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SHORT RESEARCH AND DISCUSSION ARTICLE

Bioconversion of alperujo into an alternative feed for ruminants by pretreatment with live yeasts and/or exogenous fibrolytic enzymes

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Abstract

Extraction of olive oil through a two-stage centrifugation process produces a large amount of phytotoxic waste known as alperujo. This research was performed to bioconvert alperujo into enriched ruminant feed by pretreatment with exogenous fibrolytic enzymes (EFE) or/and live yeasts (LY). These additives were used in a completely randomized design with 3 EFE doses (0, 4, and 8 µl/g dry matter) and 3 LY doses (0, 4, and 8 mg/g dry matter) in a 3 × 3 factorial arrangement. Fermented alperujo with both EFE doses converted some of their hemicellulose and cellulose to simple sugars and increased bacterial abundance in the rumen. As a result, it shortens the lag time of rumen fermentation, increases the rate and amount of rumen fermentation, and improves digestibility. This improvement provides additional energy that can be used by ruminants to produce milk and by rumen microbiota to produce short-chain fatty acids. Fermented alperujo with a high dose of LY decreased their antinutritional compounds and reduced their high content of lipid. In the rumen, this waste became rapidly fermentable, and rumen bacteria became more abundance. Fermented alperujo with a high dose of LY + EFE accelerated rumen fermentation and improved rumen digestibility, energy available for milk production, and short-chain fatty acids compared to the use of LY or EFE alone. This synergistic interaction between these two additives increased protozoa abundance in rumen and the ability of rumen microbiota to bioconvert ammonia–nitrogen to microbial protein. Ultimately, fermentation alperujo with EFE + LY is a good strategy with minimum investment for a social sustainable economy and environment.

Keywords Energy feed · Environmental · Feed additives · Fermentation · Valorization · Waste management

Introduction

The olive oil sector is one of the most important socio-economic activities in the Mediterranean region (Dahdouh et al. 2023), whose production has tripled since 1960, reaching about 3×10^6 tons in the 2021–2022 season, representing more than 90% of world production. Spain, Italy, and Tunisia are the main producers of olive oil, accounting for 42.9%, 10.2%, and 7.7% of world production, respectively (International Olive Council 2022). Three methods are used in olive oil extraction: the traditional press, the three-stage method, and the two-stage centrifugation method (Dahdouh et al. 2023). The use of the traditional method has declined and has been replaced by the three-stage and the two-stage continuous extraction methods, which use a centrifuge to separate solid from liquid streams (Dahdouh et al. 2023). The two-stage centrifugation system has become the predominant system used for olive oil extraction in many countries (Alburquerque et al. 2004). This extraction method significantly reduces water consumption and liquid waste compared to a three-stage system. However, it generates a large amount of semi-solid waste known as alperujo, which is a mixture of plant water, peels, pulp, and pits of olives and represents 80% of processed olives (Marcos et al. 2019). This waste is characterized by high acidity, phenolic components, and sulfur dioxide emissions that damage soil structure, reduce soil microbial population and plant performance, contaminate water resources, and generate undesirable odors (Alburquerque et al. 2004; Moreno-Maroto et al. 2019; Dahdouh et al. 2023). Therefore, the appropriate

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recycling process for this waste represents an economic and environmental challenge for the sustainable development of the olive oil sector.

Several studies have proved that wastes from the olive oil industry wastes can be used as a low-cost alternative feed for ruminants, improving milk and meat quality and animal health, and increasing livestock breeders' income (Zagmutt et al. 2016; Obeidat and Kridli 2021). However, their high content of lignocellulose makes them resistant to ruminal microbial enzymes, and their high content of polyphenols and tannins inhibits the growth of the ruminal microbiota, so much of their nutrients cannot be degraded in the rumen and much of their energy cannot be metabolizable by ruminants (Yáñez-Ruiz and Molina-Alcaide 2007; Marcos et al. 2019). Therefore, their use in ruminant diets remains very limited and is mainly used in non-intensive ruminant systems for animals with low productivity and mainly in periods of scarce feed resources (Obeidat and Kridli 2021). Due to the recent global crises—the COVID-19 pandemic, the Russian–Ukrainian war, and the global warming, the feed resources have become scarce, and their price has increased exponentially (Du et al. 2022; Galanakis 2023). Therefore, the valorization of agro-industrial by-products, such as byproducts of olive oil extraction, as alternative feed and the improvement of their nutritional value has become a very interesting research direction that needs to be further investigated.

Our previous studies have proved that pretreatment of solid waste from the olive oil industry using the traditional press or the three-stage method at 26 °C with exogenous fibrolytic enzymes (EFE) produced from *Trichoderma longibrachiatum* bioconvert part of their cell-wall polysaccharides into simple sugars, during preincubation. Consequently, it increases ruminal fermentation, nutrient digestibility, palatability, and growth performance of ruminants without affecting their health (Abid et al. 2020, 2022a). Recent researches have also proved that incorporation of live yeasts (LY) containing *Saccharomyces cerevisiae* in rumen feed is beneficial to animals. In the rumen, these probiotics scavenge oxygen, stabilize rumen pH, and produce several beneficial nutrient cofactors such as organic acids, peptides, and vitamins that stimulate and facilitate the growth and proliferation of the beneficial rumen microbiota (Elghandour et al. 2022), resulting in improved of nutriment digestibility, feed palatability, growth performance and milk production, animal health, and providing a clear economic advantage for sheep, buffalo, calf, and cow breeding (Anjum et al. 2018; Villot et al. 2019; Wang et al. 2022; Xue et al. 2022; Hiltz et al. 2023). In recent years, many experiments have proved that fermenting feeds such as potato peel, orange pulp, sobyan meals, and food waste with *Saccharomyces cerevisiae* before offering them to animals is a good strategy to reduce their antinutritional factors and improve their nutritional value (Hassaan et al. 2015; Maxwell et al. 2018; Guerra et al. 2021; Li et al. 2022). The LY, including the *Saccharomyces cerevisiae*, is the best microorganism that effaces fermented feeds in an aerobic environment and enhances their quality (Rai et al. 2019; Li et al. 2022). Temperature is the most important parameter affecting the growth of *Saccharomyces cerevisiae*, which grows well between 25 and 33 °C. The temperatures between 25 and 30 °C are the optimum for their cell reproduction (Yalcin and Ozbas 2008).

To our knowledge, there is no information on the pretreatment of alperujo by LY and EFE in ruminant nutrition. Our hypothesis was that fermentation of alperujo with EFE or LY would improve their nutritional value and that synergy between these two additives could be established. The aim of this study is to bioconvert this phytotoxic waste into enriched alternative feed for ruminants through EFE and LY pretreatments in order to reduce the environment pollution of olive oil mills using a two-stage centrifugation system.

Materials and methods

Collect of alperujo and pretreatment with biological additives

Fresh alperujo were harvested immediately after olive oil extraction from olive oil mills located in Sfax, Tunisia, using a two-stage centrifugation system. This waste was dried at 55 °C for 48 h and ground to 1 mm size to obtain small homogeneous particles. Then, 2 ml of sterile distilled water contended 0 (control), 4 µl EFE, 8 µl EFE, 4 mg LY, 4 mg LY + 4 µl EFE, 4 mg LY + 8 µl EFE, 8 mg LY, 8 mg LY + 4 µl EFE, 8 mg LY + 8 µl EFE was added to 1 g dry matter alperujo. These solid-state fermentations were realized at a 120 rpm shaker in an aerobic environment at a temperature of 26 °C for 24 h. The EFE used in this study was produced by *Trichoderma longibrachiatum* (Dyadic International Inc. Jupiter, Florida, USA) and consisted of 2267 UI xylanases, 1161UI endoglucanase, and 113 UI exoglucanase per ml (Abid et al. 2022a). The LY used in this study contained 5×10^{10} CFU *Saccharomyces cerevisiae* per g dry matter (Yea- Sacc1026, Alltech Inc, Nicholasville, KY, USA).

Chemical composition

The chemical composition of fresh, unfermented alperjo was determined immediately after the collection from industry and after treatment with these additives. Crude protein, ether extracts, and ash were analyzed according to the procedures of AOAC (2016). Total polyphenols were determined by the Folin-Ciocalteu colorimetric method using a spectrophotometer (Shimadzu UV-1201 UV–Vis spectrophotometer) at 725 nm absorbance (Makkar et al. 1993). Condensed tannins were analyzed by the acid-butanol-HCl-Fe method

using a spectrophotometer (Shimadzu UV-1201 UV–Vis spectrophotometer) at an absorbance of 550 nm (Makkar et al. 1993). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using the ANKOM220 fiber analyzer (ANKOM technology, Macedon, NY, USA) (Van Soest et al. 1991). Cellulose was determined by subtracting the acid detergent lignin from the acid detergent fiber (Van Soest et al. 1991). Hemicellulose was determined by subtracting the acid detergent fiber from the neutral detergent fiber (Van Soest et al. 1991). Total sugars were determined by the phenol–sulfuric acid method using a spectrophotometer (Shimadzu UV-1201 UV–Vis spectrophotometer) at an absorbance of 490 nm following the procedure of Dubois et al. (1956).

In vitro ruminal incubation

In vitro rumen fermentation was performed according to the gas production technique method of Theodorou et al. (1994). Briefly, the rumen contents of 2 slaughtered adult Holstein cows (700 kg body weight) were obtained from different parts of the rumen. Before slaughter, the cows were fed 7 kg of oat hay and 3 kg of commercial concentrate. The rumen inoculum was filtered through four layers of cheesecloth, mixed in equal volume ratio, and immediately transported to the laboratory in a thermos flask maintained at 39 °C and flushed with CO_2. In the laboratory, rumen inoculum was mixed with an artificially buffered solution in a 1:2 (v/v) ratio according to Menke and Steingass (1988). Two hundred milligrams of dry matter of alperujo was weighed into amber glass serum bottles of 120 ml and incubated with 30 ml of the buffered rumen inoculum. These bottles were immediately closed with rubber stoppers and metal ring and incubated in a water bath at 39 °C. All preparations were performed at a temperature of 39 °C and in an anaerobic environment. Gas pressure was measured after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation using a pressure transducer connected to a data logger. The gas produced was corrected for the gas produced in the negative control bottles (which contained only the buffered rumen inoculum) and converted to volume. This experience was repeated three times (3 runs). For each run, three replicates of each pretreatment were performed (3 replicates).

Fermentation kinetics parameters were determined from the adjusted cumulative gas volume using a nonlinear process from SAS (2018) following the model of France et al. (2000) described in Eq. 1:

$$\mathrm{GP}_{(t)} = B\left(1 - e^{-C(t-\mathrm{Lag})}\right) \tag{1}$$

where GP is net gas production (ml/g dry matter); t is incubation time (h); B is potential gas production (ml/g dry matter); C is constant gas production rate (ml/h); and Lag is lag phase (h).

The energy available for milk production was determined according to the equation of Menke and Steingass (1988) described in Eq. 2:

$$\begin{aligned}\text{Energy available for milk production} &= 0.101 \times \mathrm{GP24} + 0.051 \times \mathrm{CP} \\ &\quad + 0.112 \times \mathrm{EE}\end{aligned} \tag{2}$$

where energy available for milk production (MJ/kg dry matter); GP24 is net gas production (ml) from 200 mg dry matter after 24 h of incubation; CP is crude protein (% dry matter); and EE is ether extracts (% dry matter).

Total short-chain fatty acids were calculated according to the equation of Getachew et al. (1998) described in Eq. 3:

$$\text{Total short-chain fatty acids} = -0.00425 + 0.0222 \times \mathrm{GP24} \tag{3}$$

where total short-chain fatty acids (mmol/ 200 mg dry matter) and GP24 is net gas production (ml) from 200 mg dry matter after 24 h of incubation.

At the end of incubation, rumen pH was immediately measured using a pH meter (Jenway Ltd Felsted, model 3020, England) calibrated with buffers (pH 4 and 7). The contents of each serum bottle were filtered using filter paper disks (Whatman 541). The residues were collected and dried at 55 °C for 48 h to determine the digestibility of the dry matter using Eq. 4:

$$\text{Dry matter digestibility (\%)} = \frac{\text{initial weight (dry matter)} - \text{residual weight (dry matter)}}{\text{initial weight (dry matter)}} \times 100 \tag{4}$$

Microbial protein synthesis was determined according to the equation of Blümmel et al. (1997) described in Eq. 5:

$$\text{Microbial protein synthesis} = \mathrm{DMD} - 2.2 \times \mathrm{GP24} \tag{5}$$

where the microbial protein synthesis (mg/g dry matter); DMD is the amount of digestible dry matter (mg/g) at the end of incubation, and GP24 is the net gas production (ml) from 200 mg dry matter of the substrate after 24 h of fermentation.

Ruminal ammonia–nitrogen was analyzed according to the protocol of Broderick and Kang (1980). Briefly, 5 ml of the filtered liquid was preserved with 0.5 mol/L H_2SO_4, centrifuged at 16,000 × g for 15 min, and the supernatant was stored at − 20 °C. Rumen ammonia–nitrogen concentration was determined by the

Table 1 Chemical composition of fresh unfermented alperujo (g/kg dry matter)

CP	EE	NDF	ADF	ADL	Ce	HC	Ash	TP	CT	TS
117.8 ± 2.1	106.1 ± 3.3	649.4 ± 6.0	428.3 ± 5.1	223.1 ± 2.3	205.2 ± 4.2	221.1 ± 6.4	107.8 ± 3.8	55.6 ± 0.9	38.3 ± 0.3	22.6 ± 0.9

CP is crude protein. *EE* is ether extracts. N*DF* is neutral detergent fiber. *ADF* is acid detergent fiber. *ADL* is acid detergent lignin. *Ce* is cellulose. *HC* is hemicellulose. *TP* is total polyphenols. *CT* is condensed tannins. *TS* is total sugars

phenol-hypochlorite method using a spectrophotometer (Shimadzu UV-1201 UV–Vis spectrophotometer) at 630 nm absorbance. Ruminal protozoa and bacteria were counted in the filtered liquid according to the methods of Galyean (1989).

Statistical analysis

All collected data were statistically analyzed using the general linear model (GLM) procedure of SAS (2018). The statistical module used was

$$Y_{ijk} = \mu + EFE_i + LY_j + (EFE \times LY)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents the dependent variable; μ represents the overall mean; EFE_i represents the effect of the i^{th} EFE ($i = 0$, 4, and 8); LY_j represents the effect of the j^{th} LY ($i = 0$, 4, and 8); $(EFE \times LY)_{ij}$ represents the interaction between the i^{th} EFE and the j^{th} LY; and ε_{ijk} represents the residual experimental error. The differences between treatments were compared using Tukey's multiple range test. Significant was declared when $p < 0.05$.

Results and discussion

The analysis of the chemical composition of the fresh unfermented alperujo obtained from different industrial of extraction of olive oil using a two-stage centrifugation system in Tunisia is shown in Table 1. This semi-solid waste is characterized by a high content of lignocellulosic compounds similar to those of cereal straw widely used in rumen feeding, but with a higher content of lignin, protein, and EE (Datsomor et al. 2022; Sufyan et al. 2022). The chemical composition of this semi-solid waste was comparable to that found by Marcos et al. (2019) in alperujo from Spain. In addition, higher levels of polyphenols and condensed tannins were found in alperjo from Tunisia than in alperjo from Spain (Yáñez-Ruiz and Molina-Alcaide 2007). These differences can be explained by the diversity in the varieties of olive, fruit maturation, and agronomic conditions.

The effects of pre-treatment of alperujo with EFE and LY on their chemical composition are shown in Table 2. Both EFE dosages reduced ($p < 0.05$) their high lignocellulose content and increased ($p < 0.05$) their total sugar content. This result is consistent with previous researches in which

Table 2 Influence of pretreatment of alperujo with live yeasts and/or exogenous fibrolytic enzymes on chemical composition (g/kg dry matter)

	LY_0			LY_1			LY_2			ESM	*P* value		
	EFE_0	EFE_1	EFE_2	EFE_0	EFE_1	EFE_2	EFE_0	EFE_1	EFE_2		EFE	LY	EFE × LY
CP	117	119	118	120	119	121	118	120	122	2.1	0.84	0.88	0.86
EE	106^a	104^a	105^a	102^a	98^a	100^a	89^b	87^b	86^b	4.6	0.77	< 0.001	0.76
NDF	650^a	573^b	560^b	648^a	568^b	554^b	648^a	568^b	552^b	14.1	< 0.001	0.91	0.87
ADF	429^a	393^b	384^b	430^a	392^b	383^b	430^a	391^b	381^b	13.4	< 0.001	0.90	0.71
ADL	223	218	222	222	220	219	220	221	218	4.9	0.91	0.92	0.92
Ce	206^a	175^b	162^b	208^a	172^b	164^b	210^a	170^b	163^b	11.5	< 0.001	0.93	0.68
HC	221^a	180^b	176^b	218^a	176^b	171^b	218^a	177^b	171^b	10.6	< 0.001	0.88	0.65
Ash	109	108	103	107	104	103	108	103	105	5.2	0.73	0.85	0.74
TP	55.9^a	55.4^a	55.5^a	54.9^a	55.0^a	54.8^a	50.1^b	49.9^b	48.3^b	1.74	0.81	0.002	0.77
CT	38.4^a	38.2^a	38.4^a	37.9^a	38.0^a	38.1^a	33.5^b	33.4^b	33.1^b	0.63	0.83	0.001	0.78
TS	22.5^b	32.4^a	33.4^a	23.0	32.2^a	33.3^a	22.7^b	32.5^a	33.5^a	3.9	0.009	0.84	0.85

[a,b] Means of each chemical fraction with different superscripts are significantly different at $p < 0.05$ (Tukey test). EFE_0 is alperujo untreated with exogenous fibrolytic enzymes. EFE_1 is alperujo pretreated with exogenous fibrolytic enzymes at 4 µl g/dry matter. EFE_2 is alperujo pretreated with exogenous fibrolytic enzymes at 8 µl/g dry matter. LY_0 is alperujo untreated with live yeasts. LY_1 is alperujo pretreated with live yeasts at 4 mg g/dry matter. LY_2 is alperujo pretreated with live yeasts at 8 mg/g dry matter. *SEM* is the standard error of means. *CP* is crude protein. *EE* is ether extracts. *NDF* is neutral detergent fiber. *ADF* is acid detergent fiber. A*DL* is acid detergent lignin. *Ce* is cellulose. H*C* is hemicellulose. *TP* is total polyphenols. *CT* is condensed tannins. *TS* is total sugars

Table 3 Influence of pretreatment of alperujo with live yeasts and/or exogenous fibrolytic enzymes on rumen fermentation characteristics and microflora

	LY_0			LY_1			LY_2			SEM	*P* value		
	EFE_0	EFE_1	EFE_2	EFE_0	EFE_1	EFE_2	EFE_0	EFE_1	EFE_2		EFE	LY	EFE×LY
B	89.3^{c}	133.5^{b}	136.1^{b}	92.0^{c}	130.8^{b}	136.9^{b}	91.7^{c}	131.0^{b}	145.7^{a}	8.77	< 0.001	0.49	< 0.001
C	0.038^{c}	0.044^{b}	0.045^{b}	0.039^{c}	0.045^{b}	0.046^{b}	0.047^{b}	0.048^{b}	0.062^{a}	0.005	< 0.001	0.002	< 0.001
Lag	0.71^{a}	0.43^{b}	0.49^{b}	0.70^{a}	0.44^{b}	0.49^{b}	0.49^{b}	0.46^{b}	0.44^{b}	0.06	< 0.001	< 0.001	0.67
pH	6.46^{a}	6.45^{a}	6.30^{b}	6.45^{a}	6.44^{a}	6.45^{a}	6.46^{a}	6.44^{a}	6.43^{a}	0.09	0.02	0.87	0.81
NH_3-N	232^{a}	229^{a}	228^{a}	233^{a}	231^{a}	229^{a}	230^{a}	224^{a}	209^{b}	8.4	0.87	0.88	0.02
DMD	254^{c}	285^{b}	288^{b}	253^{c}	284^{b}	290^{b}	265^{c}	284^{b}	363^{a}	10.1	0.004	0.19	< 0.001
EML	2.81^{c}	3.54^{b}	3.56^{b}	2.87^{c}	3.45^{b}	3.59^{b}	2.81^{c}	3.39^{b}	3.83^{a}	0.19	< 0.001	0.47	< 0.001
SCFA	0.22^{c}	0.38^{b}	0.39^{b}	0.24^{c}	0.37^{b}	0.39^{b}	0.26^{c}	0.39^{b}	0.49^{a}	0.04	< 0.001	0.46	< 0.001
MPs	231.6^{b}	246.5^{b}	249.1^{b}	228.6^{b}	246.9^{b}	250.5^{b}	238.6^{b}	244.8^{b}	314.1^{a}	22.4	0.09	0.38	< 0.001
TPort	5.2^{b}	5.3^{b}	5.5^{b}	5.3^{b}	5.4^{b}	5.4^{b}	5.3^{b}	5.6^{b}	6.4^{a}	0.3	0.81	0.80	0.003
TBact	4.0^{b}	4.9^{a}	5.1^{a}	4.2^{b}	4.9^{a}	5.1^{a}	4.7^{a}	4.9^{a}	5.0^{a}	0.4	0.007	0.008	0.79

[a,b,c] Means of each parameter with different superscripts are significantly different at $p < 0.05$ (Tukey test). EFE_0 is alperujo untreated with exogenous fibrolytic enzymes. EFE_1 is alperujo pretreated with exogenous fibrolytic enzymes at 4 µl g/dry matter. EFE_2 is alperujo pretreated with exogenous fibrolytic enzymes at 8 µl/g dry matter. LY_0 is alperujo untreated with live yeasts. LY_1 is alperujo pretreated with live yeasts at 4 mg g/dry matter. LY_2 is alperujo pretreated with live yeasts at 8 mg/g dry matter. *SEM* is the standard error of means. *B* is potential gas production (ml/g dry matter). *C* is constant gas production rate (ml/h). *Lag* is lag phase (h). *NH_3-N* is ammonia nitrogen (mg/l). *DMD* is dry matter digestibility (g/kg DM). *EML* is energy available for milk production (MJ/kg dry matter). *SCFA* is total short-chain fatty acids (mmol/200 mg dry matter). *MP* is microbial protein synthesis (mg/g dry matter). *TProt* is total protozoa (10^5 cells/ml). *TBact* is total bacteria (10^{10} cells/ ml)

various industrial wastes such as crude olive cake and sesame seed coats were pretreated with the same EFE preparation (Abid et al. 2022a, b). In contrast, the same preparation did not modify the chemical composition of Posidonia oceanica wastes (Abid et al. 2023). This variability may be attributed to the different on chemical composition and fiber structure of the treated substrates. Fermented alperujo with a high dose of LY significantly reduced their excessive antinutritional compounds (total polyphenol and condensed tannins) responsible for low digestibility and palatability (Kelln et al. 2020). This detoxification is due to the ability of LY to extract abundant extracellular enzymes, such as tannase, which hydrolyzes tannins into glucose and gallic acid or ellagic acid (Hawashi et al. 2019). In addition, this feed additive significantly decreased the excessive EE compounds of alperujo that reduce the attachment of the rumen microbiota to carbohydrates in the rumen (Joy et al. 2021). A comparable effect was found with gum seed kernels fermented with LY (Gunun et al. 2022).

The effects of EFE and LY on rumen fermentation and the nutritional value of alperujo are shown in Table 3 and Fig. 1. Pretreated alperujo with EFE significantly improves

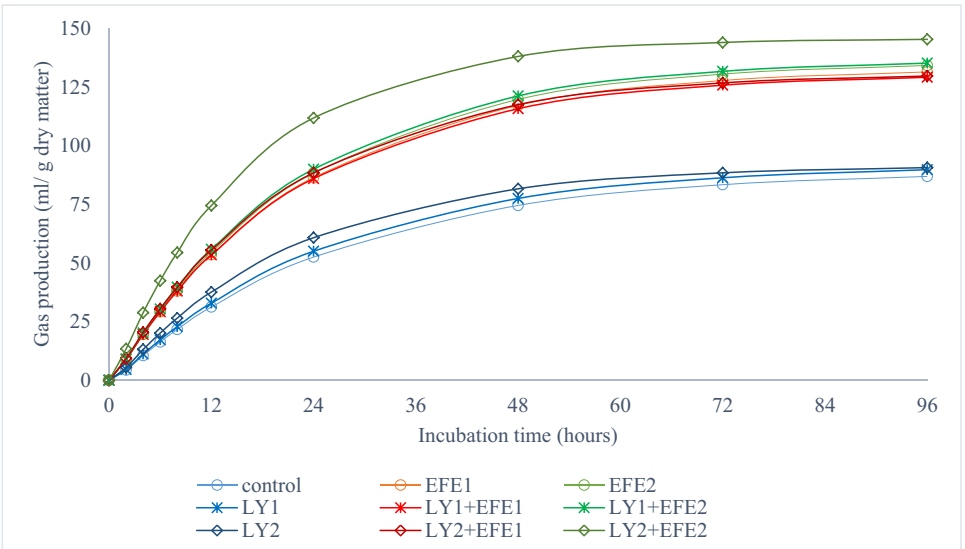

Fig. 1 Influence of pretreatment of alperujo with live yeasts and/or exogenous fibrolytic enzymes on the gas profile control is alperujo untreated. EFE_1 is alperujo pretreated with exogenous fibrolytic enzymes at 4 µl g/dry matter. EFE_2 is alperujo pretreated with exogenous fibrolytic enzymes at 8 µl/g dry matter. LY_1 is alperujo pretreated with live yeasts at 4 mg g/dry matter. LY_2 is alperujo pretreated with live yeasts at 8 mg/g dry matter

their fermentation in the rumen by shortening the delay phase of the onset of rumen formation and increasing the rate and amount of rumen fermentation, as well as improve their digestibility. This improvement provides additional energy that can be used by the ruminants for milk production and used by the rumen microbiota to form short-chain fatty acids. A comparable result was obtained with crude olive cake and sesame seed coats pretreated with the same EFE preparation (Abid et al. 2022a, b). Moreover, this feed additive significantly increased the bacterial abundance in rumen. These results are consistent with previous in vivo studies in which diets of Holstein bulls were pretreated with EFE preparations (Zhang et al. 2022). However, both doses of EFE used in this study did not improve the ability of the rumen microbiota to convert rumen ammonia–nitrogen to microbial protein. These results are consistent with previous in vivo studies in which diets of Jersey heifers and Holstein bulls were pretreated with EFE preparations (Gandra et al. 2017; Zhang et al. 2022). Fermented alperujo with a high dose of EFE decreased rumen pH due to an increase in rumen fermentation and short-chain fatty acid production without causing the risk of rumen acidosis (Van Soest 1994). A similar effect was found in vivo experiments when the EFE preparation was added to the feed of Holstein bulls (Zhang et al. 2022). Fermented alperujo with a high dose of LY improved the proliferation of rumen bacteria and accelerated the fermentation of this waste in the rumen similar to EFE. However, it did not modify the digestibility, energy available for milk production, ruminal pH, ruminal ammonia–nitrogen, and the ability of ruminal microbiota to produce short-chain fatty acids and microbial protein. These results are consistent with previous in vitro and in vivo experiments by Maamouri and Ben Salem (2022), who found improvement in rumen fermentation in fattening calves with the addition of LY without changes in rumen pH, organic matter digestibility, rumen ammonia–nitrogen, and metabolizable energy. However, Kholif et al. (2017) found that addition of the same LY to the diet of Nubian goats increased their digestibility and rumen pH, improved the capacity of rumen microbiota to produce total short-chain fatty acid, and decreased rumen ammonia–nitrogen. This difference could be due to several factors, such as the differences between the substrates treated, the animals used, the mode of using LY, and the dose of the LY.

There are few researches investigating the effect of fermented feed with a complex of EFE and LY. In this study, fermented alperujo with a high dose of complex LY and EFE accelerates rumen fermentation and improves dry matter digestibility compared to the use of LY or EFE alone. As a result, the ability of the rumen microbiota to produce short-chain fatty acids and the capacity of rumen to utilize energy from this waste for milk production increase. This improvement can be explained by the ability of this complex to promote the proliferation of rumen protozoa, which are responsible for up to 30% of fibrolytic enzyme activity in the rumen (Takenaka et al. 2004) and 20% of fiber digestion (Newbold et al. 2015). This complex also enhanced the ability of the rumen microbiota to convert ammonia–nitrogen to microbial protein. According to Fonty and Chaucheyras-Durand (2006), improving microbial crude protein synthesis is an interesting strategy to protect the environment by reducing nitrous oxide and ammonia emissions produced by ruminants. This synergistic interaction between LY and EFE has also been demonstrated in previous studies of buffalo calf growth performance (Malik and Bandla 2010). Despite the capacity of this complex to enhance rumen fermentation and stimulate short-chain fatty acids production, it did not affect rumen pH in contrast to the use of EFE alone. This result is due to the ability of LY to reduce lactate formation and stabilize rumen pH (Elghandour et al. 2022).

Conclusion

Fermented alperujo with LY and EFE is a promising option for conversion into enriched alternative feeds for ruminants. This biological complex converts dietary fiber into simple sugars and reduces nutritionally harmful compounds. This change in chemical composition increases the rumen microflora and allows an increase in the rumen fermentation process, dry matter digestibility, energy available for lactation, and total short-chain fatty acid production. In addition, this complex stimulates the bioprocess of conversion of ammonia–nitrogen into microbial protein without disturbing the pH in the rumen. This strategy provides a suitable matrix with adequate nutritional value and low cost that can compete with conventional feeds, provide additional income for the olive oil industry, and protect the environment from pollution.

Author contribution K. Abid and M. Kamoun have conceived the concept and design of the study. K. Abid and J. Jabri performed the experiment, analyzed data, and interpreted the results. H. Yaich helped to perform the experiment. K. Abid wrote the draft. M. Malek, J. Rekhis, and M. Kamoun supervised this study. All authors revised and approved to the published version of the paper.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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