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FERMENTATION QUALITY AND *IN VITRO* DIGESTIBILITY OF TOTAL MIXED RATION ENSILED WITH FRESH OLIVE CAKE

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ABSTRACT

This study aimed to investigate the fermentation characteristics, aerobic stability, and *in vitro* digestibility of the total mixed rations (TMR) ensiled with different amounts of fresh olive cake (OC). Three TMRs were ensiled with different OC percentages on DM basis; 0% OC (control); 10% OC (OC10), and 20% OC (OC20). For each group, 27 vacuum bags (silos; 81 bags in total) were prepared. Three bags from each group were opened on d 2, 4, 7, and 14 of ensiling to measure pH, and on d 21 and d 42 to evaluate the pH and aerobic stability. The remaining bags (9 from each group, 27 in total) were opened on d 60 to measure aerobic stability, fermentation quality (pH, organic acids, and NH_3-N), nutritional compositions, and *vitro* digestibility. Increasing the OC inclusion in the TMRs increased the ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations whereas, non-fibrous carbohydrates, water soluble carbohydrates, and total phenolic content decreased ($P \leq 0.05$). The control group had lower pH compared to other groups during the fermentation period ($P \leq 0.05$). No significant differences were seen among the treatments for lactic acid and acetic acid concentrations. However, propionic acid was greater in OC10 group compared to control ($P \leq 0.05$). Dry matter (DM) loss and aerobic stability were not different among the groups. Flieg score was greater in control group in comparison with that of OC20 group ($P = 0.016$). Relative feed value (RFV) was greater in OC20 group than other groups ($P \leq 0.001$) whereas, control group had lower RFV than OC10 group ($P \leq 0.001$). *In vitro* digestibility parameters were lower in OC20 group in comparison with other groups ($P \leq 0.001$). Consequently, the results demonstrated that ensiling of TMR with OC had a good quality fermentation profile and feed value. However, *in vitro* nutrient digestibility was dramatically reduced when OC was used at 20% in TMR on a DM basis. Therefore, the inclusion of OC up to 10% in ruminant diets seems liable.

Keywords: Ensiled total mixed ration, olive cake, *in vitro* digestibility, fermentations characteristics

INTRODUCTION

Olive cultivation, 98 % of which occurs in Mediterranean countries, benefits the economic and social dimensions in rural areas whilst also playing an important role in the development of the agro-industrial sector in this region. According to the International Olive Council, olive oil production has increased steadily over the last 60 years (IOC, 2024). Despite its economic importance, the olive oil industry poses environmental challenges due to the substantial amounts of waste generated during oil extraction, which exhibits high phytotoxicity and adverse impacts on soil and groundwater quality (Palmeira et al., 2023). Nonetheless, much of this waste holds potential for valorization as fuel, fertilizer, or animal feed.

The solid waste produced during olive oil extraction, commonly referred to as olive cake (OC), consists of olive pulp, skin, stones, and water. The OC is characterized by high crude fiber content (CF; 72% of DM) and ether extract (EE; 7.5% of dry matter (DM)) (Beken and Şahin, 2011), along with a low crude protein content (CP; 5.5% of DM) (Abarghoei et al., 2011). Given its nutritional profile, OC can serve as a valuable alternative forage source in ruminant diets (Foti et al., 2022). However, OC in its crude or fresh form, which contains approximately 75-80% water, is a perishable feed that requires preservation through drying or ensiling to prolong its storage life. The drying process requires additional energy costs, therefore, ensiling offers a more cost-effective method to produce OC that allows for longer storage and ease of use in ruminant diets. However, the lower water-soluble carbohydrate (WSC) content in OC presents a significant challenge for successful ensiling. Consequently, previous studies have focused on establishing the optimum ensiling conditions for OC to ensure its long-term preservation (Rowgani and Zamiri, 2007; Weinberg et al., 2008).

Ensiled total mixed ration (ETMR) comprising of forages, concentrates, byproducts, minerals, vitamins, and additives, is produced by mixing and storing these feed materials under anaerobic conditions. While ETMR is not a recent practice (Owen and Howard, 1965), there has been a resurgence of interest in its application in ruminant nutrition since the 2000s, owing to its numerous benefits, including uniform composition during storage, higher aerobic stability, and increased utilization of unpalatable or less digestible byproducts (Bueno et al., 2020). Due to these advantages, ETMR has been the focus of extensive research in recent years, presenting an effective approach to incorporate high-moisture residues into animal feed, particularly those generated by agroindustry. Additionally, ETMR can facilitate the fermentation of feeds that are otherwise difficult to ensile since it not only incorporates grains but also previously fermented feeds that aid in immediate acidification of the mixture during ensiling process (Bueno et al., 2020).

Studies have demonstrated improvements in the fermentation quality of OC ensiled with feed materials rich in WSC like molasses (Weinberg et al., 2008) or grains (Duru, 2012). However, to the best of our knowledge, no study has described the fermentation quality of total mixed ration (TMR) ensiled with fresh OC. Therefore, we hypothesized that ensiling OC with TMR would benefit from the advantages of ETMR, such as high aerobic stability and homogenous composition, thereby increasing the utilization of OC in ruminant diets. Hence, this study aimed to investigate the fermentation characteristics, aerobic stability, and *in vitro* digestibility of ETMR formulated with different inclusion levels of fresh OC.

MATERIALS AND METHODS

Experimental design, total mixed ration, and ensiling: The study was conducted in a completely randomized design comprising of three experimental groups of TMRs with three different inclusion levels of OC on DM basis as 0% OC (control), 10% OC (OC10), and 20% OC (OC20). The TMRs were adjusted to meet the nutrient requirements for Holstein dairy cattle in mid-lactation, 600 kg body weight, and 30 kg milk yield (NRC 2001).

The experimental total mixed rations (TMRs), composed of alfalfa hay, maize silage (32% DM), fresh olive cake (28% DM), and concentrates, maintained a 50:50 forage-to-concentrate ratio. Alfalfa hay was included at a fixed level of 14.4%. In the control diet, maize silage was incorporated at 35.6%, while in the experimental diets, it was partially replaced by fresh olive cake at levels of 10% (OC10) and 20% (OC20), respectively (Table 1). The concentrates were provided by a commercial feed factory. Alfalfa hay and maize silage were provided by the Center for Agriculture, Livestock and Food Research, Burdur Mehmet Akif University, Türkiye. The fresh OC was purchased from a commercial olive oil production facility with a two-phase centrifugation extraction procedure and used in the TMRs on the same day. To ensure homogeneity during ensiling, alfalfa hay was finely chopped and properly mixed with other ingredients. Approximately 300 g of each TMR was tightly packed into vacuum bags using a vacuum sealer (SMVK150G Sonkaya, İstanbul, Türkiye). A total of 81 vacuum-sealed bags (silos) were prepared (3 TMRs × 27 replicates) and stored at room temperature (~20°C) for 60 days under anaerobic conditions. On days 2, 4, 7, 14, 21, and 42, three ETMR bags were opened from each group to measure pH. Aerobic stability was also assessed on days 21 and 42. Remaining ETMR bags from each group were opened on d 60 to evaluate chemical composition, aerobic stability, fermentation quality (pH, organic acids, NH_3-N, feed value), and *in vitro* digestibility. Additionally, freshly prepared TMRs were sampled before ensiling to ascertain the pH, aerobic stability, and chemical composition at the time of ensiling.

Chemical analyses: Fresh and ensiled TMR samples were oven-dried at 65°C and ground to pass through 1 mm sieve using a lab grinder (Retsch ZM 1000 Düsseldorf, Germany). The DM (method 934.01), ash (method 942.05), and EE (method 920.39) were analyzed according to AOAC (1998) procedures. The CP was measured by the Kjedahl method, as nitrogen (N) × 6.25 (AOAC, 1998; method 984.13). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were assayed according to Van Soest et al. (1991) using an ANKOM 200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Concentration of WSC was determined according to Dubois et al., (1956). Non-fibrous carbohydrates (NFC) were calculated using the following formula: NFC, % = 100 − (NDF + CP + Ash + EE). Total phenolic content (TP) was analyzed using Folin-Ciocalteu reagent method (Ainsworth and Gilliespie, 2007) followed by measuring the absorbance with a UV-vis spectrophotometer

(Multiskan SkyHigh UV/Vis microplate Spectrophotometer, Thermo Fisher Scientific, MA, USA).

Fermentation profile and aerobic stability: To analyze fermentation characteristics, a 25 g sample of fresh and ensiled TMR was homogenized in 100 mL distilled water using a blender, sieved through four layers of cheesecloth, and pH was measured (Isolab pH/Temperature Measuring Instrument, Istanbul/Turkey). For samples from ETMR, the homogenate was centrifuged at 4000 × *g* for 10 minutes. The supernatant (5mL) was mixed with 1 mL of 25% orthophosphoric acid, chilled in an ice bath for 30 minutes, and centrifuged again. Supernatants were analyzed by gas chromatography (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) with a 30 m × 0.25 mm × 0.25 µm column (Stabilwax-DA, Restek, Bellefonte, PA, USA) and a flame ionization detector according to conditions outlined by Sacakli et al. (2023). The lactic acid was determined by using a UV-vis spectrophotometer (Multiskan SkyHigh UV/Vis microplate Spectrophotometer, Thermo Fisher Scientific, MA, USA) according to Borshchevskaya et al. (2016). The NH_3-N was measured according to the method previously described by Meeske et al. (2002). Briefly, 50 g of each ETMR was homogenized in 250 mL 0.1 N sulfuric acid, filtered through four layers of cheesecloth, and subjected to distillation and titration following the Kjeldahl method as described by AOAC (1998).

Aerobic stability was measured by recording the temperature of fresh and ensiled TMR every 30-minutes over 21 days with a multichannel data logger (TMC6-HD, CEM, Shenzen, China). Aerobic stability was denoted as the time (h) interval before the sample temperature increased by 2°C above the room temperature (Agarussi et al., 2022).

Flieg score and relative feed value: Flieg score (Kılıç ,1986), relative feed value (RFV), net energy for lactation (NE_L), and total digestible nutrients (TDN) for each ETMR were computed using equations from Horrocks and Vallentine (1999).

Flieg score = 220 + (2 × (DM% - 15)) – (40 × pH)

RFV = (120 ÷ NDF) × (88.9 – (ADF × 0.779)) × 0.775

NE_L = (1.044 – (0.0119 × ADF)) × 2.205

TDN = (–1.291 × ADF) + 101.35

In vitro true digestibility: *In vitro* true digestibility of each ETMR sample was analyzed by ANKOM Daisy^{II} incubator. Dried ETMR samples (0.25 g), prepared in triplicates, were packed in ANKOM F57 filter bags (4.5 × 4.0 mm with a pore size of 25 µm), and placed in Daisy^{II} incubator bottles. The bottles were filled with 1330 mL of buffer solution A (KH_2PO_4 10 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, NaCl 0.5 g/l, $CaCl_2 \cdot 2H_2O$ 0.1 g/l, Urea 0.5 g/l) and 266 mL of buffer solution B (Na_2CO_3 15.0 g/l, $Na_2S \cdot 9H_2O$ 1.0 g/l). Fresh rumen fluid was collected into a thermos under CO_2 gas and filtered with four layers of cheesecloth. The filtered ruminal fluid (400 mL) was introduced in each bottle. After 48 h of incubation at 39°C, the filter bags were thoroughly washed with cold tap water to stop microbial fermentation, then oven-dried at 105°C to constant weight. Then, the bags were analyzed for organic matter (OM) and NDF.

Statistical analyses: Data on pH, aerobic stability, and chemical composition of fresh TMRs, as well as chemical composition, fermentation characteristics, feed value and *in vitro* digestibility of ETMRs, were evaluated in a completely randomized design using one-way analysis of variance (ANOVA). Following general model was used to assess the effect of treatments on the selected traits of ETMRs:

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} = response variable, µ = overall mean, T = effect of treatment i, and e_{ij} = error term.

To carry out the statistical analysis, compare means procedure was applied followed by one-way ANOVA function of a computer-based statistical software package SPSS (version 22.0, IBM Corp., Armonk, NY, USA). Differences among the means were assumed significant at 95% probability ($P \leq 0.05$). Tukey's test was applied as post-hoc to compare the means exhibiting significant differences across the treatments. Results were presented as means ± standard error of the means (SEM).

RESULTS

Table 2 presents the chemical composition of the fresh, pre-ensiled TMRs. No significant differences were observed in DM, CP, and WSC contents across treatments (P>0.05). The control had significantly lower EE, NDF, ADF, and ADL levels than the OC10 treatment, while these parameters were significantly higher in the OC20 treatment compared to both control and OC10 (P≤0.001). The NFC concentration was highest in control treatment compared to OC10 and OC20 (P≤0.001). Total phenolic content was highest in control, followed by OC10, with OC20 treatment containing the lowest TP level (P≤0.001).

The chemical composition of ETMRs after 60 d of ensiling is presented in Table 3. While DM loss was greater in the control treatment (4.58%) compared to OC10 (2.69%) and OC20 (1.26%), these differences were not statistically significant (P=0.091). CP levels were similar between the control and OC10 treatments, while the OC20 had significantly lower CP than the other treatments (P=0.021). EE, ADF, and ADL concentrations in the OC10 treatment were significantly higher than in the control but lower than in the OC20 treatment

($P\leq0.001$). NDF was significantly lower in control than in both OC10 and OC20 treatments ($P\leq0.001$). The control treatment also had significantly higher NFC and WSC content than OC10 and OC20; additionally, NFC concentration in OC20 was significantly lower than in OC10 (NFC, $P\leq0.001$ and WSC, $P=0.018$). TP content was also highest in the control, significantly exceeding the levels present in the OC10 and OC20 treatments ($P\leq0.001$).

The fermentation profile of ETMRs, including NH_3-N and organic acids, is presented in Table 4. NH_3-N concentrations were significantly higher in the control and OC20 treatments than in OC10 ($P\leq0.001$). The OC10 treatment showed significantly greater levels of propionic acid compared to the control, while levels of this acid in OC20 were comparable to those in both the control and OC10 ($P=0.013$). Butyric acid and isovaleric acid concentrations were significantly higher in OC10 and OC20 compared to control ($P\leq0.001$ and $P=0.004$, respectively). Isobutyric concentrations were significantly higher in control and OC10 compared to OC20 ($P\leq0.001$). No significant differences were found across treatments for lactic acid, acetic acid and valeric acid concentrations ($P>0.05$).

Figure 1 shows the pH of the ETMRs opened on different days of ensiling. As expected, pH levels declined in all treatments as the number of silo days increased. The control treatment consistently showed the lowest pH on each opening day compared to OC10 and OC20 ($P\leq0.001$). Between OC10 and OC20, OC20 maintained a higher pH throughout the fermentation period, with the exception of day 4. The observed rates of pH reduction were -13.9% for the control, -10.9% for OC10, and -13.11% for OC20.

Tables 1. I Ingredient proportions of the total mixed rations (TMRs).

Ingredients (%DM)	Control	OC10	OC20
Forages			
Fresh olive cake	-	10	20
Maize silage	35.6	25.6	15.6
Alfalfa hay	14.4	14.4	14.4
Concentrates			
Maize, ground	16.8	16.8	16.8
Barley, ground	9.1	9.1	9.1
Cotton seed meal	8.5	8.5	8.5
Sunflower meal	12.2	12.2	12.2
Soybean meal	1.6	1.6	1.6
Milestone	0.7	0.7	0.7
Sodium bicarbonate	0.5	0.5	0.5
Salt	0.4	0.4	0.4
Premix*	0.2	0.2	0.2
Total	**100**	**100**	**100**

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR.

*Each kilogram contains 1.300.000 IU vitamin A, 260.000 IU vitamin D3, 3.000 mg vitamin E, 120.000 mg niacin, 5.000 mg Mn, 5.000 mg Fe, 5.000 mg Zn, 1.000 mg Cu, 15 mg Co, 80 mg I, and 15 mg Se

Table 2. The chemical composition of the pre-ensiled total mixed rations (TMRs).

Item	Control	OC10	OC20	SEM	*P*-value
DM, g/kg	495	518	499	8.12	0.095
CP, g/kg DM	150	148	147	1.13	0.183
EE, g/kg DM	294^{c}	505^{b}	661^{a}	21.6	≤0.001
NDF, g/kg DM	349^{c}	370^{b}	385^{a}	3.9	≤0.001
ADF, g/kg DM	254^{c}	273^{b}	297^{a}	2.78	≤0.001
ADL, g/kg DM	39.4^{c}	62.3^{b}	86.9^{a}	1.69	≤0.001
NFC, g/kg DM	402^{a}	364^{b}	341^{b}	4.77	≤0.001
WSC, g/kg DM	61.0	62.5	59.3	2.07	0.493
TP*, g/kg DM	9.40^{a}	6.22^{b}	4.56^{c}	0.26	≤0.001

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR; SEM=Standard error of mean; DM=Dry matter; CP=Crue protein; EE=Ether extract; NDF= neutral detergent fiber; ADF=acid detergent fiber; ADL= acid detergent lignin; NFC=non-fibrous carbohydrates; WSC=water soluble carbohydrate; TP=Total phenolic compounds.

* TP is presented as Gallic acid equivalent (GAE)

^{a,b,c} Means within the same row with different superscripts differ significantly according to Tukey's post hoc test.

Table 5 presents the Flieg score, along with the values of RFV, NE_L and TDN for the ETMRs opened after 60 d of ensiling. The OC20 treatment had the lowest Flieg score, significantly lower than the control but comparable to OC10 ($P=0.016$). RFV, NE_L and TDN values were highest in control, followed by OC10, with the lowest values observed in the OC20 treatment ($P\leq0.001$). Figure 2 illustrates the aerobic stability of fresh pre-ensiled TMRs (day 0) and ETMRs that were opened on days 21, 42, and 60. On day 0 and 21, the OC10 treatment exhibited greater aerobic stability compared to both control and OC20 ($P\leq0.05$). However, OC treatments did not significantly influence aerobic stability in ETMRs opened on days 42 and 60 ($P>0.05$).

The *in vitro* digestibility parameters (IVTD, IVOMD, IVNDFD) for the ETMRs opened after 60 d of ensiling ae shown in Table 6. The values for IVTD and IVOMD were significantly lower in the OC20 treatment compared to both the control and OC10 treatments (*P*≤0.001). Additionally, IVNDFD was significantly higher in the OC10 treatment, followed by the control, while the OC20 treatment exhibited the lowest value (*P*≤0.001).

Table 3. The chemical composition of the ensiled total mixed rations (ETMRs) after 60 d of ensiling.

Item	Control	OC10	OC20	SEM	*P*-value
DM loss, %	4.58	2.69	1.26	0.93	0.091
CP, g/kg DM	153^{a}	148^{a}	142^{b}	1.64	0.021
EE, g/kg DM	22.0^{c}	50.1^{b}	77.3^{a}	1.84	≤0.001
NDF, g/kg DM	378^{b}	398^{a}	407^{a}	4.07	≤0.001
ADF, g/kg DM	263^{c}	283^{b}	305^{a}	1.95	≤0.001
ADL, g/kg DM	44.1^{c}	68.7^{b}	93.1^{a}	1.11	≤0.001
NFC, g/kg DM	377^{a}	336^{b}	313^{c}	5.09	≤0.001
WSC, g/kg DM	26.3^{a}	22.9^{b}	23.4^{b}	0.70	0.018
TP*, g/kg DM	9.80^{a}	5.97^{b}	6.17^{b}	0.16	≤0.001

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR; SEM=Standard error of mean; DM=Dry matter; CP=Crue protein; EE=Ether extract; NDF= neutral detergent fiber; ADF=acid detergent fiber; ADL= acid detergent lignin; NFC=non-fibrous carbohydrates; WSC=water soluble carbohydrate; TP=Total phenolic compounds.

* TP is presented as Gallic acid equivalent (GAE)

^{a,b,c} Means within the same row with different superscripts differ significantly according to Tukey's post hoc test.

Table 4. Fermentation profile of the ensiled total mixed rations (ETMRs) after 60 d of ensiling.

Item	Control	OC10	OC20	SEM	*P*-value
NH_3-N, g/kg Total N	36.8^{a}	30.2^{b}	35.3^{a}	0.75	≤0.001
Lactic acid, g/kg DM	62.6	66.3	69.7	4.12	0.493
Acetic acid, g/kg DM	19.5	25.8	21.3	1.79	0.057
Propionic, acid g/kg DM	2.06^{bc}	3.10^{a}	2.50^{ab}	0.22	0.013
Butyric acid, g/kg DM	ND^{b}	0.08^{a}	0.08^{a}	0.003	≤0.001
Isobutyric acid, g/kg DM	0.03^{a}	0.02^{a}	ND^{b}	0.005	≤0.001
Valeric acid, g/kg DM	0.05	0.05	0.05	0.001	0.779
Isovaleric acid, g/kg DM	0.09^{b}	0.12^{a}	0.12^{a}	0.006	0.004

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR; ND= Not detected; SEM=Standard error of mean.

^{a,b,c} Means within the same row with different superscripts differ significantly according to Tukey's post hoc test.

Table 5. Flieg score and some quality parameters of the ensiled total mixed rations (ETMRs) after 60 d of ensiling

Item	Control	OC10	OC20	SEM	*P*-value
Flieg score	112^{a}	107^{ab}	100^{b}	1.70	0.016
RFV	168^{a}	155^{b}	148^{c}	1.89	≤0.001
NE$_{L}$	1.60^{a}	1.55^{b}	1.50^{c}	0.05	≤0.001
TDN	67.2^{a}	64.7^{b}	61.8^{c}	0.25	≤0.001

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR; RFV= Relative feed value; NE$_{L}$= Net energy lactation; TDN=total digestible nutrient; SEM=Standard error of mean

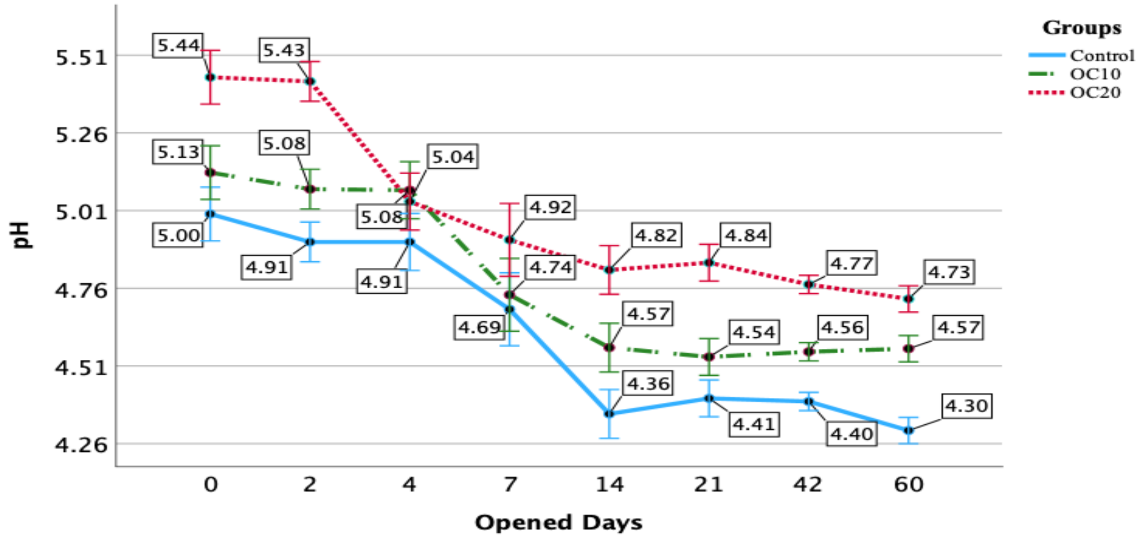
^{a,b,c} Means within the same row with different superscripts differ significantly according to Tukey's post hoc test.

Table 6. The *in vitro* true digestibility of the ensiled total mixed rations (ETMRs) after 60 d of ensiling.

Item	Control	OC10	OC20	SEM	*P*-value
IVTD, %	68.0^{a}	69.0^{a}	63.3^{b}	0.49	≤0.001
IVOMD, %	69.4^{a}	70.2^{a}	64.7^{b}	0.49	≤0.001
IVNDFD, %	24.6^{b}	30.5^{a}	18.7^{c}	1.25	≤0.001

Control= 0% olive cake in TMR; OC10= 10% olive cake in TMR; OC20= 20% olive cake in TMR; IVDM=*In vitro* true digestibility; IVOM= *in vitro* degradation of organic matter*;* IVNDF=*In vitro* degradation of neutral detergent fiber; SEM=Standard error of mean

^{a,b,c} Means within the same row with different superscripts differ significantly according to Tukey's post hoc test.



P<0.001 for opened days 2,14,21,42, and 60
P<0.05 for opened days 4, and 7

Figure 1. The pH value of the pre-ensiled TMRs (day 0) and ETMRs opened on different days.

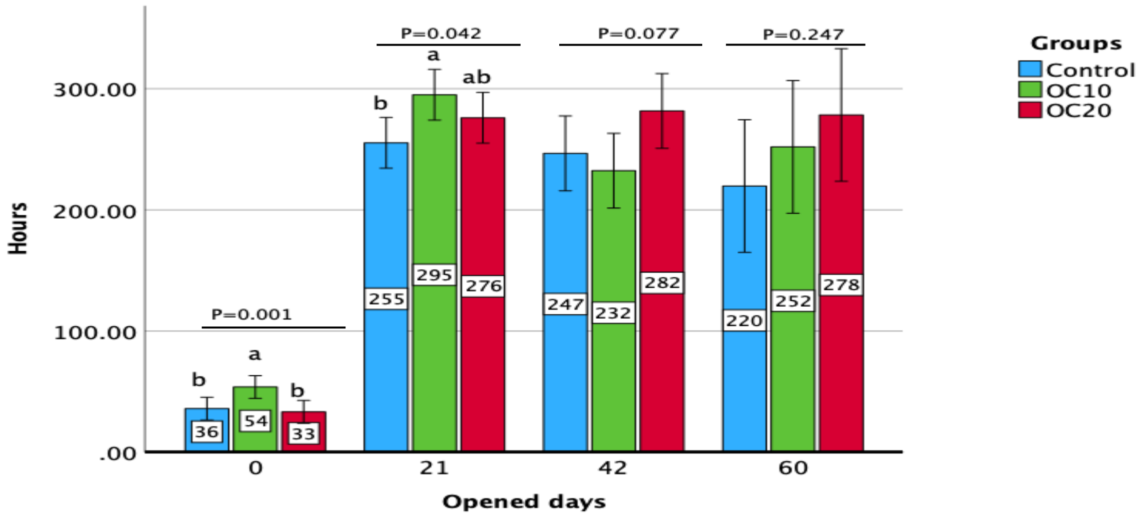


Figure 2. The aerobic stability of the pre-ensiled TMRs (day 0) and ETMRs opened on different days (21, 42 and 60).

DISCUSSION

Although OC is known as an unpalatable by-product, it is an alternative feed and can be used in place of high moisture forage in ruminant diets such as maize silage. Nonetheless, ETMR could support the increasing use of unpalatable by-products in animal diets by altering odors and flavors due to the ensiling process. Therefore, determining the fermentation characteristics and *in vitro* digestibility of ETMR ensiled with OC, may be a useful contribution to a better understanding for effective OC use in ruminant diets.

Previous studies have reported greater concentrations of EE and structural carbohydrates in OC in comparison with maize silage (Abarghoei et al., 2011; Duru, 2012). In line with these findings, inclusion of OC increased the EE, NDF, ADF, and ADL concentrations in TMRs. However, no significant differences were seen in DM, CP, and WSC contents of fresh TMRs prepared with the inclusion of OC. Various studies have demonstrated greater TP concentrations in OC (Khalili and Dinani, 2018; Foti et al., 2022). However, control group showed higher TP concentration than OC10 (-3.18 GAE g/Kg DM) and OC20 (-4.56 GAE g/Kg) groups in the present study. This can be explained by a positive correlation between lactic acid bacteria and TP content in silage (Fan et al., 2020). Although the population of lactic acid bacteria was not analyzed in maize silage in our study, it is known that maize silages contain large numbers of lactic acid bacteria (Filya and Sucu, 2010). Therefore, lower TP concentrations in fresh OC10 and fresh OC20 groups could be associated with lower inclusion levels of maize silage in these groups.

In the current study, DM loss was below 5% and numerically higher in control than in OC10 (-1.89%) and OC20 (+3.32%). Dong et al. (2011) stated that lignin, a polyphenolic polymer, not only reduces NDF digestibility but also negatively affects the digestibility of other organic fractions. However, OC inclusion increased the ADL concentration in experimental ETMRs. Therefore, lignin may prevent DM loss by reducing microbial enzyme activity during fermentation. Furthermore, the addition of maize silage containing organic acids has been suggested as a protective feed additive (Kim et al., 2021) that might have contributed to improve the DM recovery in all ETMRs. Thus, any marked differences in DM recovery did not show up among the groups in the present study. The inclusion of OC increased EE, NDF, ADF and ADL while decreasing CP, NFC and WSC in ETMRs. The present study is the first trial that describes the effects of OC in the ETMRs. Similar results were observed when OC was ensiled with different feed materials in the previously available studies. Abarghoei et al. (2011) stated that structural carbohydrates were increased by ensiling OC-molasses mixture compared to ensiled OC alone. Moreover, Duru and Kaya (2016) found that ensiled OC-maize mixture had lower CP and higher EE concentrations than ensiled maize. Control group had a higher WSC (3.3 g/kg DM) content than the other groups, although it was similar in fresh TMRs. This result could be related to the different maize silage rates in the TMRs. It is known that WSC is used in the first stage of fermentation in silages (Yang et al., 2021). However, the higher maize silage content of the control could cause a rapid reduction in pH and the elimination of bacteria that use WSC in silage.

It has been reported that the fermentation process reduces the TP content of ensiled OC (Abarghoei et al., 2011; Alhamad et al., 2017). However, in our study, TP concentrations were prevented in all groups. The different ensiling methods may explain the difference. In general, OC was ensiled in larger commercial silos (>50 kg) in previous studies. Whereas a laboratory vacuum machine was used to ensile ETMRs package (250-300 g) and the fermentation process was well controlled in the current study. In support of this idea, Fan et al. (2020) stated that ensiling artichoke by-products (200 g) by using vacuum machine increased phenolic compounds including gallic acid.

In the present study, all groups had NH_3-N concentration below 40 g/kg total N. Chamberlain and Wilkonson (2000) stated that lower NH_3-N concentrations (≤50 g/kg total N) is a crucial indicator for good quality in silages. Therefore, it can be argued that the ETMRs, with or without OC, exhibited a high level of quality in this study.

In numerous studies, ETMRs have exhibited lactic acid concentrations exceeding 80 g/kg DM (Weinberg et al., 2011; Restelatto et al., 2019; Chen et al., 2014). Nevertheless, the LA proportion of ETMRs was under 70 g/kg DM in the present study. The differing compositions of the feed materials used in TMRs may have contributed to this outcome. The inclusion of salt, sodium bicarbonate and alfalfa hay have the potential to enhance the buffering capacity (Bueno et al., 2020) and restrict the formation of lactic acid in experimental ETMRs. Moreover, the incorporation of OC into ETMRs did not influence the concentration of acetic acid and valeric acid. It is established that anaerobic bacteria reduce pH by converting WSC to organic acids during the fermentation period (Kim et al., 2021). However, the similar WSC contents in ETMRs may be associated with analogous organic acid composition. The quantity of BA in ETMRs was markedly low in OC10 and OC20 (≤0.1 g/kg DM) and was undetected in the control. Consequently, it can be considered that the fermentation process was effective in the experiment.

In all groups, pH dropped to 5 at the end of the first week during the fermentation period. Furthermore, it was observed that the drop in pH levels was not noticeable from day 14 onwards in all experimental groups. Although no previous study has investigated the fermentation profile of the ETMR ensiled with OC, similar results were found when ensiled OC with molasses (Weinberg et al., 2008) or with maize (Duru and Kaya, 2016). In addition, the use of an already fermented product (maize silage) in TMRs could contribute to the pH during the fermentation period. The maize silage not only gave acidity to fresh TMRs but also had lower WSC because of the fermentation. Therefore, it could cause the slight difference in pH after 14 days of fermentation due to the absence of WSC. Similar to our findings, Yang et al. (2021) declared that the pH reduction was slight (from 4.59 to 4.49) after day 15 in ETMR with oat silage.

In the current study, pH was positively associated with OC inclusion. Similarly, Duru (2012) explained that increasing the OC rate (from 20 to 40% DM basis) in ensiled OC-maize mixture increased the pH. Weingberg et al. (2008) observed that ensiled OC had higher pH compared to ensiled OC-maize mixture. This result may be related to the pH of fresh TMRs, which increased with increasing OC levels. In support of our study, Yang et al. (2021) observed the same differences in pH levels in both fresh and ensiled TMRs after 60 d of fermentation.

Flieg score may be a useful indicator to determine quality of ensiled feed materials. Yalçınkaya et. al (2012) reported that Flieg score > 80 suggests "excellent quality" for ensiled feed materials. However, the lowest Flieg score was 100 (in OC20) in the study. The RFW is an index that describes the quality of forage in relation to NDF and ADF concentrations. In the present study, RFW showed a negative association with OC inclusion rate in ETMRs. This can be explained by the increasing concentration of NDF and ADF with the addition of OC in ETMRs. Similar results were observed in NEL and TDN. Due to higher ADL concentration of OC, NE_L and TDN decreased with increasing OC inclusion in the ETMRs.

Like our findings, it was reported that the temperature in ETMRs was stable within 200 h after air exposure in previous studies (Hao et al., 2015; Xie et al., 2022). The OC10 had higher aerobic stability on opened days 0 and 21 compared to control and OC20. Burt (2004) indicated that the essential oils extracted from leaves, fruits, seeds are known to possess antibacterial, and antifungal properties. However, high dosage oil supplementation can elevate feed water activity and increase the risk of mold growth in the feed (Liu et al., 2017). In support of previous studies, our results reveal that the EE content had a decisive influence on the aerobic stability of the ensiled feed.

Wilkinson and Davies (2013) found that the aerobic stability of the ensiled forage was affected not only by DM, pH and organic acid but also by room temperature. However, the aerobic stability was similar in the present study, although significant differences were observed in pH value of ETMRs opened on day 42 and 60. The temperature of the laboratory where the aerobic stability was analyzed (January and February 2024) may be a reason for this result. In support of this idea, Koc et al. (2009) explained that aerobic deterioration was faster when the room temperature was above 20ºC. Whereas the temperature was 18±2ºC in the laboratory through the aerobic stability analysis.

The *in vitro* true digestibility parameters (IVTD, IVOMD, IVNDFD) were lower in OC20 than in control and in OC10 (average -5.2%, -5.1% and -8.8% respectively; Table 6). It is well known that a high oil diet is associated with lower nutrient digestibility, especially in ruminants (Klusmeyer et al., 1991; Jenkins, 1993). However, the EE concentrations of OC20 (7.7% DM basis) were at the upper critical level for optimal nutrient digestibility. Therefore, excessive EE level in OC20 may be a reason for lower DM, OM, and NDF digestibility.

Conclusions: The olive cake increased the EE and structural carbohydrates in ETMRs. The NH_3-N and butyric acid concentrations of ETMRs demonstrated that ensiled TMRs with OC had a good quality fermentation profile. DM recovery was improved with increasing OC levels in ETMRs. TP content was not affected by ensiling process. The aerobic stability was higher in ETMRs than fresh TMRs and was not affected by OC treatments. However, *in vitro* nutrient digestibility was dramatically reduced when OC 20% (DM basis) was used due to its oil content. Therefore, the OC should be added up to %10 of DM in ruminant diets. Moreover, the results from of the current study were obtained under the laboratory conditions. Further experiments are needed to describe the effects of OC inclusion in ETMRs with commercial silos and nutrient digestibility in feeding trials.

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Author Contributions

Murat ER: Conceptualization, Methodology, Writing – original draft, Funding acquisition, Formal analysis. Data curation, Validation, **Umair Ahsan:** Investigation, Methodology, Formal analysis, Data curation, Review & editing. **Eren Kuter**: Investigation, Methodology, Formal analysis, Data curation. **Hulusi Akçay:** Investigation, Methodology. **Muhammad Shazaib Ramay:** Investigation, Editing. **Derya Merve Karagöz, Mehmet Onur Takka:** Investigation.

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