

Nutritional and net energy value of fermented olive wastes in rations of lactating ewes

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ABSTRACT: In an experiment with 18 lactating Chios ewes, the replacement of barley grain, wheat grain, sugar beet pulp and lucerne meal with fermented olive wastes (FOW), maize grain and soybean meal was used to formulate diets that allowed the net energy (NE) for lactation (NE_l) density of FOW to be calculated. In the experiment, which lasted 8 weeks, ewes were allocated, after equal distribution for milk yield, body weight (BW) and lactation number, into three treatments of 6 ewes each in a Youden square experimental design with 4 consecutive periods of 14 days. Ewes in all groups were offered a concentrate mixture *ad libitum* (1.12 kg/ewe/day, dry matter (DM) basis) and lucerne hay (0.90 kg/ewe/day, DM basis). FOW were added to the concentrate mixture at inclusion levels (on an as-fed basis) of 0, 100 and 200 kg/t for treatments FOW0, FOW100 and FOW200, respectively. There were no differences among groups in milk protein (60.0 g/kg), lactose (53.0 g/kg) or ash (9.6 g/kg) contents, but milk fat content increased linearly ($P < 0.01$) with increasing dietary levels of FOW. Average milk yield (1 137 g/day) and yields of components tended ($P < 0.10$) to decrease with increased FOW feeding. The best estimate of the NE_l density of FOW was 2.55 MJ/kg DM at 2.7×M NE intake, a value only slightly lower than that of 2.62 at 2.7×M based upon a Van Soest et al. (1984) discount from equations of Van Es (1978), who suggested 3.00 MJ/kg DM at 1.1×M of NE intake. FOW is a low protein, high fibre and low NE_l feedstuff that will be of limited value in rations of high producing lactating ewes.

Keywords: fermented olive wastes; Chios ewes; milk yield; milk composition

Greece is, after Spain and Italy, the third highest olive oil producing country in the world. About 3 500 olive mills operate in Greece, and most of them are scattered in the countryside of Crete, Peloponnese, Ionian islands and Lesbos. The operation of olive oil mills is seasonal, primarily from November to March. About 214 kg olive oil, 496 kg crude olive cake, 40 kg of leaves and 1 633 kg of olive mill wastes water (OMWW) are produced from 1 tonne of fresh olives (Vlyssides et al., 2004). The high amount of OMWW produced during olive oil extraction occurs because water is added in quantities that exceed the weight of fresh olives. Olive stone wooden residue (OSWR) is a by-product of

the crude olive cake industries and is the remaining residue after the solvent extraction of stone oil. The OMWW, which amounts to 1.5 million tonnes annually, is considered toxic and may cause serious environmental problems, mainly due to its high phenolic content (Israilides et al., 1997).

In the framework of integrated management of olive oil mills, and to lessen the environmental impact of OMWW applications to soil and water, this waste can be aerobically fermented (i.e. composted) after mixing with OSWR (ratio 5:1, respectively) for 2 to 3 months. During this co-composting process, OSWR decomposes through exothermic reactions, while the loss in moisture is replenished by the addi-

tion of OMWW to maintain favourable moisture levels. This product is free from heavy metals (e.g. Pb, Cd) and aflatoxins, has an 80% lower phenolic content, and is a good soil conditioner and fertilizer (Israilides et al., 2000). However, fermented OSWR with OMWW, called fermented olive wastes (FOW), can be further dried to approximately 900 g/kg dry matter (DM) and, because of its chemical composition, can provide the ruminant industry with an alternative feedstuff.

In Greece, intensive sheep production is based on diets high in cereal grains and a protein supplement, with soybean meal (SBM) being the most common. However, using the old equations of Van Es (1978) based upon *in vivo* research, FOW would contain only 3.00 MJ/kg DM of net energy (NE) for lactation (NE_l) at about 1.1 times maintenance (M) NE intake (i.e. 1.1×M). If true, this low level would preclude the use of FOW at substantive levels in most rations of production ruminants. However no data is available on the NE_l value of modern FOW.

The objective of this study was to determine the NE_l concentration of modern FOW by feeding it to lactating Chios ewes, using diets containing barley grain, wheat grain, sugar beet pulp and lucerne meal.

MATERIAL AND METHODS

Fermented olive wastes

The experiment was conducted at the Animal Research Institute, National Agricultural Research Foundation (N.AG.RE.F.), in Giannitsa (Greece). FOW (Table 1) were obtained from the Institute of Technology of Agricultural Products (N.AG.RE.F.) in Lycovrissi, Athens (Greece). FOW were placed to a depth of 2 cm in 100 cm × 80 cm × 5 cm metal pans, and sun dried for 72 h. Due to its geographical position, the weather in Greece is fairly uniform and has mild winters and warm summers characterized by sunshine and very little rainfall. The final DM content of FOW after drying generally depends on air temperature and humidity. The amount of FOW that was exposed to the drying process was finally dried to 922 g/kg (Table 1).

Lactating ewe experiment

The NE_l level of FOW was calculated by partial replacement of barley grain, wheat grain and sugar beet pulp with FOW, maize grain and SBM in rations of lactating ewes with 18 Chios dairy

Table 1. Chemical composition^a (g/kg) of fermented olive wastes and lucerne hay (dry matter (DM) basis)

	Fermented olive wastes	Lucerne hay
Dry matter (as fed)	922.0	900
Crude protein	92.0	189
Fat	4.0	33
Neutral detergent fibre	752.0	500
Acid detergent fibre	555.0	333
Ash	81.0	117
Calcium	15.9	–
Phosphorus	2.2	–
Magnesium	1.4	–
Copper (mg/kg)	16.1	–
Zinc (mg/kg)	26.1	–
IVDOM ^b	417.0	–
Net energy for lactation ^c (1.1×M; MJ/kg DM)	3.00	–
Net energy for lactation ^d (2.7×M; MJ/kg DM)	2.62	–

^avalues represent duplicate assays of two samples for each material

^b*in vitro* digestibility of organic matter

^ccalculated from the equations of Van Es (1978)

^dcalculated from the equations of Van Es (1978) discounted to 2.7×M based upon Van Soest et al. (1984)

ewes. All ewes were cared for according to applicable recommendations of the USA NRC (1996). Milk yield and body weights (BW) of the ewes were recorded both after parturition and immediately before the commencement of the study. Ewes were allocated at weaning, on day 42 after parturition, after equal distribution relative to milk yield, BW and lactation number (i.e. 2 or 3), into three groups of 6 ewes each and accommodated in six individual floor pens/groups. The mean BW at the beginning of the experiment was 53.3 ± 1.9 kg, and the milk yield was $1\,351 \pm 54$ g/day. All 18 pens were essentially identical, with the same direction and covered area

($3\text{ m}^2/\text{ewe}$), and were equipped with similar troughs for feeding grain concentrates, hay and water. In a Youden square experimental design of four consecutive periods of 2 weeks each, all ewes were offered a concentrate mixture *ad libitum* with $0.90\text{ kg}/\text{ewe}/\text{day}$ (DM basis) of lucerne hay according to applicable nutrient requirements of sheep (NRC, 1985). The concentrate mixture (Table 2) for treatment FOW0 (control) had no FOW, while those for treatments FOW100 and FOW200 included 100 and 200 kg/t of FOW (on an as-fed basis), respectively.

BWs were measured at the start and at the end of each period, and BW change was calculated. Feed

Table 2. Concentrate composition of rations for lactating ewes

	Treatment ^a		
	FOW0	FOW100	FOW200
Ingredient composition (kg/t, as mixed)			
Maize grain, ground	320	370	410
Barley grain, ground	300	200	100
Wheat grain, ground	142.5	85.5	47.5
Fermented olive wastes	0	100	200
Lucerne meal (170 g/kg CP)	20	0	0
Sugar beet pulp	102.5	102.5	82.5
Soybean meal (440 g/kg CP)	50	80	110
Sunflower meal (290 g/kg CP)	30	30	20
Limestone	13	9	7
Monocalcium phosphate	12	13	13
Salt	4	4	4
Vitamin-trace mineral premix ^b	6	6	6
Chemical composition (g/kg DM)			
Dry matter (as fed)	888	887	894
Crude protein	135	130	132
Crude fat	23	20	22
Neutral detergent fibre	196	245	313
Acid detergent fibre	87	133	176
Ash	48	46	51
Calcium	11.4	11.5	11.6
Phosphorus	6.9	6.8	6.8
Sodium	2.4	2.4	2.3
Estimated NE for lactation (3×M; MJ/kg DM)	7.62	–	–
Estimated NE for lactation (2.7×M; MJ/kg DM) ^c	7.74	–	–

^aFOW0 = control treatment, FOW100 = treatment with 100 kg/t fermented olive wastes, FOW200 = treatment with 200 kg/t fermented olive wastes

^bpremix contained 390 g/kg Ca and 81 g/kg P and supplied/kg of concentrate: 9 000 I.U. vitamin A; 3.75 mg vitamin B₁; 1 400 I.U. vitamin D₃; 24.5 mg vitamin E; 0.5 mg Co; 2 mg Cu; 1.2 mg I; 30 mg Fe; 52 mg Mn; 0.24 mg Se; 80 mg Zn

^cpresented at 2.7×M as this was the average NE intake level of the sheep (Table 3)

intake was measured daily for each ewe. Ewes had free access to water and were machine milked twice daily at 7.00 and 18.00 h with a 2 × 24 DeLaval (Thessaloniki, Greece) milking machine. Milk yield was recorded daily for the morning and afternoon milking during days 9 to 14 for each period. Milking was conducted at a vacuum level of 42 kPa, pulsation rate of 90/min and pulsation ratio of 60/40. During 6 morning and afternoon milkings (days

9 to 14 of each period), samples for chemical analyses were collected from each ewe.

Chemical analyses

FOW, lucerne hay, and concentrate mixtures were analyzed for DM by drying at 102°C for 16 h in a forced air oven, and for crude protein (CP),

Table 3. Body weight (BW), BW change, feed intake, daily milk yield of ewes, milk composition, net energy (NE) output and concentrate NE for lactation (NE_l) density during the experiment

	Treatment ^a			Significance level ^b		
	FOW0	FOW100	FOW200	SEM	linear	quadratic
Initial BW (kg)	55.00	55.00	54.90	1.00	0.98	0.95
Final BW (kg)	56.70	56.60	56.40	1.00	0.90	0.98
BW change (g/day)	128	112	110	15.00	0.63	0.81
Feed intake (kg DM/day)						
Concentrate (Table 2)	1.10	1.12	1.15	0.03	0.51	0.96
Lucerne hay	0.90	0.90	0.90	– ^c	–	–
Total	2.00	2.02	2.05	0.03	0.51	0.96
Yield (g/day)						
Milk	1 175	1 125	1 110	16.10	0.10	0.60
Fat	66	69	70	1.00	0.09	0.88
Protein	69	67	65	0.80	0.08	0.85
Lactose	63	60	59	0.90	0.09	0.65
Ash	12	11	11	0.20	0.04	0.24
Milk content (g/kg)						
Fat	57.10	61.0	63.8	0.28	<0.01	0.29
Protein	59.80	60.5	59.7	0.21	0.87	0.07
Lactose	52.90	53.0	53.1	0.10	0.45	0.92
Ash	9.80	9.5	9.6	0.08	0.20	0.12
NE output (MJ/day)						
NE for maintenance	4.94	4.94	4.93	0.09	0.96	0.98
NE for milk production	4.97	4.92	4.95	0.20	0.97	0.93
NE for BW change	3.51	3.05	3.02	0.54	0.73	0.86
Total NE	13.42	12.92	12.91	0.50	0.69	0.82
Concentrate NE _l density (MJ NE _l /kg DM at 2.7×M) ^d	7.74	7.16	6.96			
FOW NE _l density (MJ NE _l /kg DM at 2.7×M) ^e	–	0.50	2.55			

^aFOW0 = control treatment, FOW100 = treatment with 100 kg/t fermented olive wastes, FOW200 = treatment with 200 kg/t fermented olive wastes

^bnumbers are probability values

^cdue to the equal limit feeding among treatments, no statistical analysis was done

^dassumes NE_l of lucerne hay to be 5.32 MJ/kg DM at 3×M and 5.45 MJ/kg DM at 2.7×M

^eassumes NE_l of concentrate feeds to be the same as those used to estimate the NE_l level of the control concentrate in Table 2, and to be the same in all diets

fat and ash according to methods 976.06, 920.39, and 942.05 of AOAC (1990), respectively. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. (1991). NDF was analyzed without sodium sulphite or alpha amylase, and NDF and ADF were expressed without residual ash. FOW were also analyzed for Ca, Cu, Mg and Zn according to method 968.08 and for P according to method 965.17 of AOAC (1990).

In vitro digestibility of organic matter (OM) in DM (IVDOM) of FOW was determined according to Tilley and Terry (1963), as modified by O'Shea and Wilson (1965), for 96 h.

Milk samples were analyzed for fat, protein, lactose and solids-not-fat (SNF) with IR spectroscopy (Milkoscan 4000; TESCO, Denmark) according to method 972.16 of AOAC (1990). Ash was calculated as SNF minus protein and lactose.

Energy calculations

The NE_1 content of FOW was calculated by the equations of Van Es (1978) to be 3.00 MJ NE_1 /kg DM at approximately $1.1 \times M$. However, based upon a Van Soest et al. (1984) discount of 0.08 per unit maintenance (estimate from feeds with similar NDF levels) from equations of Van Es (1978), the NE_1 content of FOW was calculated to be 2.62 MJ NE_1 /kg DM at $2.7 \times M$.

The NE requirements of lactating ewes (i.e. NE for maintenance, NE for milk production and NE for BW change) were estimated according to McDonald et al. (1990). For the milk production NE requirement, the average day in milking used was 70.

The NE_1 density of the control diet (Table 2) was calculated using the NE_1 values of concentrate feeds based upon the most recent NRC (2001) feed tables at $3 \times M$, and increased slightly to $2.7 \times M$ by extrapolating the values listed at $3 \times M$ and $4 \times M$, as this was the actual average NE intake of the ewes in the study.

The NE_1 density of lucerne hay was estimated from NRC (2001) tables by matching its NDF, ADF and CP values to table values which suggested the NE_1 value of 5.32 MJ/kg DM at $3 \times M$ and 5.45 MJ/kg DM at $2.7 \times M$. Based upon the measured NE output of the ewes and the NE_1 estimate of the lucerne hay, the actual NE_1 density of the concentrate was estimated. The calculated and estimated NE_1 density

of the concentrate was 7.74 MJ/kg DM from both procedures, which allowed the NE_1 density of FOW in both FOW diets to be estimated by assuming that the NE_1 values of all other feeds in the FOW100 and FOW200 diets were the same as in the FOW0 diets, and that residual NE_1 could be attributed to the FOW. These values are listed by treatment (i.e. FOW100 and FOW200) in Table 3.

Statistical analysis

Performance of ewes, milk composition, NE output and concentrate NE_1 density were statistically analyzed by one-way analysis of variance. Differences between treatment means were tested using linear and quadratic contrasts at the 5% probability level (Steel and Torrie, 1980). Statistical analysis used the SPSS (1999).

RESULTS

Analysis of feeds and diets

The chemical composition of FOW is in Table 1, and that of lucerne hay is consistent with NRC (2001) values. The diets were formulated to be similar in all nutrients, but this was not possible due to the high NDF level of FOW. However, due to the NE_1 estimation technique used for FOW, this was not essential and increases in NDF and ADF occurred as the levels of added FOW increased.

Lactating ewe experiment

There were very few feed refusals, and the feed consumption of the concentrate and lucerne was similar among diets with increasing FOW inclusion levels (Table 3).

Overall BW and BW change of the ewes were not affected by treatments (Table 3), and milk, fat, protein and lactose yield, and milk protein, lactose and ash content were not affected either (Table 3). In contrast, milk ash yield decreased linearly ($P = 0.04$) and milk fat content increased linearly ($P < 0.01$) with increasing level of FOW in the concentrate.

The estimated NE_1 density of FOW in treatment FOW100 was only 0.50 MJ/kg of DM, whereas in treatment FOW200 it was 2.55 MJ/kg of DM (both at $2.7 \times M$).

DISCUSSION

FOW are a low CP feed that has high NDF and ADF levels and can be a good source of Ca, Mg, Cu and Zn, and a moderate source of P. Although it is known that the microbial fermentation of feeds can enrich them with vitamins, enzymes and growth factors (Israilides et al., 1994), this was not examined.

The measured performance parameters of ewes were largely unaffected by increasing the feeding level of FOW, maize grain and SBM in replacement for barley grain, wheat grain, sugar beet pulp and lucerne meal. However, milk, protein and lactose yields tended ($P < 0.10$) to decrease linearly.

The estimated NE_1 density of FOW differed between the FOW100 diet, where it was calculated to be only 0.50 MJ/kg DM, and the FOW200 diet where it was calculated to be 2.55 MJ/kg DM (both at $2.7 \times M$). The technique we used to estimate the NE_1 density of feeds allocates all unaccounted NE output to the unknown feed (in this case FOW) and so the error associated with the NE_1 estimate tends to be reduced as the level of the unknown feed in the diet increases. However low-quality feeds, similarly like unknown feeds, may substantially reduce the animal's intake and/or performance if included in the diet at levels that are too high, and so impact the estimated NE output by causing high losses of BW. As the calculation technique is designed to estimate the NE_1 of the unknown feed, as its NE_1 density is not known a priori, its most appropriate levels to add to the diet can only be surmised. In this case, it would appear that the FOW100 diet contained a too low incorporation level to gain an accurate estimate of the NE_1 value of FOW, while the incorporation level of FOW in the FOW200 diet may have been more appropriate.

CONCLUSIONS

Partial replacement of barley grain, wheat grain, sugar beet pulp and lucerne meal with fermented olive wastes, maize grain and soybean meal in diets of lactating ewes resulted in similar productive performance, although there were trends to reductions. There is no evidence that the calculation of 3.00 MJ/kg DM at approximately $1.1 \times M$ NE intake for FOW (or 2.62 MJ/kg DM at $2.7 \times M$ assuming a 0.08 discount per unit maintenance (estimate from feeds with similar NDF levels as listed by Van Soest

et al., 1984)) based upon the equations of Van Es (1978) is too low for modern FOW, as the best estimate from this study is 2.55 MJ/kg DM at $2.7 \times M$ NE intake. FOW will likely be of limited value in rations of high producing lactating ewes due to its low protein, high fibre and low NE_1 levels.

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REFERENCES

- AOAC (1990): Official Methods of Analysis of the AOAC. Helrich K. (ed.): 15th ed. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.
- Israilides C.J., Iconomou D., Kandylis K., Nikokyris P. (1994): Fermentability of sugar beet pulp and its acceptability in mice. *Bioresour. Technol.*, 47, 97–101.
- Israilides C.J., Vlyssides A.G., Mourafeti V.N., Karrouni G. (1997): Olive oil wastewater treatment with the use of an electrolysis system. *Bioresour. Technol.*, 61, 163–170.
- Israilides C.J., Vlyssides A.G., Pashalides C., Dimirkou A., Papadopoulos P., Couloubis P., Iconomou D., Papayanopoulou D., Giouzelyannis A., Christodoulou V. (2000): Utilization of composted olive stone wooden residue with olive mill waste waters as a soil conditioner. In: *Inter. Conf. Protection and Restoration of the Environment*. 3–6 July, Thassos, Greece, 561–568.
- McDonald P., Edwards R.A., Greenhalgh J.F.D. (1990): *Animal Nutrition*. 4th ed. Longman Scientific and Technical, Essex, England, 368.
- NRC (1985): National Research Council. *Nutrient Requirements of Sheep*. 6th rev. ed. National Academy Press, Washington DC, USA.
- NRC (1996): National Research Council. *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington DC, USA.
- NRC (2001): National Research Council. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academy Press, Washington DC, USA.
- O'Shea J., Wilson R.K. (1965): Relationship between *in vitro* and *in vivo* dry matter digestibility. *Irish J. Agric. Res.*, 4, 235–237.

- SPSS (1999): Statistical Package for the Social Sciences. Release 10.0. SPSS Inc., Chicago, USA.
- Steel R.G.D., Torrie J.H. (1980): Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York, USA.
- Tilley J.M.A., Terry R.A. (1963): A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grsl. Soc., 18, 104–111.
- Van Es A.J.H. (1978): Feed evaluation for ruminants. I. The systems in use from May 1977 onwards in the Netherlands. Livest. Prod. Sci., 5, 331–345.
- Van Soest P.J., Fox D.G., Sniffen C.J. (1984): Discounts for net energy and protein. In: 4th ed. Proc. Cornell Nutr. Conf. Feed Manuf. Dept. of Anim. Sci., Cornell University, Ithaca, USA, 121–136.
- Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583–3597.
- Vlyssides A.G., Loizides M., Karlis P.K. (2004): Integrated strategic approach for reusing olive oil extraction by-products. J. Cleaner Prod., 12, 603–611.

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