Effects of Juniper tar (Juniperus oxycedrus) aromatic water supplemented to whole milk on oxidative stress, immune response, intestinal flora, and kidney-urinary system of suckling Holstein calves

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Abstract

Digestive system and respiratory tract diseases, which are frequently seen in calves during the suckling period, suppress the development of the calves or cause deaths and great economic losses. In the present study, it was investigated whether Juniper aromatic water (JOW) would be suitable for promoting the health of suckling Holstein calves. Sixteen newborn calves (n = 4, in each group) were randomly selected and assigned to the following four treatments; G1: control group, fed with whole milk (WM) and calf starter (CS), G2: fed with 1.25% JAW supplemented WM and CS, G3: fed with 2.5% JAW supplemented WM and CS, G4: fed with 5% JAW supplemented WM and CS. The supplementation of JAW significantly reduced the incidences of digestive system and respiratory tract diseases in calves. The supplementation of JAW suppressed the growth of intestinal pathogenic bacteria at weaning age but did not affect the growth of lactic acid bacteria. It did not affect the urinary and kidney systems of the calves. JAW decreased oxidative stress concentrations while a non-significant increase occurred in antioxidant defence mechanism enzymes. It showed a significant increase in Immunoglobulin A, G, and M. The best result was observed in the supplementation of 1.25% JAW. The finding of the study showed that JAW, a by-product, can be used safely in the health rearing of calves.

Introduction

The health and management of calves, which are the future of the dairy herd, are important components of overall herd profitability. The productivity of the dairy herd may be adversely affected by the negative growth of calves, the decreased future milk yield of animals with chronic diseases during childhood, the spread of infectious diseases from calves to adult cows, increased veterinary costs and limited genetic selection due to high mortality of replacement animals (Lorenz et al. 2011). Of all animals on a dairy farm, the highest morbidity and mortality rates usually occur in calves before they are weaned. Because the placental transfer of immunoglobulins (Ig) is minimal in ruminants and calves born hypogammaglobulinemic, their resistance to disease is low (Senturk 2013). 7.8% of calf deaths occur during the pre-weaning periods and 1.8% occur post-weaning period. Diarrhoea and digestive problems account for 56.5% of pre-weaning deaths, followed by respiratory problems (22.5%) and other or unknown causes (21.0%). Respiratory problems account for 46.5% of post weaning deaths, followed by diarrheal problems (12.6%) and other or unknown problems (40.9%) (NAHMS 2007). Antibiotics used in the treatment of these diseases reduce non-pathogenic microorganism counts as well as pathogenic microorganisms. Thus, it causes to stop or to regress of growth in the early stages of ruminants (Postema et al. 1987; Soltan 2009). The World Health Organization has suggested that, with the misuse of antibiotics, the microorganism gains immunity to specific antibiotics over time and they cannot be effective in protecting human and animal health. For this reason, the use of antibiotics in animal production is prohibited and restricted in EU countries (Anadon 2006). As a result, researchers began to research growth factors that could be alternatives to antibiotics. For this purpose, extensive researches on medicinal and aromatic plants and essential oils obtained from them have been carried out and are still

being undertaken. Essential oils have been shown to have no health hazards when consumed by humans and animals, and have been classified as safe additives (FDA 2003).

Juniperus oxycedrus L. type junipers naturally found in Turkiye (Ansin and Ozkan 1993) are rich in essential oils, tannins, flavonoids, resins, lignin and triterpenes (Hegnauer 1986). Extracts of *J. oxycedrus* L. are used in traditional medicine in Europe and many countries of the world (Loizzo et al. 2007). Aromatic oil prepared from this plants is used as alternative medicine due to its anti-inflammatory and anti-cancer properties as well as in soap, cream and lotion production due to its dermatological properties (Loizzo et al. 2007; Skalli et al. 2013; Zhang et al. 2015). In addition, medicines prepared with roots, fruits and leaves of *J. oxycedrus* L. have been used antiseptics in diseases such as pain, cough, and rheumatism, tuberculosis (Tumen and Hafizoglu 2003). It is also known to be good for diabetes, digestive tract diseases, and respiratory tract diseases such as bronchitis and asthma, kidney and urinary tract diseases, jaundice, sciatica, sinusitis, liver disorders, metabolism disorders and is used against these disorders (Koc 2002; Gurkan 2003).

It was stated that essential oil had higher antiradical activity and iron-reducing properties than aromatic water in a study comparing essential oil and aromatic water obtained from Juniper fruit and leaves. It was also stated that aromatic water showed selective antibacterial properties whereas essential oil completely stopped the growth of both pathogenic and non-pathogenic bacteria at increasing concentrations (Isik et al. 2020). In the study conducted with JAW, it was reported that JAW improves growth performance, increase feed consumption, reduces the incidences of diarrhoea and diseases, and allows healthy calves to be raised (Isik and Ozkaya 2021).

In our previous studies, we investigated the effects of JAW on the growth and general health parameters of calves, as well as the total phenolic content, antioxidant, antibacterial and iron-reducing properties (Isik et al. 2020; Isik and Ozkaya 2021). No studies have been found on the effects of JAW, which is a by-product that is released when extracting essential oil, on the performance, immunity, oxidative stress and antioxidative defense mechanism, intestinal flora, kidney-urinary system of suckling Holstein calves. Therefore, in this study, it was aimed to test the hypothesis that whether or not JAW improved the health of calves as a by-product without a negative impact on these parameters.

Material And Methods

The study was carried out in the dairy cattle farm of Muzaffer Yilmaz in the village Yassigume, Burdur. In the power analysis, it was determined that the highest value of the enterobacter count in the intestinal flora in the literature reviews was 6.3, the lowest value average was 4.9 and the standard deviation was 0.92, and it was determined that 4 animals should be present in each group for 95% power.

A commercially available calf starter was used in the study. During the study, standard feeding was given to the groups. The calves were given starter feed *ad libitum*. The calves were given a total of 4L of milk (2L in the morning-2L in the evening) in two meals.

Calves born on the farm were fed with colostrum for the first 3 days. Then, 4-day-old the calves were randomly divided into 4 groups by taking their live weight and body measurements and housed in individual boxes. The doses of Juniper tar were determined as a result of the minimum inhibition concentration (MIC) analysis and presented as a % of the amount of milk fed to the calves by mixing in the milk (Isik et al. 2020).

Experimental groups were designed as follows; G1: CS and WM (Control group), G2: fed with 1.25% JAW supplemented WM and CS, G3: fed with 2.5% JAW supplemented WM and CS, G4: fed with 5% JAW supplemented WM and CS (Isik and Özkaya, 2021).

Measurements and Sample Collection.

Crude protein, fat (AOAC, 2006), fibre, moisture and ash (AOAC, 2005) content of CS used in the study were determined (Table 1). Fat, protein, lactose, and dry matter of milk were performed with a HasVet Milk analyser and somatic cell count was performed using SOMATOS Mini (Has Vet Medical, Antaly, Turkiye).

Table 1
Chemical composition of calf starter and milk

	CS	**Milk**			
		G1	G2	G3	G4
DM, %	90.05	12.00	11.90	11.90	11.90
CP, %	18.17	3.40	3.30	3.30	3.30
Crude Fat, %	2.79	3.50	3.60	3.60	3.60
CF, %	9.41				
Moisture, %	9.95				
CA, %	7.52				
Starch, %	28.25				
ME, kcal/kg	2797.59				
Intensity, %		31.90	31.50	31.10	30.90
Lactose, %		5.10	5.00	5.00	5.00
pH		7.00	7.10	7.10	7.10
SSC		364.10	223.50	247.50	226.50

DM: Dry matter, CP: Crude protein, CF: Crude fibre, CA: Crude ash, ME: Metabolic energy, SSC: Somatic cell count

Faeces samples were collected from all animals at 28-day-old and weaning age. After washing the rectums of the calves with betadine solution, faeces samples were taken into sterile faeces containers (3-5g) before the morning meal. Coliform, *E.coli*, *Enterobacteriaceae*, and lactic acid bacteria counts in faeces were performed using ready-made media (3M Health Care, St. Paul, MN, USA) whose results were internationally accepted.

Urine samples were collected from all animals at 28-day-old and weaning age. Urine samples were taken into sterile urine containers by massaging the vagina and penis of the calves. Blood bilirubin, urobilinogen, ketone bodies, glucose, protein, nitrite, leukocytes, pH and specific gravity were measured in the urine. Urine analyses were performed using urinary sticks (Acon Laboratories, Inc. San Diego, CA, USA), of which the results are internationally recognized.

Blood samples from calves in each group were taken from the vena jugular of calves at the beginning of the experiment, at weaning age, and on the 5th day of the weaning program. The blood was collected in gel tubes and centrifuged at 3000 rpm for 10 min. Obtained blood serum was analyzed using the Mindray BS-300 (Mindray, Shenzhen, P.R. China) biochemical blood analyzer. Total antioxidant status (TAS), paraoxonase-1 (PON-1), total thiol (TTL), native thiol (NTL), thiol/disulfide homeostasis (TDH), catalase (CAT), superoxide dismutase (SOD) which are markers of antioxidative defence mechanisms and Malondialdehyde (MDA), total oxidant status (TOS) and oxidative stress index (OSI) which are markers of oxidative stress were examined. Immunoglobulins (IgA, IgE, IgG) were also examined. TDH (µmol/L), TAS (mmol/L), TOS (µmol/L), and PON-1 (U/L) tests were measured using a commercially available kits (Rel Assay Diagnostics, Baran Medical, Ankara, Turkiye). OSI (the ratio of TOS to TAS was accepted as the OSI). OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (µmol H_2O_2 equivalent/L)/TAS (µmol Trolox equivalent/L (Yumru et al. 2009). MDA (nmol/L) level was determined by a method based on the reaction with thiobarbituric acid. SOD (U/ml) activity is measured by the inhibition of xanthine and xanthine oxidase reaction. CAT (U/L) is measured at 405 nm wavelength. IgA is measured at the wavelength of 600 nm. IgG is measured at the wavelength of 600 nm. Antioxidative defence mechanisms, oxidative stress markers and immune system were determined by the spectrophotometric method (Baran Medical, Ankara, Turkiye).

Digestive system and respiratory tract diseases of calves recorded daily. When the faeces score (Larson et al. 1977) was ≥ 3 for 2 consecutive days, it was recorded as digestive system diseases in calves. The calves were checked by the veterinarian when the respiratory score (Heinrichs et al. 2003) was ≥ 3. Those diagnosed with respiratory tract disease were recorded.

Statistical Analysis

The data obtained in the study were analyzed with the repeated measurements analysis of variance technique, the differences between the groups means were examined with the Turkey test (MINITAB v20, Minitab LLC, State College, Pennsylvania, USA).

Results

Non-significant statistical differences were observed among the age of groups to start ruminating and consuming CS (Table 2). The calves in G2 started consuming CS earlier than the other groups and accordingly started ruminating early.

Table 2
Effect of Juniper aromatic water supplementation on health parameters and intestinal flora

28-day-old					
Coliforms	6.82 ± 0.13	6.37 ± 0.15	6.42 ± 0.09	6.55 ± 0.22	0.22
E. coli	6.82 ± 0.13	6.46 ± 0.17	6.36 ± 0.15	6.52 ± 0.18	0.24
Enterobacter	6.85 ± 0.18	6.46 ± 0.17	6.43 ± 0.16	6.42 ± 0.14	0.23
Lactic acid	6.85 ± 0.03	6.96 ± 0.05	6.92 ± 0.05	6.81 ± 0.08	0.24
Weaning age					
Coliforms	7.13 ± 0.10^{a}	6.58 ± 0.14^{b}	6.36 ± 0.15^{b}	6.39 ± 0.05^{b}	0.00
E. coli	7.10 ± 0.06^{a}	6.59 ± 0.08^{b}	6.54 ± 0.16^{b}	6.23 ± 0.13^{b}	0.00
Enterobacter	7.09 ± 0.11^{a}	6.63 ± 0.12^{ab}	6.13 ± 0.12^{c}	6.33 ± 0.11^{b}	0.00
Lactic acid	6.89 ± 0.05	6.96 ± 0.05	6.93 ± 0.03	6.94 ± 0.05	0.21
	G1 **Mean ± S.E.**	**G2** **Mean ± S.E.**	**G3** **Mean ± S.E.**	**G4** **Mean ± S.E.**	**P**
DD	8.25 ± 2.39^{a}	0.25 ± 0.25^{b}	2.75 ± 2.43^{ab}	4.50 ± 1.44^{ab}	0.04
ID	2.00 ± 1.15^{a}	0.00 ± 0.00^{b}	0.75 ± 0.75^{b}	0.00 ± 0.00^{b}	0.03
SRA, day	16.25 ± 0.95	14.00 ± 1.47	14.50 ± 2.10	16.00 ± 3.57	0.78
SFCA, day	4.78 ± 0.85	4.00 ± 1.00	5.75 ± 2.43	8.50 ± 1.94	0.30

DD: Diarrhoea day, ID: Illness day, SR: Starting rumination age, SFCA: Starting feed consumption age

The supplementation of JAW significantly ($P < 0.05$) reduced the incidence of diarrhoea. The highest incidence of diarrhoea was in G1, and lowest in G2 (Table 2). Similarly, JAW also significantly ($P < 0.05$) reduced respiratory diseases. The diagnosis of respiratory diseases was highest in G1, while the lowest was in G2 and G4 (Table 2).

The supplementation of JAW did not have a significantly effect on the pathogen bacteria count such as coliform, *E.coli*, *Enterobacteriaceae* and non-pathogen bacteria such as lactic acid in calf intestine at the 28-day-old (Table 2). However, while JAW significantly ($P < 0.05$) suppressed the growth of pathogenic bacteria at weaning age, it did not affect the growth of non-pathogenic bacteria.

JAW did not affect the urinary and kidney system of calves (Table 3).

Table 3
The effect of supplementation of Juniper aromatic water kidney and urinary system.

	G1 Mean ± S.E.	G2 Mean ± S.E.	G3 Mean ± S.E.	G4 Mean ± S.E.	P value
Color					
28-day-old	Light yellow	Light yellow	Light yellow	Light yellow	
Weaning age	Yellow	Light yellow	Light yellow	Light yellow	
Blood					
28-day-old	18.75 ± 6.25	6.25 ± 6.25	12.50 ± 7.22	15.00 ± 6.12	0.14
Weaning age	125.00 ± 72.20	8.75 ± 5.91	0.00 ± 0.00	127.50 ± 7.08	
Bilirubin					
28-day-old	-	-	-	-	
Weaning age	-	-	-	-	
Urobilinogen					
28-day-old	0.15 ± 0.05	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.41
Weaning age	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	
Ketone bodies					
28-day-old	-	-	-	-	
Weaning age	-	-	-	-	
Glucose					
28-day-old	-	-	-	-	
Weaning age	-	-	-	-	
Protein					
28-day-old	22.50 ± 7.50	15.00 ± 8.66	7.50 ± 7.50	15.00 ± 8.66	0.51
Weaning age	40.00 ± 2.12	30.00 ± 0.00	30.00 ± 0.00	22.50 ± 7.50	
Nitrite					
28-day-old	-	-	-	-	
Weaning age	-	-	-	-	

[ab] difference between the means in the same row

	G1 Mean ± S.E.	G2 Mean ± S.E.	G3 Mean ± S.E.	G4 Mean ± S.E.	P value
Leukocyte					
28-day-old	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.41
Weaning age	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
pH					
28-day-old	7.00 ± 0.58^{b}	8.50 ± 0.29^{a}	8.25 ± 0.48^{ab}	8.13 ± 0.32^{ab}	0.03
Weaning age	8.00 ± 0.41^{b}	9.00 ± 0.00^{a}	8.00 ± 0.41^{b}	8.00 ± 0.41^{b}	
Specific gravitt					
28-day-old	1.02 ± 0.00	1.01 ± 0.00	1.01 ± 0.00	1.01 ± 0.00	0.62
Weaning age	1.02 ± 0.01	1.02 ± 0.01	1.02 ± 0.00	1.01 ± 0.00	
^{ab} difference between the means in the same row					

Antioxidative defence mechanism markers and immune responses of the groups were not significant at the beginning of the experiment (Table 4). However, while MDA, an oxidative stress marker, was significant ($P < 0.05$), TOS and OSI were not. The MDA was lowest in G3.

Table 4
Effect of Juniper aromatic water on oxidative stress, antioxidative defence mechanism and immune system

		G1	G2	G3	G4	P
		Ort. ±S. H.	Ort. ±S. H.	Ort. ±S. H.	Ort. ±S. H.	
TAS, mmol/L	1	0.78 ± 0.06	0.96 ± 0.08	1.01 ± 0.08	0.78 ± 0.07	0.09
	2	0.87 ± 0.03^{bc}	0.97 ± 0.03^{ab}	1.00 ± 0.06^{a}	0.85 ± 0.03^{c}	0.03
	3	0.99 ± 0.04	1.06 ± 0.04	1.00 ± 0.07	0.93 ± 0.03	0.30
TOS, µmol/L	1	3.72 ± 0.90	5.82 ± 1.65	10.83 ± 8.14	4.81 ± 1.12	0.68
	2	3.57 ± 0.43	2.46 ± 0.73	3.08 ± 0.17	2.90 ± 0.72	0.66
	3	6.31 ± 1.17	3.61 ± 3.17	12.65 ± 9.61	4.17 ± 0.84	0.60
OSI	1	0.46 ± 0.07	0.64 ± 0.22	0.97 ± 0.68	0.63 ± 0.16	0.81
	2	0.41 ± 0.04	0.25 ± 0.07	0.31 ± 0.00	0.34 ± 0.09	0.34
	3	0.63 ± 0.12	0.34 ± 0.28	1.27 ± 0.78	0.44 ± 0.10	0.63
PON-1, U/L	1	89.80 ± 55.30	22.00 ± 5.69	64.50 ± 23.90	40.00 ± 10.90	0.54
	2	369.50 ± 59.90	558.00 ± 44.20	447.00 ± 59.50	487.00 ± 123.00	0.43
	3	462.30 ± 82.10	671.30 ± 37.60	568.80 ± 95.90	577.00 ± 195.00	0.49
TTL, µmol/L	1	483.90 ± 17.70	612.00 ± 109.00	827.00 ± 262.00	634.70 ± 24.40	0.44
	2	452.20 ± 51.20	558.30 ± 71.10	409.00 ± 54.40	428.50 ± 74.80	0.41
	3	502.20 ± 59.40	584.30 ± 94.80	904.00 ± 414.00	453.30 ± 51.70	0.48
NTL, µmol/L	1	375.00 ± 21.80	364.00 ± 76.70	488.80 ± 96.30	451.80 ± 52.60	0.52
	2	334.00 ± 53.60	469.20 ± 47.40	304.70 ± 47.20	344.60 ± 72.90	0.25
	3	373.40 ± 76.40	482.20 ± 52.60	574.00 ± 157.00	362.60 ± 56.90	0.44
TDH	1	54.50 ± 17.40	124.20 ± 16.20	169.30 ± 93.90	91.40 ± 21.10	0.47

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, PON-1: Paraoxonase-1, TTL: Total thiol level, NTL: Native thiol level, TDH: Thiol/Disulfide Homeostasis, CAT: Catalase, SOD: Super oxide dismutase, MDA: Malondialdehyde, Initial, 2: Weaning age, 3: On the 5th day of the weaning program, ^{abc} Difference between averages in the same row.

		G1	G2	G3	G4	P
		Ort. ±S. H.	Ort. ±S. H.	Ort. ±S. H.	Ort. ±S. H.	
	2	44.60 ± 14.30	59.10 ± 11.20	52.20 ± 12.00	41.90 ± 10.80	0.74
	3	51.10 ± 22.50	64.39 ± 9.02	40.90 ± 9.05	45.30 ± 25.60	0.63
CAT, kU/L	1	89.80 ± 20.70	37.00 ± 14.00	111.50 ± 59.70	72.80 ± 30.90	0.62
	2	57.00 ± 15.50	104.80 ± 16.40	59.30 ± 26.70	85.00 ± 17.60	0.26
	3	94.00 ± 6.36	227.00 ± 145.00	83.50 ± 17.80	112.00 ± 11.80	0.52
SOD, U/ml	1	706.0 ± 176.0	813.0 ± 283.0	1698 ± 1049	855.00 ± 344.00	0.64
	2	781.0 ± 351.0	1290.0 ± 393.0	541.70 ± 35.20	511.00 ± 208.00	0.29
	3	769.0 ± 166.0	1328 ± 945.00	992.0 ± 448.00	387.00 ± 126.00	0.64
MDA, µmol/ml	1	21.42 ± 4.18^{b}	22.48 ± 5.99^{b}	38.97 ± 5.99^{a}	10.12 ± 1.84^{c}	0.01
	2	15.90 ± 9.11	5.91 ± 0.85	11.31 ± 4.24	9.69 ± 3.96	0.64
	3	8.20 ± 2.99	6.36 ± 0.60	16.00 ± 6.47	18.40 ± 6.90	0.14
IgE, IU/ml	1	56.50 ± 28.00	37.27 ± 4.29	59.20 ± 36.70	31.93 ± 8.06	0.83
	2	25.75 ± 5.98	29.23 ± 2.93	22.33 ± 5.77	21.20 ± 6.06	0.71
	3	23.82 ± 5.49	24.63 ± 3.75	24.63 ± 3.02	24.90 ± 3.12	0.97
IgA, mg/dL	1	10.48 ± 4.57	5.60 ± 2.11	8.32 ± 3.25	9.17 ± 5.08	0.88
	2	1.35 ± 0.68	4.80 ± 1.84	1.38 ± 0.58	1.38 ± 0.81	0.09
	3	1.58 ± 0.54^{c}	7.23 ± 2.65^{a}	1.95 ± 0.75^{c}	3.67 ± 3.67^{b}	0.03
IgG, mg/dL	1	4.83 ± 1.05	6.70 ± 1.46	5.50 ± 1.33	6.08 ± 0.94	0.74
	2	3.95 ± 1.07	5.15 ± 0.69	3.25 ± 0.92	3.52 ± 1.07	0.23
	3	3.53 ± 0.88^{b}	7.08 ± 3.14^{a}	3.23 ± 0.50^{b}	4.00 ± 1.40^{b}	0.04
IgM, mg/dL	1	3.48 ± 0.67	3.63 ± 0.91	3.60 ± 3.47	4.63 ± 4.39	0.78
	2	1.35 ± 0.94	3.20 ± 3.20	2.80 ± 2.80	2.08 ± 2.08	0.20

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, PON-1: Paraoxonase-1, TTL: Total thiol level, NTL: Native thiol level, TDH: Thiol/Disulfide Homeostasis, CAT: Catalase, SOD: Super oxide dismutase, MDA: Malondialdehyde, Initial, 2: Weaning age, 3: On the 5th day of the weaning program, ^{abc} Difference between averages in the same row.

	G1 Ort. ±S. H.	G2 Ort. ±S. H.	G3 Ort. ±S. H.	G4 Ort. ±S. H.	P
3	3.23 ± 1.26[b]	7.00 ± 4.04[a]	3.02 ± 3.02[b]	2.00 ± 2.00[b]	0.44

TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, PON-1: Paraoxonase-1, TTL: Total thiol level, NTL: Native thiol level, TDH: Thiol/Disulfide Homeostasis, CAT: Catalase, SOD: Super oxide dismutase, MDA: Malondialdehyde, Initial, 2: Weaning age, 3: On the 5th day of the weaning program, [abc] Difference between averages in the same row.

Antioxidative defence mechanism except for TAS value and oxidative stress markers were not significant (Table 4). The effect of JAW on immune responses was not significant.

On the 5th day of the weaning program, JAW had no significant effect on the antioxidative defence mechanism, oxidative stress markers, however, supplementation of JAW increased the concentration of IgA, G, and M, but resulted in a non-significant increase in IgE (Table 4).

Discussion

Calves start consuming concentrate feed at approximately 1 week old and increase feed consumption at 2 week old (Morittu et al. 2021) and start to ruminate approximately 14 days after birth (Lopreiato et al. 2018). There is a relationship of the calf's rumen and digestive abilities affects rumination (Lopreiato et al. 2018). Rumination affects feed consumption and feed efficiency, which is associated with calf weight gain, health and welfare (Ambriz-Vilchis et al. 2015). Indeed, all calves in the JAW supplemented groups started CS intake and rumination at an early age. This can be explained by the fact that CS intake encourages rumination (Beauchemin 2018). Tapki et al. (2020) reported that the supplementation of oregano oil started CS consumption in calves at an early age. Similarly, green tea and oregano extract have been reported to promote CS intake at an early age (Heisler et al. 2020).

JAW supplemented to milk improved the health of calves by reducing the incidence of diarrheal and respiratory diseases. The antiseptic and antibacterial activities of plant extracts increase feed efficiency by affecting gastrointestinal system development, rumen microbiological activity, and reduce digestive and respiratory diseases in newborn calves (Cobellis et al. 2016; Siefzadeh et al. 2017; Campolina et al. 2021). The reason why JAW reduces digestive and respiratory diseases may be that the active components of the extracts have positive effects on the secretion of endogenous enzymes, improvement of the intestinal environment, balanced intestinal flora, and increased liver functions for better utilization of fats and proteins (Seifzadeh et al. 2017). As a matter of fact, it has been reported that plants oil and extracts reduce the incidence of respiratory (Sastir Koroglu and Kocabagli 2019) and digestive system diseases (Campolina et al. 2021) of calves.

Extracts obtained from plant leaves, flowers, and fruits suppress the growth of pathogenic microbes by reducing their effectiveness (Özkaya et al. 2018; Hassan et al. 2020). The supplementation of JAW suppressed the growth of pathogens at 28-day-old not significantly, but significantly at weaning age. The

decrease in the count of pathogens in feces can be attributed to the fact that plant extracts have various antibacterial mechanisms, such as enzyme inhibition, cell membrane disruption, substrate deficiency, and inhibition of bacterial colonization (Damjanovic-Vratnica et al. 2011; Bahadar et al. 2016; Hassan et al. 2020). In many studies, it has been reported that plant oil and liquid extracts suppress the growth of pathogenic bacteria (Ünlü and Erkek 2013; Bi et al. 2017; Özkaya et al. 2018; Hassan et al. 2020).

The presence of high levels of non-pathogenic bacteria in the intestine compared to pathogens improves the intestinal health of calves (Bi et al. 2017; Özkaya et al. 2018). The supplementation of JAW did not affect the growth of lactic acid bacteria in the gut. This can be explained by the fact that lactic acid bacteria adhering to the intestinal surface produce lactate and acetate, which reduces the adhesion and colonization of pathogenic bacteria to the surface of intestinal epithelial cells and improves intestinal health (Seo et al. 2010).

Juniper berries and leaves or extract have traditionally been used as a diuretic. However, oral use for a long time or in high doses may not be safe and may lead to kidney-urinary tract problems (Raina et al. 2019). Despite the fact, there was no negative effect of JAW supplementation on kidney-urinary tract functions of calves. It was thought that this was due to the fact that the doses administered were not very high.

While TOS and MDA concentrations in G2 decreased at the weaning age and on the 5th day of the weaning program, the levels of antioxidative defence mechanism enzymes increased. Concentrations of TOS and MDA which are lipid peroxidation products, indicate the degree of lipid peroxidation (Davey et al. 2005; Hassan et al. 2020; Özkaya et al. 2022). A decrease in TOS and MDA concentrations means a decrease in lipid peroxidation. The increase in antioxidant defence mechanism enzymes confirms the antioxidant property of 1.25% JAW. The increase in antioxidant defence mechanism enzymes improves the antioxidant capacity of calves by increasing the scavenging of free radicals (Wei et al. 2020). Compounds of herbal extracts disrupt radical reactions by combating reactive host components, which stops the propagation of the oxidation chain (Amorati et al. 2013; Hassan et al. 2020; Özkaya et al. 2022). Endogenous antioxidant enzymes contribute to the intracellular defence against oxidative stress (Ding et al. 2021).

OSI, which is an indicator of the degree of oxidative stress, was low in G2, although not significant. High OSI is a result of high TOS and low TAS value (Ogut et al. 2013; Özkaya et al. 2022). Many researchers have stated that OSI increases in parallel with the increase in TOS value (Marcil et al. 2013; Dagulli et al. 2014; Yucel et al. 2015; Özkaya et al. 2022).

Immunoglobulins (IgA, IgG, IgM, and IgE) provide defence for all tissues that blood reaches and help protect against blood-borne infections, septicemia and spread by neutralizing microorganism entering the circulatory system (Roomruangwong et al. 2017). Calves fed 1.25% JAW had improved IgA, IgG, and IgM concentration compared to other calves. Extracts from plants improve the immune system with the effect of secondary compounds (Qiao et al. 2013; Özkaya et al. 2018; Lakhani et al. 2019; Kozyr et al. 2019; Wafa et al. 2021; 2022).

Juniper aromatic water supplement allowed calves to start rumination and intake of starter at an early age. It reduced the incidence of digestive and respiratory diseases. Juniper aromatic water suppressed the growth of pathogenic bacteria while did not affect the growth of lactic acid bacteria. Application of JAW 1.25%/calf/day increased the release of antioxidant defence mechanism enzymes and decreased the concentration of oxidative stress markers. Supplementation of 1.25%/calf/day Juniper aromatic water significantly improved the immune response. These results, which were obtained due to the negative effects of synthetic antibiotics and antioxidants on both human and animal health, showed that Juniper aromatic water can be used as a preservative. It was determined that Juniper aromatic water, whose effects on health parameters, pathogenic bacteria, antioxidant defence mechanism enzymes and immune system were examined, can be defined as an additive that increases the health of calves.

Declarations

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Authors' contributions Serkan Ozkaya: Designed and conducted the experiment, wrote the first draft of the article and statistical analyzes. Sabri Erbas: Obtained aromatic water in oregano leaves. Kanber Kara: Performed blood analyzes and evaluated the blood results and revised the article.

Statement of animal rights All procedures were reviewed and approved by the Burdur Mehmet Akif Ersoy University, Animal Experiments Local Ethics Committee, Burdur, Turkey (Protocol number: 507 and Approval Date: 10.04.2019) and was conducted in accordance with guidelines of Committee.

Conflict of interest statement The authors declared that there is no conflict of interest.

Data availability Datasets obtained and/or analyzed during the current study are available from corresponding after upon reasonable request.

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