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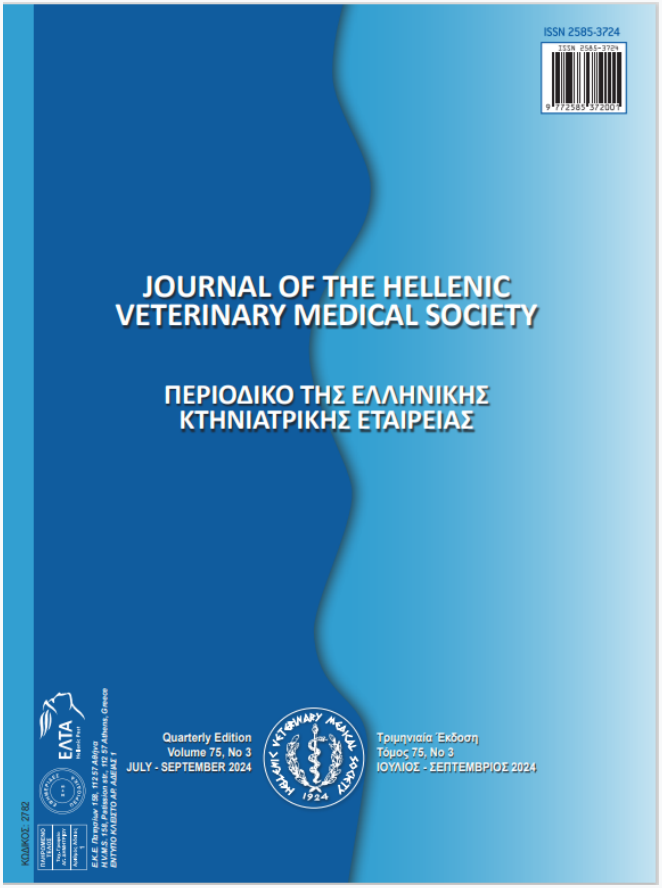

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Effects of Oregano Aromatic Water (*Oreganum onites* L.) Supplementation on Diets of Lactating Dairy cows on Milk Production Performance, Metabolic Health and Antioxidative Defense Mechanism

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ABSTRACT: This study aimed to validate the effect of oregano aromatic water (OAW) on blood parameters, oxidative stress, and the production and composition of milk when used as a feed additive. Thirty lactating Holstein cows (average 570 kg), with an average of 74 days in milk and a lactation number of 2.44, were randomly divided into three groups for a 60-day period. These groups included: (G1) control ration, (G2) control ration + OAW (10 mg/kg of DM), (G3) control ration + OAW (20 mg/kg of DM). Milk production (MP) was unaffected by OAW doses ($P>0.05$). The supplementation of OAW did not affect the milk composition and somatic cell count (SCC) ($P>0.05$). OAW supplementation did not affect blood biochemical values except for ketone and glucose. The glucose value increased as the ketone level decreased with OAW supplementation ($P<0.05$). The hematological blood values were not found to be significantly affected. OAW significantly decreased the Total Oxidant Capacity (TOC) and Oxidative Stress Index (OSI) while increasing the Total antioxidant capacity (TAC). The findings of the current study indicated that OAW can be used as a feed additive to improve cow health and milk production.

Keywords: Blood metabolites; Holstein cows; Milk composition; Oregano aromatic water; Oxidative stress; Production performance

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INTRODUCTION

The pressure exerted by consumer organizations on the livestock industry to decrease the use of antibiotics, coupled with increased customer awareness, has led to restrictions and prohibitions on antibiotics usage in this sector. Later discoveries revealed that plant extracts and their secondary metabolites could serve as natural alternatives to feed additives for high-yielding cows. Furthermore, its application as a rumen moderator in ruminant animals has garnered significant attention among researchers studying dairy cows, owing to its capacity to enhance feed efficiency, diminish methane gas production, and mitigate environmental pollution (Abdel Hakim et al., 1989; Huang et al., 2018). Previous studies have demonstrated that plant extracts and their secondary metabolites not only support the immune system and antioxidative defense mechanisms but also exhibit antimicrobial activities (Oh et al., 2017; Braun et al., 2019). Specifically, it has been suggested that the antimicrobial activity attributed to oregano is a result of increased permeability in the bacterial cell wall caused by thymol and carvacrol (Lambert et al., 2001).

The first lactation period following calving is a pivotal phase significantly influencing both the health and milk production of a cow. This period, characterized by heightened milk production, amplifies the susceptibility to diseases such as mastitis, metritis, and ketosis. These conditions contribute to metabolic stress in cows due to escalated nutrient and energy demands associated with milk synthesis, and secretion. Consequently, this metabolic stress not only exacerbates animal welfare issues but also results in decreased yield. Throughout this critical phase, diseases and metabolic stress are known to manifest (Sharma et al., 2011; Sordillo and Mavangira, 2014). Metabolic stress in the first lactation phase is typified by a combination of disrupted nutrient metabolism, dysfunctional inflammatory responses, and oxidative stress characterize metabolic stress (Sharma et al., 2011). This period often witnesses an imbalance between heightened reactive oxygen species (ROS) and the capacity of the antioxidant defense system to mitigate their accumulation. Such an imbalance, vital for reducing the buildup of ROS, can result in oxidative stress and subsequent diseases in cows during this lactation. During this phase, the demand for safe additives has prompted an inclination toward exploring plant-based additives. Nevertheless, the diverse effects of essential oils on animals underscore the inherent variability in the bioactivity and bioavailability of different essential oils (Giannenas et al., 2011). Silva Filho et al. (2017) indicated that essential oils have the potential to decrease the somatic cell count in milk, thereby potentially mitigating the risk of mastitis and preserving the properties. Additionally, Giller et al. (2020) reported that mixtures of thyme, eucalyptus and anise essential oil do not adversely affect the milk yield or composition of cows.

It is well-established that carvacrol and thymol, two phenolic compounds present in oregano aromatic water, exhibit notable antimicrobial and antioxidant properties (Ozkaya et al., 2018). Despite this knowledge, studies investigating the effects of oregano aromatic water, a by-product released during essential oil extraction, on the performance, metabolic health, and antioxidative defense mechanisms of lactating cows are lacking. Hence, the primary objective of this study was to assess whether the utilization of oregano aromatic water as a by-product would positively influence the performance and health of dairy cows without exerting any adverse effects on these parameters.

MATERIAL AND METHODS

Animals and Rations

In this study, thirty lactating Holstein dairy cows, averaging 74 days in milk, 2.44 lactation numbers, and an average live weight of 570 kg, were utilized. The determination of the number of cows was conducted through the power analysis method. Based on this analysis, it was established that for 95% power, the highest milk yield average was 31.09 kg/d, the lowest milk yield average was 29.75 kg/d, and the standard deviation was 1.285, there should be 8 cows in each group; however, to ensure a more robust statistical analysis, 10 cows were included in each group. The duration of the experiment lasted 60 days. The cows were randomly divided into three groups, each consisting of 10 cows, to facilitate a valid comparison between the groups. All groups were provided similar access to feed, clean water, and sand sleeping beds throughout the study. The treatments administered were as follows;

G1) Control group: Basal ration

G2) Basal ration + OAW at a dosage of 10 mg/kg of dry matter (DM)

G3) Basal ration + OAW at a dosage of 20 mg/kg of DM.

The OAW was meticulously mixed with the vita-

min-premix daily spraying method. Subsequently, after the basal ration was distributed to the feeder, the OAW was added to the basal ration and presented to the cows for consumption.

The chemical composition of the ration utilized in the study is presented in Table 1. Notably, the management of feeding and rations for animals on the farm remained unaltered throughout the study. The cows were fed twice daily, immediately after milking, at 07:00 and 18:30. Daily weighing of residual was conducted.

The determination of crude protein in both concentrate and roughage was executed using the Kjeldahl method (AOAC 2000, method: 955.04), while ether extract was obtained utilizing the Soxhlet method (AOAC 2000, method: 948.22). Neutral detergent fiber (NDF) analysis was performed using the ANKOM220 Fibre Analyzer (Ankom Technology, Macedon, NY, USA). Additionally, the metabolic energy (ME) was calculated based on data provided by the Turkish Standards Institution (TSE, 1991).

Table 1. Diet ingredients and chemical composition of total mixed ration for dairy cows

	TMR
Chemical profile	
Dry Matter g/kg	604.2
Crude ash g/kg	50.60
Crude protein g/kg	152,10
Ether extract g/kg	42.30
Neutral Detergent Fiber g/kg	206.30
Acid Detergent Fiber g/kg	132.00
Acid Detergent Lignin g/kg	27.30
Starch g/kg	146.60
Metabolic Energy kcal kg^{-1}	1567.93
Feed items (% of dry matter)	
Corn silage	47.24
Alfalfa	11.81
Wheat straw	2.36
Concentrate feed*	11.81
Grain of maize	9.45
Cottonseed meal	3.54
Sun flower seed meal	3.54
Brewer's grain	9.45
Vitamin premix	0.18
Salt	0.24
Sodium carbonate	0.14
Calcium carbonate	0.24

TMR: Total mixed ration, *Ingredients: Wheat bran, Corn, Sunflower meal, Barley, Soybean meal, Boncalite, Molasses, Calcium carbonate, Sodium chloride, Vitamin premix

Measurements and Sample Collection

Milk yields of the cows were continuously monitored using automatic milk counters (Te-Ta Tech. Agric. Com., Izmir, Turkey) for a duration of two months, encompassing measurements at 06:00 and 18:00 hours. Milk samples were collected twice a week and stored at 4^{o}C until analysis.

The analysis of milk composition included determining fat % (Method: IDF 141C:2000), protein % (Method: IDF 141C:2000), lactose % (Method: IDF 141C:2000). Additionally, the freezing point (Method: ISO 13366-2:2006/IDF 142:2006) and somatic cell count (ISO 13366-2/AC: 2008) of the milk samples were assessed. These analyses were conducted using a Bentley B150 milk analyzer (Bentley Combi 150, Bentley Instruments, Inc. Minnesota, Chaska, USA).

The total mixed ration (TMR) provided to the cows was weighed on a daily basis before being offered to them. The intake of the TMR was recorded on a weekly basis. It's worth noting that all groups of cows consumed the entire amount of TMR offered to them without ant refusal.

The dry matter intake (DMI) of the groups was calculated based on the recorded intake data. To calculate feed efficiency, the formula used was;

Feed Efficiency (FE) = Kilograms of Milk / Kilograms of DMI

This formula allows for the determination of feed efficiency by evaluating the amount of milk produced per unit of DM consumed by the cows.

Blood samples were collected from the jugular veins of the cows at the conclusion of the study for further analysis of biochemical and hematologic parameters. The analysis of biochemical blood parameters was performed using a Mindray BS120 analyzer (Mindray Corporation, Nanshan, China). Blood samples, intended for biochemical parameters such as Ketone, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyltransferase (GGT), Lactate dehydrogenase (LDH), Glucose (GLU), Creatinine (CREA), Urea, Total Bilirubin (TBIL), Direct Bilirubin (DBIL), Total Protein (TP), were collected in non-additive tubes and transferred to the laboratory on ice. Subsequently, these blood samples were centrifuged at 3000 rpm per minute for 10 min.

For the analysis of hematologic parameters (WBC: White blood cell, RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PCT: Procalcitonin), blood samples were collected into EDTA tubes. The analyses was carried out using an ABACUS 38 analyzer (Diatron Corporation, Budapest, Hungary). Specifically, 25 µl of blood from the open tube was drawn by the device's automatic sample rotor, diluted with 50 µlof diluent, and then analyzed using the volumetric impedance method for blood cell counting.

Total antioxidant capacity levels were assessed using commercially available kits (Rel Assay Diagnostics, Gaziantep, Türkiye). The method employed for this measurement is based on the principle of decolorization of the stable ABTS (2, 2Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay demonstrates exceptional precision with values lower than 3%. Results were quantified and expressed as millimoles of Trolox equivalent per liter (mmol Trolox equivalent/L) following the methodology developed by Erel (2004). Total oxidant capacity levels were determined using commercially available kits (Rel Assay Diagnostics, Gaziantep, Türkiye). In this method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to the ferric ion. Glycerol molecules, abundant in the reaction medium, enhanced the oxidant reaction. The resulting ferric ion formed a colored complex with xylenol orange in an acidic environment. The intensity of this color, measurable spectrophotometrically, correlated with the total amount of oxidant molecules in the sample. The assay was calibrated using hydrogen peroxide, and results were expressed as a micromolar hydrogen peroxide equivalent per liter (µmol H2O2 equivalent/L) according to the methodology developed by Erel (2005). To calculate the oxidative stress index (OSI), the ratio of TOC to TAC was determined. For this calculation, the TAC values were converted to micromoles per liter (µmol/L), and the OSI was calculated using the following formula:

OSI (arbitrary unit)= TOC (µmol H2O2 equivalent/L)/TAC (µmol Trolox quivalent/L)

This approach to calculating OSI, as described by Yumru et al. (2009) allows for the assessment of oxidative stress by evaluating the balance between TOC and TAC in the biological sample.

Oregano Aromatic Water Production

The process for obtaining OAW involved drying 500 grams of oregano leaves in room conditions. These dried leaves were then placed in a 5-liter bottle connected to a Clevenger hydrodistillation device along with 2.5 liters of water, following the guidelines outlined in the European Pharmacopoeia (1975). Through hydrodistillation, aromatic water was collected after separating it from the oregano oil. The essential oil within the aromatic water was extracted using n-hexane to identify the odor components using a GC/MS (Gas Chromatography/Mass Spectrometry) device (QP Shimadzu 2010 Plus, Shimadzu Corp. Kyoto, Japan). The identification of these components was conducted by referencing data from Wiley, NIST, and Tutor libraries, cross-referencing retention times of standard substance against the composition of mass spectra. The analysis revealed the presence of carvacrol and thymol in the OAW. The composition analysis indicated a carvacrol ratio of 99.4% and a thymol ratio of 0.05% within the OAW obtained from the oregano leaves.

Statistical Analysis

The statistical analysis of the results were performed using one-way ANOVA (Analysis of Variance) with Minitab Statistical Software Package (Version 17; Minitab Ltd., Coventry, UK). A Tukey test was utilized to distinguish between treatment means. Significance was established at $P<0.05$, while a trend was considered for $0.05<P<0.10$.

The mathematical model for one-way ANOVA was represented as followa;

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

Where:

Y_{ij} represents the *j*-th observation value in the *i*-th experiment,

μ signifies the overall mean,

T_i denoted the effect of the *i*-th experiment,

ε_{ij} represents random error terms associated with each observation.

RESULTS

It appears that due to the housing of cows in groups, individual calculations for DMI and FE were not feasible. Instead, weekly recordings were made per experimental group. Consequently, statistical cal-

culations for DMI and FE were not performed. The recorded weekly values for DMI were as follows; 20.78 for G1, 21.60 for G2 and 25.46 for G3. Similarly, the recorded weekly values for FE were 1.09 for G1, 1.12 for G2 and 1.09 for G3. These values denote the average weekly DMI and FE for each experimental group, providing insights into the intake and efficiency parameters within the specified groups.

The comparison between initial and final milk production (MP) did not statistically significant differences. However, an observable trend was noted, suggesting that the supplementation of OAW led to an approximate increase of 3 kilograms MP (Table 2). This increase in MP exhibited a proportional relationship with the doses of supplemented OAW.

It was observed that OAW supplementation did not exert a statistically significant effect on the DM, fat, protein, and lactose content within the milk (Table 2).

Additionally, while not statistically significant, there was an observed trend indicating a reduction in SSC with OAW supplementation (Table 2). This implies that the supplementation of OAW potentially contributed to a decrease in SSC, albeit not meeting the threshold for significance.

In the biochemical assessment of blood parameters, the supplementation of OAW did not exhibit a notable effect on various parameters, except for blood ketone concentration and serum glucose concentration (Table 3). Interestingly, the doses of OAW demonstrated a significant effect: they effectively reduced the concentration of ketones in the blood while concurrently increasing the concentration of glucose in serum ($P<0.05$). This indicates that the administration of OAW resulted in lowered blood ketone concentration and elevated serum glucose concentration compared to the control group.

Upon hematological examination of blood parameters, numerical differences between the various groups did not significant (Table 4). This suggests that the supplementation of OAW did not lead to significant variances in the measured hematological parameters among the experimental groups.

The supplementation of OAW resulted in notable effects on oxidative parameters, as in Table 5. Specifically, OAW supplementation led to a significant reduction ($P<0.05$) in oxidant capacity while concurrently increasing ($P<0.05$) the antioxidant capacity. Additionally, the OSI exhibited a significant decrease ($P<0.05$) with OAW supplementation. These findings indicate that the administration of OAW played a role in diminishing oxidant capacity, enhancing antioxidant capacity, and consequently reducing the overall OSI, implying a potential antioxidative effect of OAW.

DISCUSSION

Indeed various feed additives, such as monensin and essential oils, have demonstrated the potential to enhance the digestibility of dry matter and crude protein in animals. Additionally, these additives are known to influence the concentration of volatile fatty acids (VFAs) in the rumen, thereby increasing feed efficiency and nitrogen utilization (Silva et al., 2018).

Table 2. Milk production and composition of lactating cows

Items	G1	G2	G3	P
	Mean±SE	Mean±SE	Mean±SE	
DIM	69.83±0.60	76.83±3.22	75.83±1.96	0.14
LN	2.67±0.33	2.33±0.33	2.33±0.33	0.73
Initial MP	20.13±0.30	18.17±1.18	17.87±0.24	0.13
Final MP	28.13±0.88	29.43±0.23	29.43±0.58	0.31
Differences	8.00±1.16	11.27±1.41	11.57±0.72	0.06
MP	22.75±0.37	24.12±0.75	26.25±1.11	0.06
Fat, %	3.80±0.05	3.82±0.02	3.85±0.01	0.52
Protein, %	4.37±0.04	4.37±0.04	4.42±0.01	0.52
Lactose, %	4.96±0.06	4.97±0.05	5.04±0.01	0.58
DM, %	14.50±0.12	14.51±0.12	14.58±0.05	0.83
SCC, cell/mL	401000±10017	387333±9821	385667±8373	0.49

DIM: Days in milk, LN: Lactation number,; MP: Average Milk production, kg/d;; SCC: Somatic cell count, G1: Control ration, G2: Control ration+10cc/head/day OAW, G2: Control ration+20cc/head/day OAW.

Table 3. Blood serum parameters of lactating cows

Items		G1	G2	G3	P
		Mean±SE	Mean±SE	Mean±SE	
Ketone	Initial	1.43±0.03	1.47±0.13	1.50±0.00	0.77
	Final	0.83±0.83[a]	0.67±0.03[ab]	0.57±0.07[b]	0.02
ALT	Initial	52.00±5.77	59.67±8.17	70.67±3.84	0.18
	Final	45.67±4.41	35.00±4.04	39.67±1.67	0.19
ALP	Initial	97.70±6.72	63.33±7.54	81.67±7.13	0,32
	Final	119.00±15.30	83.33±4.91	47.30±31.30	0.12
GGT	Initial	32.67±3.67	21.00±4.73	18.33±1.76	0.06
	Final	22.67±2.03	27.67±4.37	14.33±3.33	0.08
GLU	Initial	42.67±1.20	42.33±0.88	43.33±3.67	0.88
	Final	44.33±1.45[b]	53.67±4.06[a]	57.67±2.60[a]	0.01
CREA	Initial	1.84±0.01	1.72±0.14	1.78±0.02	0.60
	Final	1.49±0.28	1.79±0.08	0.92±0.32	0.12
UREA	Initial	18.10±1.24	18.60±0.31	16.87±3.46	0.84
	Final	15.87±2.96	21.20±1.47	9.57±4.87	0,13
TBIL	Initial	0.39±0.30	0.01±0.01	0.15±0.15	0.43
	Final	0.15±0.10	0.04±0.04	0.01±0.01	0.28
TP	Initial	7.91±0.15	6.98±0.24	7.24±0.33	0.10
	Final	6.69±0.25	6.77±0.32	6.61±0.27	0.92

Initial: Beginning of experiment, Final: End of experiment after 60 days, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyltransferase, GLU: Glucose, CREA: Creatinine, TBil: Total bilirubin, TP: Total protein, [a,b] Means in a line with different lower case letters differ significantly

Table 4. Hematologic parameters of lactating cows

Items		G1	G2	G3	P
		Mean±SE	Mean±SE	Mean±SE	
WBC, 10^3/ul	Initial	9.37±0.80	8.08±1.55	8.51±0.18	0.67
	Final	11.82±2.43	8.93±0.66	9.32±0.57	0.37
Lymphocytes, 10^3/ul	Initial	4.30±0.70	3.16±0.92	3.86±0.22	0.52
	Final	4.37±0.99	4.01±0.61	3.83±0.23	0.85
Monocytes,10^3/ul	Initial	1.06±0.12	1.03±0.16	0.82±0.11	0.40
	Final	2.11±1.00	0.97±0.17	0.93±0.17	0.32
Granulocytes, 10^3/ul	Initial	4.03±0.65	3.90±0.68	3.84±0.07	0.97
	Final	5.34±1.31	3.95±0.30	4.57±0.38	0.50
RBC, 10^6/ul	Initial	6.24±0.31	6.20±0.45	5.99±0.19	0.86
	Final	6.76±0.49	6.49±0.52	6.82±0.21	0.85
Hemoglobin, g/dl	Initial	9.65±0.20	9.80±0.44	8.88±0.25	0.14
	Final	10.55±0.49	10.35±0.38	10.50±0.22	0.93
Hematocrit, %	Initial	27.85±0.71	28.00±1.28	26.18±0.43	0.32
	Final	28.55±1.23	28.03±0.85	28.01±0.78	0.91
MCV, fl	Initial	45.00±1.73	45.50±1.50	43.70±0.85	0.68
	Final	42.75±1.93	43.50±2.25	41.25±1.80	0.73
MCH, pg	Initial	15.53±0.62	15.98±0.81	14.88±0.33	0.48
	Final	15.70±0.60	16.10±0.80	15.45±0.51	0.78
MCHC, g/dl	Initial	34.55±0.21	35.00±0.67	33.95±0.52	0.37
	Final	36.88±0.28	36.93±0.23	37.58±0.34	0.21
PCT, %	Initial	0.15±0.04	0.19±0.04	0.21±0.03	0.62
	Final	0.20±0.03	0.17±0.02	0.19±0.02	0.69
Platelets, 10^3/ul	Initial	255.30±54.40	235.00±60.60	313.00±42.00	0.58
	Final	308.30±55.00	247.50±35.50	296.80±34.30	0.58

Initial: Beginning of experiment, Final: End of experiment after 60 days, WBC: White blood cell, RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PCT: Procalcitonin,

Table 5. Effect of OAW on oxidative stress

Items	G1	G2	G3	P
	Mean±SE	Mean±SE	Mean±SE	
TOC	2.84±0.54[a]	0.71±0.25[b]	1.19±0.25[b]	0.02
TAC	2.26±0.29[b]	5.46±1.96[a]	5.98±1.75[a]	0.02
OSI	0.14±0.04[a]	0.01±0.01[b]	0.02±0.01[b]	0.02

OAW, TOC: Total oxidative capacity, mmol H2O2 Eq/L; TAC: Total antioxidant capacity, mmol Trolox Eq/L; OSI: Oxidative stress index, TOC/TAC*10; [a,b] Means in a line with different lower case letters differ significantly

Monensin, for instance, is recognized as an ionophore feed additive that can modify rumen fermentation, leading to increased propionate production and subsequent enhancement of feed efficiency. Essential oil, on the other hand, possesses bioactive compounds like phenolic compounds, terpenes, and other constituents that can positively affect rumen fermentation, leading to improved nutrient utilization and increased VFA production, thereby contributing to enhanced animal performance. Indeed, DMI is a crucial determinant, particularly in high-yielding dairy cows, affecting their MP. The physical occupancy within the rumen plays a significant role in regulating DMI. The relationship between DMI and MP is closely linked to nutrient supply. As DMI increases in dairy cows, there is often a parallel increase observed in MP. Studies conducted by Hassanat et al. (2017) and Giller et al. (2020) support this relationship, indicating that higher DMI correlates positively with increased MP in dairy cows. Essentially, the greater the intake of nutrients via increased DMI, the more substantial the supply of nutrients available for MP, contributing to enhanced milk yield in high-yielding cows. The effects of herbal feed additives, particularly essential oils on dairy cows' MP and composition seem to vary across different studies, leading to conflicting results. Benchaar (2021) reported findings where supplementation of thyme oil did not result in significant changes in both DMI and MP in cows. Similarly, the supplementation of mixtures of various essential oils, as noted in the study by Giller et al. (2020), did not affect the milkfat ratio, reflecting the findings in our study. This lack of effect on the milk fat ratio might be linked to the absence of an effect on the molar ratio of acetate, a primary precursor for fat synthesis in mammary glands (Benchaar, 2021). Moreover, various studies, such as those conducted by Meiring (2014), Hashemzadeh-Cigari et al,(2015), Drong (2016), Kuester (2016), Nowers (2016), Silva et al. (2018), Prayitno et al. (2019), Stivanin et al. (2019), Braun et al. (2019), Silvestre et al. (2022) have reported that herbal feed additives generally do not significantly affect MP or composition in dairy cows. Similarly in our study, OAW application did not affect milk composition, which tended to increase average MP. However, there are studies, such as those by Hashemzadeh-Cagari et al. (2014) and Braun et al. (2019) that reported contradictory results. These divergent outcomes across studies might indeed be attributed to variations in the types of herbal feed additives employed, the dosages administered, or potentially other factors related to experimental design, animal physiology, or environmental conditions, influencing the observed effects on milk production and composition in dairy cows.

SCC in milk serves as a significant indicator of udder health and milk quality. Elevated SCC, typically above 500.000 cells per milliliter (Hashemzadeh-Cigari et al., 2014), are considered indicative of poor milk quality and potential udder health issues. In our study, the SCC obtained were reported to be lower than legal counts considered acceptable for human consumption, which are generally below 500.000 cells per milliliter (De Villiers et al., 2000). Lower SCC is indicative of better udder health and improved milk quality. Therefore, in this context, the obtained SCC below the legal threshold suggest that the milk obtained from cows in our study met the standard requirements for human consumption, signifying food udder health and quality MP. Indeed, various studies have explored the effect of herbal products and essential oils on SCC in milk, reporting differing outcomes. Silva Filho et al. (2017) reported that supplementation with thyme essential oils showed tendencies to reduce SCC. Similarly, Giannenas et al. (2011) observed a significant reduction in SCC in sheep milk upon the use of essential oils. Additionally, several other researchers, including Hashemzadeh-Cigari et al. (2014), Meiring (2014), Moller (2015), and Kuester (2016), have reported reductions in SCC when experimenting with herbal products and their respective essential oils. Silvestre et al. (2022) found that the combination of Capsicum oleoresin and clove essential oil did not

have a significant effect on SCC when administered to dairy cows. Similarly, another study conducted by Hausmann et al. (2018) reported that the combination of plant bioactive lipid compounds and biotin did not influence milk composition or SCC in dairy cows. Our indicated an insignificant decrease in SCC with the application of OAW. Conversely, Giller et al. (2020) reported a significant increase in SCC upon the use of thyme oil in cows, although this increase did not reach a level indicative of mastitis. The diverse results across studies could be attributed to the intricate nature of essential oils, which contain numerous compounds. Sometimes, the biological activity of an essential oil may be ascribed to one or two major compounds present within it. However, in other instances, the collective action or synergy of multiple compounds in the essential oil could prove to be more effective than any single major compound. This complexity in essential oil composition and the interaction between various compounds might lead to disparate effects on SCC observed in different studies, thereby contributing to the variability in outcomes regarding the impact of essential oils on SCC in dairy cows (Bakkali et al., 2008; Giller et al., 2020).

Absolutely, analyzing hematological and biochemical blood variables is a crucial approach used to assess the metabolic and health status of animals, particularly dairy cows. These variables provide valuable insights into the physiological condition of the animal and aid in diagnosing potential health issues (Klinkon and Jezek, 2012). In the early lactation stages, dairy cows often experience a negative energy balance, leading to an increased risk of ketosis. Ketosis occurs when there's an elevation in blood ketone concentration and a reduction in serum glucose concentration. Subclinical ketosis is typically recognized when the blood ketone concentration falls between 1.2 - 1.4 mmol/L (Oetzel, 2013). This condition can be indicative of an impending or mild form of ketosis without necessarily displaying overt clinical symptoms. Monitoring these blood concentrations is crucial to detect subclinical ketosis and address metabolic imbalances early to prevent more severe health complications in dairy cows. Indeed, ketosis is a metabolic condition that arises when energy demands, particularly due to high MP, surpass the intake of energy, resulting in a negative energy balance (Nowers, 2016). This imbalance triggers a series of significant changes, notably a substantial decline in carbohydrate availability, including reduced serum glucose concentration and diminished levels of liver glucose and glycogen content (Bergman, 1971). As a result of this energy deficit, the body begins to utilize stored fats for energy, leading to the breakdown of fats and subsequent accumulation of ketone bodies. These ketones, including acetone, acetoacetate, and beta-hydroxybutyrate, accumulate in the bloodstream, reflecting the metabolic shift toward increased fat metabolism as an alternative energy source (Nowers, 2016). Addressing this glucose deficit is pivotal in preventing excessive ketone production. Major preventive strategies involve providing gluconeogenic precursors, such as specific feed additives, or altering ruminal fermentation processes to enhance ruminal propionate production (Hausmann et al., 2018). Increasing the availability of gluconeogenic substrates helps replenish glucose concentration and aids in preventing the onset of ketosis by providing an alternative energy source to counterbalance the negative energy balance seen in high-producing dairy cows. Indeed, various studies have explored different strategies and supplements aimed at preventing ketosis in dairy cows by addressing the underlying metabolic imbalance and improving glucose concentration, thus mitigating excessive ketone production. Nowers (2016) highlighted the anti-ketogenic effect of monensin supplementation in dairy cows, which resulted in decreased serum ketone concentration and increased glucose concentration. This demonstrates the potential of certain supplements, like monensin, to positively influence the metabolic state in cows and reduce the risk of ketosis. Additionally, Moosavi-Zadeh et al. (2023) reported that supplementation with fennel (*Foeniculum vulgare*) seed powder in early lactation dairy cows exhibited preventative effects against metabolic diseases such as fatty liver and ketosis. This supplementation appeared to improve far mobilization, decrease milk ketone bodies levels, and enhance milk fat mobilization, thereby contributing to maintaining a healthier metabolic profile in dairy cows. Moreover, the study by Hausmann et al. (2018) showed promising results with a combination of plant-bioactive lipid compounds and biotin which reduced the incidence of ketosis in dairy cows. This combination decreased beta-hydroxybutyrate levels, a key ketone body, without affecting glucose concentration. By targeting ketone reduction and maintaining adequate glucose concentration, these supplements demonstrate potential to prevent ketosis in dairy cows. Overall, these studies emphasize the significance of providing gluconeogenic precursors through feed additives or modulating ruminal fermentation to enhance propionate production, as ef-

fective strategies to prevent ketosis in dairy cows by addressing glucose deficits, which are the fundamental trigger for excessive ketone production. Research has highlighted that propionate possesses an anti-ketogenic effect, as supported by studies conducted by Bush and Milligan (1971) and Henno et al. (2021). Moreover, some studies have indicated that OAW can increase propionate concentration, as documented by Ozkaya (2020) and Ozkaya et al. (2020). However, Tassoul and Shaver (2009) reported that essential oils did not notably affect glucose and ketone concentration in cows during the early lactation. These differing outcomes could indeed be attributed to variations in the specific herbal feed additives used and their dosages across studies. In the current study, the observation that OAW led to a decrease in serum ketone concentration and simultaneous reduction in glucose concentration is noteworthy. The initial ketone and glucose concentrations of the cow were above the reference concentration. By the end of the experiment, the decrease in ketone concentration observed in the OAW-supplemented groups, coupled with increased glucose concentration, might be attributed to the potential anti-ketogenic effects of the plants and their extracts. This suggests that OAW supplementation might have influenced metabolic pathways, potentially triggering an anti-ketogenic response, leading to reduced ketone concentration, while concurrently affecting glucose metabolism in the cows. However, further research and detailed investigations are essential to understand the precise mechanism by which OAW.

The effects of thyme and oregano extracts on hematological parameters, particularly hemoglobin (Hb), red blood cell (RBC) levels, and white blood cell (WBC) counts, have been discussed in various studies. Franchini et al. (2007) suggested that thyme and oregano extracts might contribute to increased Hb and RBC due to their low saponin content, which could potentially enhance the permeability of large molecules like ferritin through intestinal villi. Our study found that the addition of OAW did not lead to significant changes in Hb and RBC. Interestingly, some studies have suggested that when plant extracts induce Hb and RBC along with a decrease in WBC, it might signify an influence on the immune system and potentially antioxidant properties (Seirafy and Sobhanirad, 2017). Similar to the study conducted by Drong (2016), did not identify a significant difference in the average of hematological blood parameters. However, there was a non-significant decrease observed in WBC in cows in this study with the addition of OAW. While the observed changes in hematological parameters were not statistically significant in our study, such alterations in WBC, even if not significant, might hint at potential immune-modulatory effects. These variations could suggest that OAW supplementation might have a subtle influence on the immune system, albeit not strong enough to reach statistical significance in our specific experimental conditions. The intricate relationships between plant extracts and their effects on hematological parameters, especially in relation to immune responses and antioxidant properties, often require more in-depth investigations and diverse experimental setups to draw concrete conclusions. Further research in crucial system and antioxidant status in dairy cows.

The research conducted by Sharma et al. (2011) highlighted that cows experience heightened oxidative stress and diminished antioxidative defense systems during early lactation, rendering them more susceptible to diseases like mastitis and metritis. Our study's findings suggest that supplementing cows with OAW might contribute to bolstering the antioxidative defense mechanism during this critical early lactation period, potentially offering protection against the effects of oxidative stress. Similarly, in a study involving calves, Seirafy and Sobhanirad (2017) reported that supplementation with thyme extract improved the antioxidant defense mechanism, demonstrating the potential of specific plant extracts to enhance antioxidative responses in animals. However, Hashemzadeh-Cagari et al. (2015) and Mazur et al. (2019) observed no significant effects on the antioxidative defense system when supplementing cows with a herbal mixture. Discrepancies in outcomes among studies exploring the impact of herbal supplementation on antioxidative defense mechanisms might arise from variations in the specific herbal compositions used, dosages, animal breeds, and individual differences in physiology.

The current study implies that OAW supplementation potentially supports antioxidative defense mechanisms aligned with the notion that certain plant extracts or herbal supplements might aid in fortifying antioxidative responses in animals, particularly during critical phases like early lactation. Nonetheless, further comprehensive research is crucial to ascertain the consistent effects underlying mechanisms of herbal supplementation and antioxidative defense mechanism in dairy cows.

CONCLUSIONS

Our conclusion offers a comprehensive summary of the observed effects and potential benefits of Oregano Aromatic Water supplementation in dairy cows during early lactation. It highlights several key findings, including:

Milk Production: While there was a trend toward improvement in milk production with Oregano Aromatic Water supplementation, the current study found that the doses used were not sufficient to exhibit significant positive effects on milk quality.

Ketosis Prevention: The significant decrease in blood ketone concentration and increase in serum glucose concentration with Oregano Aromatic Water supplementation indicate its potential in preventing ketosis caused by negative energy balance during early lactation.

Udder Health: Although there was a non-significant decrease in somatic cell count, which indicates udder health, it suggests a potential positive impact of Oregano Aromatic Water on udder health.

Metabolic Health: The decrease in total oxidant capacity and increase in total antioxidant capacity imply that Oregano Aromatic Water might have a beneficial role in improving metabolic health by mitigating the effects of oxidative stress.

We rightfully note the challenges associated with studying natural extracts like essential oils. The diverse composition of essential oils within plant species due to geographical variations poses a challenge in ensuring consistency in scientific research.

To better comprehend the effects of individual essential oils compounds and their combinations on dairy cow metabolism and production-related parameters, future studies should focus on identifying promising compounds and specific mixtures in precise dosages. Such research would help in elucidating dairy cow health and productivity.

Our conclusion provides valuable insights and underscores the need for further research to better harness the potential benefits of essential oil compounds, like those found in Oregano Aromatic Water, in improving dairy cow health and production parameters.

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CONFLICT OF INTEREST

None declared

REFERENCES

Abdel Hakim NF, Hilali EA, Amer AA, Younis TM (1989) Effect of some antibiotics as growth promoters on performance of broiler chicks fed different protein levels. Arch Anim Nutr 39 (1-2):97-104.

AOAC (2000) Official methods of analysis method 955.04 and method 948.22, protein (crude) and fat (crude) or ether extract in animal feed. Official Methods of Analysis of AOAC International.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils - a review. Food Chem Toxicol 46 (2):446-475.

Benchaar C (2021) Diet supplementation with thyme oil and its main component thymol failed to favorably alter rumen fermentation, improve nutrient utilization, or enhance milk production in dairy cows. J Dairy Sci 104 (1):324-336.

Bergman EN (1971) Hyperketonemia-ketogenesis and ketone body metabolism. Journal of dairy science, 54 (6):936-948.

Braun HS, Schrapers KT, Mahlkow-Nerge K, Stumpff F, Rosendahl J (2019) Dietary supplementation of essential oils in dairy cows: evidence for stimulatory effects on nutrient absorption. Animal 13 (3):518-523.

Bush RS, Milligan LP (1971) Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. Can J Anim Sci 51 (1):121-127.

De Villiers P, Petzer IM, Erasmus L (2000) Chapter 8: the use of milk recording information as a herd management tool. In: Dairy Herd Improvement in South Africa. ARC-Animal Improvement Institute, Irene, RSA.

Drong C (2016) Effects of monensin and essential oils on ruminal fermentation, performance, energy metabolism and immune parameters of dairy cows during the transition period. Doctora thesis, University of Veterinary Medicine, Hannover, Germany.

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38 (12):1103-1111.

Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37 (4):277-285.

European Pharmacopoeia (1975) Vol. 3, Maisonneuves S. A. Sqante- Ruffine.

Franchini M, Salvagno GL, Montagnana M, Lippi G (2007) Serum ferritin levels correlate with haemoglobin concentration: a report on 589 outpatients from a single centre. Blood Transfus 5 (4):244-245.

Giannenas I, Skoufos J, Giannakopoulos C, Wiemann M, Gortzi O, Lalas S, Kyriazakis I (2001) Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. J Dairy Sci 94 (11):5569-5577.

Giller K, Rilko T, Manzocchi E, Hug S, Bolt R, Kreuze M (2020) Effects of mixed essential oils from eucalyptus, thyme and anise on composition, coagulation properties and antioxidant capacity of the milk of dairy cows. J Anim Feed Sci 29 (1):3-10.

Hashemzadeh-Cigari F, Ghorbani GR, Khorvash M, Riasi A, Taghizadeh A, Zebeli Q (2015) Supplementation of herbal plants differently modulated metabolic profile, insulin sensitivity, and oxidative stress in transition dairy cows fed various extruded oil seeds. Prev Vet Med 118 (1):45-55.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR, Kadivar M, Riasi A, Zebeli Q (2014) Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. J Dairy Sci 97 (12):7487-7497.

Hassanat F, Gervais R, Benchaar C (2017) Methane production, ruminal fermentation characteristics, nutrient digestibility, nitrogen excretion, and milk production of dairy cows fed conventional or brown midrib corn silage. J Dairy Sci 100 (4):2625-2636.

Hausmann J, Deiner C, Patra AK, Immig I, Starke A, Aschenbach JR (2018) Effects of a combination of plant bioactive lipid compounds and biotin compared with monensin on body condition, energy metabolism and milk performance in transition dairy cows. PLoS One 13 (3):e0193685.

Henno M, Ling K, Kaart T, Ariko T, Karis P, Jaakson H, Kuusik S, Ots M (2021) Effect of monensin on milk fatty acid profile in dairy cows and on the use of fatty acids for early diagnosis of elevated blood plasma concentrations of nonesterified fatty acids and hyperketonemia. J Dairy Sci 104 (9):10355-10362.

Huang Q, Liu X, Zhao G, Hu T, Wang Y (2018) Potential and challenges of tannins as an alternative to in-fed antibiotics for farm animal production. Anim Nutr 4 (2):137-150.

Klinkon M, Jezek J (2012) Values of blood variables in calves. A Bird's-Eye View Of Veterinary Medicine, 1.

Kuester OJ (2016) An evaluation of feeding a blend of essential oils and cobalt lactate to lactating dairy cows. MSc Thesis, South Dakota State University, USA.

Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91 (3):453-462.

Mazur M (2019) Zwyrzykowska-Wodzinska A, Wojtas E, Zachwieza A, Salejda AM. Effect of yerba mate (Ilex paraguariensis A. St. -Hil.) supplementation on oxidative stress in ruminants. Chil J Agric Res 79:316-322.

Meiring P (2014) Effects of essential oils, ionophore and live yeast supplementation on rumen fermentation dynamics in Jersey cows. Doctora Thesis, University of Pretoria, Hatfield, South Africa.

Moller Z (2015) The use of oregano oil extract as feed-additive for Jersey cows grazing on ryegrass pasture in spring. Doctora Thesis, Stellenboch University, Matieland, South Africa.

Moosavi-Zadeh E, Rahimi A, Rafiee H, Saberipour H, Bahadoran R (2023) Effects of fennel (Foeniculum vulgare) seed powder addition during early lactation on performance, milk fatty acid profile, and rumen fermentation parameters of Holstein cows. Front Anim 4:1097071.

Nowers N (2016) The effect of on oregano oil extract in a lactating dairy cows diet on production responses of Holstein cows. Doctora Thesis, Stellenboch University, Matieland, South Africa.

Oetzel GR (2013) Understanding the impact of subclinical ketosis. University of Wisconsin, Madison: pp 15-26.

Oh J, Wall EH, Bravo DM, Hristov AN (2017) Host-mediated effects of phyto-nutrients in ruminants: a review. J Anim Sci 100 (7):5974-5983.

Ozkaya S, Almali OE, Ayan Y, Erbas S, Aksu T (2020) Effects of oregano (Oreganum onites L.) aromatic water on rumen microbial fermentation of Holstein calves. Turkish J Vet Anim Sci 44 (5):1110-1114.

Ozkaya S, Erbas S, Ozkan O, Baydar H, Aksu T (2018) Effect of supplementing milk replacer with aromatic oregano (Oreganum onites L.) water on performance, immunity and general health profiles of Holstein calves. Anim Prod Sci 58 (10):1892-1900.

Ozkaya S. (2020) Effect of oregano aromatic water supplementation to rumen fluid on in vitro digestion and rumen parameters of starter in calves. Indian J Anim Sci 90(6):903-906.

Prayitno CH, Subagyo Y, Jayanegara A (2019) Nutrient utilization and milk yield of dairy cows fed a diet containing monensin, garlic peel and organic minerals during the lactation period. Adv Anim Vet Sci 7(5):412-416.

Seirafy H, Sobhanirad S (2017) Effects of oregano (Origanum vulgare) and thyme (Thymus vulgaris) oils on growth performance and blood parameters in Holstein suckling calves. Indian J Anim Sci 7 (4):585-593.

Sharma N, Singh NK, Singh OP, Pandey V, Verma PK (2011) Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Australas J Anim Sci 24 (4):479-484.

Silvestre T, Räisänen SE, Cueva SF, Wasson DE, Lage CFA, Martins LF, Wall E, Hristov A N (2022) Effects of a combination of Capsicum oleoresin and clove essential oil on metabolic status, lactational performance, and enteric methane emissions in dairy cows. J Dairy Sci 105 (12):9610-9622.

Silva Filho EC, Roma Junior LC, Salles MSV, Salles FA, Ezequiel JMB,

Van Cleef EHCB (2017) Thyme essential oil supplementation on performance and milk quality of lactating dairy cows. In: Book of Abstract of the 68th Annual Meeting of the European Federation of Animal Science, Tallin, Estonia, Wageningem (TheNetherlands): pp 204.

Silva GG, Takiya CS, Del Valle TA, de Jesus EF, Grigoletto NTS, Nakadonari B, Cortinhas CS, Acedo TS, Renno FP (2018) Nutrient digestibility, Ruminal fermentation, and milk yield in dairy cows fed a blend of essential oils and amylase. J Dairy Sci 101 (11):9815-9826.

Sordillo LM, Mavangira V (2014) The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 54 (9):1204-1214.

Stivanin SCB, Vizzotto EF, de Paris M, Zanela MB, Passos LT, Angelo IDV, Fischer V (2019) Addition of oregano or green tea extract into the diet for Jersey cows in transition period. Feeding and social behavior, intake and health status. Plant extracts for cows in the transition period. Anim Feed Sci Technol 257:114265.

Tassoul MD, Shaver RD (2009) Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cow. J Dairy Sci 92 (4):1734-1740.

TSE (1991) Animal Feed-Metabolic (Reversible) Energy Determination (Chemical Method). TSE No: 9610, Turkish Standards Institute, Ankara.

Yumru M, Savas HA, Kalenderoglu A, Bulut M, Celik H, Erel O (2009) Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog Neuropsychopharmacol Biol Psychiatry 33 (6):1070-1074.