Journal of Agriculture and Rural Development in the Tropics and Subtropics
Vol. 116 No. 1 (2015) 91–97

urn:nbn:de:hebis:34-2015031947751 ISSN: 2363-6033 (online); 1612-9830 (print) – website: www.jarts.info

Effect of rosemary (*Rosmarinus officinalis*) extract on weight, hematology and cell-mediated immune response of newborn goat kids

Borhan Shokrollahi [a,*], Fardin Amini [a], Shahin Fakour [b], Mohammad Amiri Andi [a]

[a] *Department of Animal Science, Faculty of Agriculture, Sanandaj Branch, Islamic Azad University, Sanandaj, Kurdistan, Iran*
[b] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Kurdistan, Iran*

Abstract

This study aimed at evaluating the effects of different levels of rosemary (*Rosmarinus officinalis*) extract on growth rate, hematology and cell-mediated immune response in Markhoz newborn goat kids. Twenty four goat kids (aged 7±3 days) were randomly allotted to four groups with six replicates. The groups included: control, T1, T2 and T3 groups which received supplemented-milk with 0, 100, 200 and 400 mg aqueous rosemary extract per kg of live body weight per day for 42 days. Body weights of kids were measured weekly until the end of the experiment. On day 42, 10 ml blood samples were collected from each kid through the jugular vein. Cell-mediated immune response was assessed through the double skin thickness after intradermal injection of phyto-hematoglutinin (PHA) at day 21 and 42. No significant differences were seen in initial body weight, average daily gain (ADG) and total gain. However, significant differences in globulin ($P < 0.05$), and white blood cells (WBC) ($P < 0.001$) were observed. There were no significant differences in haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), lymphocytes and neutrophils between the treatments. Skin thickness in response to intra dermal injection of PHA significantly increased in the treated groups as compared to the control group at day 42 ($P < 0.01$) with the T3 group showing the highest response to PHA injection. In conclusion, the results indicated that aqueous rosemary extract supplemented-milk had a positive effect on immunity and skin thickness of newborn goat kids.

Keywords: rosemary extract, growth rate, hematology, cell-mediated immune response, goat kids

1 Introduction

Immunity of newborn animals is low in the first weeks after birth due to a variety of physiological and environmental stressors (Shokrollahi *et al.*, 2013). The mortality rate is high during this period because of various inducements including pneumonia and diarrhea (Peeler & Wanyangu, 1998). There is a high degree of variability in the quality of passive immunity acquired by kids. Scientists are exploring additives that improve animal health, passive immunity and productivity. The use of phytogenic ingredients have gained prominence and received attention in animal industry because of their broad antioxidative actions (Wei & Shibamoto, 2007), antimicrobial actions (Özer *et al.*, 2007), as well as their growth and immune booster actions. Rosemary (*Rosmarinus officinalis* L.), as an aromatic herb, is one of the most widely commercialised plants administered in animal diets as a source of natural polyphenols such as rosmanol, genkwanin, carnosol, rosmadial, caffeic

* Corresponding author
Pasdaran St. Se Rahe Adab, Sanandaj branch,
Islamic Azad University, Sanandaj, Iran,
Postal code: 6616935391,
Email: Borhansh@gmail.com
Phone: +98 8733627007; Fax: +98 8733288677

Published online: June 2, 2015

acid, rosmarinic acid and carnosic acid (Anderson *et al*., 2008; Costa *et al.*, 2007; Cuvelier *et al.*, 1996; Moran *et al.*, 2005). Its powerful antioxidant activity supports protection against damage induced by free radicals (Ramírez *et al.*, 2004). Moreover, rosemary has been demonstrated to have anticancer (Tai *et al.*, 2012), anti-inflammatory and antimicrobial effects (Aruoma *et al.*, 1996; Seydim & Sarikus, 2006; Suong *et al.*, 2011). The effect of rosemary or its essential oils on rumen fermentation and on milk fatty acids compositions has been investigated (Nudda *et al.*, 2014; Sahraei *et al.*, 2014). Extracts of rosemary leaves fed to lactating Murciano-Granadina goats increased polyunsaturated fatty acids in milk with increasing dosage (Boutoial *et al.*, 2013) but this effect was not observed in cattle (Benchaar *et al.*, 2007; Hristov *et al.*, 2013). Moujahed *et al.* (2013) reported that rosemary essential oils had no effect neither on total gas production nor on volatile fatty acid accumulation in rumen of sheep.

Markhoz goats are reared in western Azerbaijan, Kurdistan and Kermanshah provinces of Iran and produce mohair (Bahmani *et al.*, 2011; Farshad *et al.*, 2008). The mohair obtained from these animals has an important cultural role and is used for making local clothes in Kurdistan; the animals are also major source of red meat (102,000 ton per year) in Iran (Farshad *et al.*, 2008). Nutritional strategies are necessary to reduce the immunosuppressive consequences of physical or pathological stresses which cause mortality among newborn kids.

The effect of herbs on immunity enhancement and subsequent growth of goat kids remained to be surveyed. Taking account of a possible immune stimulant effect of rosemary due to its antimicrobial, antioxidant, flavour enhancing properties and potential therapeutic benefits, the objective of the current study was to determine the effect of rosemary extract at different supplemental levels on growth rate, haematology and cell mediated immune response in newborn goat kids.

2 Materials and methods

2.1 Rosemary aqueous extract preparation

Rosemary plants were collected in January from fresh herbs grown under greenhouse condition. The extract was prepared by submerging 5 or 10 g L^{-1} of fresh leaves in boiling water for 10 minutes. Once the water had cooled off to 25 °C, the solution was filtered to remove the leaves before use (Malo *et al.*, 2011).

2.2 Animal management and experimental design

The experiment was conducted on the Markhoz goat research station in Sanandaj in the Kurdistan province of West Iran. A total of twenty-four Markhoz newborn male goat kids (about 7±3 days of age) were randomly divided into four groups (Control, T1, T2 and T3) and kept under an approved protocol by the research station. These animals were selected according to the parity of dams (all dams were homogeneous for parity), weight and nutrition during their pregnancy. The treated groups (T1, T2 and T3) were fed with supplemented milk containing 100, 200 or 400 mg rosemary extract per kg of live body weight per day for 42 days, respectively. The control group was fed by an equal amount of milk without rosemary extract.

Uniform management standards were applied in all groups. Health status of kids was checked by diagnosing pneumonia and diarrhoea and treated if pneumonia and diarrhoea were recorded at the beginning of the experiment. Each kid was kept with its mother in a separate cage (2 ×1.5 m) equipped with feeders and water until the end of the experiment. Before feeding supplemented milk, goat kids were separated from their mothers and fasted for 3 h after which they were fed with rosemary extract supplemented milk using pacifiers. Rosemary extract supplemented milk was prepared by dissolving the required amount of rosemary extract into 70 cc of fresh goat milk. During the experimental period, kids were closely monitored to ensure enough suckling. All kids received colostrum during the first days after birth.

2.3 Weighing and sampling

Kids were weighed weekly from the beginning to the end of the experiment (6 weeks). About 10 ml of blood were collected from each kid through the jugular vein at the end of the study (day 42). Two and half millilitres of blood anticoagulated with EDTA were used for blood cells count and 7.5 ml transferred to a plane tube for serum separation. All tubes were instantly kept at 4 °C and then centrifuged (3,000 × g for 10 min); the obtained serum was separated and all samples transferred to the laboratory and stored at –20 °C until analyses.

Anticoagulated blood was analysed for hematocrit (PCV), haemoglobin (Hb) and leukocyte and erythrocyte counts (WBC and RBC) by micro-hematocrit, cyanmethaemoglobin and standard manual methods, respectively. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique (Jain, 1986).

Serum globulin was measured using a commercial kit (Pars Azmun R2 11005). Average daily gain (ADG) ratios were calculated daily throughout the experimental period.

2.4 Skin-testing of cell-mediated immunity with PHA

Cell-mediated immune response was evaluated by determining double skin thickness in response to phytohaemagglutinin (PHA) using the test procedure reported by Lacetera *et al.* (1999). Skin tests were performed on days 21 and 42 after the beginning of the experiment. To this end, 250 μg PHA diluted in 0.1 ml phosphate buffer was intradermal injected to a shaved area on the right lumbar back using an automatic injector. Double skin thickness was measured using a digital calliper before (time 0) and 8, 16, and 24 h after injection of PHA. Sterile phosphate buffer saline (PBS) was injected nearby approximately 10 cm from the injection site of PHA to test of any skin responses to PBS alone.

2.5 Statistical analysis

Data for haematology parameters and weights were analysed according to a completely randomised design using the General Linear Models procedure of SAS (version 9.2). Double skin thickness data were submitted to the MIXED procedure, considering the skin thickness before PHA injection (time 0) as covariate. Means were separated by LSD, and least squares means and SEM for all data are presented. Main effects were discussed if $P < 0.05$.

3 Results

3.1 ADG, total gain and haematology parameters

The mean initial weight, total gain and ADG did not differ significantly among groups (Table 1), however, the highest ADG and total gain were observed in group T2.

Significant differences in globulin ($P < 0.05$) and WBC ($P < 0.001$) were found among the groups. Globulin was significantly increased in T3 kids as compared to the control. WBC was the highest in the T2 and T1 groups and differed significantly from the control group. However, no significant differences were observed for Hb, PCV, RBC, lymphocytes and neutrophils levels among groups (Table 2).

Table 1: *Mean (±SEM) of total gain and average daily gain (ADG) in the different groups.*

Item	*Control*	*T1*	*T2*	*T3*	*P-value*
Initial weight (kg)	5.60 ±0.377	5.49±0.326	5.50 ±0.250	5.90 ±0.278	NS
ADG (g/day)	68.8±11.36	62.9±13.27	75.3±7.89	70.2±5.69	NS
Total gain (kg)	2.89±0.477	2.64±0.557	3.58±0.332	2.95±0.239	NS

The Control, T1, T2 and T3 groups were supplemented with 70 cc of milk containing 0, 100, 200 or 400 mg rosemary extract per kg of live body weight per day.

Table 2: *Mean (±SEM) concentration of different blood biochemical and haematology factors for the different groups.*

Item	*Control*	*T1*	*T2*	*T3*	*P-value*
Globulin (g/dl)	2.65 ± 0.083^{b}	2.82 ± 0.030^{ab}	2.95 ± 0.048^{a}	2.82 ± 0.076^{ab}	*
Hb (g/dl)	9.26±0.435	8.75±0.389	8.26±0.388	8.86±0.443	NS
PCV (%)	26.8±2.00	22.9±1.53	23.7±1.32	23.8±1.57	NS
RBC ($\times10^6/\mu$l)	16.2±1.08	13.5±0.96	13.5±1.20	14.2±0.97	NS
WBC ($\times10^6/\mu$l)	17.0 ± 0.92^{c}	20.9 ± 1.16^{b}	24.1 ± 1.18^{a}	17.8 ± 1.06^{c}	***
Neutrophils (%)	34.5±1.60	35.5±1.85	34.2±1.48	35.5±1.25	NS
Lymphocyte (%)	64.2±1.79	62.7±1.95	64±1.39	63.5±1.39	NS

The Control, T1, T2 and T3 groups were supplemented with 70 cc of milk containing 0, 100, 200 or 400 mg rosemary extract per kg of live body weight per day.
Means in the same row with different letter are significantly different. NS = P>0.05, * = P<0.05, *** P<0.001.

Table 3: *Mean (±SEM) of double skin thickness (millimetre) measurements for the different groups after PHA injection at day 21 and day 42 of experiment.*

Item	*Control*	*T1*	*T2*	*T3*	*P-value*
21-day	6.98±0.166	6.80±0.141	6.74±0.222	6.65±0.159	NS
42-day	6.22 ±0.218^{b}	6.93±0.266^{a}	6.69±0.128^{ab}	7.17±0.221^{a}	**

The Control, T1, T2 and T3 groups were supplemented with 70 cc of milk containing 0, 100, 200 or 400 mg rosemary extract per kg of live body weight per day.
Means in the same row with different letter are significantly different. NS = P>0.05, ** P<0.01.

3.2 Cell-mediated immune response

Skin thickness was not significantly different among groups on day 21 of the experiment. However, significant differences were observed after injection of PHA among groups on day 42 of the experiment (P<0.01) with the T3 group showing the highest response to PHA injection. Significant differences were observed in skin thickness among groups after 8, 16 and 24 hours post PHA injection on day 42 (Table 3).

4 Discussion

Medicinal herbs or phytogenic products seem to be valuable for enhancing immunity and subsequently, by their broad-spectrum activity, they may lead to an elevated growth. Rosemary being an important medicinal herb has many reported beneficial effects. Some researchers have shown the beneficial effect of rosemary on growth performance. Ghazalah & Ali (2008) showed that the addition of 0.5 % rosemary leaves to the basal diet of broilers enhanced weight gain as compared to the control group. Moreover, different levels of rosemary remarkably increased growth performance for 42 days in broiler chicks (Yesilbag *et al.*, 2011). Findings of the current research revealed that there were no substantial differences in growth rate among the treated and control groups, as ADG and total weight gain non-significantly increased in kids given rosemary extract. No effect of rosemary on growth performance was detected alike in chicks (Basmacıoğlu *et al.*, 2004; Yasar *et al.*, 2011), sea bass (Turi *et al.*, 2009), rat (Afonso *et al.*, 2013) and rabbit (Beghelli *et al.*, 2012). Similarly, Janz *et al.* (2007) reported that rosemary had no substantial effect on growth performance in treated pigs in comparison with the control group. Contrary, Ibarra *et al.* (2011) suggested that carnosic acid-rich rosemary leaf extract in a high-fat diet limited weight gain in mice. These, partial contradictory results may be due to differences in the type, quality or quantity of supplemental rosemary and also due to species and age of animals.

Changes in the physiological state of an animal are often reflected by changes in haematology parameters. Hence, blood indices are important tools used to confirm the effects of nutritional and environmental management in animals. In the current study, we have not seen any significant differences in the rate of Hb, PCV and RBC among groups, although, the rate of these parameters tended to decrease in the treated kids. There is no reported study about the effect of rosemary on Hb, PCV and RBC in newborn or adult ruminants. In contradiction with our results, in a study with quails, Yesilbag *et al.* (2012) showed that 100 mg of rosemary per kg of diet caused an increase in levels of RBC, Hb and PCV.

Milk supplemented with rosemary extract (T1 and T2) had a statistically significant effect on WBC counts but had no effect on lymphocyte or neutrophil amounts. Contrary to these results, in a study on quails, rosemary plus oregano volatile oil mixture had no remarkable effect on WBC counts but significantly enhanced the levels of lymphocyte and neutrophil (Yesilbag *et al.*, 2012). Moreover, Savoini *et al.* (2003) reported that dietary rosemary extract markedly decreased the counts of WBC and blood neutrophils percentage compared to the control group in organically managed dairy goats.

The amount of globulin significantly increased in T3 kids compared to other groups. Serum proteins especially globulin contribute profoundly to the immunity and growth rate of newborn kids, not only because of the immunoglobulin content, but also because of other nutritional and physiological effects (Chen *et al.*, 1999). Rosemary supplementation increased blood total protein content of organically managed dairy ewes (Chiofalo *et al.*, 2012). Ghazalah & Ali (2008) showed that rosemary leaves meal (at 0.5 and 1 % of the diet) increased the globulin level in broilers as compared to the control group. Because of the close correlation between globulin and immunoglobulins levels, it could be concluded from the present study that rosemary extract had a positive effect on immunity due to its role in developing and protecting cells and inhibiting non-enzymatic oxidation (Ghazalah & Ali, 2008).

Measurement of immune reactivity is an important tool (Hessing *et al.*, 1995), and is also beneficial as a complement to diagnostic tests based on the immune response. PHA, a lectin from *Phaseolus vulgaris*, causes agglutination of erythrocytes; and growth, division and non-specific activation of T-cells. The skin test involves injecting PHA and measuring the change in skin thickness. The results of the current study showed that milk supplemented with rosemary extract had an effect on the double skin thickness in response to PHA injection in comparison with control kids at day 42 of the experiment. Similar to these results, 200 ppm rosemary extracts increased mitogenic response of spleen cells to concanavalin A and PHA in rats fed 10 % casein (Babu *et al.*, 1999). Furthermore, Al-Sheyab *et al.* (2012) detected that concanavalin A-stimulated proliferation of spleen cells from mice fed with 100 mg/kg body weight rosemary extract was significantly higher by 57 % than that of cells from the corresponding control animals, these researchers also showed that mice treated with 10, 50, 100 mg of rosemary extract per kg body weight had significantly higher Immunoglobulin M (IgM) in comparison with the control group. Rosemary because of its powerful antioxidant activity may help to alleviate the oxidative stress conditions like protein or antioxidant deficiency (Babu *et al.*, 1999).

In conclusion, the rosemary supplementation to newborn kids significantly improved the WBC counts and double skin thickness in response to PHA injection on day 42. Future studies involving the investigation of the bioavailability of rosemary phenolic elements and their effect on immune enhancing parameters may help to clarify their role in physiological and environmental stressors in newborn animals.

Acknowledgements

This research was supported by Sanandaj branch, Islamic Azad University, Kurdistan, Iran.

References

Afonso, M. S., Silva, A. M. D. O., Carvalho, E. B., Rivelli, D. P., Barros, S. B. M., Rogero, M. M., Lottenberg, A. M., Torres, R. P. & Mancini-Filho, J. (2013). Phenolic compounds from Rosemary (*Rosmarinus officinalis* L.) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats. *Nutrition and Metabolism*, 10, 1–9.

Al-Sheyab, F. M., Abuharfeil, N., Salloum, L., Hani, R. B. & Awad, D. S. (2012). The Effect of Rosemary (*Rosmarinus officinalis* L.) Plant Extracts on the Immune Response and Lipid Profile in Mice. *Journal of Biology and Life Science*, 3, 37–58.

Anderson, D., Cheng, Y. & Duan, R. D. (2008). Ursolic acid inhibits the formation of aberrant crypt foci and affects colonic sphingomyelin hydrolyzing enzymes in azoxymethane-treated rats. *Journal of Cancer Research and Clinical Oncology*, 134, 101–107.

Aruoma, O., Spencer, J. P., Rossi, R., Aeschbach, R., Kahn, A., Mahmood, N., Munoz, A., Murcia, A., Butler, J. & Halliwell, B. (1996). An evaluation of the antioxidant antiviral action of extracts of rosemary and provencal herbs. *Food and Chemical Toxicology*, 34, 449–456.

Babu, U. S., Wiesenfeld, P. L. & Jenkins, M. Y. (1999). Effect of dietary rosemary extract on cell-mediated immunity of young rats. *Plant Foods for Human Nutrition*, 53, 169–174.

Bahmani, H., Tahmoorespur, M., Aslaminejad, A. A., Ali Abbasi, M. & Ebnabbas, R. (2011). Assessment of Demographic, Geographical and Genetic Risks in Markhoz Goat Population. *Journal of Animal and Veterinary Advances*, 10, 162–168.

Basmacıoğlu, H., Tokuşoğlu, Ö. & Ergül, M. (2004). The effectof oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFA's inbroilers. *South African Journal of Animal Science*, 34, 197–210.

Beghelli, D., Cardinali, R., Caterbi, S., Bistoni, O., Alunno, A., Gerli, R. & Castellini, C. (2012). Phytoderivates in rabbit diet and immune responses. In *Proceedings 10th World Rabbit Congress, Sharm El-Sheikh – Egypt* (pp. 1019–1023). World Rabbit Science Association.

Benchaar, C., Petit, H. V., Berthiaume, R., Ouellet, D. R., Chiquette, J. & Chouinard, P. Y. (2007). Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *Journal of Dairy Science*, 90, 886–897.

Boutoial, K., Ferrandini, E., Rovira, S., García, V. & Belén López, M. (2013). Effect of feeding goats with rosemary (*Rosmarinus officinalis* spp.) by-product on milk and cheese properties. *Small Ruminant Research*, 112, 147–153.

Chen, J. C., Chang, C. J., Peh, H. C. & Chen, S. Y. (1999). Serum protein levels and neonatal growth rate of Nubian goat kids in Taiwan area. *Small Ruminant Research*, 32, 153–160.

Chiofalo, V., Liotta, L., Fiumanò, R., Benedetta, E. R. & Chiofalo, B. (2012). Influence of dietary supplementation of *Rosmarinus officinalis* L. on performances of dairy ewes organically managed. *Small Ruminant Research*, 104, 122–128.

Costa, S., Utan, A., Speroni, E., Cervellati, R., Piva, G., Prandini, A. & Guerra, M. C. (2007). Carnosic acid from rosemary extracts: A potential chemoprotective agent against aflatoxin B1. An in vitro study. *Journal of Applied Toxicology*, 27, 152–159.

Cuvelier, M. E., Richard, H. & Berset, C. (1996). Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *Journal of American Oil Chemist's Society*, 73, 645–652.

Farshad, A., Akhondzadeh, S., Zamiri, M. J. & Sadeghi, G. (2008). The Estrous Cycle of the Markhoz Goat in Iran. *Asian-Aust Journal of Animal Science*, 21, 1411–1415.

Ghazalah, A. A. & Ali, A. M. (2008). Rosemary Leaves as a Dietary Supplement for Growth in Broiler Chickens. *International Journal of Poultry Science*, 7, 234–239.

Hessing, M. J., Coenen, G. J., Vaiman, M. & Renard, C. (1995). Individual differences in cell-mediated and humoral immunity in pigs. *Veterinary Immunology Immunopathology*, 45, 97–113.

Hristov, A. N., Lee, C., Cassidy, T., Heyler, K., Tekippe, J. A., Varga, G. A., Corl, B. & Brandt, R. C. (2013). Effect of *Origanum vulgare* L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science*, 96, 1189–1202.

Ibarra, A., Cases, J., Roller, M., Chiralt-Boix, A., Coussaert, A. L. & Ripoll, C. (2011). Carnosic acid-rich rosemary (*Rosmarinus officinalis* L.) leaf extract limits weight gain and improves cholesterol levels and glycaemia in mice on a high-fat diet. *British Journal of Nutrition*, 106, 1182–1189.

Jain, N. C. (1986). *Schalm's veterinary hematology*. Lea & Febiger, Philadelphia, U.S.

Janz, J. A., Morel, P. C., Wilkinson, B. H. & Purchas, R. W. (2007). Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. *Meat Science*, 75, 350–355.

Lacetera, N., Bernabucci, U., Ronchi, B. & Tardone, A. (1999). The effect of injectable sodium selenite on immune function and milk production in Sardinian sheep receiving adequate dietary selenium. *Veterinary Research*, 30, 363–370.

Malo, C., Gil, L., Cano, R., Martínez, F. & Galé, I. (2011). Antioxidant effect of rosemary (*Rosmarinus officinalis*) on boar epididymal spermatozoa during cryopreservation. *Theriogenology*, 75, 1735–1741.

Moran, A. E., Carothers, A. M., Weyant, M. J., Redston, M. & Bertagnolli, M. M. (2005). Carnosol Inhibits{beta}-Catenin Tyrosine Phosphorylation and Prevents Adenoma Formation in the C57BL/6J/Min/+(Min/+) Mouse. *Cancer Research*, 65, 1097–1104.

Moujahed, N., Bouaziz, Y. & Khelfa, A. (2013). Effects of essential oils from *Rosmarinus officinalis* and *Thymus capitatus* on in vitro rumen fermentation in sheep. In H. Ben Salem, & A. López-Francos (Eds.), *Feeding and management strategies to improve livestock productivity, welfare and product quality under climate change* (pp. 35–38). CIHEAM / INRAT / OEP / IRESA / FAO, Zaragoza.

Nudda, A., Battacone, G., Neto, O. S., Cannas, A., Helena, A., Francesconi, D., Atzori, A. S. & Pulina, G. (2014). Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira de Zootecnia*, 43, 445–456.

Özer, H., Sökmen, M., Gülüce, M., Adigüzel, A., Sahin, F., Sökmen, A., Kiliç, H. & Bariş, Ö. (2007). Chemical composition and antimicrobial and antioxidant activities of the essential oil and methanol extract of *Hippomarathrum microcarpum* (Bieb) from Turkey. *Journal of Agriculture and Food Chemistry*, 55, 937—942.

Peeler, E. J. & Wanyangu, S. W. (1998). Infectious causes of small ruminant mortality in Kenya: A review. *Small Ruminant Research*, 22, 1–11.

Ramírez, P., Senorans, F. J., Ibáñez, E. & Reglero, G. (2004). Separation of rosemary antioxidant compounds by supercritical fluid chromatography on coated packed capillary columns. *Journal of Chromatography A*, 1057, 241—245.

Sahraei, M., Pirmohammadi, R. & Payvastegan, S. (2014). The effect of rosemary (*Rosmarinus officinalis* L.) essential oil on digestibility, ruminal fer-

mentation and blood metabolites of Ghezel sheep fed barley-based diets. *Spanish Journal of Agricultural Research*, 12, 448–454.

Savoini, G., Cattaneo, D., Paratte, R., Varisco, G., Bronzo, V., Moroni, P. & Pisoni, G. (2003). Dietary rosemary extract in dairy goats organically managed: effects on immune response, mammary infections and milk quality. *Italian Journal of Animal Science*, 2 (SUPPL. 1), 548–550.

Seydim, A. C. & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International*, 39, 639—644.

Shokrollahi, B., Mansouri, M. & Amanlou, H. (2013). The effect of enriched milk with selenium and vitamin E on growth rate, hematology, some blood biochemical factors, and immunoglobulins of newborn goat kids. *Biological Trace Element Research*, 153, 184–190.

Suong, N. T. N., Desmond, B. W. & Richard, J. H. (2011). Rosemary and cancer prevention: preclinical perspectives. *Critical reviews in food science and nutrition*, 51, 946–954.

Tai, J., Cheung, S., Wua, M. & Hasman, D. (2012). Antiproliferation effect of Rosemary (*Rosmarinus officinalis*) on human ovarian cancer cells in vitro. *Phytomedicine*, 19, 436—443.

Turi, L. D., Ragni, M., Jambrenghi, A. C., Lastilla, M., Vicenti, A., Colonna, M. A., Giannico, F. & Vonghia, G. (2009). Effect of dietary rosemary oil on growth performance and flesh quality of farmed seabass (*Dicentrarchus labrax*). *Italian Journal of Animal Science*, 8, 857–859.

Wei, A. & Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils. *Journal of Agriculture Food Chemistry*, 55, 1737–1742.

Yasar, S., Namik, D., Fatih, G., Gokcimen, A. & Selcuk, K. (2011). Effects of inclusion aeriel dried parts of some herbs in broiler diets. *Journal of Animal and Plant Science*, 21, 465–476.

Yesilbag, D., Eren, M., Agel, H., Kovanlikaya, A. & Balci, C. (2011). Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum SOD activity. *British Poultry Science*, 52, 472–482.

Yesilbag, D., Gezen, S. S., Biricik, H. & Bulbul, T. (2012). Effect of a rosemary and oregano volatile oil mixture on performance, lipid oxidation of meat and haematological parameters in Pharaoh quails. *British Poultry Science*, 53, 89–97.