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The Effects of Olive Pomace on the Fatty Acid Profile and Weight Gain in *Capra Aegagrus Hircus* as a Model for Ruminants

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ABSTRACT

One objective was to determine if olive pomace could be used as an acceptable feedstuff to maintain weight during the colder winter months. Twenty-eight Spanish-influenced goats were fed (2% of BW) varying amounts of fermented pomace with a protein pellet to meet NEm requirements. Four test groups (n = 7) consisted of a 3:1, 1:1, and 1:3 olive pomace-to-concentrate ratio (O:C) and a control containing no pomace. All groups received molasses at 0.5% BW and were fed for 49 days. The ADG for the 1:3, 1:1, 3:1, and control groups were 0.0370, 0.0166, 0.0119, and 0.0262 kg/day, respectively with no difference detected between groups (P > 0.88). A difference (P < 0.001) in consumption rates was detected between treatments with the 3:1 group consuming more feed with an average of 0.785 kg/day compared to the control at 0.694 kg/day. The second objective was to determine if olive pomace could be supplemented at a level that increases the C18:1 concentration in blood. Mature Spanish-influence goats (n = 14; 41.6 kg) were fed ad libitum olive pomace, starting at 2% of their BW for 28 days. There were significant changes in C18:1 and C18:2 over time with mean consumption of 0.55 kg/d over the duration of the trial. From d0 to d28, serum concentration of C18:1 increased (P < 0.001) by 8.67% and C18:2 increased (P < 0.03) by an average of 4.38%. Serum concentration of C18:0 increased by 3.59% but was not significant.

KEYWORDS: Olive; Goats; ADG; Palatability; Oleic; Serum

INTRODUCTION

Livestock used in food production require certain nutrients from feedstuffs for body functions and growth. Producers supplement domestic animals in order to accelerate their natural growth curve, thus making a more plentiful and profitable harvest in order to feed the ever-growing population. Ruminants are efficient at digesting forages due to the

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vast population of micro-flora, bacteria, and other small organisms that reside in their gastrointestinal tract.

Olive pomace is the by-product of processing olives through a hammer mill. This by-product is one of the few items created from the production of olive oil and has no predetermined or widely known purpose. This study examined the effects of this by-product to help olive producers find a long term and profitable supplement to reduce feed costs. Olive pomace consists of up to 80% oleic acid (C18:1 cis-9), along with linoleic acid (C18:2), and palmitic acid (C16:0) (Ranalli et al. 2002). Small traces of stearic acid (C18:0) and α-linolenic acid (C18:3 cis-9,12,15) can also be found in the pomace and olive residue (Ranalli et al. 2002). Due to the price of feedstuffs high in fat, the feeding of these materials is not widely practiced throughout the commercial feeding industry. It is more economical to feed concentrates that are much higher in carbohydrates, yet fats have a higher energy level and can therefore be fed in lower quantities to achieve the same endpoint when compared to the carbohydrate based concentrates. By analyzing the effects of olive pomace, producers may be able to utilize this by-product as a high-energy feedstuff with minimal costs.

The fatty acid profile of goats can change dramatically depending on age, intake type, and other variables as determined by Banskalieva et al. (2000). The effects of oleic acid ((9*E*)-Octadec-9-enoic acid or 18:1 cis-9) are of particular interest. Scollan et al. (2001) stated that it is possible to introduce various feedstuffs with desired fatty acid chains and raise their presence in the tissue of beef cattle. Over 120 d, Scollan et al. (2001) found that only a select few fatty acids could be directly influenced by feeding excessive levels. Of these, α-linolenic, docosapentaenoic, and docosahexaenoic are the main ones addressed. Oleic acid, however, remained at a level comparable to d 0 in the control and decreased in each test group. This can be partially explained by the fact that their feedstuffs, linseed and fish oil, were not high enough in oleic acid to sustain the oil in tissues. It is also noted that the study conducted by Velasco et al. (2004) found similar results to Scollan et al. (2001). A key difference in these studies was unweaned lambs left to pasture exhibited lower levels of stearic (C18:0) and oleic fatty acids. However, the level of fatty acids containing less than 18 carbons all increased. It is known that the health benefits of olive oil are correlated with the high oleic acid content (Terés et al. 2008). As the oleic acid intake increases, it becomes a regulator of membrane lipid structure in such a way as to control protein-mediated signaling, causing a reduction in blood pressure (Terés et al. 2008). This project focused on defining the feed efficiency and performance of the pomace in a commercial setting, and secondly, how the fatty acid (FA) profiles of goats are affected by a high concentration of different polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) found in olive pomace.

METHODOLOGY

This study was approved by the Sam Houston State University Institutional Animal Care and Use Committee (Protocol #15-11-30-1008-3-01). This study was divided into two feeding periods to accomplish the objectives. The first period sought to evaluate performance characteristics. The objective of the second period was to analyze the deposition, if any, of the oleic acid found in the olive pomace in the circulating serum of goats.

Evaluating the olive pomace. Olive pomace was collected in 400-gallon containers directly from the milling processes at the time of harvest from producers in Elmendorf, Dripping Springs, and Madisonville, Texas. The material was transported to Huntsville, Texas in temporary storage containers before processing. The fresh pomace was processed by either fermentation, drying, or storage when wet before sending off for near-infrared spectroscopy at SDK Laboratories (Hutchinson, KS). The analyses helped determine which of the possible methods of feeding the pomace is the most practical regarding longevity of the material and efficiency in nutrient requirements for livestock producers.

Supplementation trial[1]. Twenty-eight Spanish does were fed treatment rations in a commercial type setting to determine the feed efficiency of varying levels of pomace mixed with a commercially available pelleted feed. Four separate groups (n = 7 per group) were fed 3:1 olive:feed ration, 1:1 olive:feed ration, 1:3 olive:feed ration, and a control (ration consisting of strictly the pelleted ration). The 16% pellet ration was a commercially available feed from Producers Cooperative Feed Company in Bryan, TX. The diets were balanced using the nutrient requirements for maintenance over a 49-day trial. Dietary treatments were offered at 2% BW, with molasses incorporated at an additional 0.5% of BW to homogenize the ration and discourage selective browsing. Dietary components were offered at 24-hour intervals each morning for 49 days. Body weights were obtained weekly and intake adjusted accordingly.

The weights taken at seven-day intervals; average daily gain (ADG), feed efficiency (FE), and the cost efficiency of each ration were determined. The rations were balanced for net energy for maintenance (NEm) based on the current National Research Council guidelines.

[1]Trial results presented at 2016 American Society of Animal Science Joint Annual Meeting in Urso, P. M., M. M. Beverly, S. F. Kelley, M. J. Anderson, J. L. Leatherwood, K. J. Stutts, and S. Nair. 2016. Effects of supplementing olive pomace as a feed additive on weight gain in Capris aegagrus hircus. *Journal of Animal Science*. 94, E-Supplement. doi:10.2527/jam2016-1721 and at the 2017 American Society of Animal Science Southern Section Meeting and published in Urso, P. M., M. M. Beverly, S. F. Kelley, M. J. Anderson, J. L. Leatherwood, K. J. Stutts, and S. Nair. 2016. Effects of supplementing olive pomace as a feed additive on weight gain in Capris aegagrus hircus. *Journal of Animal Science*. doi:10.2527/ssasas2017.0101

Table 1. Selected feed values of the rations utilized in the supplementation trial as well as analysis strictly of olive pomace.

	DM (%)[1]	CP (%)[2]	EE (%)[3]	ADF (%)[4]	NeM (Mcal/lb)[5]	ME (Mcal/lb)[6]
3:1 Ration	60.02	9.82	8.95	28.25	0.80	1.16
1:1 Ration	67.32	11.88	7.05	21.44	0.83	1.19
1:3 Ration	74.58	13.38	5.48	18.65	0.83	1.19
Control	84.09	15.23	4.23	16.02	0.83	1.20
Olive Only	33.07	6.85	15.60	50.47	0.73	1.08

[1]Percent Dry Matter
[2]Percent Crude Protein
[3]Percent Ether Extract
[4]Percent Acid Detergent Fiber
[5]Net Energy for Maintenance in megacalories per pound
[6]Metaboliazable Energy in megacalories per pound

Fatty acid deposition in meat and muscle tissue. Fourteen mature Spanish does were utilized in a randomized design for a 28-day trial. The does were given *ad libitum* feed of strictly olive pomace for 28 days to allow the fatty acids in the pomace to be absorbed, should microbial activity allow. Blood was collected bi-weekly to determine if the circulatory system is a pathway where the fatty acids frequent once consumed. Weights were taken weekly to determine at what levels feed should be increased to maintain a 2% feed intake based on the animals' body weight, or if the pomace is not an adequate feedstuff and causing animals to lose weight.

Blood samples were obtained every 7 days via jugular venipuncture and stored at -20 °C for analysis of fatty acid methyl esters concentrations and to extract any residual C18:1 and other select fatty acids that may be present.

Table 2. Select fatty acid concentration levels in olive pomace as seen in Zambiazi et al. (2007).

Fatty Acid	C16:0	C18:0	C18:1	C18:2	Total
% in Olive Pomace	9.31	3.2	74.0	10.33	96.84

The does were kept in a dry lot setting at Gibbs Ranch in Huntsville, Texas to minimize unforeseen and unaccounted nutrients from forage and to mimic the conditions in which most cattle and other meat producing livestock are fed.

This trial was conducted with the upmost regard and dedication to producing results that were dependent on the variables tested and not negligence of following protocol. However, there were external factors that were not in the control of the researchers. The goats utilized in this trial were purchased from an independent auction in San Angelo, Texas. These goats were ideal for the conducting of this trial. All of the animals were previously from a single producer, were of relatively the same age, and were fed the same diet before the introduction into this trial. Ten does from the herd were covered by a buck days before purchase by Sam Houston State University and therefore were in different physiological stages during the trial. These individuals were excluded from both trials so their rapid weight gain would not skew the true results of the olive pomace as a feed variable.

Statistical Analysis. All data was analyzed using the PROC MIXED procedure of SAS (v9.4). Models contained effects for dietary treatment, time, and treatment by time interaction with a repeated measures statement. All samples were sent to an independent laboratory for fatty acid methyl ester analysis. The laboratory identified the fatty acid profile of the blood taken in order for the investigators of this study to attempt to find any correlations using PROC CORR in SAS with the amount of olive pomace fed and the profiles present.

RESULTS

Supplementation trial. Within the commercial based feeding trial, there was no significant correlation between the feeding groups over time. Across all groups, 3:1 olive to feed, 1:1 olive to feed, 1:3 olive to feed, and the control, the average daily gain (ADG) remained constant ($P > 0.88$) as seen in Figure 1.

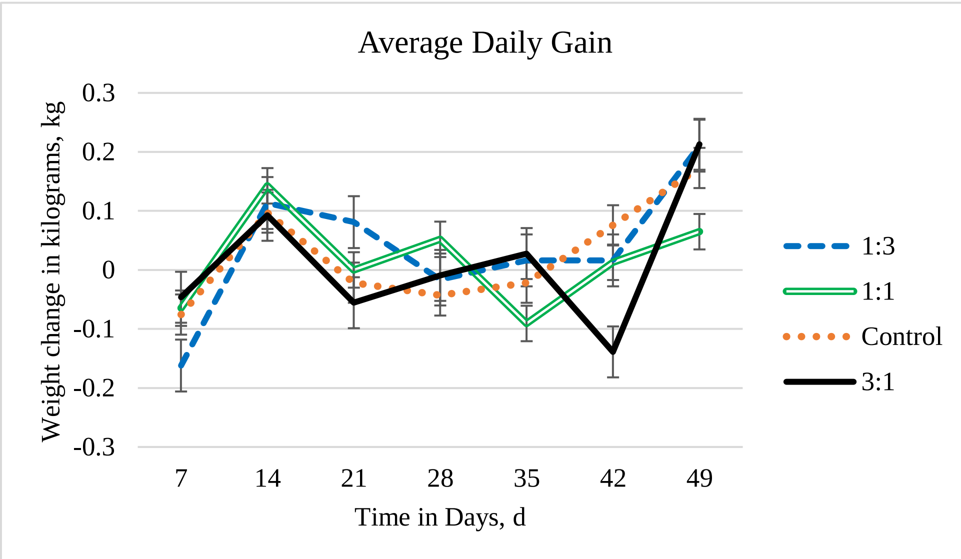

Figure 1. The average daily gain of goats fed 3:1 olive:feed ration, 1:1 olive:feed ration, 1:3 olive:feed ration, and a control over 49 days.

The average consumed in each group varied significantly across treatment ($P < 0.0001$) as seen in Figure 2. On average, the control group consumed 0.693 kg/d, the 1:3 group consumed 0.7311 kg/d, the 1:1 group consumed 0.6823 kg/d, and the 3:1 group consumed 0.7847 kg/d.

The average efficiency (Fig. 3) showed no significant difference between treatment groups ($P > 0.96$). The LS mean of each group is as follows: 1:3 gained 0.31 kg of weight for 1 pound of feed, 1:1 gained 0.16 kg, 3:1 gained 0.14 kg, and the control gained 0.26 kg.

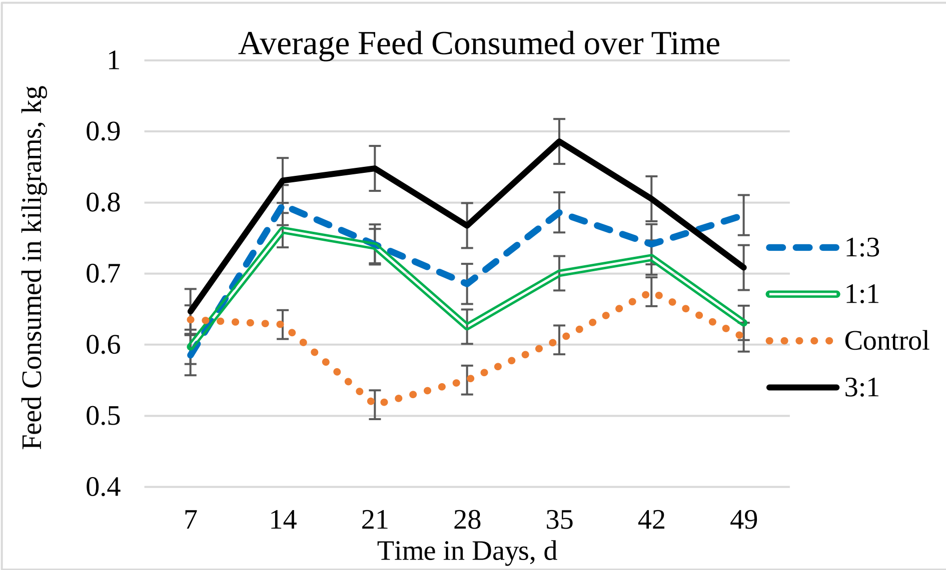

Figure 2. The average amount of feed consumed per day by each group (3:1 olive:feed ration, 1:1 olive:feed ration, 1:3 olive:feed ration, and a control) over a 49-day period.

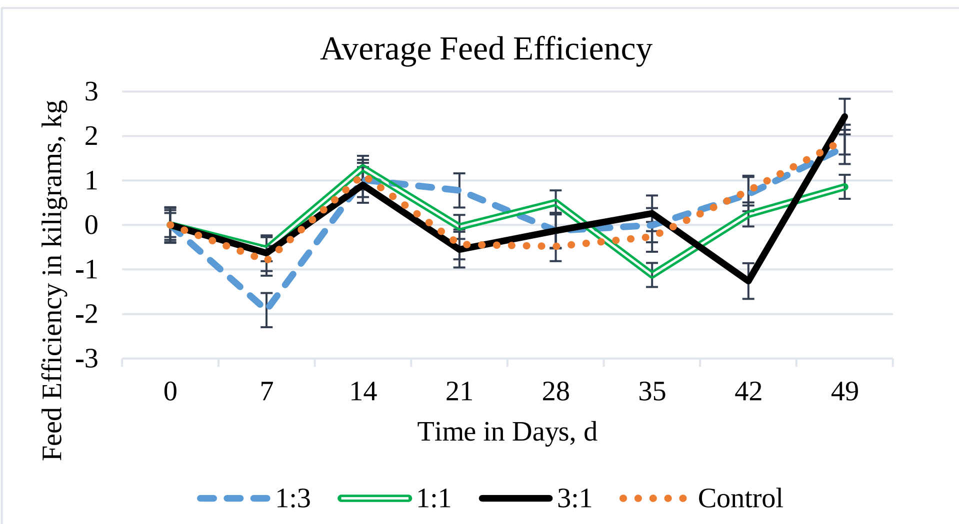

Figure 3. The average feed efficiency of goats fed 3:1 olive:feed ration, 1:1 olive:feed ration, 1:3 olive:feed ration, and a control over 49 days.

Fatty acid trial. Regarding fatty acid composition, there were significant changes in C18:1, C18:2 and C18:0 over time as seen in Figure 4. Over 28 days, the concentration of C18:1 in blood increased by 8.67% ($P < 0.004$). There was a tendency of C18:0 to increase by 3.59% ($P < 0.09$) and a significant increase in C18:2 by an average of 4.38% ($P < 0.035$). C16:0 was also recorded with no significant change noticed ($P > 0.2$).

The average amount of feed consumed (Fig. 5) over time increased ($P < 0.001$). At day 7, animals were consuming an average of 0.44 kg/day whereas by day 28, consumption was up to 0.66 kg/day.

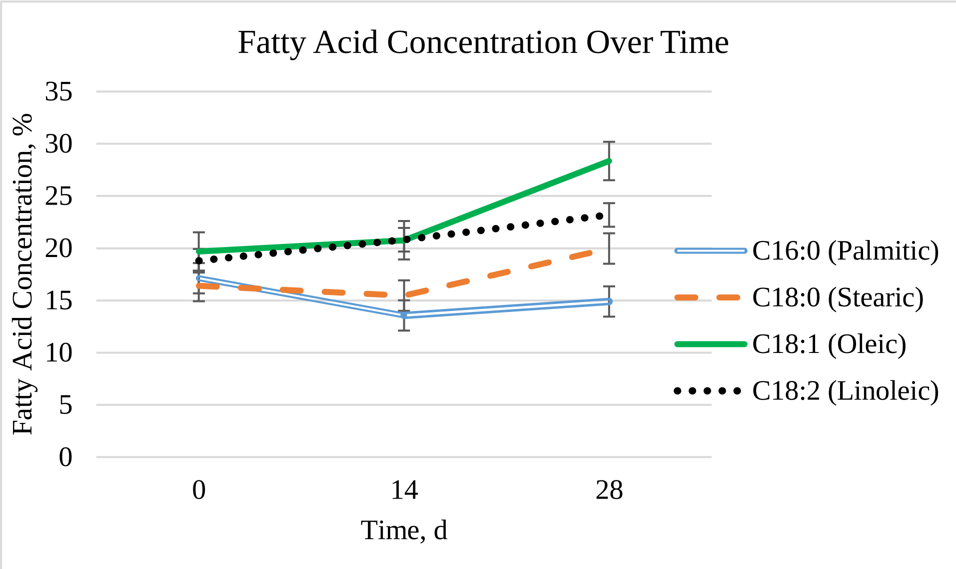

Figure 4. The concentration of fatty acids in goat serum fed *ad libitum* olive pomace over time.

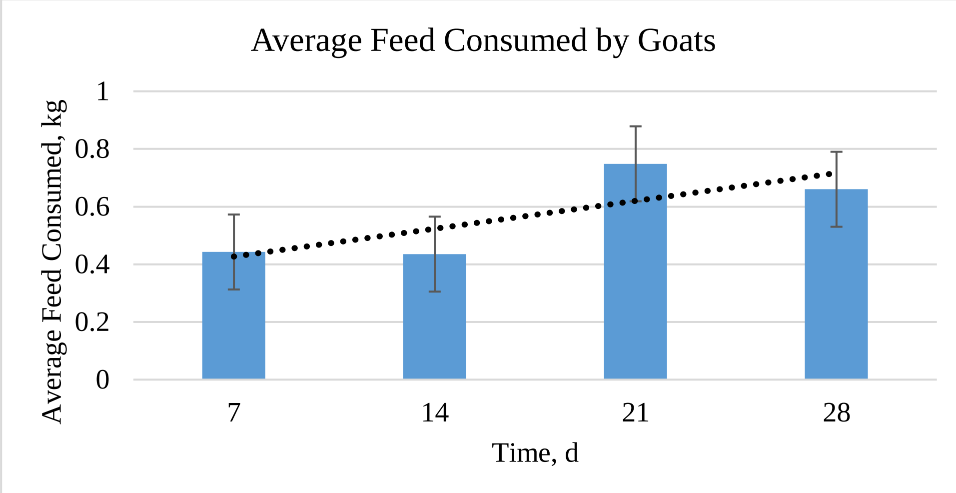

Figure 5. The average feed consumption of does over a 28-day period of *ad libitum* olive pomace. Days are averaged into a single week format.

DISCUSSION

Supplementation trial. The average daily gain for each group remained consistent in the commercially based trial. While this ration was balanced for NEm, dramatic weather shifts and stress associate with frequent handling could have influenced weight gain or loss overtime. By understanding that olive pomace can be supplemented at inclusion rates of 3:1 olive to feed, the mostly olive ration can be considered as efficient in managing weight over the wintering period when compared to a commercially available ration. This is partially due to the amount of fat content in the predominantly olive ration.

The amount consumed potentially portrays a difference in palatability. The 3:1 group consumed significantly more feed per week than their control counterparts on average. This difference can potentially be explained by the sweetness of scent and flavor observed in the olive pomace after the fermentation process. After a brief adjustment period to the pomace, the subjects in the pomace groups were more eager to consume their daily ration, thus leaving less waste. Buchanan-Smith (1990) found different results when feeding sheep varying qualities of silage to increase consumption. It was observed that sheep decrease consumption when fed silage. In this study, goats were much more likely to consume higher amounts of feed if there were higher levels of pomace included. The pomace might have a different biochemical makeup when compared to Lucerne silage that allows it to be more palatable.

Fatty Acid Trial. With the significant change in fatty acid composition over time, it can be noted the biochemical processes in the gastrointestinal tract alter fatty acid composition over time. It was noticed that both C18:0 and C18:1 followed a similar pattern of change ($r = 0.682$). C18:1, the fatty acid that is the most prominent in olive pomace, increased by 44% from day 0 to day 28 on a normalized distribution ($P < 0.002$). C18:0 also had a tendency to increase by a total of 22% from day 0 to day 28 ($P < 0.092$). This correlation was observed previously by Mosley et al. (2002), who stated that biohydrogenation of C18:1 by mixed ruminal microbes involves the formation of several positional isomers of *trans* monoenes, as well as the contributing to the direct biohydrogenation to C18:0. It is also observed in the trial that C18:2 increased at a constant level throughout feeding. This is likely contributed to C18:2 being the second most prominent FA in the pomace. However, a slight amount can potentially be contributed classifying olive pomace as a plant-based product because of the minimal processing since its harvest. Marín et al. (2015) showed that goats fed plant-based diets showed a linear increase in C18:0, C18:1, and C18:2. The spike from day 14 to day 28 and consistent climb in C18:2 is consisted with the theory that C18:2 is saturated into C18:1 and then further into C18:0. The consistent 10.33% concentration of C18:2 supplemented in the feed can potentially explain the gradual linear increase of C18:2. While previous research has shown similar results, it is not demonstrated that biohydrogenation of C18:2 into C18:1 and eventually C18:0 occurred in this study.

It should also be noted that the consumption of the pomace increased over time. The consistency of the consumption can also contribute to the gradual rise in C18:2. The goats on trial did decrease consumption between day 7 and 14. This is likely due to the acclimation period accompanied with feeding a silage-based product as well as adverse weather conditions during that time. As their feed intake increased, the amount of the observed fatty acids increased as well.

Another saturated fatty acid that was observed in this trial was C16:0. There was no significant difference in the concentration of C16:0 from day 0 to day 28. This can lead to the observation that while saturation occurs with the 18 carbon fatty acids, β-oxidation is not as prevalent.

Implications. By increasing the incidence of C18:1 in the blood, it is possible that further saturation or loss of carbons does not occur and the fatty acids are deposited in the muscle tissue. A continuation of this trial at a later date will examine the concentration of the fatty acid profile of muscle biopsies. Jones et al. (2014) stated that C18:1 enriched canola oil can contribute to a lower risk of cardiovascular disease. If C18:1 is deposited in the muscle tissue as evident in the blood of this trial, feeding olive pomace can be a healthy alternative to high starch ingredients such as corn and barley.

The reduced cost of the ration in the commercial based trial can be a benefit to not only producers of olive oil but also livestock producers. Other by-products such as dried distiller's grains have contributed a substantial economic benefit in the animal agriculture industry. With further research, olive pomace may contribute to the economic wellbeing to producers as well.

CONCLUSION

With the conclusion of the pilot feeding trial for olive pomace, it was observed that supplementation of the ingredient can be beneficial to the animals that consume it as well as observing an increase in select fatty acid concentrations in serum. It can also be a benefit to the producers that feeding this medium can potentially be obtained cheaper than a commercially available ration. Future studies may reveal more specific inclusion rates to determine the olive pomaces' optimum efficiency level as well as determining if olive C18:2 and C18:1 undergoes biohydrogenation or β-oxidation before being deposited in tissue. Further economic investigation can potentially reveal the pomaces' true economic value in future studies.

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