADG), and gain-to-feed ratio (GF) were not affected. Therefore, ZEO increased ($P < 0.01$) the observed-to-expected diet NE by 3.9%. Compared to CON and ZEO lambs, ZH lambs showed greater ($P < 0.01$) ADG (12.1%) GF (11.5%), and dietary NE (7.3%) values. As in the non-supplemented ZH diets, the presence of ZEO in the ZH diet increased ($P < 0.05$) the observed-to-expected dietary NE by 4.6% compared to the diet with ZH alone. Based on the results, ZEO similarly affects diet NE in lambs when included in the diet alone or with ZH. Further, ZEO is not an antagonist to ZH, and the energy dilution due to ZEO inclusion did not affect the benefits of ZH supplementation.

Key words: Dietary energy, energy efficiency, lambs, performance, zeolite clay, zilpaterol.

INTRODUCTION

Globally, there are approximately 50 natural zeolites (ZEOs), volcanic clays of the tectosilicates class, the most common are the clinoptilolites (Roque-Malherbe, 2001). Due to its molecular structure, ZEO has a great ion exchange ability and absorbability and is therefore largely used in the industry and in animal feed (Iswarya and Beulah, 2021). In ruminant diets, ZEO clay has been gaining popularity as a feed ingredient in the last decades, largely in fattening diets (Tánori-Lozano et al., 2022). According to earlier findings, ZEO is an effective captor of NH_3-N molecules in the rumen environment, favoring N retention, mainly in animals fed diets supplemented with high-rumen-degradable N (Roque-Jiménez et al., 2018). Because of its high buffering capacity (Navia et al., 2005), ZEO can be supplemented when high-concentrate diets are offered during fattening. Recent studies have shown that ZEO increased ruminal organic matter (OM) digestion, mainly by increasing starch digestion, favoring a greater proportion of ruminal propionate (Urías-Estrada et al., 2017). The changes in the digestion and fermentation rates can partly be explained by the retarding of the passage of the digesta through the gastrointestinal tract via slightly increasing viscosity, also promoting nutrient absorption (Spotti et al., 2005). Although in most reports, the inclusion of ZEO up to 4.5% did not affect feed efficiency (gain-to-feed ratio), it should be considered that ZEO itself does not provide energy. From an energetic perspective, ZEO consistently increases dietary energy efficiency (Urías-Estrada et al., 2021) and can therefore be defined as a feed additive and as functional ingredient in finishing diets.

The beta-agonist zilpaterol hydrochloride (ZH) is another additive extensively used in feedlots in countries in which it is approved (Cayetano-De-Jesus et al., 2020). It is well known that the optimal benefits on growth performance, weight gain, and dietary energetics of lambs are when ZH was supplemented at a rate of 4 to 8 mg kg^{-1} diet during the last 20 to 40 d of fattening (Ortiz-Rodea et al., 2016). Although, several factors could affect the magnitude of responses when ZH is supplemented, such as dose (Estrada-Angulo et al., 2008; Cayetano-De-Jesus et al., 2020), supplementation period (Vahedi et al., 2015), withdrawal period (Robles-Estrada et al., 2009a), gender (Montgomery et al., 2009), slaughter weight (Castro-Pérez et al., 2021), type of ZH (patented or generic, Rivera-Villegas et al., 2019), and environmental factors (Macías-Cruz et al., 2013), few studies have reported the effects of combining ZH with other additives such antibiotics in feedlot diets (Hilton et al., 2009; Montgomery et al., 2009). However, to our knowledge, the potential interactions between ZH and other ingredients widely used in animal diets have not been investigated so far. Because ZEO, partly replace ingredients such as corn or soybean meal, which reduces diet energy concentration, the livestock industry has considerable interest regarding the impact of presence of ZEO clay in diets supplemented with ZH. In this context, the objective of this study was to evaluate the impact of ZEO clay in finishing diets supplemented with ZH and offered during the last 34 d of the finishing phase to feedlot lambs on growth performance, gain efficiency, and dietary energetics.

MATERIAL AND METHODS

This experiment was conducted at the Universidad Autónoma of Sinaloa Feedlot Lamb Research Unit, located in the Culiacán City (24°46’13” N, 107°21’14” W; 55 m a.s.l), México. Culiacán City has a tropical climate. Average daily minimum and maximum air temperatures during the trial were 28.1 and 34.4 °C (average 31.2 °C), with an average relative humidity of 53.5% (minimum and maximum of 42.3% and 64.4%, respectively). All animal management procedures were conducted according to the Mexico Federal guidelines for animal use and care and approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Sinaloa (Protocol #09032022).

Animals, treatments, and experimental design

Forty-eight Pelibuey × Katahdin (36.2 ± 4.4 kg initial body weight (BW)) intact male lambs were used in a 34-d growth-performance experiment to evaluate the treatment effects on growth performance, gain efficiency, and dietary energetics. Prior to the experiment, lambs were treated for endoparasites (Closantil, 5%, CHINOIN LAB, México City, México), and injected with 1×10^6 IU vitamin A (Synt-ADE, Fort Dodge, Animal Health, México City, México). Lambs were fed the control diet (Table 1) for a 4 wk period before the experimental period started. At the start of the experiment, lambs were individually weighed (electronic scale; TORREY TIL/S: 107 2691, TOR REY electronics Inc., Houston Texas, USA), grouped by weight into six uniform weight blocks, and assigned to 24 pens (two lambs per pen). Individual pens were 6 m^2 with overhead shade, automatic waterers, and 1 m fence-line feed bunks.

Table 1. Composition (dry matter basis) of dietary treatments offered to lambs. ZEO: Zeolite include at 3% in diet; ZH: zilpaterol hydrochloride included at 6 mg ZH kg^{-1} diet. [a]ZH used was a generic zilpaterol under de commercially name GROFACTOR (VIRBAC México, Mexico); according to the label, the product contains 4.8% ZH. Thus, the dosage of 125 mg product kg^{-1} diet corresponds to a dietary ZH concentration of 6 mg kg^{-1} diet (as feed basis). The ZEO source was calcic clinoptilolite with a purity of 92% (ZEO-SIL; Grupo TCDN, Puebla, México). [b]Mineral-protein supplement contained (%): $CoSO_4$, 0.068; $CuSO_4$, 1.04; $FeSO_4$, 3.57; ZnO, 1.24; $MnSO_4$, 1.07; KI, 0.052; limestone, 56.96%; urea, 18%, and NaCl, 18%. [c]Values determined in laboratory. [d]Calculated from tabular values for individual feed ingredients published by NRC (2007).

	Treatments[a]			
Item	Control	ZEO	ZH	ZH+ZEO
Dry-rolled corn, g kg^{-1}	560.0	545.0	560.0	545.0
Sudan grass hay, g kg^{-1}	105.0	105.0	105.0	105.0
Soybean meal, g kg^{-1}	65.0	50.0	65.0	50.0
Yellow grease, g kg^{-1}	25.0	25.0	25.0	25.0
Dried distillers' grain with soluble, g kg^{-1}	130.0	130.0	130.0	130.0
Cane molasses, g kg^{-1}	90.0	90.0	90.0	90.0
Zeolite, g kg^{-1}	0.0	30.0	0.0	30.0
Zilpaterol hydrochloride, g kg^{-1}	0.0	0.0	0.006	0.006
Mineral-protein supplement[b]	25.0	25.0	25.0	25.0
Dry matter (DM), g kg^{-1}	882.2	884.7	880.8	885.8
Chemical composition[c], g kg^{-1} DM				
Total crude protein	140.5	132.5	141.2	133.1
Ether extract	63.4	53.4	62.9	53.4
Neutral detergent fiber	199.2	194.0	196.1	193.4
Calculated net energy[d], Mcal kg^{-1}				
Maintenance	2.11	2.04	2.11	2.04
Gain	1.44	1.39	1.44	1.39

Dietary treatments (Table 1) consisted of a corn (*Zea mays* L.) grain-based finishing diet supplemented as follows: 1) Basal diet without zeolite (ZEO) and zilpaterol hydrochloride (ZH) (CON); 2) inclusion of 3% of ZEO replacing in equal parts (1.5% each) cracked corn and soybean (*Glycine max* (L.) Merr.) in CON diet (ZEO), 3) CON diet supplemented with 6 mg ZH kg^{-1} diet (ZH), and 4) ZEO diet supplemented with 6 mg ZH kg^{-1} diet (ZEO+ZH). The ZH used was a generic zilpaterol (GROFACTOR, Laboratorios Virbac México, Guadalajara, México); according to the label, the product contains 4.8% ZH. Thus, the dosage of 125 mg product kg^{-1} diet corresponds to a dietary ZH concentration of 6 mg kg^{-1} diet (as feed basis). Supplemental ZH was hand-weighed using a precision balance (mod AS612, OHAUS, Pine Brook, New Jersey, USA), and premixed for 5 min with the mineral-protein supplement before incorporation into the complete mixed basal diet using a 2.5 m^3 capacity paddle mixer (mod 30910-7, JOPER, Malce, Coyoacán, México). To avoid contamination, the mixer was thoroughly cleaned between each treatment. Zilpaterol hydrochloride was supplemented for 31 d followed by a 3-d pre-harvest withdrawal in which lambs continued to receive the corresponding treatment without ZH. The ZEO source was calcic clinoptilolite with a purity of 92% (ZEO-SIL; Grupo TCDN, Puebla, México); the level of ZEO used corresponded to the level for which better responses of growth performance and dietary energy in finishing lambs have been reported (Estrada-Angulo et al., 2017a; 2017b; Urías-Estrada et al., 2017). The four dietary treatments were randomly assigned to pens within each weight block in a randomized complete block design. Lambs were allowed *ad libitum* access to both dietary treatments and clean water. Fresh feed was provided twice daily at 08:00 and 14:00 h at approximately 40:60 proportion of the daily registered intake (as feed basis). Daily feed allotments to each pen were adjusted to allow for approximately 5% residual feed remaining in the feed bunk at the time of the morning feeding. Feed bunks were checked between 07:40 and 07:50 h each morning, and residual feed was collected and weighed for the determination of daily feed intake. Adjustments in daily feed delivery were made at the afternoon feeding. Lambs were individually weighed at the beginning of the trial and at the end of the experiment (day 34). The initial shrunk body weight (SBW) was determined as full body weight × 0.96 (adjustment for gastrointestinal fill). Upon completion of the study, all lambs were weighed following an 18 h fast (feed but not drinking water was withdrawn) to obtain final SBW. Sampling of feed and refusals, as well as determination of DM, crude protein and neutral detergent fiber were performed according to the techniques and procedures described by Urías-Estrada et al. (2017).

Calculations

Estimates of average daily weight gain (ADG), and dietary net energy (NE) were based on the initial SBW and the final (d 34) fasted SBW. Average daily gain was computed by subtracting the initial SBW from the final SBW and dividing the result by the number of days on feed. Feed efficiency was computed as ADG/daily DM intake (DMI) and expressed as gain-to-feed ratio (GF). One approach for the evaluation of 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 the estimated diet NE concentration and measures of growth performance, there is an expected energy intake. This estimation of expected DMI is performed based on the observed ADG, average SBW, and NE values of the diet (Table 1):

$$\text{Expected DMI, kg d}^{-1} = (EM/EN_m) + (EG/EN_g),$$

where EM is energy required for maintenance (Mcal d^{-1}) = 0.056 × $SBW^{0.75}$, EG is energy gain (Mcal d^{-1}) = 0.276 × ADG × $SBW^{0.75}$, and NE_m and NE_g are the NE contained in the experimental diets, those values were calculated based on the ingredient composition (NRC, 2007) of the basal diet (Table 1). The coefficient (0.276) was taken from the NRC (1985) assuming a mature weight of 113 kg for Pelibuey × Katahdin male lambs. The observed dietary NE was calculated using the EM and EG values, and DMI observed during the experiment by means of the quadratic formula:

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

where x is NE_m (Mcal kg^{-1}), a = -0.41 EM, b = 0.877 EM + 0.41 DMI + EG, and c = -0.877 DMI (Estrada-Angulo et al., 2017b).

Statistical analysis

Growth performance data (gain, DMI, gain efficiency, and dietary energetics) were analyzed as a randomized complete block design, using initial weight as the blocking criterion and pen as the experimental unit, in a 2 × 2 factorial arrangements using the MIXED procedure according to SAS version 9.0 (SAS Institute, Cary, North Carolina, USA). Treatment effects were separated into the following orthogonal contrasts: (1) CON *vs*. ZEO, (2) CON *vs*. ZH, and (3) ZEO× ZH interaction. In addition, means separations were performed using the honestly significant difference test (Tukey's HSD test). Contrasts are considered significant when the P value was ≤ 0.05 and as tendencies when the P-value was > 0.05 and ≤ 0.10.

RESULTS AND DISCUSSION

There were no mortality or health problems that forced the elimination of some lamb from the experiment. There were no treatments interactions (P ≥ 0.21) on lamb growth performance or dietary energetics (Table 2). Even so, the interaction P-values as well as the main effects of treatments are presented in Table 2. Even when the replace of corn plus soybean due by ZEO inclusion in diet diluted 2.9% the diet NE (Table 1); compared to CON, there were no difference (P ≥ 0.21) by ZEO supplementation on DMI, ADG, GF, and observed dietary energy. Therefore, ZEO increased (P < 0.01) the observed-to expected diet NE by 3.9%. Similarly, most of the reports indicate that the inclusion of ZEO from 2.0% up to 4.5% did not affect ADG and feed efficiency (Toprak et al., 2016; Estrada-Angulo et al., 2017a; 2017b). Despite this, it is important to consider that ZEO itself does not contribute energy, in contrast to the replaced ingredient; therefore, analyzed under an energetic perspective, ZEO consistently increases the dietary energy efficiency (Urías-Estrada et al., 2021). As reported previously (Castro-Pérez et al., 2022), the estimation of dietary NE based on measures of growth-performance provides important insights into the effects of additives (or other factors) on dietary energy use efficiency. An observed-to-expected dietary NE ratio of 1.00 indicates that performance is consistent with dietary NE values, based on tables of feedstuff standards (NRC, 2007) and the observed DMI. A ratio greater than 1.00 indicates a greater dietary energy use efficiency, whereas a ratio below 1.00 indicates a lower dietary energy use. Therefore, lambs fed the CON diet showed an observed-to-expected ratio of 0.992, whereas with the inclusion of ZEO, this ratio was 1.036. This 3.9% increase is in agreement with the average increase in the observed-to-expected dietary NE of 3.7% (1.5% to 7.0%, n = 6) reported in previous experiments conducted at these research facilities, using the same breed of lambs and similar diets (Estrada-Angulo et al., 2017a; 2017b). However, it is lower than the value reported by Urías-Estrada et al. (2021), who analyzed the data of 10 studies (ZEO inclusion in diets from 1.2% to 9.0%) in which diet composition, average initial weight, final weight, DMI, ADG, and the type (breed and sex) of animal used were specified. Using the energetic approach, the authors estimated an average increase of 4.5% in observed-to-expected dietary NE when ZEO replaced corn grain, soybean meal, or both. However, the mechanism underlying the improvement in dietary energy when ZEO was included in the diets needs to be elucidated in future studies. We assume that it can be attributed to the improved ruminal N economy, by enhanced digestible and metabolizable energy of diet, and by leaner tissue deposit. An alternative approach for expressing treatments effects on animal energetics in the present experiment is to keep the net energy (NE) value of the diet constant and present treatment effects solely as a function of changes in the maintenance coefficient (MQ), as follows: $MQ = (NE_m \times [DMI–\{EG/NE_g\}])/SBW^{0.75}$, where NE_m and NE_g correspond to the NE of diet (Table 1), EG = $(0.276\ SBW^{0.75})$ ADG1.097 and SBW is the average SBW. Accordingly, the maintenance coefficient by ZEO was reduced by 6%.

Table 2. Main effects on performance and dietary net energy (NE) during last 34-d of fattening. ZEO: Zeolite include at 3% in diet; ZH: zilpaterol hydrochloride included at a rate of 6 mg ZH kg^{-1} diet; SEM: standard error of the mean. ZH used was a generic zilpaterol under de commercially name GROFACTOR (VIRBAC México, Mexico); according to the label, the product contains 4.8% ZH. Thus, the dosage of 125 mg product kg^{-1} diet corresponds to a dietary ZH concentration of 6 mg kg^{-1} diet (as feed basis). The ZEO source was calcic clinoptilolite with a purity of 92% (ZEO-SIL; Grupo TCDN, Puebla, México).

	ZEO, % diet DM		ZH, mg kg^{-1} DM			P-value		
Item	0	3	0	6	SEM	CON *vs.* ZEO	CON *vs.* ZH	ZEO× ZH
Live weight, kg								
Initial	36.212	36.225	36.212	36.225	0.10	0.90	0.90	0.85
Final	44.835	45.028	44.365	45.498	0.27	0.62	< 0.01	0.24
Weight gain, kg d^{-1}	0.253	0.259	0.240	0.270	0.01	0.63	< 0.01	0.21
DM intake, kg	1.168	1.167	1.165	1.170	0.043	0.98	0.90	0.25
Gain to feed ratio	0.218	0.223	0.207	0.234	0.006	0.25	< 0.01	0.84
Maintenance	2.17	2.20	2.11	2.27	0.022	0.34	< 0.01	0.62
Gain	1.49	1.52	1.44	1.58	0.027	0.34	< 0.01	0.62
Maintenance	1.029	1.079	1.015	1.093	0.011	< 0.01	< 0.01	0.56
Gain	1.038	1.094	1.015	1.116	0.019	0.01	< 0.01	0.56
Observed-to-expected								
DM intake	1.001	0.930	0.987	0.877	0.016	< 0.01	< 0.01	0.73

Compared to CON and ZEO lambs, lambs that received ZH showed greater ADG values (12.1%, $P < 0.01$) but similar values of DMI ($P > 0.89$), thus improved ($P < 0.01$) GF (11.5%) and dietary NE (7.3%). This is a common response when ZH is supplemented in high-energy diets for feedlot lambs (Ortiz-Rodea et al., 2016). According to the observed DMI, the total daily intake of ZH was 7.9 mg $lamb^{-1}$, which corresponds to an average dose of 0.19 mg kg^{-1} BW. Better responses to ZH supplementation in finishing Pelibuey × Katahdin lambs have been obtained for doses of approximately 20 mg kg^{-1} BW (Estrada-Angulo et al., 2008; Ríos-Rincón et al., 2010). The absence of effects on DM intake is the most common response to ZH supplementation in feedlot lambs (Estrada-Angulo et al., 2008; Robles-Estrada et al., 2009b; Rivera-Villegas et al., 2019) and in feedlot cattle (Lean et al., 2014; Castro-Pérez et al., 2021). However, some reports indicate decreases (Cayetano-De-Jesus et al., 2020) or increases (Ríos-Rincón et al., 2010) in DM intake with ZH supplementation. According to Reinhardt et al. (2014), the main factors that could affect DMI when ZH is supplemented are the season and the intake level pattern prior to ZH supplementation. The apparent increase in energy use efficiency is consistent with the findings of previous studies in which lambs received ZH supplementation in high-energy diets (> 1.95 Mcal NE_m kg^{-1}; Rojo-Rubio et al., 2018; Rivera-Villegas et al., 2019). The ADG and dietary energy efficiency improvements with ZH supplementation are mainly due to the fact that ZH acts as repartitioning agent, increasing protein instead of fat deposition in muscle tissues, changing the rate and composition of gain during the finishing phase of fattening (Rivera-Alegría et al., 2022). The energy cost for fat accretion is nearly two times greater than that for muscle accretion; therefore, the change composition of gain is a tool to increase the energy use efficiency during the finishing phase (Galyean and Hales, 2023). Using the same alternative approach mentioned above for ZEO, lambs received ZH supplementation decrease its net energy of maintenance requirement by 16%.

Similar to the response to supplemental ZEO in the CON diet, the presence of ZEO in the ZH-supplemented diet increased the observed-to-expected dietary NE by 4.6% ($P < 0.05$) compared to ZH alone. This increase was 18.0% greater when compared with the 3.9% increase when ZEO was present in the diet without ZH supplementation but similar to the average increase of 4.5% reported by Urías-Estrada et al. (2021). The use of feed additives is increasingly widespread in the livestock industry. Currently, it is not uncommon that two or more additives are used simultaneously. However, it is essential to determine not only the possible antagonistic effects but also the possible reduction of the expected benefits when such an approach is used. In this sense, the main concern of the feedlot industry is whether energy and protein dilution resulting from replacing ingredients such as corn grain or soybean meal (or both) by inorganic ingredients such as ZEO can negatively affect the magnitude of the response to ZH supplementation. There is little information on the energy level effect on the response of ZH. Still, compared to non-supplemented cattle, ZH significantly increased the ADG and dietary energy efficiency when the animals were fed *ad libitum* (high-energy available), but it had no effect on ADG nor feed efficiency when it was supplemented in cattle that were fed at maintenance level (low-energy level; Walter, 2015). Similarly, in diets with a moderate-to-low energy concentration (i.e., < 1.80 Mcal EN_m kg^{-1}), ZH supplementation did not result in increases in ADG or feed efficiency when compared to the controls (O'Neill et al., 2010). In North America, the energy concentration in finishing diets during the last stage of fattening in feedlot systems ranges from 2.00 to 2.20 Mcal EN_m kg^{-1}. This range appears to be optimal regarding the response to ZH supplementation; however, there is no information about the minimal energy level to obtain an optimal response to ZH supplementation, requiring further studies. Based on our results, the slight reduction in energy from 2.11 to 2.04 Mcal EN_m kg^{-1} diet because of ZEO inclusion did not negatively affect growth performance or dietary energy use in ZH-supplemented lambs. Overall, the results show that ZEO is not an antagonist to ZH and that energy dilution due to ZEO inclusion does not affect the benefits of ZH supplementation.

CONCLUSIONS

The addition of zeolite (ZEO) to diets at inclusion levels of up to 3.0% did not affect the magnitude of the response of growth performance, increasing, by dilution effect, the observed-to-expected dietary energy. The relative increase in the observed-to-expected dietary energy with ZEO+zilpaterol hydrochloride (ZH) supplementation was similar to that when ZEO was supplemented alone. In summary, ZEO is not an antagonist to ZH, and the energy dilution due to ZEO inclusion at 3.0% level in diet did not affect the benefits of ZH supplementation.

Author contributions

Conceptualization: JUS, AEA, AP. Data curation: AEA, AB, YVG, AP. Formal analysis: AB, YVG. Methodology: AEA, AP. Investigation: JUS, EPB, BICP, YAW, JRM. Writing-original draft: JUS, AP. Writing-review & editing: AP. All co-authors reviewed the final version and approved the manuscript before submission.

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