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SHORT COMMUNICATION

Impact of dietary inclusion of clinoptilolite as substitute of soybean meal on growth performance, dietary energetics and carcass traits of feedlot ewes fed a corn-based diet

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ABSTRACT. Forty ewes (31.725±1.44 kg initial LW) were used to evaluate the effects of clinoptilolite (ZEOL) supplementation (0, 1, 2, and 3%, DM basis of diet) as substitute of soybean meal (SBM) in a finishing corn-based diet on growth performance, dietary energetics, and carcass traits. The experiment lasted 56 days. For each percentage of substitution of SBM by ZEOL, the crude protein and net energy (NE) of basal diet were decreased in 0.37 percentage units and 0.02 Mcal/kg, respectively. There were no treatment effects (P>0.27) on average daily gain (ADG), dry matter intake (DMI), and gain efficiency (ADG/DMI). Dietary NE was not affected by ZEOL supplementation (P≥0.69). However, due to the inertness (it does not provide energy) of ZEOL, itself, the ratio of observed-to-expected dietary NE linearly increased (P=0.02) and the ratio of observed-to-expected DMI linearly decreased (P=0.02) with ZEOL supplementation. Clinoptilolite supplementation linearly decreased fat thickness (P=0.02) and visceral fat (P=0.03) with no effects (P≥0.12) on other carcass measures or the organ tissue weights (as proportion of g/kg of empty body weight). Dilution of CP and dietary NE by substitution of SBM by zeolite up to 3% did not negatively affect growth performance and carcass traits. This result suggests that the inclusion of up to 3% of clinoptilolite in substitution of a high protein source (SBM) on finishing diets has a positive effect on the utilization of dietary energy.

Key words: clay, lambs, efficiency, visceral, finishing.

RESUMEN. Se utilizaron 40 ovejas (31,725 ± 1,44 kg PV inicial) para evaluar la suplementación (0, 1, 2 y 3%, en base seca de la dieta) de clinoptilolita (ZEOL) en sustitución de la harina de soya (SBM) en una dieta de finalización a base de maíz en el crecimiento, balance energético de la dieta y características de la canal. El experimento duró 56 días. Para cada porcentaje de sustitución de SBM por ZEOL, la proteína cruda y la energía neta (EN) de la dieta basal se redujo en 0,37 unidades porcentuales y 0,02 Mcal/kg, respectivamente. No hubo efecto de los tratamientos en la ganancia diaria de peso (GDP), el consumo de materia seca (CMS) o la eficiencia alimenticia (GDP/CMS). La EN de la dieta no se vio afectada por la administración de ZEOL, pero debido a la característica inerte de ZEOL la relación de EN dietética observada-a-esperado aumentó linealmente y la proporción de observado-a-esperado del CMS disminuyó con la suplementación ZEOL. La clinoptilolita disminuyó linealmente el espesor de la grasa y la grasa visceral, sin efectos en otras medidas de la canal o del peso de los órganos (como proporción de g/kg de peso corporal vacío). La sustitución de SBM por zeolita hasta 3% no afectó negativamente el crecimiento y las características de la canal, resultando que la inclusión de hasta 3% de clinoptilolita en sustitución de una fuente de alta proteína (SBM) en dietas de acabado tuvo un efecto positivo en la utilización de energía de la dieta.

Palabras clave: arcillas, corderos, eficiencia, masa visceral, finalización.

INTRODUCTION

Zeolites are a family of minerals of volcanic origin that are composed of crystalline aluminosilicates. Clinoptilolite is the most abundant natural zeolite. Its dimensional structure is characterised by the ability to lose and gain water reversibly and to exchange cations without major change of their structure (Trckova *et al* 2011). Because of its sorbent properties that modify ruminal fluid viscosity (Spotti *et al* 2005), and binding capacity with NH_3-N, clinoptilolite may have application as a feed additive in ruminant nutrition (Mumpton and Fishman 1977). In several studies (Pond 1984, Ghaemnia *et al* 2010), effects of clinoptilolite supplementation resulted in positive responses on growth performance. Whereas in others, no effects, or even negative effects were observed (Gaylean and Chabott 1981, Pond 1989). Inconsistencies in response to supplementation could be attributed to factors such as level of supplementation (McColumm and Galyean 1983), method of addition (by substitution for one or more ingredients in the diet that change chemical composition between controls and zeolite diets, Câmara *et al* 2012), or by type of diet (forage level, degradable intake protein level; Pond 1984,1989). Limited information is available about of role of clinoptilolite on growth performance and carcass characteristics of ruminant fed with a high-energy finishing diet (McCollum and Galyean 1983). The objective of this experiment was to evaluate the influence of level of clinoptilolite supplementation as substitute of soybean meal on growth-performance, dietary energy, and carcass traits of feedlot ewes fed a corn-based finishing diet.

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MATERIAL AND METHODS

This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in the Culiacán, México (24° 46' 13" N and 107° 21' 14"W). Culiacán is about 55 m above sea level, and has a tropical climate. All animal management procedures were conducted within the guidelines of locally approved techniques (Mexican Official Rules, NOM-025, 033 and 051-ZOO-1995) for animal use and care. Forty ewes (¼Pelibuey × ¾ Katahdin, 31.73 ± 1.44 kg initial LW) were used in a randomised complete block design to evaluate the effects of treatments. Three weeks before starting of the experiment, ewes were treated for endoparasites (Albendaphorte 10%, Animal Health and Welfare, México City, México), injected with $1x10^6$ IU vitamin A (Synt-ADE®, Fort Dodge, Animal Health, México City, México), and vaccinated for *Mannheimia haemolityca* (One shot Pfizer, México City, Mexico). Two weeks before starting the study all ewes were fed the same basal diet (no clinoptilolite supplementation, table 1). At the beginning of the experiment, ewes were weighed individually prior to the morning (08:00 h) meal (electronic scale; TORREY TIL/S: 107 2691, TORREY electronics Inc., Houston, TX, USA), blocked by weight into five uniform weight groupings and assigned within weight grouping to 20 pens (2 ewe/pen, 5 pen/treatment). Pens were 6 m^2 with overhead shade, automatic waterers and 1 m fence-line feed bunks. Dietary treatments consisted in a dry-rolled-corn-based finishing diet supplemented with either 0, 1, 2, or 3% of clinoptilolite (ZEOL). These levels were selected to reflect the range in supplementation of ZEOL used in prior research wherein positive responses were observed (Pond 1984, Trckova *et al* 2004. Since one of the properties of zeolites is their ability to binding with NH3-N that favour the ruminal N retention, it was decided that the zeolite inclusion would be carried out by replacement of a high-protein ingredient in the diet (SBM). The source of ZEOL used was clinoptilolite-Ca (ZEO-SIL; Grupo TCDN, Puebla, México). Supplemental ZEOL replaced soybean meal (SBM) in the basal diet. The physicochemical composition of SBM replaced by ZEOL is shown in the footnote of table 1. Dietary treatments were randomly assigned to ewes within weight groupings. The experiment lasted 56 days and ewes were weighed at the beginning of the trial and every 28 days thereafter. The initial live weight (LW) was converted to shrunk body weight (SBW) by multiplying the weight by 0.96 to adjust for the gastrointestinal fill (NRC 2007) and all ewes were fasted (drinking water was not withdrawn) for 18 hours before recording the final LW. Ewes were allowed *ad libitum* access to dietary treatments. In order to determine the feed intake on a daily basis, the ewes were fed twice daily at 08:00 and 14:00 h, the feed bunks were checked 10 minutes before the morning feed was offered, then the refusals were collected and weighed. To maintain a minimal feed refusals, adjustments of daily feed delivery, were provided at the afternoon feeding. The feed and refusal samples were collected daily for DM analysis, which involved oven-drying the samples at 105°C until no further weight loss occurred (AOAC 2000). Complete mixed diets and refusals were subjected to the following analyses: Dry matter (DM, oven drying at 105°C until no further weight loss; method 930.15, AOAC 2000); crude protein (CP, N× 6.25, method 984.13, AOAC 2000); ash (method 942.05, AOAC 2000); NDF [Van Soest *et al* 1991, corrected for NDF-ash, incorporating heat stable α-amylase (Ankom Technology, Macedon, NY) at 1mL per 100mL of NDF solution (Midland Scientific, Omaha, NE)]; acid detergent fiber (ADF, residuals direct sulphuric acid method; method 973.18, AOAC 2000), and ether extract (method 920.39; AOAC 2000).

All ewes were slaughtered on the same day. After sacrifice, ewes were skinned, and the gastrointestinal organs were separated and weighed. Carcasses (with kidneys and internal fat included) were chilled in a cooler at -2 °C to 1 °C for 48 h, then the following measurements were obtained: 1) fat thickness perpendicular to the *m. longissimus thoracis* (LM), measured over the center of the ribeye between the 12th and 13th rib; 2) LM surface area, measure using a grid reading of the cross sectional area of the ribeye between 12th and 13th rib, and 3) kidney, pelvic and heart fat (KPH). The KPH was removed manually from the carcass, and then weighed and reported as a percentage of the cold carcass weight (USDA 1982). Each carcass was split along the vertebrae into two halves. Shoulders were obtained from the forequarter. The procedures for obtaining shoulders and the measurement of their composition, as well as the dissection of the organs and the estimation of visceral organ mass were done following the procedures described by Rios-Rincon *et al* (2010).

The estimations of performance, expected dry matter intake (DMI), and dietary energetic were calculated based on SBW. Average daily gains (ADG) were estimated as follows: (Initial SBW- final SBW)/56. Feed efficiency was calculated as ADG/DMI. Expected DMI was determined based on observed ADG and average SBW according to the following equation: expected DMI, kg/d = $(EM/NE_m) + (EG/NE_g)$, where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$ (NRC 1985), EG (energy gain, Mcal/d) = $0.276 \times ADG \times SBW^{0.75}$ (NRC 1985), and NE_m (dietary net energy of maintenance) and NE_g (dietary net energy of gain) are 1.94 and 1.29; 1.92 and 1.28; 1.90 and 1.26, and 1.88 and 1.25 Mcal/kg, for 0, 1, 2, and 3% ZEOL, respectively [derived from tabular values (NRC 2007) based on the ingredient composition of the experimental diets (table 1)]. The coefficient (0.276) was estimated assuming a mature weight of 113 kg for Pelibuey × Katahdin crosses (Estrada-Angulo *et al* 2013). Observed dietary NE was estimated by of the quadratic formula: $x = (-b - \sqrt{b^2 - 4ac})/2c$, where $x = NE_m$, Mcal/kg, a =-0.41EM, b = 0.877EM + 0.41DMI + EG, c = -0.877DMI,

Table 1. Ingredients and composition of experimental diets[1].

Item	Clinoptilolite level, % of DM[2]			
	0	1	2	3
Ingredient composition, % DMB				
Cracked corn	36.00	36.00	36.00	36.00
Dry distillers grain with solubles	28.00	28.00	28.00	28.00
Soybean meal[3]	7.00	6.00	5.00	4.00
Alfalfa hay	10.00	10.00	10.00	10.00
Sudangrass hay	10.00	10.00	10.00	10.00
Cane molasses	7.50	7.50	7.50	7.50
Trace mineral salt [4]	1.50	1.50	1.50	1.50
Clinoptilolite	0.00	1.00	2.00	3.00
Net energy concentration[5], Mcal/kg of DM basis				
EN_m, Mcal/kg	1.94	1.92	1.90	1.88
EN_g, Mcal/kg	1.29	1.28	1.26	1.25
Nutrient composition, % of DM[6]				
Crude protein	16.46	16.02	15.78	15.35
NDF	21.91	21.75	21.60	21.45
ADF	8.55	8.35	8.28	8.02
Ether extract	4.11	4.03	4.00	3.96
Ash	7.94	8.96	10.02	10.88
Calcium	0.81	0.81	0.82	0.83
Phosphorus	0.61	0.61	0.61	0.61

[1]Prices of diets (US dollars/kg) were: $0.264., $0.259, $0.254, and $0.250 for 0,1, 2, and 3% of clinoptilolite inclusion, respectively; [2]Calcium clinoptilolite (Zeo-Sil, Grupo TCDN, Puebla, Puebla); [3]Composition of SBM were (%): 91.7 DM; 92.5 OM; 48.4 CP; 12.1 NDF, and 2.8% ether extract; [4]Trace mineral salt contained: $CoSO_4$, 0.068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$, 1.07%, KI 0.052%; and NaCl, 92.96%; [5]Based on tabular net energy (NE) values for individual feed ingredients (NRC 2007),[6]Dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

and NEg = 0.877NEm – 0.41 (Estrada-Angulo *et al* 2013). The chemical composition of SBM used in the trial, and the complete mixed diets were determined following the procedures of AOAC (2000).

Performance (DMI, gain, gain efficiency, observed dietary NE, observed-to expected dietary NE ratio, and observed-to-expected DMI ratio) and carcass data were analysed as a randomised complete block design considering the pen as the experimental unit (n = 5 repetitions/treatment). The MIXED procedure of SAS (SAS 2004) was used to analyse the variables. Shoulder composition was analysed as a general complete block design, including the effect of block × treatment interaction, together with the effect of CCW as covariate. When the covariate represented a non-significant (P>0.05) source of variation it was not included into the model. The analysis was performed using the MIXED procedure (SAS 2004). Relative visceral organ mass data were analysed as a general complete block design, including the effect of block × treatment interaction considering ewe as experimental unit (n = 10 observations/treatment). The MIXED procedure of SAS (SAS 2004) was used to analyse the variables. Treatment effects were tested for linear, quadratic and cubic components of the ZEOL supplementation level. Orthogonal polynomials were considered significant when P≤0.05, and tendencies were identified when P>0.05 and ≤0.10.

RESULTS AND DISCUSSION

Cubic effects were not significant (P≥0.10). Thus, the P-values for this component are not presented in the tables.

The zeolite used in this experiment (San Juan Raya deposit, Puebla region; Grupo TCDN, Puebla, México), was comprised (g/kg of product) of SiO_2 (662), Al_2O_3 (146), and CaO (27.3), in good agreement with tabular mineral composition of natural zeolite (clinoptilolite-Ca) reported by EFSA (2013).

The effects of ZEOL supplementation on 56-d feedlot growth-performance of ewes are shown in table 2. There were no treatment effects (P>0.27) on daily gain, dry matter intake, and gain efficiency (ADG/DMI). Effects of ZEOL inclusion on DMI and ADG in growing-finishing ruminants has not been consistent (i.e. negative effects on feed efficiency of lambs was noted by Pond *et al* 1989 when ZEOL replaced ground corn since 2% of a finishing diet),

Table 2. Influence of supplementation clinoptilolite level on growth performance and dietary energetics.

Item	Clinoptilolite, % in diet DM				Contrast *P*-value[1]		
	0	1	2	3	SEM[2]	L	Q
Pens replicates	5	5	5	5			
Days on feed	56	56	56	56			
Weight, kg[3]							
Initial	31.67	31.67	31.75	31.82	0.305	0.35	0.44
Final	42.53	42.93	43.62	42.80	0.520	0.59	0.30
Dry matter intake, kg	1.053	1.034	1.103	0.996	0.040	0.58	0.29
ADG, kg/d	0.194	0.201	0.212	0.196	0.010	0.67	0.27
Gain for feed, kg/kg	0.184	0.189	0.193	0.192	0.006	0.30	0.64
Dietary net energy, Mcal/kg							
Maintenance	1.924	1.953	1.958	1.994	0.029	0.15	0.90
Gain	1.278	1.303	1.307	1.338	0.026	0.15	0.90
Observed to expected dietary ratio							
Maintenance	0.992	1.017	1.030	1.060	0.016	0.02	0.88
Gain	0.990	1.018	1.037	1.071	0.025	0.02	0.88
Observed-to-expected daily DM intake[4]	0.998	0.975	0.957	0.928	0.017	0.02	0.87

[1]*P*=observed significance level for linear and quadratic effect of supplementation level of ZEOL. Since cubic effects were not significant (*P*>0.10) the *P*-values for those components are not presented in the tables, [2] SEM, standard error of mean; [3]Initial weight was reduced 4% to account for fill, and all ewes were fasted (food but not drinking water was withdrawing) for 18 h before recording the final BW; [4]Expected DMI was computed as follows: DMI, kg/d = $(EM/NE_m) + (EG/NE_g)$, where EM=maintenance coefficient of 0.056 Mcal/LW $^{0.75}$ (NRC 1985) and EG is the daily energy deposited (Mcal/d) estimated by equation: $EG = 0.276 \times ADG \times SBW^{0.75}$; NRC 1985. The divisor NE_m and NE_g are the NE of each diet (calculated from tables of composition of feed; NRC 2007).

but similar to our results (McColumm and Galyean 1983), ZEOL supplementation up to 2.5% did not affect ADG, or gain efficiency in feedlot steers fed a finishing diet.

Even though diets were diluted with ZEOL inclusion, the observed dietary NE was not affected by ZEOL supplementation ($P \geq 0.69$). Thus, the ratio of observed-to-expected dietary net energy linearly increased (P=0.02) and the ratio of observed-to-expected DMI linearly decreased (P=0.02) with ZEOL supplementation. Across the entire 56-day period, the average observed-to-expected DMI of ewes fed the reference diet was 99% of the expected value, based on tabular (NRC 2007) estimates of diet energy density and observed SBW and ADG values (table 2), which supports the suitability of the prediction equations proposed by the NRC (1985) for the estimation of DMI in relation to SBW and ADG in feedlot lambs. On the other hand, the NE_m (Mcal/kg) of the diets can be estimated from chemical analyses using the following equation (adapted from NRC [1984] using all feedstuffs with an $NE_m \geq 1.70$ and for which all pertinent analyses are tabulated excluding fat, which was assigned a NE_m value of 6.00; R^2 =0.97, N=36) (Zinn and Plascencia 1993): 0.0255ADF + 0.0325CP + 0.0704EE + 0.034NFE - 1.18, where nutrient concentration are expressed as g/100g, EE is ether extract and NFE (nitrogen free extract) is equivalent to 100 - (ADF + CP + EE + ash). By applying the above equation to the chemical composition determined by analyses for the experimental diets (table 1), the net energy values of diets were 2.00, 1.97, 1.93 and 1.90 Mcal ENm/kg (averaging 1.95). This estimate is in good (102%) agreement with the estimated NE value based on tabular net energy (NE) values for individual feed ingredients (table 1, NRC 2007). The above supports that the comparison between observed to expect performed here is valid.

We expect that dietary NE ratio (observed-to-expected) would be to 1.0 [this mean that animals were performed as expected. Or stated differently, animal performance is consistent with DMI and dietary energy density (NRC 2007)]. If ratio is greater than 1, the observed dietary NE represent a greater value (concentration) than expected according to NRC (2007), therefore the energy was better utilized by the animal, thus, the efficiency was improved. In contrast, if ratio is less than 1, energetic efficiency was less than expected (contrary to the observed:expected DMI in which values greater than 1 represent lower efficiencies). The basis for the increases on energy utilization to ZEOL supplementation observed in the present experiment is uncertain. A few studies revealed that ruminants that were fed zeolites had better utilization of N compounds (Ghaemnia *et al* 2010) and/or greater utilization of digestible energy (Stojković *et al*. 2012), or by a more efficient ruminal fermentation (McColumm and Galyean 1983).

The effects of ZEOL supplementation on carcass characteristics, tissue composition and organ mass are shown

Table 3. Treatment effects on carcass characteristics and shoulder tissue composition.

Item	Clinoptilolite, % in diet DM				*P* value[1]		
	0	1	2	3	SEM[2]	L	Q
Pens replicates	5	5	5	5			
Hot carcass weight (kg)	24.89	25.28	26.08	24.96	0.52	0.74	0.19
Dressing percentage	58.53	58.88	59.78	58.31	0.97	0.96	0.37
Cold carcass weight (kg)	24.41	24.87	25.84	24.71	0.60	0.99	0.22
LM area (cm^2)	18.15	17.35	17.40	17.56	0.56	0.77	0.56
Fat thickness (cm)	0.56	0.57	0.45	0.45	0.033	0.02	0.93
Kidney pelvic and heart fat (%)	4.15	3.79	3.69	3.99	0.43	0.78	0.47
Shoulder weight (kg)	2.432	2.375	2.364	2.320	0.072	0.31	0.94
Shoulder composition (%)							
Muscle	64.99	64.36	65.33	65.06	0.496	0.61	0.72
Fat	18.04	18.46	17.93	17.63	0.855	0.66	0.68
Bone	16.96	17.16	16.74	17.29	0.694	0.86	0.81
Muscle to fat ratio	3.60	3.52	3.65	3.74	0.192	0.57	0.68
Muscle to bone ratio	3.86	3.76	3.92	3.78	0.158	0.93	0.91

[1]*P*=observed significance level for linear and quadratic effect of supplementation level of ZEOL. Since cubic effects were not significant (*P*>0.10) the *P*-values for those components are not presented in the tables; [2]SEM, standard error of mean.

Table 4. Treatment effects on relative visceral organ weight (n = 10 observations/treatment).

Item	Clinoptilolite, % in diet DM				*P* value[1]		
	0	1	2	3	SEM[2]	L	Q
GIT fill (kg)[3]	3.16	3.10	3.29	3.08	0.24	0.97	0.75
Empty body weight, kg	39.36	39.82	40.32	39.71	0.69	0.36	0.44
Empty body weight (% of full weight)	92.55	92.75	92.46	92.78	0.59	0.90	0.90
Full viscera (kg)[4]	7.40	7.63	7.69	7.56	0.24	0.64	0.78
Organs (g/kg, empty body weight)							
Stomach complex[5]	28.06	28.86	28.96	28.08	0.66	0.87	0.45
Intestines [6]	48.51	48.15	49.45	48.57	1.82	0.86	0.69
Liver/spleen	19.04	18.56	18.58	17.41	0.60	0.12	0.80
Heart/lungs	18.79	19.27	20.10	20.26	0.91	0.23	0.87
Visceral fat	43.83	42.25	40.16	36.62	1.57	0.03	0.54

[1]*P*=observed significance level for linear and quadratic effect of supplementation level of ZEOL. Since cubic effects were not significant (*P*>0.10) the *P*-values for those components are not presented in the tables; [2]SEM, standard error of mean; [3]GTI, gastrointestinal tract; [4]Full viscera= full viscera mass = (stomach complex + small intestine + large intestine + liver + lungs + heart) including digesta;[5]Stomach complex = (rumen + reticulum + omasum + abomasums), without digesta; [6]Without digesta.

in tables 3 and 4. Clinoptilolite supplementation linearly decreased fat thickness (*P*=0.02) and visceral fat (*P*=0.03) with no effects ($P \geq 0.12$) on other carcass measures or the organ tissue weights (as proportion of g/kg of empty body weight). Absence of effects of ZEOL supplementation on HCW, dressing, LM are a common results in finishing ruminants (McColumm and Galyean 1983, Pond 1984).

As expected (based on a similar daily gains, Mahgoub *et al* 2000), tissue composition was not affected by treatments. The decrease in fat thickness as result of ZEOL inclusion in finishing lambs had been previously reported (Forouzani *et al* 2004). The reduction of some body fat depots (fat thickness, visceral fat) could be by a reduction of energy density of diet by dilution effect of ZEOL. In lambs, there is very limited information related to the effects of supplementation of ZEOL on organ weights. It has been argued that ZEOL essentially is not absorbed and is excreted with the faeces (EFSA 2013). Because of their density, it is reasonable to expect that clay particles would accumulate along the digestive tract, particularly in the forestomach regions. However, this potential effect was not reflected on relative organ weight in this experiment.

It is concluded that dilution of CP and dietary NE by substitution of SBM by zeolite up to 3% did not negatively affect growth performance and carcass traits. The results showed that the inclusion of up to 3% of clinoptilolite as substitute of a high protein feed (SBM) on finishing diets has a positive effect on the utilization of dietary energy.

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