

Effects of ecotrofin™ on milk yield, milk quality and serum biochemistry in lactating goats

Nadia Musco¹  | Valeria Maria Morittu² | Vincenzo Mastellone¹ |
 Anna Antonella Spina² | Giuseppe Vassalotti² | Biagio D'Aniello³ |
 Raffaella Tudisco¹  | Federico Infascelli¹ | Pietro Lombardi¹

¹Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Napoli, Italy

²Department of Health Sciences, Magna Graecia University of Catanzaro, Catanzaro, Italy

³Department of Biology, University of Naples Federico II, Naples, Italy

Correspondence

Raffaella Tudisco, Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Via F. Delpino, 1, 80137 Napoli, Italy.
 Email: tudisco@unina.it

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Abstract

A nutritional supplement (Ecotrofin™, by Vetoquinol Italia S.r.l.) recommended in ruminants feeding to strengthen the physiological condition and improve digestive performance was tested in 20 pluriparae grazing goats divided in two groups (control and treated) to assess its possible effects on milk yield and quality and to assess eventual adverse effects. Animals from both groups also received 400 g/day of corn meal, and the treated group was supplemented with 20 g/head/day of the nutritional supplement. At the doses suggested by the manufacturer, despite a transient increase after 30 days of supplementation, Ecotrofin™ did not show significant effects on milk yield and, although some changes were found in the fatty acids profile, no significant improvement of MUFA and PUFA, as well as of omega-6:omega-3 ratio and CLA content were seen. Therefore, in our experimental conditions the supplementation of diet with Ecotrofin™ did not appear useful to improve goat's performance. A significant effect on kidney health markers (27 vs. 22.5 for urea and 0.83 vs. 0.76 for creatinine, $p < 0.05$) suggested a beneficial effect on renal function but, since levels fell in the normal ranges in both groups, such hypothesis would need further studies to be addressed.

KEYWORDS

CLA, fatty acids, goat, milk yield, nutritional supplement

1 | INTRODUCTION

Nutrition plays an important role in determining the metabolic status of the animals, thus also reflecting on animal welfare as well as on livestock performance (Celi et al., 2017). Indeed, in addition to the ration, natural substances can be also used as a supplement in order to modulate the rumen fermentation and to improve milk yield

(Arowolo & He, 2018). Goats fed on high-quality and valuable forage provide the production of high-quality milk and dairy products. Natural substances can be also used as nutritional supplements to this purpose, but most of the research has been performed on housed animals (Olagaray & Bradford, 2019). Nevertheless, the influence on the performance can be variable, depending on many factors such as the dosage, diet composition, forage to concentrate

Federico Infascelli and Pietro Lombardi, contributed equally to this work.

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ratio, feeding strategy, physiological state of the animals. Plants are serving several purposes whether health, nutrition and medicinal. With the development in techniques and recent research, it has been proved that certain non-nutritive chemicals in plants, which were earlier thought to be of no importance in the diet can possess beneficial properties for animal's health.

In such contest, the Meliaceae are largely used in folk medicine in Brazil and other countries encompassing the Amazon rainforest for their therapeutic properties. In particular, the seed's oil of *Carapa guianensis* L., is reported to exert anti-inflammatory activity due to the presence of limonoids, which are mainly tetranortriterpenoids (Henriques & Penido, 2014). Moreover, the andiroba oil, which contains myristic, palmitic, oleic, linoleic, stearic and arachidic fatty acids, tetraterpenoids and flavonoids is extracted from *C. guianensis* seeds (Costa-Silva et al., 2007).

The goal of this research was to assess the influence of a nutritional supplement on milk yield and health status by assaying biochemical parameters in grazing goats. Ecotrofin™ is a commercial product by Vetoquinol S.r.l. that, basing on an association of limonoids, bioflavonoids and PUFA, is claimed to strengthen the physiological condition and to improve digestive performance in ruminants.

2 | MATERIALS AND METHODS

2.1 | Animals

The trial was carried out from February to August 2018 in a farm located at Casaletto Spartano (832 m s.l.; 40°09' N; 15°37'E; 35 to 70 mm rainfall; 9.6 to 21°C temperature; province of Salerno, Italy). Twenty pregnant Cilentana dairy goat's homogeneous for parity (3rd), body weight (50.2 ± 3.4 kg) and daily milk yield at the previous lactation (1.220 ± 0.105 kg) were recruited for the trial. They were fed oat hay ad libitum plus 100–200 or 300 g/day of corn meal, 45–30 or 15 days up to kidding (first week of February), respectively. Successively, the goats were randomly divided into two groups (CTR, control and ECO, treated, $n = 10$ each). Both groups had free access to pasture composed by *Trifolium alexandrinum*, *Vicia* spp., *Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne* for 7 h (9.00 a.m. to 16.00 p.m.) on a 10 hectares area. After grazing, the animals returned to the stall and were individually stabled in a 1 × 2 m box and fed with 400 g/head/day of corn meal. From April to August, the goats of the ECO group were daily supplemented with 20 g/head/day of Ecotrofin™ a commercial product by Vetoquinol (Vetoquinol Italia S.r.l.) basing on an association of limonoids, bioflavonoids and PUFA, is recommended in ruminants feeding to strengthen the physiological condition and to improve digestive performance. Body weight was monthly measured as well as health status assessed by clinical examination from the start of the experiment. Corn refusals were measured each day. From day 0 to 60, milk was suckled only by kids; then, goats were milked twice a day for 5 months (April to August), and milk yield was recorded daily. All the animal procedures

were conducted according to the Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and after the approval of the local Bioethics Committee (protocol number: PG20200016570).

2.2 | Diets

In order to reduce the differences in chemical composition previously observed (D'Urso et al., 2008; Tudisco et al., 2010, 2014) by using a monthly collection, pasture samples were collected once per week, and a composite sample was monthly used for analysis. The collection was done as follows: The grass of four different 2.5 m² areas was cut at 3 cm from the ground; after weighing, 4 representative samples (1 kg each, obtained balancing the amount from the 4 different areas) were air-oven dried at 65°C, milled through a 1-mm screen and stored. The chemical composition of pasture, corn and supplement was analysed according to AOAC (2012) for dry matter (DM, ID 934.01), crude protein (CP, ID 984.13) and ether extract (EE, ID 920.29); the structural carbohydrates were determined as suggested by Van Soest et al. (1991) and nutritive value (UFL = 1,700 kcal of net energy for lactation) was calculated according to INRA (1978).

Fatty acids (FA) profile of pasture, corn and supplement was determined as described by Tudisco et al. (2014). Briefly, total fat was extracted according to Folch et al. (1957) and FA transmethylated by a base-catalysed procedure reported by Christie (1982), modified by Chouinard et al. (1999). The methyl esters were quantified by gas chromatograph (ThermoQuest 8000TOP gas chromatograph, equipped with flame ionization detector; Thermo Electron Corporation) equipped with a CP-SIL 88 fused silica capillary column [100 m 0.25 mm (internal diameter) with 0.2- μ m film thickness; Varian] set according to Zicarelli et al. (2016). FA peaks were identified by comparing the retention times of commercial standard containing 37 methyl esters of fatty acids (Sigma-Aldrich). The retention times of the CLA isomers were controlled by the elution of commercial standards (Larodan AB-SE-171 65 Solna) of these fatty acids. The area of each individual fatty acid identified in the sample was quantified by percentage calculation on the total area of the eluted peaks.

2.3 | Milk

Representative individual milk samples from the two daily milking were monthly analysed for protein, fat and lactose using a MilkoScan FT+ (Foss) standardized for goat milk. In addition, as described by Hara and Radin (1978), total fat of milk was separated using a mixture of hexane-isopropane (3/2 v/v). FA transmethylation and methyl esters quantification were effected as above described for the feeds using a gas chromatograph (ThermoQuest Focus, equipped with flame ionization detector; Thermo Electron Corporation) equipped with a CP-SIL 88 fused silica capillary column (100 m × 0.25 mm (i.d.) with 0.2- μ m film thickness; Varian). Gas chromatograph conditions

were those described by Cavaliere et al. (2018). Fatty acid peaks were identified using pure methyl ester standards and CLA isomers (Larodan Fine Chemicals, AB, Limhamnsgårdens Malmö).

2.4 | Blood analyses

Blood samples were collected from fasted goats via jugular venepuncture. Individual blood samples were collected into plastic tubes. The serum was separated by centrifugation at $1,500 \times g$ for 15 min and stored at -20°C until the analyses were performed. Blood samples were collected before delivery and every month up to the end of the experiment and analysed for biochemical parameters in order to determine: urea (UREA), creatinine (CREA), total protein (TP), albumin (ALB), Albumin/Globulin ratio (A/G), aspartate amino transferase (AST), bilirubin (BIL), non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA). Blood chemistry analyses on serum aliquots were performed by an automatic biochemical analyser (AMS Auto lab, Diamond Diagnostics) using reagents from Spinreact (Girona).

2.5 | Statistical analyses

Pasture analyses were performed using the one-way ANOVA using the procedure of the JMP software (SAS Institute, NC, USA, 2000) according to this model:

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

where Y_{ij} = mean of response variable, μ = population mean, T_i = effect of sampling time ($i = 5$; April, May, June, July, August) and e_i = experimental error.

Body weight, milk and blood analyses were performed using the two-way ANOVA for repeated measures over time using the procedure of the JMP software (SAS Institute, NC, USA, 2000) according to this model:

$$Y_{ijk} = \mu + G_i + T_j + (DT + ST)_{ijk} + e_{ijk}$$

where Y_{ijk} = mean of response variable, μ = population mean, G_i = effect of the group ($i = 2$; CTR and ECO), T_j = effect of sampling time ($j = 5$; April, May, June, July, August), $(G \times T)_{ijk}$ = fixed effect of interaction between dietary treatment and sampling time, and e_{ijk} = experimental error.

The comparison among the mean values was performed by using Tukey's test. Differences were considered statistically significant at $p < 0.05$.

3 | RESULTS

In Table 1, feeds and Ecotrofin™ chemical composition and fatty acids profile are reported. The pasture showed lower contents of

TABLE 1 Chemical composition, nutritive value and fatty acids profile of feeds

Chemical composition (g/kg DM)	Corn	Pasture	Ecotrofin™
CP	99.6	153.2	159.3
EE	41.2	20.1	130.8
NDF	123.3	438.5	176.6
ADF	31.2	300.2	92.2
ADL	6.0	38.0	8.4
UFL/kg DM	1.1	0.76	0.83
Fatty acid profile (% total FA)			
ΣSFA	15.31	16.22	29.59
ΣMUFA	27.99	5.52	16.02
ΣPUFA	56.69	78.26	54.39
C18:3n3	1.45	44.03	24.64
C18:2n6	54.88	25.13	29.29

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; EE, ether extract; MUFA, monounsaturated fatty acids; NDF, neutral detergent fibre; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFL, unit feed for lactation.

crude protein and NDF than those reported in studies previously effected in the same geographical area by Tudisco et al. (2012), Tudisco, Morittu, et al. (2019); the nutritive value was lower as well. The PUFA (56.69% of total FA) recorded in the corn meal were almost completely due to the linoleic acid (C18:2n6; 96.8% of PUFA) whereas the alpha-linolenic acid (C18:3n3) was 56.3% of total PUFA in the pasture. Ecotrofin™ showed a slight prevalence of C18:2n6 on C18:3n3 (53.8 vs. 46.2% of total PUFA, respectively).

Table 2 shows the changes in chemical composition of the pasture. No significant differences were seen for all the chemical parameters throughout the trial.

No corn meal refusals were detected, and goat's body weight (BW) did not change throughout the trial, and also, no statistical difference was found for milk yield and milk composition between the groups (Table 3).

Regarding the milk fatty acids profile (Table 4), significantly lower levels were seen for C4:0, C6:0, C11:0, C12:0, C15:0 and C17:0 in the ECO group, but no difference was seen for total SFA. Similarly, C15:1, C18:1 cis9 were significantly lower in the ECO group, but no differences were seen for total MUFA. Total PUFA resulted lower (3.84 vs. 4.10 for ECO and CTR, respectively) due to the significant ($p < 0.05$) difference registered in PUFA omega 6 (2.29 vs. 2.75 for ECO and CTR groups, respectively). No difference was seen for the omega-6:omega-3 ratio, as well as for CLA content.

As seen in Table 5, urea (22.5 vs. 27.0 mg/dl) and creatinine (0.76 vs. 0.83 mg/dl) were significantly ($p < 0.05$) lower in the ECO group, and also, significant ($p < 0.01$) change was seen for the time effect for both parameters.

Chemical composition	April	May	June	July	August	p	RMSE
CP	156.0	157.0	151.0	149.0	158.8	NS	11.34
EE	20.12	20.00	19.89	20.14	19.70	NS	9.80
NDF	477.5	470.5	491.5	515.5	453.5	NS	41.67
ADF	300.2	323.4	341.9	345.9	313.5	NS	22.19
ADL	38.9	40.1	39.3	43.7	38.2	NS	8.41
UFL/Kg DM	0.77	0.76	0.75	0.75	0.77	NS	0.06

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; NS, Not Significant; RMSE, root mean square error; UFL, unit feed for lactation.

	CTR	ECO	Group effect	Time effect	G × T	RMSE
Body weight kg	50.1	50.2	NS	NS	NS	5.12
Milk yield g/head/day	1282.6	1500.0	NS	**	*	462.32
Fat %	3.65	3.93	NS	NS	NS	1.03
Protein %	3.30	3.16	NS	NS	NS	0.278
Lactose %	4.13	4.08	NS	NS	NS	0.180

Abbreviations: NS, Not Significant; RMSE; root mean square error.

* $p < 0.05$; ** $p < 0.01$.

TABLE 2 Pasture chemical composition (% dry matter basis) throughout the trial

TABLE 3 Body weight, milk yield and chemical composition in treated group (ECO) and control group (CTR)

4 | DISCUSSION

The body weight (BW) did not change throughout the trial in both groups, and thus, the energy requirements were satisfied. Indeed, the pasture intake of the local genotype goats in Mediterranean area account to 20 g DM/kg BW and their energy requirements to 0.0365 UFL/kg metabolic weight ($MW = BW^{0.75}$) for maintenance and 0.41 UFL/kg fat-corrected milk (4% fat) (Rubino, 1996). The goats of both groups weighed 50.2 (± 3.4) kg BW; thus, pasture intake was 1 kg DM (0.76 UFL, see Table 1). Their average daily milk yield was 1.390 kg with 3.79% of fat, and therefore, energy requirement was 1.23 UFL (0.69 UFL maintenance, plus 0.54 UFL milk production). Corn meal (1.1 UFL/kg DM) supplied the differences (0.47).

Ecotrofin™ did not affect milk yield and milk composition; nevertheless, the ECO group showed a slight increase while CTR a significant decrease ($p < 0.05$) of milk yield in May (Figure 1). Actually, such effect was not seen at the successive samplings.

Since no difference in pasture composition was seen throughout the trial, this result cannot be ascribable to the worsening of pasture chemical composition with consequent support of Ecotrofin™ for milk yield, and on the other hand, according to Oba and Allen (1999) and Souza et al. (2017), in terms of milk yield, the stage of lactation is a plausible factor which can affect the response of ruminants to nutritional manipulations.

Conflicting results are reported supplementing ruminants' diet with flavonoids, limonoids, or PUFA. Gessner et al. (2015) observed an increase of milk yield in goats whose diet was supplemented with grape seed and grape marc meal containing bioflavonoids. According to Gobert et al. (2009), flavonoids, with high antioxidative potential,

determined an increase of the antioxidative status and reduction of lipid peroxidation in plasma, thus improving health and performance by reducing oxidative stress. Kholifa et al. (2015) reported an increase of milk yield in goats fed *Moringa Oleifera*, rich in limonoids, while Ying et al. (2017) did not find this effect in dairy cows by adding Citrus pulp, limonoids rich as well, in the diet. Renna et al. (2016) supplementing goats' diet with rumen unprotecting Echium oil, rich in omega-3 fatty acids, found a negative effect on milk yield. In contrast, Tudisco, Chiofalo, et al. (2019) observed an increase of milk yield when diet of lactating goats was supplemented with PUFA rich feed.

It has been reported that, by altering the feeding strategy, including by the use of feed additive supplementation, the fatty acid profile of goat milk can be changed dramatically (Rojo et al., 2015).

In this trial, PUFA omega-6 showed significant lower levels in the ECO group. Such result caused a lower level of total PUFA in the ECO group, but was not able to affect the omega-6:omega-3 ratio, which is considered the main critical marker of milk (Simopoulos, 2002).

In contrast, in terms of health-promoting effects for humans, milk of ECO group showed significantly lower levels of short-chain fatty acids (C4:0 and C6:0) that inhibited bacterial and viral growth and dissolve cholesterol deposits (Sun et al., 2003).

Santos-Silva et al. (2016) reported that the administration of feeds rich in limonoids, resulted in a decrease of cis-9-18:1 compensated by an increase of cis-9, trans-11-18:2. Such increase was not seen in our experiment, suggesting that some modification occurred during the biohydrogenation process. Indeed, it has been described that some plant secondary products are able to affect rumen

TABLE 4 Milk fatty acids profile (% of total FA) of treated group (ECO) and control group (CTR)

Fatty acids profile	CTR	ECO	Group effect	Time effect	G × T	RMSE
C4:0	0.005	0.001	**	**	**	0.018
C6:0	0.130	0.062	*	**	**	0.142
C8:0	0.750	0.663	NS	**	**	0.438
C10:0	7.210	7.079	NS	**	**	2.076
C11:0	0.060	0.042	*	*	NS	0.029
C12:0	4.270	3.626	**	*	NS	0.789
C14:0	11.730	11.445	NS	NS	NS	0.036
C14:1 cis 9	0.060	0.062	NS	NS	NS	1.058
C15:0	0.930	0.848	*	**	NS	0.132
C15:1	0.224	0.199	*	*	NS	0.052
C16:0	32.54	33.93	NS	**	NS	3.195
C16:1 cis9	0.673	0.639	NS	NS	NS	0.123
C17:0	0.710	0.654	**	*	NS	0.073
C17:1	0.149	0.159	NS	NS	NS	0.051
C18:0	14.440	14.480	NS	NS	NS	2.592
C18:1 cis9	0.373	0.304	**	**	*	0.064
C18:1trans 11	21.630	21.697	NS	NS	NS	0.266
C18:2 trans9 trans 12 omega-6	0.370	0.277	*	NS	NS	0.084
C18:2 cis9 cis 12 omega-6	2.339	1.807	**	NS	NS	0.456
C20:0	0.108	0.118	NS	NS	NS	0.248
C18:3 omega-3	0.922	0.900	NS	NS	NS	0.204
C22:0	0.016	0.016	NS	NS	NS	0.006
C24:0	0.001	0.001	NS	NS	NS	0.003
C22:6 omega-6	0.039	0.030	NS	NS	NS	0.011
cis-9 trans-11 CLA	0.395	0.391	NS	NS	NS	0.131
trans-10 cis-12 CLA	0.035	0.028	NS	NS	NS	0.010
SFA	73.00	73.10	NS	NS	NS	3.073
MUFA	22.89	23.06	NS	NS	NS	3.819
PUFA	4.10	3.84	*	NS	NS	0.770
∑CLA	0.43	0.41	NS	NS	NS	0.119
PUFA omega-3	1.20	1.12	NS	NS	NS	0.260
PUFA omega-6	2.75	2.29	*	NS	NS	0.649
Omega-6/Omega-3	2.29	2.04	NS	NS	NS	0.917

Abbreviations: CLAs, conjugated linoleic acids; CTR, Control Group; ECO, Ecotrofin™ group; MUFA, monounsaturated fatty acids; NS, Not Significant; PUFA, polyunsaturated fatty acids; RMSE, root mean square error; SFA, saturated fatty acids.

* $p < 0.05$; ** $p < 0.01$.

biohydrogenation pathways (Vasta & Bessa, 2012). During the biohydrogenation of fatty acids the ingested cis-9, cis-12 C18:2 and cis-9, cis-12, cis-15 C18:3 are isomerized and saturated to finally form C18:0 (Bessa et al., 2007) by the rumen microorganisms (Kemp et al., 1975). Overall, the results on milk FA did not show beneficial effects of Ecotrofin™ at the dose suggested.

More interesting results came from the blood profile, even if the biochemical status fell in the reference ranges in both groups (Piccione et al., 2010). UREA and CREA, which are well known markers of renal function, were significantly lower in the ECO group, and

such results may be due to the combination of PUFA, limonoids and bioflavonoids present in the Ecotrofin™ formula. Therefore, the ECO group showed an improvement of renal function compared to the Control one, and this could be attributable to the presence of PUFA in the nutritional supplement. Lipids derived from the omega-6 PUFA and arachidonic acid play important roles in renal physiology (Imig et al., 2005). Studies have shown that flavonoids were potent enough to maintain the normal renal function and exert effective nephroprotective action against the different nephropathies reviewed suggested that their main mechanism of action is due to their impact

Blood profile	Unit	CTR	ECO	Group effect	Time effect	G × T P	RMSE
UREA	mg/dl	27.0	22.5	*	**	NS	7.074
CREA	mg/dl	0.83	0.76	*	**	NS	0.064
TP	g/dl	7.73	7.51	NS	NS	NS	0.61
ALB	g/dl	2.86	2.95	NS	NS	NS	0.22
A/G		0.534	0.561	NS	NS	NS	0.092
AST	U/L	98.8	107.6	NS	*	NS	29.94
BIL	mg/dl	0.37	0.37	NS	*	NS	0.067
NEFA	mmol/l	0.39	0.36	NS	NS	NS	0.15
BHBA	mmol/l	0.38	0.42	NS	NS	NS	0.13

TABLE 5 Blood chemistry parameters of treated group (ECO) and control group (CTR)

Abbreviations: A/G, Albumin/Globulin ratio; ALB, albumin; AST, aspartate amino transferase; BHBA, beta-hydroxybutyric acid; BIL, bilirubin; Creatinine, CREA; NEFA, non-esterified fatty acids; NS, Not Significant; RMSE, root mean square error; TP, total protein.

* $p < 0.05$; ** $p < 0.01$.

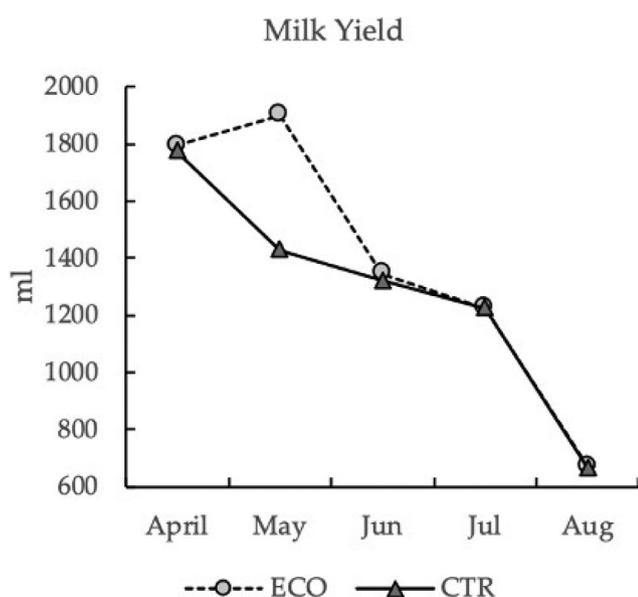


FIGURE 1 Milk yield in CTR and ECO groups throughout the trial

on the inflammatory cascade and oxidative perturbations, affecting several pathways (Calder, 2013). Moreover, clinical evidence demonstrated that omega-3 PUFA dietary supplementation has beneficial effects in acute and chronic inflammatory conditions in humans and in animals. Indeed, since the animals used in this study were healthy and nutritional requirements were satisfied, further studies are needed to address the possible beneficial effects of Ecotrofin™ on renal function. Finally, no differences were seen for the other biochemical parameters, thus suggesting no adverse effects were seen in the group treated with Ecotrofin™.

5 | CONCLUSIONS

In this study, the supplementation with Ecotrofin™ did not exert the claimed positive effects in terms of production performances, as

well as of milk quality in terms of human health. Also, the significant decrease of renal parameters cannot be considered a renal function improvement since the levels of both parameters fall in the physiological range for goats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization PL, FI, RT and NM; Formal analysis NM, RT, VM, GV and AAS. Methodology PL, BD, RT, VMM and NM; Writing – original draft NM, PL and FI; Writing – review and editing NM, FI and PL.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

ORCID

Nadia Musco  <https://orcid.org/0000-0001-9490-9933>

Raffaella Tudisco  <https://orcid.org/0000-0003-1378-0328>

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