SHORT COMMUNICATION

Energy value of unfermented dried grape pomace as substitute of alfalfa hay in diets for growing lambs

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ABSTRACT. The energy value of unfermented dried grape pomace (DGP) was evaluated in an 84 d feeding trial on 16 Pelibuey×Dorper lambs (17.8 ± 2.0 kg). The control diet contained 90% mature alfalfa hay and 10% concentrates. Treatments consisted in the replacement (DM basis) of alfalfa hay by DGP at 0, 10, 20 or 30%. Lambs were blocked by weight and assigned individually to pens. Increasing DGP level in diet tended (P=0.06) to decrease daily gain and linearly increased (P=0.04) dry matter intake (DMI); thus, feed efficiency, dietary energy, and observed-to-expected diet NE were linearly decreased (P<0.01), increasing linearly (P<0.01) observed-to-expected DMI coefficient as DGP level was increased in the diet. There were no effects of treatments on carcass characteristics. Using the replacement technique, the energy value of DGP were 0.80, 0.75 and 0.67 Mcal EN_m/kg DM, for 10, 20 and 30% of inclusion, respectively. It is concluded that the estimated NE of DGP at 10% inclusion level in the diet is very similar to the NE value assigned by the NRC (2007) for the fermented DGP. Increasing the inclusion of DGP levels beyond 10% decreases its energy value, maybe due to the negative associative effects of chemical compounds (such as phenols) naturally contained in DGP.

Key words: wine industry by-product, efficiency, energy value, carcass, ruminants.

INTRODUCTION

Viticulture is an important industry in Baja California, Mexico, and a large proportion of their organic by-products are fresh grape pomace (Bustamante *et al* 2008). Generally, grape pomace is constituted by stalks, seeds, skin and pulp in similar proportions, and dried grape pomace (DGP) is characterised by being a source of high fiber content with moderated concentration of CP and lipids, rich in organic acids as tannins and phenols (Nistor *et al* 2014). Based on fiber and protein concentrations, grape pomace is similar to mature alfalfa hay which is a common forage source for growing lambs. However, grape pomace is mainly considered as a waste product, being commonly collected and eventually used as soil improver. Some studies on the use of grape pomace as an ingredient for ruminant rations reported poor results on animal performance (Manterola *et al* 1997, Nistor *et al* 2016) and those results have been associated to the type of DGP, because DGP commonly used was a by-product of the alcoholic fermentation, and therefore it was low in sugars and energy value. The unfermented by-products of viticulture have a greater concentration of sugars, and theoretically more energy concentration (Baumgärtel *et al* 2007), however, they maintain higher concentrations of the original compounds such as tannins and phenols (Xu et al 2016), which may represent negative associative effects with other components of the diet that could affect the feeding value. Therefore, it is possible that the expected feeding value of GP could

be decreased as its inclusion increases in the diet. To our knowledge, there is no information available regarding the influence of unfermented dried grape pomace (DGP) inclusion level in partial replacement of forage in diets for growing lambs on the performance, dietary energy and carcass characteristics. Therefore, the objective of this trial was to determine the energy value of unfermented DGP included at different levels as a substitute of mature alfalfa hay in diets for growing lambs.

MATERIAL AND METHODS

Sixteen Pelibuey×Dorper lambs (initial weight at start of experiment=17.8 ± 2.0 kg) were allocated (four lambs/treatment) to indoor facilities in individual pens of 3.4 m^2 with automatic waterers and 0.50 m fence-line feed bunks. Before the start the experiment, lambs had a 14 days adaptation period to the basal diet (diet without dried grape pomace; table 1) and the facilities. The animals were also treated for internal parasites (Ivermectin, Vetoquinol, México), received injections of vitamins A, D, E (Synt-ADE, Fort Dodge, Animal Health, México), and were given ear tags. Treatments consisted of replacement (DM basis) of alfalfa hay in the basal diet by DGP at 0, 10, 20 or 30%. The grape pomace, obtained from red grape after the juice was extracted by crushing and pressing, was brought from a local viticulture. The material was first conveyed over a screen to separate liquids and solids. Solids were then spread over a concrete surface and allowed to air-dry. The final dried material was carefully mixed, grounded and sampled before it was incorporated to diets. The alfalfa hay used was obtained from a batch obtained from the same cut-harvest which was produced in Mexicali valley. Both, alfalfa hay and DGP were ground in a hammer mill (Bear Cat #1A-S, Westerns Land and

Roller Co., Hastings, NE, USA) with a 7.62 cm screen, before adding it to the complete mixed diet. Lambs were allowed *ad libitum* access to dietary treatments. Daily feed allotments to each pen were adjusted to allow minimal feed refusals (~50 g/kg of feed offered). The amounts of feed offered and refused were weighed daily. Lambs were provided fresh feed daily at 08:00 and 14:00 h in a 30:70 proportion (as fed basis). Refusals were collected and weighed prior to the morning feeding and feed intake was determined daily. The experiment lasted 84 days. Lambs were individually weighed in the morning (07:00 h) at the start of experiment and before harvest. Alfalfa hay, DGP and complete mixed diets 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); NDF[corrected for NDF-ash, incorporating heat stable α-amylase (Ankom Technology, Macedon, NY, USA) at 1 mL per 100 mL of NDF solution (Midland Scientific, Omaha, NE, USA), Van Soest *et al* 1991], ether extract (method 920.39, AOAC 2000), and crude fiber (method 962.09, AOAC 2000). Dry matter content of feed and feed refusal was determined daily. The estimations of dietary energetic and expected DMI were performed based on the estimated initial shrunk body weight (SBW), to convert to a SBW basis is assuming that SBW is 96% of the full LW and final LW (Cannas *et al* 2004). Average daily gains (ADG) were computed by subtracting the initial SBW from the final SBW and dividing the result by the number of days on feed. Gain to feed ratio was determined by dividing ADG by the daily DMI. Expected DMI was determined based on observed ADG and average SBW according to the following equation (NRC 1985): expected DMI, kg/d = $(EM/NE_m) + (EG/NE_g)$, where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$ and EG (energy gain, Mcal/d) = $0.276 \times ADG \times SBW^{0.75}$ estimated accordingly to NRC (1985), estimated NE_m (dietary net energy of maintenance) and NE_g (dietary net energy of gain) are based on the ingredient composition of each experimental diets as described in table 1. The coefficient (0.276) was estimated assuming a mature weight of 113 kg for Pelibuey×Dorper male lambs (Sánchez-Mendoza *et al* 2016). Observed dietary NE was estimated by of the quadratic formula: $x = (-b - \sqrt{b^2 - 4ac})/2c$, where $x = NE_m$, $a = -0.41EM$, $b = 0.877 EM + 0.41 DMI + EG$, $c = -0.877 DMI$, and $NE_g = 0.877 NE_m - 0.41$ (Estrada-Angulo *et al* 2008). Hot carcass weights (HCW) were obtained for all lambs at the time of slaughter. After carcasses were chilled in a cooler at -2 to 1 ºC for 48 h, measuring of fat thickness (FT) was taken perpendicular to the m. *longissimus thoracis*, measured over the center of the ribeye between the 12th and 13th rib. All animal management procedures were conducted within the guidelines of of locally approved techniques (Mexican Official Rules, NOM-025, 033 and 051-ZOO-1995) for animal use and care. Performance (DMI, ADG, gain to feed ratio, dietary NE, observed-to-expected dietary NE ratio, observed-to-expected DMI ratio), and carcass data were analysed as a randomised complete block design

Table 1. Composition of experimental diets.

Item	Grape pomace level (% diet DM)			
	0	10	20	30
Ingredient composition (%)				
Alfalfa hay (mature)	90.00	80.00	70.00	60.00
Dried grape pomance	00.00	10.00	20.00	30.00
Ground corn	6.00	6.00	6.00	6.00
Molasses cane	3.30	3.28	3.25	3.23
Urea	0.30	0.32	0.35	0.37
Trace mineral salt[1]	0.40	0.40	0.40	0.40
Chemical composition[2] (%DM basis)				
Crude protein	13.95	13.88	13.88	13.86
Ether extract	1.41	1.89	2.37	2.85
Crude fiber	43.08	43.55	43.80	44.16
Neutral detergent fiber	51.94	52.07	52.72	53.44
Calculated net energy[3] (Mcal/kg)				
Maintenance	1.14	1.11	1.09	1.06
Gain	0.59	0.56	0.55	0.52

[1]Mineral premix contained: Calcium, 28%; Phosphorous, 0.55%; Magnesium, 0.58%; Potassium, 0.65%; NaCl, 15%; vitamin A, 1,100 IU/kg; vitamin E, 11 UI/kg.
[2]Dietary composition of crude protein, neutral detergent fiber and ether extract were 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.
[3]Based on tabular net energy (NE) values for individual feed ingredients (NRC 2007).

considering lamb as the experimental unit. The MIXED procedure of SAS (2007) was used to analyse the variables. The fixed effect consisted of treatment, and lamb as the random component. Treatment effects were tested for linear, quadratic and cubic components of the DGP supplementation level. Contrasts are considered significant when the $P \leq 0.05$, and tendencies are identified when the P-value was > 0.05 and ≤ 0.10.

RESULTS AND DISCUSSION

Chemical composition of alfalfa hay mature and DGP are shown in table 2. Due to differences in chemical composition between alfalfa hay and DGP, the inclusion of DGP slightly increased fiber and lipid concentration of experimental diets (table 1). The chemical composition of alfalfa corresponds closely to those reported by NRC (2007) for alfalfa mature hay. The NE of alfalfa hay used as reference ingredient in our experiment can be estimated from its chemical analyses in our laboratory according to the equation (adapted from NRC (2007), using all fibrous feedstuffs and for which all pertinent analyses are tabulated): NE_m, Mcal/kg = 0.00137CF% + CP0.0237CP% + 0.0377EE% + NFE× 0.0229NFE%) - 0.1072, R^2=0.96, n=29, where CF=crude fiber, CP= crude protein, EE= ether extract and NFE= 100 - (CF%+CP%+EE%+ ash%). Accordingly, the estimated EN value of alfalfa hay is 1.06 which corresponds closely to the value of 1.10 Mcal EN_m/ kg assigned by NRC (2007). Chemical composition of DGP was consisted with previous reports (NRC 2007, Nistor *et al* 2014), which is characterised as a source of high fiber content (47-50%) with moderated concentration of CP (13-15%) and lipids (5-8%).

Since there were no quadratic and cubic treatment effects ($P \geq 0.10$); thus, P-values for this component are not presented in the tables.

Treatment effects on growth performance and dietary energetics are shown in table 3. Based on average DMI, supplemental DGP daily intake averaged 0, 114, 224 and 372 g/lamb (average lamb weight in experiment=22.42 kg).

Table 2. Chemical composition (as percentage of DM) of alfalfa hay (ingredient of reference) and dried grape pomace determined by analyses[1].

Item	Alfalfa hay	Dried grape pomace
Dry matter content	93.80	93.87
Chemical composition		
Crude protein (N×6.25)	13.43	12.64
Ether extract	1.35	5.80
Crude fiber	45.80	49.40
NDF	42.54	43.82
Ash	7.29	7.76

[1] n = 6 samples of each ingredient.

The increases of DGP level in diet tended (linear component, P=0.06) to decrease average daily gain (ADG) and linearly increased (P=0.04) dry matter intake (DMI); therefore, as replaces of alfalfa hay by DGP increases, feed efficiency, dietary energy, and observed-to-expected diet NE were linearly (P<0.01) decreased, and observed-to-expected DMI was linearly increased (P<0.01). The average observed-to-expected DMI of controls was 101% of the expected value based on tabular estimates of diet energy density (NRC 2007) and observed SBW and ADG values (table 1), lending support for suitability of the prediction equations proposed by the NRC (1985) for the estimation of DMI in relation to SBW and ADG in feedlot lambs. The linearly increases on DMI on DGP supplemented diets suggest a lower energy (Galyean and Defoor 2003) contained in DGP diets; however, increases of DMI were more pronounced than expected as a result of the estimated energy dilution by growing inclusion of DGP in those diets (table 1). The above is confirmed because the expected-to-observed DMI ratio increased from 1.02 to 1.10 when the level of DGP increased in diet from 10 to 30%. The estimation of dietary net energy and the ratio of observed-to-expected DMI revealed differences on the efficiency of energy utilisation of the diet itself. We expect that observed:expected DMI ratio would be to 1.0 (this mean that animals 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 DMI represent a lower energy value (concentration) than expected according to NRC (2007), therefore the energy was poorly used by the animal, thus, the efficiency was negatively affected. In contrast, if ratio is less than 1, energetic efficiency was greater than expected (contrary to the dietary NE in which values lower than 1 represent greater efficiencies). Based on observed NE (determined through the observed performance of lambs), the comparative NE_m values of GDP can be estimate using the replacement technique. Given that NE_m value of mature alfalfa hay is 1.10 Mcal/kg (NRC 2007), the estimated comparative NE values for the DGP are estimated using the replacement technique (Zinn and Plascencia 1993) as follows: DGP NE_m, Mcal/kg= [(Test diet NE-Control diet NE)/DGPy] +1.10, where DGPy represents the proportion of DGP that replaced alfalfa hay in the basal diet (0.10, 0.20 and 0.30), and 1.10 represents de NE_m of alfalfa hay replaced by DGP. Dietary NE_g can be derived from NE_m by the equation NE_g=0.877 NE_m -0.41 (Zinn *et al* 2008). Accordingly, the NE_m and NE_g values of DGP at the 10, 20 and 30% inclusion level were 0.80 and 0.29, 0.75 and 0.25, and 0.67 and 0.18 Mcal/kg, respectively. Those values represent 72, 69, and 63%, respectively, of the relative NE values of mature alfalfa hay. The average NEm value of DGP at the 10% inclusion level was identically (0.80 Mcal/kg) with the NEm value assigned by NRC (2007) for fermented DGP, and it reasonably agrees with the 0.93 Mcal NEm value determined by difference digestible

Table 3. Treatment effects on growth performance, dietary energy, and carcass in drylot hairy lambs fed different levels of unfermented dried grape pomace.

| Item | Grape pomace level (% diet DM) | | | | SEM | P-value |
	0	10	20	30		Linear
Days on test	84	84	84	84		
Replicates	4	4	4	4		
Live weight[1] (kg)						
Initial	17.64	17.85	17.74	17.97	0.35	0.58
Final	27.72	27.53	26.50	26.98	0.57	0.34
Average daily gain (kg)	0.120	0.115	0.104	0.107	0.004	0.06
Dry matter intake (kg/d)	1.109a	1.142ab	1.120a	1.240b	0.034	0.03
Gain to feed (kg/kg)	0.108a	0.101ab	0.093bc	0.087c	0.004	<0.01
Observed dietary Net energy (Mcal/kg)						
Maintenance	1.13a	1.09ab	1.05bc	0.99c	0.020	<0.01
Gain	0.57a	0.54ab	0.51bc	0.46c	0.018	<0.01
Observed to expected dietary Net energy ratio						
Maintenance	0.99a	0.98a	0.96ab	0.94b	0.015	<0.01
Gain	0.97a	0.97a	0.93ab	0.90b	0.020	<0.01
Observed to expected daily dry matter intake	1.01a	1.02a	1.06ab	1.10b	0.024	<0.01
Estimated Net energy of DGP [2] (Mcal/kg)						
Maintenance		0.80	0.75	0.67		
Gain		0.29	0.25	0.18		
Carcass						
Hot carcass weight (kg)	13.58	12.98	12.57	12.82	0.37	0.14
Dressing percentage	49.42	47.15	47.44	47.52	0.013	0.47
Fat thickness (cm)	0.33	0.33	0.27	0.24	0.051	0.18

a,b,c Numbers in the same row with different superscript letters differ.
[1] Initial and final live weight (LW) was reduced by 4% to adjust for the gastrointestinal fill.
[2] Estimated by replacement technique (Zinn and Plascencia 1993).

coefficient of fresh red grape pomace by Baumgärtel *et al* (2007) and with the energy concentration of 0.87 Mcal ENL/kg DM (approximate concentration of 0.95 Mcal of energy of maintenance/kg) of grape marc meal extract determined by digestion trial (Winkler *et al* 2015). On the other hand, using *in vitro* gas technique, the NE value of GP have been determined in 0.85 Mcal/kg (Alipour and Rouzbehan 2007, Besharati *et al* 2007), but in other study, using the same technique a value of 2.05 Mcal NEm/kg was determined (Moghaddam *et al* 2013).

The increases in observed-to-expected DMI and reductions in dietary NE at high levels of DGP reveal that supplementation at that level had negative effects, independently of its chemical composition; therefore, the reduction of the efficiency on energy utilisation of lambs fed with increasing levels of DGP in this experiment can be due to possible negative effects of DGP on nutrient use, or negative associative effects of DGP on other components of the diet. Based on DGP chemical composition, the estimated NE values of DGP are similar (1.04 Mcal/kg) to the corresponding NE values of the mature alfalfa

hay it replaced. However, this value is 30, 39, and 55% greater, than the NE value estimated for the inclusions levels of 10, 20 and 30%, respectively, obtained from the observed performance of lambs using the replacement technique. The negative associative effects by presence of compound as phenols (not measure in this experiment) could be affected the efficiency on the energy use. Xu *et al* (2016) reported an average concentration of 102 mg phenolic/kg of red grape pomace, and the anti-nutritional effects of phenolic compounds are well known (Waghorn and McNabb 2003). The above could be supported by previous findings (Bahrami *et al* 2010, Nistor *et al* 2016) in which it was observed that intake of DGP increased beyond 10% while feed efficiency of lambs was notably decreased. On the other hand, Baumgärtel *et al* (2007) reported a low digestion (32%) of organic matter of fresh grape pomace included at level 55.5% in the diet which contained 29% concentrates.

Even when a numerically differences were observed between controls vs. high level inclusion of DGP in hot carcass weight (5.5%), dressing percentage (3.8%), and

fat thickness (26%), those differences were not significant ($P \geq 0.14$).

It is concluded that the estimated NE value of DGP at low level of inclusion in diet (10%) agrees with the NE value for fermented GDP assigned by the NRC (2007). The increases of inclusion level of DGP beyond 10% decreases its energy value, maybe due by negative associative effects of chemical compounds (as phenols) naturally contained in DGP. The potential use of this by-product as feed ingredient for lambs would decrease the level of inclusion of high cost forages (such as alfalfa hay), and would contribute to decrease the amount of waste of this material in viticulture industry. However, further studies are needed on the presence of anti-nutritional factors in this by-product in order to have more elements to decide on its inclusion in lamb diets.

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