Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 214-217

Combination of vitamin A, E, C and volatile oils improves somatic cell counts status and antioxidant parameters in dairy cows

Hams Almohammed[1], Mohamed Marzok[1,2*], Adel Almubarak[1], Hussam Ibrahim[3], Sabry El-khodery[3], Alshimaa Farag[3]

[1]*Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia.*
[2]*Department of Surgery, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.*
[3]*Department of Internal Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Manosura, Egypt.*

ARTICLE INFO

Recieved: 16 December 2023

Accepted: 29 December 2023

***Correspondence:**

Corresponding author: Mohamed Marzok
E-mail address: mmarzok@kfu.edu.sa

Keywords:

SCC
Mammary gland
Antioxidants
TAC
MDA

ABSTRACT

The objective of the present study was to evaluate the effect of supplementation with a combination of vitamins A, E, and C and volatile oils (Superimmune®) on milk production, somatic cell count (SCC), and antioxidant parameters in dairy cows. For this purpose, 30 lactating Holstein cows were used at an early stage of lactation. The cows were randomly allocated to five groups (six each). The first group was supplemented daily with 20 ml of Superimmune® (200,000 IU vit. A, 100mg vit E and 20mg vit. C, Eucalyptus oil, lavender oil, and peppermint oil). The second group was supplemented with 200,000 IU vit. A: third group with 100 mg vit. E), and the fourth group (20 mg vit. C. The fifth group was maintained without treatment (control). Milk production, somatic cell counts, and antioxidant parameters were measured before treatment and at one- and two-months post-treatment. Cows that received Superimmune® showed a significant decrease in somatic cell count compared with the other groups at one-month post-treatment ($p<0.05$) and onward. There was also a significant increase in total antioxidant capacity (TAC) ($p<0.01$), glutathione reductase (GR) ($p<0.05$), and glutathione-S-transferase (GST) ($p<0.01$) in cows that received a combination compared to the other groups). However, there was a significant decrease in the malondialdehyde (MDA) levels ($p<0.01$). Milk production showed a non-significant increase in cows receiving a combination of vitamins compared to the other groups. The results of the present study indicate that a combination of vitamins and volatile oils may have a better effect on somatic cell counts and milk antioxidant parameters in dairy cows than supplementation with a single vitamin.

Introduction

Nowadays, there is great interest in using natural feed additives as sustainable feed additives, such as probiotics, organic acids, exogenous enzymes, propolis, and essential oils (EOs) (Deyno *et al*., 2019). These compounds could be used instead of antibiotics to alter ruminal fermentation and enhance feed efficiency in ruminants (Herago and Agonafir, 2017). Natural products including medicinal plants, essential oils (EOs), and herbal extracts are promising alternatives (Al-Maqtari *et al*., 2021). Essential oils (Eos) are considered the most economically relevant plant-derived products and are likely to have health-promoting properties (Nehme *et al*., 2021). Essential oils have unique properties and offer a huge potential benefit for animal performance by improving feed efficiency, nutrient utilization, and animal health as an alternative to antibiotics (Salem *et al*., 2019). Essential oil (EO) supplementation could improve the feed utilization and performance of high-yielding dairy Chios ewes (Giannenas *et al*., 2011). Also, a blend of essential oils could alter in vitro ruminal fermentation and improve animal performance when fed directly to cows (Kung Jr *et al*., 2008).

Somatic cells (SC) in milk are important indicators of udder health and play a crucial role in the defense of the mammary gland against environmental pathogens. Moreover, the number of cells in raw milk reflects the general health status of an animal (Albenzio *et al*., 2019) the number and distribution of various cell types are influenced by physiological and pathological conditions. Differences between species have also been reported (Paape *et al*., 2001). The effect of the combination of essential oils on SCC in small ruminants has been studied and should positively affect both milk yield and SCCs (Nudda *et al*., 2023).

Selenium and vitamin E are the most potent antioxidants responsible for the protection of membrane polyunsaturated fatty acids against lipid peroxidation (Xiao *et al*., 2021). The total protein, globulin, and cytokine levels were higher in cows supplemented with mineral complexes. Therefore, we concluded that subcutaneous mineral supplementation improves the immune response and minimizes oxidative stress in dairy cows during lactation (Warken *et al*., 2018). Cows diagnosed with mastitis had decreased serum SOD activity and trace mineral supplementation increased serum SOD activity, although leukocyte function was not affected by supplementation (Machado *et al*., 2014). The combination of essential volatile oils and vitamins improves the antioxidant status of growing animals (Almuayli *et al*., 2023).

Little is known about the effect of a combination of vitamins and volatile oils on the somatic cell count and oxidant/antioxidant balance in dairy cows. Therefore, the objective of the present investigation was to assess the effect of supplementation with a combination of vitamins E, A, and C and volatile oils (Super Immune®) on milk production, somatic cell counts, and antioxidant parameters in dairy cows. We hypothesized that a combination of vitamins and volatile oils could ameliorate redox status and improve the somatic cell count in dairy cows.

Materials and methods

Cows

Thirty apparently healthy Holstein dairy cows weighing 420-510 kg body weight at two months post parturition were used in this study. cows were raised at a commercial dairy farm in Damietta Governorate, Egypt.

ISSN: 2090-6277/2090-6269/

The cows were examined clinically, and the findings were recorded simultaneously. All animals were clinically healthy, had no history of ailments, and were kept under identical housing and veterinary supervision throughout the study. All applicable international guidelines for animal care and the use of animals for scientific purposes were followed. The present study was approved by the Animal Care and Use Committee of Mansoura University (VM.R.21.216). Informed consent was obtained from the farm owner about the proposed treatment protocol, and the farmer was given information about the potential beneficial effects of the products.

Experimental Design

The study cows were randomly allocated to five equal-sized groups (six each). The first group was supplemented daily with 25 ml of Superimmune® (Mapco Pharmaceutical Industries, Egypt containing 100,000 IU vit. A, 50mg vit E and 10mg vit. C, Eucalyptus oil, lavender oil, and peppermint oil). The second group was administered 100,000 IU Vit A (Aviton, Kahira Pharmaceutical & Chemical Industry Company, Egypt) intramuscularly. The third group was supplemented with vitamin V. E (Vitaselen, Adwia Pharmaceuticals Company, Egypt) at a dose rate of 6.66 IU/kg BW subcutaneously, and the fourth group was supplemented with Vit. C (Cevarol, Memphis Co. for pharmaceutical and chemical industries, Cairo, Egypt) at a dose rate of 30 mg/kg intravenously. The former formula was administered daily by oral route, while the latter supplements were administered twice, 72 hours apart. Cows in the fifth group received a sterile saline solution (0.9 % NaCl) and served as control group.

Following up protocol

The cows were clinically monitored throughout the study period. Vital signs were routinely monitored before and during the study. The milk yield for each cow was also recorded before and one- and two-months post-treatment.

Blood samples

Via jugular venipuncture, ten ml blood samples were collected from each cow under investigation at (T0) time, one month (T1) and two months (T2) post-administration. Blood was collected in plain tubes (i.e., without anticoagulants) to obtain serum. Only clear non-hemolyzed sera were harvested and aliquoted for biochemical analyses of malondialdehyde (MDA), total antioxidant capacity (TAC), reduced glutathione (GR), and glutathione S-transferase (GST), which were measured spectrophotometrically (Photometer 5010, Germany) following standard methods using commercially available test kits (Biodiagnostics, Cairo, Egypt). Somatic cell count (SCC) in milk was determined using DELAVAL Cell Counter DCC for Somatic Cell Count (DEL-10-92740080).

Statistical analysis

All analyses were performed using a commercial statistical software program (SPSS for Windows, Chicago, IL, USA). The data were assessed for normality using the Kolmogorov–Smirnov test. As data proved to be normally distributed, the mean ±SD for each result was presented. Subsequently, a general linear model with repeated measures ANOVA was used to calculate the effect of within-treatment (time), between-groups (treatment), and evidence of time × treatment interaction. For this purpose, sphericity was assumed, and Wilks' lambda tests were the main parameters used. Whenever the tests were found to be significant ($p<0.05$), a one-way ANOVA with post hoc Duncan multiple comparison test was used at each time point to detect which group varied from others. For all tests, the results were considered significant at $p < 0.05$.

Results

All cows in this study were clinically healthy and showed no detectable clinical signs of illness. Throughout the study period, the cows were healthy, and all vital signs were within normal reference intervals. The results of this experiment are shown in Figures 1-5.

Cows that received a combination of vitamins and volatile oils (Superimmune®) showed a significant decrease in somatic cell count compared to those supplemented with single nutrients (Wilks' Lambda, $p<0.05$). Regarding antioxidants, there was a significant increase in TAC (Wilks' Lambda, $p< 0.01$), glutathione reductase (Wilks' Lambda, $p< 0.05$), and GST (Wilks' Lambda, $p< 0.01$) in cows supplemented with superimmune compared to those supplemented with single nutrients. MDA levels showed a significant decrease (Wilks' Lambda, $p<0.01$) in cows that received Superimmune® compared with the other groups. A non-significant change in milk production was observed among the different cattle groups ($p=0.09$).

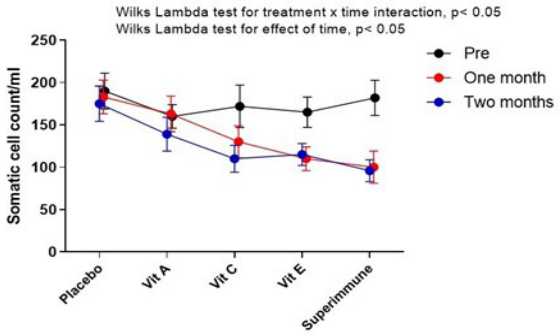

Fig. 1. Milk somatic cell count (SCC) in dairy cows supplemented with Superimmune versus single use of vit. A, E and C.

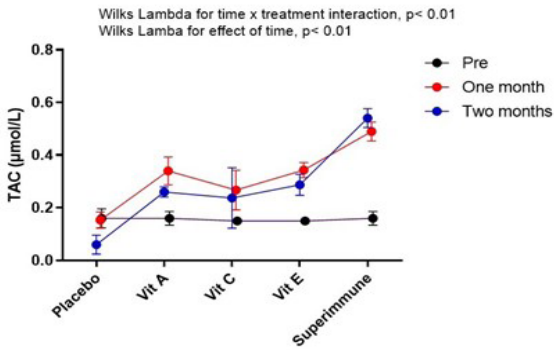

Fig. 2. Plasma total antioxidant capacity in dairy cows supplemented with Superimmune versus single use of vit. A, E and C.

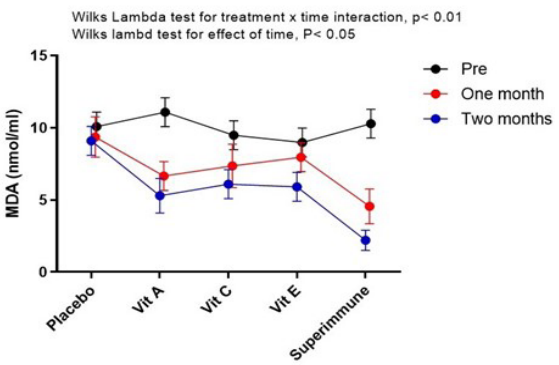

Fig. 3. Plasma MDA in dairy cows supplemented with Superimmune versus single use of vit. A, E and C.

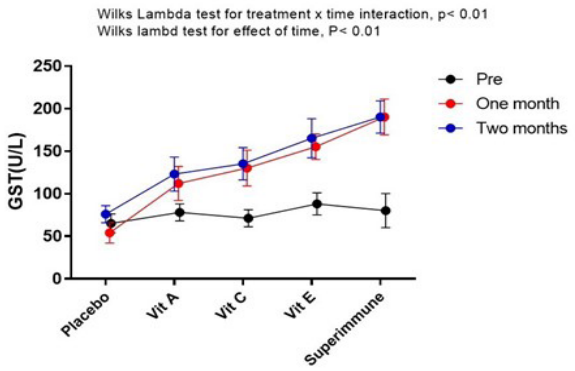

Figure 4. Plasma glutathione S-transferase (GST) in dairy cows supplemented with Superimmune versus single use of vit. A, E and C.

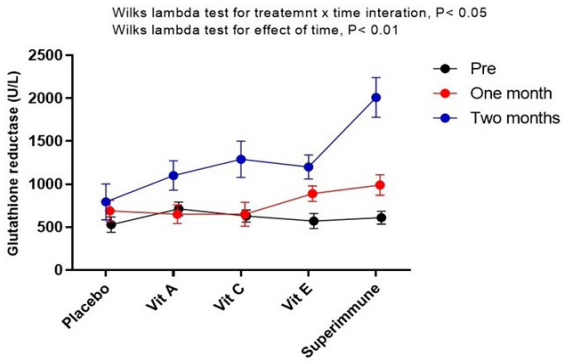

Fig. 5. Plasma glutathione reductase (GR) in dairy cows supplemented with Superimmune versus single use of vit. A, E and C.

Discussion

Currently, the use of essential oils in animal farming as a natural and sustainable alternative for improving animal health and reducing the use of antimicrobials is an area of ongoing research. However, it is essential to approach this practice with caution, prioritize animal welfare, and base decisions on sound scientific evidence and expert advice (Nehme *et al*., 2021). In dairy farms, antioxidant supplementation has been found to enhance the health of cows in a sensitive stage, such as the transition period, but can also have an additional value, giving the final product (milk/meat) value added that benefits consumers' health (Castillo *et al*., 2013).

In the present study, the combination of vitamins and volatile oils (Superimmune®) significantly reduced SCC in the treated cows compared with other cow groups. This suggests that there may be a synergistic or additive effect when these nutrients are combined in the context of SCC antigen levels in dairy cows. The superior antioxidant and immunomodulatory effects of Superimmune® compared to a single supplement may also explain this finding. Similarly, supplementing the diets of Friesian cows with aromatic plant oils, particularly black seed oil, has been found to improve milk yield, udder health, and some immune parameters (Salem *et al*., 2019). In one study, there was a −10% reduction in SCC in dairy sheep supplemented with Se and vitamin E (Morgante *et al*., 1999). Moreover, a reduction in milk SCC was also observed in Lacaune sheep supplemented with 80 mg/day of curcumin, which had anti-inflammatory and antioxidant effects on the animals (Jaguezeski *et al*., 2018). Supplements fed through the diet (vitamins and trace elements such as selenium, zinc or α-flavonoids, and manganese fundamental chain of enzymatic antioxidants) have been proven useful to reduce the occurrence of udder infections and improve the quality of their production, in terms of fat, protein, and somatic cell count (Sretenović *et al*., 2007; Navarro Ruiz *et al*., 2010). It is proposed that the fatty acids present in these oils play important roles in anti-inflammatory processes, thereby improving the immunity status of udder (Karageorgou *et al*., 2023). A positive effect on mammary gland health has been also presented by an EO complex containing thymol, eugenol, vanillin, guaiacol, and limonene; the complex significantly reduced milk SCCs in sheep (Giannenas *et al*., 2011). In general, it has been reported that most EOs have the feature of being non-toxic, residue-free, and thought to be ideal growth promoters for both milk and beef production and quality (Amorati *et al*., 2013).

On the other hand, Superimmune® did not significantly affect milk production compared to other supplements. In contrast, aromatic plant oils have been reported to improve milk yields in Holstein Friesian cows (Salem *et al*., 2019). Other studies have indicated that an increase in SCCs is associated with a reduction in milk yield and worsening of milk quality, represented by a decrease in lactose and casein content and an increase in serum protein, sodium, chloride, pH, and proteolytic activity (Bobbo *et al*., 2016; Bisutti *et al*., 2022).

Numerous studies have documented that the benefits of essential oils or supplementing a single nutrient on the overall performance of animals may vary. It is important to note that the effectiveness of essential oils varies depending on various factors, including the specific oils used and the study (Dhifi *et al*., 2016). The same authors reported an average improvement in weight gain, feed intake, and feed conversion induced by essential oils. These improvements have significant implications for livestock production and health.

In the present study, a combination of vitamins and EO (Superimmune®) supplementation significantly affected the redox status, as indicated by increased antioxidant parameters (including TAC, GR, and GST) and decreased markers of oxidative stress (MDA) and somatic cell count compared with other groups. Similarly, it has been reported that supplementation with juniper oil in dairy cows is more effective on antioxidant parameters than on performance parameters and may be used as a natural antioxidant product (Yesilbag *et al*., 2017). Some studies have suggested that vitamin E supplementation can reduce MDA levels, indicating a potential protective effect against oxidative stress (Bergin *et al*., 2021). Vitamin E injection increased plasma α-tocopherol levels in a time-dependent manner, but to a lower level than that in the oral administration and oral–parenteral administration groups (Njeru *et al*., 1992). In contrast, it has been revealed that higher levels of antioxidative enzymes, particularly SOD and glutathione peroxidase, were found in aging rats supplemented with thyme oil or thymol (Youdim and Deans, 2000). Other studies have reported that essential oil (EO) supplementation increases the activity of antioxidant enzymes in the body and improves the growth performance and immune functions of animals (Elbaz *et al*., 2022). SOD and GPx can directly counteract oxidative attack and protect cells from DNA damage. Therefore, appropriate mineral and vitamin supplementation in the diets of sheep and goats has been shown to have an inhibitory effect on milk SCC (Nudda *et al*., 2023).

Selenium and vitamin E are the most potent antioxidants responsible for the protection of membrane polyunsaturated fatty acids against lipid peroxidation in dairy cows (Xiao *et al*., 2021). Mineral complex supplementation can improve immune response and minimize oxidative stress in dairy cows during lactation (Warken *et al*., 2018). Moreover, exogenous vitamin C delivered via intramuscular injection is easy to administer, affordable, and has been shown to increase plasma vitamin C concentration (Deters and Hansen, 2020) . In bull calves, supplementation with an EO could improve the calf 's gut development, especially the lower gut, as well as improve the immunological cells' response to health challenges during early life (Campolina *et al*., 2023) . Indeed, the antioxidative properties of essential oils (EOs) have been an area of interest, not only from a human health perspective but also in the context of livestock and animal nutrition. Antioxidants play a crucial role in neutralizing free radicals, which can cause oxidative stress and damage to cells. If essential oils possess antioxidative properties, they can potentially contribute to the overall health and well-being of animals.

Conclusion

Supplementation of dairy cows with combined volatile oils and vitamins would reduce the somatic cell count, enhance the antioxidant status, and subsequently improve the health of dairy Holstein cows. Further studies are needed to assess the effect of supplementation with combined volatile oils and vitamins on milk quality, such as serum protein, sodium, chloride, pH, and proteolytic activity.

Acknowledgments

The authors would like to acknowledge the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia for the financial support of this research through Grant Number 5338.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Al-Maqtari, Q.A., Rehman, A., Mahdi, A.A., Al-Ansi, W., Wei, M., Yanyu, Z., Phyo, H.M., Galeboe, O., Yao, W., 2021. Application of essential oils as preservatives in food systems: challenges and future prospectives–a review. Phytochemistry Reviews 21, 1209–1246.

Albenzio, M., Figliola, L., Caroprese, M., Marino, R., Sevi, A., Santillo, A., 2019. Somatic cell count in sheep milk. Small Ruminant Research 176, 24-30.

Almuayli, O., Marzok, M., Hamad, Y., Ibrahim, H., El-ashker, M., Kandeel, M., Eljalii, I., El-khodery, S., 2023. Supplementation with a Combination of Vitamin A, E, C and Essential Volatile Oils Improves Growth Performance and Ameliorates Antioxidant Status in Holstein Calves. Journal of Advanced Veterinary Research 13, 288-291.

Amorati, R., Foti, M.C., Valgimigli, L., 2013. Antioxidant Activity of Essential Oils. Journal of Agricultural and Food Chemistry 61, 10835-10847.

Bergin, P., Leggett, A., Cardwell, C.R., Woodside, J.V., Thakkinstian, A., Maxwell, A.P., McKay, G.J., 2021. The effects of vitamin E supplementation on malondialdehyde as a biomarker of oxidative stress in haemodialysis patients: a systematic review and meta-analysis. BMC Nephrology 22, 126.

Bisutti, V., Vanzin, A., Toscano, A., Pegolo, S., Giannuzzi, D., Tagliapietra, F., Schiavon, S., Gallo, L., Trevisi, E., Negrini, R., 2022. Impact of somatic cell count combined with differential somatic cell count on milk protein fractions in Holstein cattle. Journal of Dairy Science 105, 6447-6459.

Bobbo, T., Cipolat-Gotet, C., Bittante, G., Cecchinato, A., 2016. The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery. Journal of Dairy Science 99, 5104-5119.

Campolina, J.P., Coelho, S.G., Belli, A.L., Neves, L.F.M., Machado, F.S., Pereira, L.G.R., Tomich, T.R., Carvalho, W.A., Daibert, R.M.P., Reis, D.R.L., Costa, S.F., Voorsluys, A.L., Jacob, D.V., Campos, M.M., 2023. Potential benefits of a blend of essential oils on metabolism, digestibility, organ development and gene expression of dairy calves. Scientific Reports 13, 3378.

Castillo, C., Pereira, V., Abuelo, Á., Hernández, J., 2013. Effect of supplementation with antioxidants on the quality of bovine milk and meat production. The Scientific World Journal 2013, 616098.

Deters, E.L., Hansen, S.L., 2020. Pre-transit vitamin C injection improves post-transit performance of beef steers. Animal : an international journal of animal bioscience 14, 2083-2090.

Deyno, S., Mtewa, A.G., Abebe, A., Hymete, A., Makonnen, E., Bazira, J., Alele, P.E., 2019. Essential oils as topical anti-infective agents: A systematic review and meta-analysis. Complementary therapies in medicine 47, 102224.

Dhifi W, Bellili S, Jazi S, Bahloul N, W., M., 2016. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines 3, 25.

Elbaz, A.M., Ashmawy, E.S., Salama, A.A., Abdel-Moneim, A.-M.E., Badri, F.B., Thabet, H.A., 2022. Effects of garlic and lemon essential oils on performance, digestibility, plasma metabolite, and intestinal health in broilers under environmental heat stress. BMC Veterinary Research 18, 430.

Giannenas, I., Skoufos, J., Giannakopoulos, C., Wiemann, M., Gortzi, O., Lalas, S., Kyriazakis, I., 2011. Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. Journal of Dairy Science 94, 5569-5577.

Herago, T., Agonafir, A., 2017. Growth promoters in cattle. Advances in Biological Research 11, 24-34.

Jaguezeski, A., Perin, G., Bottari, N., Wagner, R., Fagundes, M., Schetinger, M., Morsch, V., Stein, C., Moresco, R., Barreta, D., Danieli, B., Defiltro, R., Schogor, A., Silva, A., 2018. Addition of curcumin to the diet of dairy sheep improves health, performance and milk quality. Animal Feed Science and Technology 246, 144-157.

Karageorgou, A., Tsafou, M., Goliomytis, M., Hager-Theodorides, A., Politi, K., Simitzis, P., 2023. Effect of Dietary Supplementation with a Mixture of Natural Antioxidants on Milk Yield, Composition, Oxidation Stability and Udder Health in Dairy Ewes. Antioxidants 12, 1571.

Kung Jr, L., Williams, P., Schmidt, R., Hu, W., 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. Journal of Dairy science 91, 4793-4800.

Machado, V., Oikonomou, G., Lima, S., Bicalho, M., Kacar, C., Foditsch, C., Felippe, M., Gilbert, R., Bicalho, R., 2014. The effect of injectable trace minerals (selenium, copper, zinc, and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows. The Veterinary Journal 200, 299-304.

Morgante, M., Beghelli, D., Pauselli, M., Dall'Ara, P., Capuccella, M., Ranucci, S., 1999. Effect of administration of vitamin E and selenium during the dry period on mammary health and milk cell counts in dairy ewes. Journal of Dairy Science 82, 623-631.

Navarro Ruiz, A., Hernández Aguilar, M., Codoñer Franch, P., López Jaén, A., Valls Bellés, V., Gallardo Alés, M., 2010. C-13. Actividad antioxidante de la leche humana: Relación con factores dietéticos. Pediatría Atención Primaria 12, e69-e69.

Nehme, R., Andrés, S., Pereira, R.B., Ben Jemaa, M., Bouhallab, S., Ceciliani, F., López, S., Rahali, F.Z., Ksouri, R., Pereira, D.M., 2021. Essential oils in livestock: From health to food quality. Antioxidants 10, 330.

Njeru, C.A., McDowell, L.R., Wilkinson, N.S., Linda, S.B., Williams, S.N., Lentz, E.L., 1992. Serum alpha-tocopherol concentration in sheep after intramuscular injection of DL-alpha-tocopherol. Journal of Animal Science 70, 2562-2567.

Nudda, A., Carta, S., Battacone, G., Pulina, G., 2023a. Feeding and Nutritional Factors That Affect Somatic Cell Counts in Milk of Sheep and Goats. Vet. Sci. 10, 454.

Paape, M., Poutrel, B., Contreras, A., Marco, J.C., Capuco, A., 2001. Milk somatic cells and lactation in small ruminants. Journal of Dairy Science 84, E237-E244.

Salem, A.Y., El-Awady, H.G., El-Dein, M.A.T., Eisa, D.A., 2019. Effect of supplementation of aromatic plants oils on immunity, udder health and milk production of Friesian cows. Slovenian Veterinary Research 58, 523-530.

Sretenović, L., Aleksić, S., Petrović, M.P., Miščević, B., 2007. Nutritional factors influencing improvement of milk and meat quality as well as productive and reproductive parameters of cattle. Biotechnology in Animal Husbandry 23, 217-226.

Warken, A.C., Lopes, L.S., Bottari, N.B., Glombowsky, P., Galli, G.M., Morsch, V.M., Schetinger, M.R.C., SILVA, A.S., 2018. Mineral supplementation stimulates the immune system and antioxidant responses of dairy cows and reduces somatic cell counts in milk. Anais da Academia Brasileira de Ciências 90, 1649-1658.

Xiao, J., Khan, M.Z., Ma, Y., Alugongo, G.M., Ma, J., Chen, T., Khan, A., Cao, Z., 2021. The antioxidant properties of selenium and vitamin E; their role in periparturient dairy cattle health regulation. Antioxidants 10, 1555.

Yesilbag, D., Biricik, H., Cetin, I., Kara, C., Meral, Y., Cengiz, S., Orman, A., Udum, D., 2017. Effects of juniper essential oil on growth performance, some rumen protozoa, rumen fermentation and antioxidant blood enzyme parameters of growing Saanen kids. Journal of Animal Physiology and Animal Nutrition 101, e67-e76.

Youdim, K.A., Deans, S.G., 2000. Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. The British Journal of Nutrition 83, 87-93.