Effects of Rosemary distillation residues substitution to oat hay on diet digestibility, metabolic profile and growth performance of Barbarine ewe lambs

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SUMMARY

Introduction - The Rosemary is an aromatic and medicinal plant widely available in the Mediterranean area of which industry distillation generates voluminous residues that could be an alternative animals' feed in harsh conditions.

Aim - This study aimed to evaluate the effect of hay substitution by rosemary distillation residues (RR) in crude form (CRR) or as pellets (PRR) on lamb's performance, digestibility and nitrogen retention. The metabolic profile and the rumen fermentation parameters were also determined.

Materials and methods - 24 ewe-lambs (average body weight: 24 ± 1.3 kg) were divided into 3 groups receiving 500 g of concentrate plus 600 g of oat hay for control group (C), 600 g of crude RR for CRR group and 600 g of RR enriched pellets in PRR group.

Results and Discussion - Dry matter (DM) intake was similar for hay, CRR and PRR. The DM and crude protein digestibility was not affected by the dietary treatment. The nitrogen balance was positive for all regimens. The rumen ammonia concentration was higher for C and PRR groups, while the volatile fatty acids concentration was similar among groups. The lamb's growth was similar among groups. The cost of gain was reduced (P<0.05) for lambs fed RR diets. The glycaemia and uraemia were higher (p<0.001) for PRR.

Conclusion - In conclusion, the RR can be used in lamb's feeding at reduced cost without altering lamb's growth and rumen functionality.

KEY WORDS

Rosemary residues; Lamb's growth; Metabolic profile; Rumen fermentation; Nutrient digestion.

INTRODUCTION

Sheep and goat feeding in semi-arid areas is based on natural resources, rangeland and stubble. The biodiversity loss and climate change with water shortage in these regions resulted in land degradation and uncertain availability of such resources¹. Consequently, the livestock use common concentrates and other feeds often imported and expensive. In the Southern-Mediterranean region, the hay and straw represent the main basal diets for ruminant nutrition; however, for some part of this region, the availability of hay is a greater problem than the availability of concentrate. The transport of hay, fibrous and voluminous, is more expensive than that of concentrate. In order to surmount the feed availability and cost problem, several alternative feed resources, in particular agro-industrial by-products and shrubs are used in goat, sheep and cattle feeding^{2, 3}. In last decades, the distillation of forest plants to produce es-

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sential oils became an important industry and generated a great quantity of by-products. The Rosemary is the most forest plant exploited in artisanal or modern distillation industry in the Mediterranean area. In Tunisia, it covers large areas estimated to 346000 ha. The annual collected amount of rosemary is 15600 tons leaving 5460 tons of distilled rosemary residues (RR) whereas the potential of annual RR is around 9000 tons⁴. This important quantity of RR can be valued to partially overcome the problem of animal-feed shortage. However, they can be considered as low quality roughage but the adaptation of some easy feed technologies such multi-nutritional blocks or pellets lead to obtain effective mix balanced in proteins, fibre and minerals that can enhance animal's productivity⁵.

The use of rosemary essential oil and the RR as additive for sheep and goats was extensive, but ha d only concerned their antioxidant properties and their effects on meat quality ^{5,6}. Nevertheless, to our knowledge, the information about the use of RR as basal diet is very scarce. Therefore, the aim of this work was to study the effects of its substitution, in crude state (CRR) or pellet form (PRR), to oat hay on nutrient digestibility, ruminal fermentation, growth and haematological parameters of Barbarine lambs.

MATERIAL AND METHODS

All procedures employed in this study meet ethical guidelines and adhere to Tunisian legal requirements (The Livestock Law No. 2005-95 of 18 October 2005, Chapter II; Section 1).

Experimental diets

The fresh RR were collected, after essential oil extraction, in the forest in the semi-arid region of the country. They were transferred in plastic bags and dried under ambient conditions for one week. Then, the RR was included in the diet as crude (CRR) or pellets (PRR) forms. To make the pellets, the RR (60%) were ground and mixed with wheat bran (32%) and soybeans (8%) to obtain enhanced roughage. The oat hay was considered as control feed compared to diets containing RR. The chemical composition and feed costs of all experimental foods were given in Table 1.

Experimental design and feeding management

Twenty-four Barbarine ewe-lambs $[24\pm1.3 \text{ kg of body weight}$ (BW)] were housed in individual pens and randomly assigned to one of three diets. They averagely received 600 g of basal diets: oat hay, crude RR or pellets RR for Control, CRR and PRR group, respectively. Lambs of all groups were complemented by 500 g of local concentrate (80% of barley, 17.5% of soybean meal and 2.5 of mineral and vitamin supplement) and had free access to water. Animals were allowed 15 days as adaptation to experimental conditions where roughages amounts were progressively increased from 300 to 600 g for CRR and PRR and from 400 to 600 g for oat hay, and then the growing trial lasted 67 days. The refusals were removed and weighed daily before the morning feeding. The lambs were weekly weighed to calculate the average daily gain (ADG).

Digestibility and metabolism

A metabolism trial was performed at the end of the growing trial. Six lambs from each dietary treatment were transferred to metabolism cages for 5 days of adaptation and 5 days of measurement of nutrient digestibility and nitrogen (N) balance. The feed intake and refusals and faecal output were daily recorded and sampled for further analysis prior to morning feeding. Representative samples were used for dry matter (DM) determination. At the end of the collection period, all faeces samples for each lamb were mixed and homogenized; representative samples were stored for DM, Ash, fibre and CP analyses. The urine was daily collected from each animal in a plastic basin containing 50 ml of 10% H₂SO₄ solution. Then 10% of the to-

tal daily amount of urine was conserved at -4 °C after homogenization to determine the Nitrogen content.

Rumen fermentation

Samples of rumen fluid were collected from 6 animals from each group using a stomach tube before the morning feeding (0h), at 2, 5 and 8 hours post feeding. Ruminal pH was immediately measured using a portable pH meter (330i SET; WTW; Germany) after calibration with two buffers (7.00 and 4.01). For ammonia nitrogen analysis, the ruminal liquid was filtered through 4 layers of sterile gaze strips and a part of the filtered liquid was conserved with concentrated H₂SO₄. The NH₃-N was determined according to⁷ method. For volatile fatty acid (VFA) analysis, another part of ruminal liquid was fixed by a solution containing 0.25 ml H₃PO₄+0.25 ml Hg Cl₂ and frozen. The VFA were analysed using a Gas Chromatography Bruker Scion 460. The separation process was carried out with acapillary column (30 m x 0.25 mm D.I x 0.25 μ m film thickness). The injector temperature was at 220°C while the detector temperature at 230 °C. VFA were then identified by comparison of retention times with the patterns.

Haematological parameters

Blood samples were collected, at morning prior feeding, into heparinised vacutainers and immediately centrifuged at 3000 g for 15 minutes. Serum samples were transferred into Eppendorf tubes and frozen until analyses. Then, glucose, triglycerides, cholesterol, urea and creatinine concentrations were measured by specific Kits supplied by Phase Biomaghreb (Tunisia) and the absorbance reading was performed by a spectrophotometer (Rayto: RT-1904 C, Germany).

Chemical analyses

The DM of oat hay, concentrate, CRR, PRR, feed refusals and faeces was determined by drying at 75 °C until constant weight and then milled through 1 mm screen. Ash was determined at 550 °C for 6 hours; CP content was determined by the Kjeldahl method on distributed feed, refusals, faeces and urine. Acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin were determined according to⁸ method using an ANKOM220 fiber analyzer (ANKOM Technology Corp, Macedon, NY, USA). The total phenolic compounds (TPC) were extracted and determined as described by⁹ using the Folin-Ciocalteu's assay.

Calculations and statistical analysis

The digestibility of the nutrients was measured as the difference between the amount of nutrient ingested minus the

Table 1 - Chemical composition (g/kg DM) and cost of experimental feeds (Tunisian Dinar, TND).

	Concentrate	Oat hay	Pellets rosemary residues	Crude rosemary residues
Dry Matter (g/kg)	923	860	928	849
Crude Protein (g/kg)	119	50	116	73
NDF (g/kg)	342	667	386	385
ADF (g/kg)	44	353	175	273
Lignin (g/kg)	6.5	38	88	160
TPC (mg tannic acid/gDM)	2.6	8.1	33.8	47.3
Feed cost/ton (TND)	580	400	300	100

NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; TPC: Total Phenolic Compounds.

amount of nutrient excreted in the feces, expressed as a percentage of the nutrient ingested: 100 x (intake - excreted)/intake. The feed conversion rate was calculated as the ratio of dry matter intake (DMI) to ADG (g DMI / g BW gain). The feed cost/kg gain (TND) was calculated as the ratio of lambs' total ration cost to the whole weight gain during the experiment. The PROC GLM of SAS (2004)¹⁰ was applied. For nutrient intake, lamb's growth, daily feed cost, nutrient digestibility, nitrogen balance, haematological parameters and the rumen fermentation parameters, a one-way analysis of variance for diet effects was used. The significance was declared at *p* < 0.05. The differences between groups was compared by the Duncan's test. The following contrasts were used:

*Ct1: The RR presence effect [C vs. CRR + PRR]

*Ct2: The administration form of RR effect [CRR vs. PRR]

RESULTS

Feed chemical composition

The oat hay presented the lowest, the pelleted RR had the highest CP content (50 vs. 116 g/kg DM), while the crude RR had an intermediate value (73 g/kg DM). The NDF and ADF contents were higher for oat hay than both forms of RR; however the RR diets had the highest content of lignin and total phenolic compounds (33.8 and 47.3 vs. 8.1 mg tannic acid/g DM) for PRR, CRR and C, respectively) (Table 1).

Metabolism trial: lintake, digestibility and nitrogen balance

For all groups, the distributed amount of concentrate was entirely consumed. However, there was some refusal in roughages for all groups and the DM intake averaged 459, 455 and 494 g/day for C, CRR and PRR, respectively (p>0.05). The total DM intake was similar for all lambs (p>0.05), while the CP intake was higher for PRR than for both other groups. The nutrient digestibility for C and PRR groups was similar however; it was significantly lower in CRR diet (Table 2). The CP digestibility was unaffected (P>0.05).

The RR consumption in both forms (CRR and PRR) increased significantly the N intake (*p*<0.05; Table 2). The faecal nitrogen (FN) was higher for CRR than C, whereas PRR was intermediate. The urinary nitrogen (UN) was higher for the PRR group compared with both the other groups (C and CRR), but it was low for all groups. Given the high N intake and low FN, the highest retained nitrogen value was presented by PRR (7.66 vs. 6.05 and 4.1 g/d for CRR and C, respectively). The nitrogen efficiency (RN/NI) was higher for lambs receiving PRR (44%) than both other groups (37 and 39% for C and CRR, respectively).

Ruminal fermentation

The mean ruminal pH was higher (6.35) for C than both RR diets (5.95; Table 2). Before the morning feeding, average pH was comparable among diets (p>0.05, Figure 1); two hours after feeding, it decreased (p<0.005) for all groups; then at 8 hours post feeding, the pH value increased slightly for all groups. The ammonia concentration was lower for CRR than the rest of groups (p<0.05). It increased two hours post feeding for all groups (Figure 2).

Total VFA were unaffected by the regimens (p>0.05). The evolutions of total and individual VFA are presented in Figures 3



Figure 1 - Ruminal pH evolution.

and 4, respectively. The intake of RR decreased the proportion of acetic acid and increased the propionic acid in the total VFA for CRR and PRR compared to C diet (p<0.05). However, the butyric acid proportion was similar among diets (p>0.05).

Growth trial: feed intake and growth performance

During the growth trial, the whole offered amount of concentrate was consumed by all lambs, while the intake of different roughages (oat hay, CRR and PRR) increased progressively especially for CRR. The total dry matter intake was similar for all groups (Table 3). The Final BW, average daily gain (ADG) and feed conversion ratio (FCR; DM intake / ADG) did not differ among groups (p>0.05). The daily feed cost was reduced (p<0.05) for CRR and PRR compared to control group (Table 3). It was 0.47, 0.30 and 0.39 Tunisian Dinars for C, CRR and PRR, respectively.

Hematological parameters

The levels of glucose, triglycerides, cholesterol and urea were affected by the dietary treatment (p<0.05). Glucose and urea concentrations increased for PRR treatment compared to other diets. The lowest triglyceride and cholesterol levels were recorded for CRR group; however, no diet' effect was observed on the creatinine level (Table 3).

DISCUSSION

The CRR and PRR roughages had higher CP and lower NDF and ADF than oat hay, which could improve the efficiency of rumen activity¹¹. Nevertheless, both forms contain high level



Figure 2 - Ammonia-Nitrogen concentration evolution.

Table 2 - Nutrient intake (g/day), nutrient digestibility (g/kg), nitrogen balance (g/day) and ruminal fermentation parameters of Barbarine ewe	-
lambs.	

	С	CRR	PRR	SEM	Р	Ct1	Ct2		
Nutrient Intake									
Dry Matter (DM)	919	915	954	26.29	0.78	0.77	0.53		
Crude Protein (CP)	76 ^b	87 ^b	112ª	2.51	0.001	0.001	0.001		
Nutrient Digestibility									
DM	636ª	523 ^b	633ª	0.94	0.001	0.07	0.001		
Organic Matter	668ª	568 ^b	683ª	0.95	0.001	0.05	0.001		
NDF	595ª	465 ^b	566ª	1.13	0.001	0.004	0.002		
CP	541	460	601	2.66	0.12	0.85	0.04		
		Ν	itrogen balanc	Э					
Nitrogen Intake (NI)	11.2 ^b	15.5ª	17.4ª	1.54	0.001	0.001	0.06		
Faecal Nitrogen (FN)	5.7 ^b	8.35ª	7.04 ^{ab}	0.41	0.07	0.04	0.21		
Urinary Nitrogen (UN)	1. 4 ^b	1.1 ^b	2.7ª	0.20	0.01	0.26	0.008		
Retained nitrogen (RN)	4 .1 °	6.05 ^b	7.66ª	0.25	0.001	0.001	0.001		
RN/NI	0.37 ^{ab}	0.39 ^b	0.44ª	0.03	0.07	0.39	0.03		
Ruminal fermentation parameters									
Ruminal pH	6.35ª	5.95 ^b	5.95 ^b	0.03	0.001	0.001	1.0		
NH ₃ -N (mg/100 ml)	13.4ª	9.5 ^b	12.1ª	0.43	0.007	0.01	0.02		
TVFA (mol/100 ml)	62.7	70.8	59.5	2.74	0.25	0.67	0.11		
Acetic acid (%TVFA)	58.3ª	54.1 ^{ab}	49.0 ^b	1.12	0.01	0.01	0.08		
Propionic acid (%TVFA)	21.9 ^b	29.9ª	31.9ª	0.95	0.001	0.001	0.40		
Butyric acid (%TVFA)	14.2	13.5	14.2	0.96	0.94	0.85	0.77		

NDF: Neutral Detergent Fiber; NH₃-N: ammonia nitrogen; TVFA: Total Volatile fatty acid; Ct1: C vs. CRR+PRR; Ct2: CRR vs. PRR;

a, b, c: Means in the same row with different superscripts differ (P<0.05). SEM: Standard error of means.

of lignin and TPC, which could compromise the efficiency of these roughages. The CP digestibility was lower for CRR (rich in TPC) than PRR diet; the last was enriched in soya and wheat bran, so it contained more protein. An evident effect of protein supplementation on CP digestibility in case of low quality forages was shown¹². Generally, the use of CRR to substitute oat hay altered the nutrient digestibility for this group, however the PRR consumption that is the enhanced roughage (PRR) presented similar nutrient digestibility as the control roughage (hay). Probably the richness of these pellets on soya and wheat bran and the administration form (pellets vs. Crude form) was at the origin of this variance of nutrient digestibility. However, the administration of rosemary essential oil or its equivalent in leaves did not alter the DM digestibility¹³. In the same context, the RR intake did not alter the OM and CP digestibility until the rate of 70% of hay substitution for Queue Fine de l'Ouest lambs¹⁴. In the current study, the low digestibility in CRR group could be the consequence of their richness on lignin, which may result in a bound of their proteins and lead to a negative effect on nitrogen digestibility¹⁵.

The RR consumption in both forms increased the nitrogen intake as previously found for the lambs from Queue Fine de l'Ouest breed at the substitution rates of 30 and 70% to oat hay¹⁴. The higher faecal N output for CRR and PRR compared to C group resulted from the higher N intake in the first given the high correlation between N intake and faecal N. In addition, it could be explained by the fact that some of the RR were not digested and therefore excreted in faeces in relationship with the presence of the condensed tannins in the RR, which could complex proteins making them unprofitable to the microflora and therefore the host animal and could be excreted in faeces¹⁵. This hypothesis is particularly valuable for CRR group having the highest FN loss, for which RR with a high TPC content were intake in crude form (Table 1). The urinary N loss was feeble for all animals, but higher for PRR than other diets. This increase as well as the increase of the uraemia for this group resulted from the surplus in N availability. The relationship between high availability of nitrogen and high urinary N was established in previous studies¹³. The N balance was the highest for the PRR and lowest for control group; so it was proportional to the roughage content of N, a logical result given the same amount of concentrate¹¹. The richness of rosemary pellets in CP resulted in higher nitrogen balance, which was positive for all groups; this is curious for the control group given the low CP content in the oat hay (50 g/kg DM). Furthermore,



Figure 3 - Total volatile fatty acid concentration evolution.

	С	CRR	PRR	SEM	Р	Ct1	Ct2	
Roughage DMI (g/d)	469	392	427	6.5	0.17	0.09	0.39	
Total DMI (g/d)	910	833	868	16.5	0.17	0.09	0.39	
Total DMI (g/kg w ^{0.75})	67	62	64	1.65	0.27	0.08	0.39	
Final Body Weight (kg)	30.7	29.9	31.4	0.41	0.35	0.94	0.15	
Average Daily Gain (g)	85	83	93	4.53	0.61	0.76	0.34	
Feed Conversion Ratio	10.7	10.03	9.3	0.58	0.87	0.61	0.89	
Cost of daily feed (TND)	0.47 ^a	0.30°	0.39 ^b	0	0.001	0.001	0.001	
Feed cost/kg gain (TND)	5.78ª	3.87 ^b	4.71 ^{ab}	0.29	0.03	0.01	0.23	
Haematological parameters (mmol/l)								
Glucose	2.96 ^b	2.78 ^b	3.37ª	0.06	0.007	0.42	0.002	
Triglycerides	0.23ª	0.18 ^b	0.22 ^{ab}	0.006	0.03	0.03	0.08	
Cholesterol	1.55ª	1.10 ^b	1.50ª	0.05	0.005	0.04	0.006	
Urea	7.93 ^b	6.36°	11.92ª	0.29	0.001	0.06	0.001	
Creatinine	106	119	115	7.37	0.76	0.48	0.86	

SEM: Standard error of means; Ct1: C vs. CRR+ PRR; Ct2: CRR vs. PRR; TND: Tunisian Dinar.

 $^{\rm a,\,b,\,c}$: Means in the same row with different superscripts differ (P<0.05).





the retained N as proportion of N intake was higher for PRR than C and CRR groups. This is a logical result since the nitrogen retention is primarily a function of N intake and in this case the higher nitrogen intake was presented by PRR group. Nitrogen utilization was subsequently improved by wheat bran and soya bean inclusion in the RR pellets (PRR).

The average pH for C group ranged within previously reported values (6.1 to 6.8) for sheep¹⁶. A ruminal pH varying between 6.5 and 7 is optimal for microbial digestion of fibre and protein¹⁷. The decrease of ruminal pH two hours after feeding confirmed other results¹⁸ showing the pH decrease during the five hours after feeding in relationship with concentrate intake and VFA concentration increase. In addition, the decrease in pH could be a consequence of dietary N increasing¹².

The ammonia-Nitrogen concentration for all diets was within the range of requirements for rumen bacteria¹⁹; it is sufficient for an optimum digestion and microbial growth²⁰. The ammonia concentrations increased 2 hours after feeding and then decreased for all groups. This tendency was previously reported¹² in relationship with the high microbial activity and a favourable pH to the proliferation of protozoa. For PRR diet, the NH3-N augmentation 2 hours after feeding was spectacular corresponding to 150% ammonia concentration of CRR. This difference resulted from CP content and digestibility difference between both RR diets. Five hours post feeding, ammonia concentration decreased for all lambs in relationship with reduced microbial activity.

The VFA are the end products of rumen digestion of carbohydrate that depend on the feed energy and starch quality²⁰. Overall, the total VFA was similar for all diets that could be originated by the similarity among diet intake. The concentration before the morning feeding was the lowest. Two hours post feeding, the total VFA concentrations increased significantly among groups, this could result from the rapid rumen degradability of feed' starch. Five hours after feeding, the VFA concentration in C and PRR decreased in comparison with CRR, but without significant difference, where VFA remained still stable. The acetic and propionic acids had the same pattern for C and PRR. However, the acetic acid was higher with C diet based in hay containing the highest NDF level, which confirm the acetic acid increase with diet rich in fiber. The lower value of this acid with PRR diet was compensated by an increase in propionic acid, even though in some cases could happen that the increase of acetate occurred at the expense of butyrate and not of propionate²⁰. Nevertheless, generally, the high proportion of acetic acid compared with the propionic acid for all groups suggests that the diets are rich in fiber²¹.

The inclusion of RR in animal diets did not affect feed intake, as reported by some earlier works where the inclusion of rosemary as an additive or as the partial or total replacement to oat hay, did not affect the feed intake^{13, 14, 22}. It was reported that rosemary is an odorous herb which can limit its use in animal feeding at high levels of addition²³; however, in the current study, the total substitution of hay by RR as roughage was a success. Therefore, RR was appreciated by the ewe-lambs and its consumption was not affected in both forms. The ADG was low for all diets (< 100g/day). The weakness of growth rate could be due to (i) the lamb's sex, females had lower growth than males and (ii) the climatic conditions, since the experiment occurred in summer, with high temperatures. The similarity of ADG was obtained despite the higher N retention for PRR group (>7 g/d). This could be due to an unbalanced energy: protein ratio; for such nitrogen supply more energy should be available. Hence, the CRR, without wheat bran and soya, resulted in similar performance as PRR sparing these ingredients. Our results are consistent with other studies²⁴ using alternative foods (feedblocs, acacia and Atriplex).

The use of RR as roughages in the current study decreased the daily feed costs, which respond to the objective of the study confirming previous results on lower cost of by-products diet²⁴. The CRR diet recorded the highest economical feed; this result is pertinent showing the possibility of entire replacement of oat hay, expensive especially in the dry years, by crude rosemary by-products as collected in the forest. Hence, feeding RR to sheep could reduce the feed cost without affecting lamb's performance. In addition, the feed cost per kg of gain was reduced by 30% and 18.5% for CRR and PRR, respectively compared with C diet.

For plasma metabolites, the glucose, triglycerides and creatinine concentrations for all groups were within the ranges reported for small ruminant^{25, 26}. The higher glycaemia with PRR can be related to the the wheat bran presence, rich in starch as a polymer of glucose, can originate this augmentation²⁷. The uraemia was higher for PRR group (11.92 mmol/l) being higher than common values but similar to that resulting from carob intake28. This increase resulted from the excess of N availability for this group; similar conclusions were reported concerning the high level of dietary N and high uraemia12. The cholesterol concentration was low for CRR group. This decrease can be due to the high level of tannins in crude RR; in fact, it was shown that the blood cholesterol decreases with intake of carob rich in tannins²⁸. The triglycerides are lipids that store energy in adipose tissue of the animal. The higher triglycerides concentrations observed for C and PRR groups showed that both diets resulted in similar amount of lipids. The rate of blood' creatinine may be considered as an index of endogenous protein catabolism²⁹. Besides, the concentration of creatinine in blood can be in connection with the degradation of muscle³⁰, in the current study, it could be concluded that all lambs produced the same muscle quantity, had the same protein degradation and consequently the same creatinine rates. Generally, the haematological parameters were not affected by the inclusion of RR in both forms; these results can approve other earlier results¹³ showing no supply effect of rosemary leaves.

CONCLUSIONS

The results of the current study indicated that distillate rosemary residues can be used as unconventional roughage that can substitute oat hay for growing ewe-lambs with similar growth rates. The pellet's form, enriched with wheat bran and soya, resulted in high nitrogen retention without consecutive better growth; it is a waste of these ingredients, soya bean in particular. Such RR form should be used with higher energetic diet or formulated without soya. Using RR in both forms reduced the total feed cost without altering animal performance; it could be an economically advantageous practice for farmers.

Acknowledgements

We wish to acknowledge (i) Dr Margalida Joy and the staff of CITA in Zaragoza (Spain) where some analyses were performed, (ii) the ESAK staff for their collaboration, (iii) Dr Noura Ben Youssef, researcher, and Fatma Boussen, technician, in INRAT for their help in some analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

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