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Juniper Tar Liquid Extract in Whole Milk: Effect on Oxidative Stress, Immune Response, Gut Flora and Renal Health in Holstein Calves

ABSTRACT

Objective: Calves frequently suffer from digestive and respiratory diseases during the suckling period, leading to developmental issues, mortality, and economic losses. This study aimed to evaluate the effects of Juniper liquid extract (JLE) supplementation in whole milk on the health and growth performance of suckling Holstein calves.

Material and Methods: Sixteen newborn Holstein calves were randomly assigned to 4 treatment groups (n=4 per group): Control (G1): Whole milk (WM)+ Calf starter (CS); G2: 1.25% JLE+WM+CS; G3: 2.5%JLE+WM+CS; G4: 5% JLE+WM+CS. Calves were monitored for digestive and respiratory diseases, gut microbiota, renal health, oxidative stress and immune response parameters. The experiment was terminated by weaning the calves when they consumed 800 g/d of calf starter for 3 consecutive days.

Results: Juniper tar liquid extract supplementation significantly reduced digestive and respiratory disease incidence. It suppressed the growth of gut pathogenic bacteria at weaning without affecting lactic acid bacteria. No adverse effects of JLE supplementation were observed on renal functions. JLE decreased oxidative stress levels, while antioxidant defence enzyme activity showed a non-significant increase. Immunoglobulin A, G, and M levels significantly increased, with the best results observed in the 1.25% JLE group.

Conclusion: JLE, a by-product, can be safely used in calf nutrition to improve health by reducing disease incidence, modulating gut flora, and enhancing immune response. The 1.25% JLE supplementation provided the most effective results.

Keywords: Holstein calves, juniper, liquid extract, oxidative stress, immune response, gut flora

Katran Ardıcı Sıvı Ekstraktı Ilavesinin Holstein Buzagılarda Oksidatif Stres, Bagısıklık Tepkisi, Bagırsak Florası ve Böbrek Saglıgı Üzerine Etkisi

ÖZ

Amaç: Buzağılar, emme dönemi boyunca sindirim ve solunum sistemi hastalıklarına sıkça maruz kalmaktadırlar, bu durum gelişim sorunlarına, ölümlere ve ekonomik kayıplara neden olmaktadır. Bu çalışma, tam yağlı süte ilave edilen katran ardıcı sıvı ekstraktının (JLE) emme dönemindeki Holstein buzağıların sağlığı ve büyüme performansı üzerine etkilerini değerlendirmeyi amaçlamıştır.

Materyal ve Metot: Yeni doğan 16 Holstein buzağı rastgele dört deneme grubuna ayrılmıştır (n=4/grup): Kontrol (G1): Tam yağlı süt (TYS)+Buzağı başlangıç yemi (CS); G2: %1.25 JLE+TYS+CS; G3: %2.5 JLE+TYS+CS; G4: %5 JLE+TYS+CS. Buzağılar sindirim ve solunum sistemi hastalıkları, bağırsak mikrobiyotası, böbrek sağlığı, oksidatif stres ve bağışıklık sistemi parametreleri açısından takip edilmiştir. Buzağılar ardışık 3 gün 800 g/gün buzağı başlangıç yemi tükettiklerinde sütten kesilerek deneme sonlandırılmıştır.

Bulgular: JLE takviyesi, sindirim ve solunum hastalıklarının görülme sıklığını önemli ölçüde azaltmıştır (P<0.05). Sütten kesim döneminde bağırsaktaki patojenik bakteri gelişimi baskılanırken laktik asit bakterilerinin gelişimini ise etkilememiştir. Böbrek fonksiyonları üzerine JLE ilavesinin olumsuz etkisi gözlemlenmemiştir. Oksidatif stres seviyeleri azalırken, antioksidan savunma enzim aktivitesinde önemli olmayan bir artış gözlemlenmiştir. IgA, IgG ve IgM seviyeleri önemli ölçüde artış göstermiştir. En iyi sonuçlar %1.25 JLE grubunda elde edilmiştir.

Sonuç: Bir yan ürün olan JLE, hastalık görülme sıklığını azaltarak, bağırsak florasını düzenleyerek ve bağışıklık sistemini güçlendirerek sağlığı iyileştirmek için buzağı beslenmesinde güvenle kullanılabilir. En etkili sonuçlar %1.25 JLE takviyesinde gözlemlenmiştir.

Anahtar Kelime: Holstein buzağı, ardıç, sıvı ekstrakt, oksidatif stres, bağışıklık sistemi, bağırsak florası

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#This article is summarized from the first author's master thesis.

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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 the milk suckling period, 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 disease resistance is low (Senturk 2013). Calf deaths' 7.8% 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 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 as feed additives in animal production is prohibited and its use in treatment is 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 research on medicinal and aromatic plants and essential oils obtained from them has been carried out and is 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 Türkiye (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 these 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 as 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, renal 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 liquid extract in a study comparing essential oil and liquid extract obtained from Juniper fruit and leaves, and the liquid extract 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 JLE, it was reported that JLE improves growth performance, increases 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 JLE 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 JLE, which is a by-product that is released when extracting essential oil, on the performance, immunity, oxidative stress and antioxidative defence mechanism, intestinal flora, and renal system of suckling Holstein's calves. Therefore, in this study, it was aimed to test the hypothesis of whether or not JLE improved the health of calves as a by-product without a negative impact on these parameters.

MATERIAL and METHODS

The study was conducted at the dairy cattle farm owned by Muzaffer Yılmaz, registred in Yassigume Village, Burdur province. Sixteen Holstein calves were included in the experiment. The number of calves was determined by power analysis. 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. The calves were given starter feed ad libitum. The calves were given a total of 4L of milk (2L in the morning and 2L in the evening) in two meals. Calves were weaned and the experiment was terminated when they consumed 800 g/d of calf starter for 3 consecutive days.

Calves born on the farm were fed with colostrum for the first 3 days. Then, 4-day-old calves were randomly divided into 4 groups by taking their live weight and body measurements and housed in individual boxes. Juniper tar liquid extract was obtained according to the European Pharmacopoeia (1975) as reported by Isik et al. (2020). The doses of Juniper tar were determined as a result of the minimum inhibition concentration (MIC) analysis and presented as a percentage 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: Whole milk (WM) and Calf starter (CS) (Control group), G2: fed with 1.25% JLE supplemented WM and CS, G3: fed with 2.5% JLE supplemented WM and CS, G4: fed with 5% JLE supplemented WM and CS (Isik and Özkaya, 2021).

The total phenolic substance amount of JLE used in the study is 1.85±0.04 mg GAE/g. Juniper liquid extract content includes 55.43% α-cedrol, 20.20% Verbenone, 14.72% Verbenol, 6.07% Borneol and 3.59% Trans-pinocarveol (Isik et al., 2020).

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, Antalya, Turkiye).

Table 1. Chemical composition of calf starter and whole milk
Tablo 1. Buzağı başlangıç yemi ve tam yağlı sütün kimyasal kompozisyonu

	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, cell/ml		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. The test stick was completely immersed in the mixed urine sample. Excess urine on the stick removed from the container was cleaned. The strip was allowed to stand for 2 minutes for the reaction to occur. The resulting colours were compared with the chromatic scale provided by the manufacturer. 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. The obtained blood serum was analyzed using the Mindray BS-

300 (Mindray, Shenzhen, P.R. China) biochemical blood analyzer. Creatinine (CREA), Urea, 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 commercially available kits (Rel Assay Diagnostics, nufacturer Mega Tip, Gaziantep, Türkiye). OSI value was calculated according to the following formula: OSI (arbitrary unit)=TOS (µmol H2O2 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 (Otto Scientific Medical, Ankara, Turkiye).

Digestive system and respiratory tract diseases of calves are recorded daily. When the faeces score (Larson et al. 1977) was ≥3 for 2 consecutive days, it was recorded as a digestive system disease 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

Data were analyzed using ANOVA analysis of variance technique. Starting feed consumption age, which did not show normal distribution, was analyzed with the Kruskal Wallis test, and the differences between the groups' means were examined with the Turkey test (MINITAB v20, Minitab LLC, State College, Pennsylvania, USA). The significance level was taken as $P<0.05$.

RESULTS

No significant statistical differences were observed among the groups in terms of the age at which they started ruminating and consuming CS (Table 2). However, calves in G2 began consuming CS earlier than those in the other groups and, consequently, started ruminating earlier.

Table 2. Effect of juniper liquid extract supplementation on health parameters and gut flora of calves

Tablo 2. Ardıç sıvı ekstraktı ilavesinin buzağıların sağlık parametreleri ve bağırsak florası üzerine ekileri

	G1	G2	G3	G4	P
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	
DD, day	8.25±2.39^{a}	0.25±0.25^{b}	2.75±2.43^{ab}	4.50±1.44^{ab}	0.02
ID, day	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
					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

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

Table 3. The effect of supplementation of juniper tar liquid extract on the kidney-urinary system of calves

Tablo 3. Ardıç sıvı ekstraktı ilavesinin buzağıların böbrek-üriner sistem üzerine etkisi

	G1	G2	G3	G4	P value
	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
Crea, mg/dL 1	1.26±0.11	1.29±0.05	1.30±0.07	1.41±0.16	0.80
2	1.20±0.04	1.37±0.06[b]	1.37±0.09	1.17±0.06	0.06
3	1.33±0.06	1.23±0.05	1.27±0.07	1.46±0.02	0.31
Urea, mg/dL 1	8.86±2.14	7.51±0.72	7.65±0.86	11.31±1.62	0.28
2	14.80±4.71	13.27±2.88	13.4±4.11	11.18±2.12	0.88
3	17.12±8.93	10.84±1.08	15.70±8.56	9.25±2.76	0.90
Color					
28-day-old	Light yellow	Light yellow	Light yellow	Light yellow	
Weaning age	Yellow	Light yellow	Light yellow	Light yellow	
Blood, Ery/µL					
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	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Urobilinogen, mg/dL					
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	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Glucose					
28-day-old	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Protein, g/L					
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	NO	NO	NO	NO	
Weaning age	NO	NO	NO	NO	
Leukocyte, µL					
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 gravity					
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, NO: Not observed

Supplementation with JLE significantly (P<0.05) reduced the incidence of diarrhoea. The highest incidence of diarrhoea was observed in G1, and the lowest in G2 (Table 2). The average number of days with respiratory diseases was 2 days in G1 and 0.75 days in G3. However, respiratory diseases were not observed in G2 and G4 (Table 2). Supplementation with JLE did not have a significant effect on the pathogen bacteria count, such as coliform, E.coli, Enterobacteriaceae or non-pathogenic bacteria like lactic acid bacteria in the gut of the 28-day-old calves (Table 2). However, while JLE significantly (P<0.05) suppressed the growth of pathogenic bacteria at weaning age, it did not affect the growth of non-pathogenic bacteria.

No significant differences were observed between serum Urea and CREA concentrations. JLE did not affect the urinary and renal systems of calves (Table 3). The colour of the urine samples taken from the calves was almost the same. Bilirubin, ketones, glucose, and nitrites were not observed in the urine samples of any of the groups. Additionally, no difference was found in blood, urobilinogen, protein, leukocyte, pH and Specific gravity values.

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

Antioxidative defence mechanisms, except for the TAS value, and oxidative stress markers were not significantly different (Table 4). The effect of JLE on immune responses was not significant. On the 5th day of the weaning program, JLE had no significant effect on the antioxidative defence mechanism or oxidative stress markers. However, supplementation with JLE increased the concentration of IgA, IgG, and IgM, while resulting in a non-significant increase in IgE (Table 4).

Table 4. The effect of juniper tar liquid extract on oxidative stress, antioxidative defence mechanism and immune response of calves

Table 4. Ardıç sıvı ekstraktının buzağıların oksidatif stress, antioksidan savunma mekanizması ve bağışıklık sistemi üzerine etkisi

		G1	G2	G3	G4	P value
		Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
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, arbitary	1	0.46±0.07	0.64±0.22	0.97±0.68	0.63±0.16	0.81
unit	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

		G1	G2	G3	G4	P value
		Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	
TDH, %	1	54.50±17.40	124.20±16.20	169.30±93.90	91.40±21.10	0.47
	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
	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.

DISCUSSION and CONCLUSIONS

Calves begin consuming concentrate feed at approximately 1 week of age and increase feed consumption at 2 weeks of age (Morittu et al. 2021). They typically start ruminating about 14 days after birth (Lopreiato et al. 2018). The development of the calf's rumen and digestive abilities influences the onset of rumination (Lopreiato et al. 2018). Rumination affects feed consumption and feed efficiency, which are associated with calf weight gain, health and welfare (Ambriz-Vilchis et al. 2015). Indeed, all calves in the JLE-supplemented groups began CS intake and rumination at a non-significantly earlier age. Tapki et al. (2020) reported that supplementation of oregano oil led to earlier CS consumption in calves. Similarly, green tea and oregano extract have been shown to promote early CS intake (Heisler et al. 2020).

Milk supplemented with JLE improved the health of calves by reducing the incidence of diarrhoea and respiratory diseases. The antiseptic and antibacterial activities of plant extracts enhance feed efficiency by affecting gastrointestinal system development, rumen microbiological activity, and by reducing digestive and respiratory diseases in newborn calves (Cobellis et al. 2016; Siefzadeh et al. 2017; Campolina et al. 2021). The reduction in digestive and respiratory diseases due to JLE supplementation may be attributed to the positive effects of the active components of the extracts on the secretion of endogenous enzymes, improvement of the intestinal environment, maintenance of a balanced intestinal flora, and enhanced liver functions for better

utilization of fats and proteins (Seifzadeh et al. 2017). In fact, it has been reported that plant oil and extracts reduce the incidence of respiratory (Sastir Koroglu and Kocabagli 2019) and digestive system diseases (Campolina et al. 2021) in 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). Plant extracts reduce the activity of pathogenic microorganisms, primarily due to their bioactive substances such as phenolic compounds, flavonoids, alkaloids, tannins and essential oils. These compounds may exert antimicrobial effects against pathogens through various mechanisms. They disrupt the structural integrity of bacteria by altering cell membrane permeability, inhibit proliferation by interfering with protein synthesis and DNA replication, and disrupt metabolic processes by inhibiting enzyme functions (Hassan et al. 2020). Furthermore, some plant components inhibit bacterial communication by suppressing the quorum-sensing mechanism, thus reducing virulence factors (Çepni and Gürel, 2011). Supplementation of JLE suppressed the growth of pathogens at 28-day-old, though not significantly, but had a significant effect at weaning age. The decrease in pathogen count in faeces can be attributed to the fact that plant extracts possess various antibacterial mechanisms, such as enzyme inhibition, cell membrane disruption, substrate deficiency, and inhibition of bacterial colonization (Hassan et al. 2020; Damjanovic-Vratnica et al. 2011; Bahadar et al. 2016). Numerous studies have reported that plant oils 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 gut, compared to pathogens, improves the gut health of calves (Bi et al. 2017; Özkaya et al. 2018). Supplementation with JLE did not affect the growth of lactic acid bacteria in the gut. Secondary metabolites such as flavonoids, terpenes and phenolic compounds present in JLE exhibit antimicrobial effects primarily against pathogenic bacteria, which are beneficial components of the gut microbiota. In particular, phenolic compounds of plant origin have been shown to suppress the growth of pathogens by disrupting the cell membrane or inhibiting bacterial enzymatic pathways (Cushnie and Lamb, 2011; Daglia, 2012). However, lactic acid bacteria may be more resistant to such compounds or metabolize them in a way that does not adversely affect their growth. Lactic acid bacteria may have developed natural resistance to antimicrobial compounds present in plant extracts. For example, some lactic acid bacteria strains have “efflux pumps” that pump toxic compounds out of the cell (Makarova et al. 2006). They have also evolved mechanisms to reduce the membrane permeability of antimicrobial agents by altering the lipid composition of cell membranes (Papadimitriou et al., 2016). Such adaptations may minimize the effect of potential antibacterial compounds in JLE on lactic acid bacteria. Lactic acid bacteria adhere to the intestinal epithelial surface and produce organic acids such as lactate and acetate. These acids lower the pH of the intestinal environment and prevent the colonization of many pathogens (Seo et al., 2010). However, since lactic acid bacteria are better adapted to this low-pH environment, this may also protect them from the potential effects of antimicrobial compounds in JLE (Gänzle, 2015). Furthermore, the bacteriocins produced by lactic acid bacteria may further promote their growth by reducing the competitiveness of pathogenic bacteria in the gut (Dobson et al., 2012).

Juniper berries and leaves, or their extracts, have traditionally been used as a diuretic. However, prolonged oral use or high doses may not be safe and may lead to kidney and urinary tract problems (Raina et al. 2019). In this study, however, no adverse effects of JLE supplementation on the kidney-urinary tract functions of calves were observed. This finding is thought to be attributed to the fact that the doses administered were not high. No significant changes or adverse effects on kidney function were detected in the JLE supplementation investigations. Specifically, biochemical parameters indicating kidney function, such as CREA and ürea, remained within normal ranges. These parameters are used to assess kidney damage or dysfunction, as they reflect the filtration capacity of the kidneys and overall health status (Özçelik et al. 2014). Other parameters observed showed that the volume of urine was within normal levels, and there were no abnormal changes in urine pH (Akpolat, 2018). These results support the conclusion that supplementation, when used at low doses, does not have harmful effects on the kidneys and does not have a positive impact on kidney function.

Blood serum TOS and MDA concentrations in G2 decreased at the weaning age and on the 5th day of the weaning program, while 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. 2023). A decrease in TOS and MDA concentrations reflects a reduction in lipid

peroxidation. The increase in antioxidant defence mechanism enzymes supports the antioxidant properties of 1.25% JLE. The rise in antioxidant defence mechanism enzymes enhances the antioxidant capacity of calves by improving the scavenging of free radicals (Wei et al. 2020). Compounds in herbal extracts disrupt radical reactions by combating reactive host components, thereby halting the propagation of the oxidation chain (Amorati et al. 2013; Hassan et al. 2020; Özkaya et al. 2023). Endogenous antioxidant enzymes contribute to intracellular defence against oxidative stress (Ding et al. 2021).

OSI, an indicator of the degree of oxidative stress, was low in G2, although not significantly. High OSI is a result of high TOS and low TAS values (Ogut et al. 2013; Özkaya et al. 2023). 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. 2023).

Immunoglobulins (IgA, IgG, IgM, and IgE) provide defense to all tissues reached by the blood and prevent the spread of blood-borne infections, septicemia and microorganisms by neutralizing their entry into the circulatory system (Roomruangwong et al. 2017). In this context, adequate levels of IgA, IgG and IgM are critical for a healthy immune response. These immunoglobulins enable the body to mount a more effective defence against pathogens. Notably, this study found that calves fed 1.25% JLE exhibited a significant increase in IgA, IgG, and IgM concentration compared to the control group, suggesting that JLE has immune response-enhancing effects.

Plant extracts are recognized as valuable sources for immune system enhancement. Secondary metabolites in plants, particularly bioactive components such as flavonoids, alkaloids, and terpenes, have been frequently reported to promote immune function. For instance, Qiao et al. (2013) and Özkaya et al. (2018) investigated the antimicrobial and anti-inflammatory properties of plant extracts and demonstrated their positive effects on the immune system. The beneficial effects of plant-derived compounds on immune function may be attributed to various mechanisms, including the neutralization of microorganisms, activation of immune cells, and protection against free radical damage (Lakhani et al. 2019; Kozyr et al. 2019). Furthermore, Wafa et al. (2021; 2022) reported that plant extracts enhance multiple components of the immune response and strengthen defense mechanisms against microorganisms.

The supplementation of JLE enabled calves to initiate rumination and starter intake at an earlier age while reducing the incidence of digestive and respiratory diseases. JLE suppressed the growth of pathogenic bacteria without affecting the proliferation of lactic acid bacteria. The administration of JLE at a dosage of 1.25% per calf per day enhanced the release of antioxidant defense enzymes and reduced oxidative stress markers concentrations. Additionally, JLE supplementation significantly improved immune response. These findings, considering the adverse effects of synthetic antibiotics and antioxidants on both human and animal health, suggest that JLE can be utilized as a natural preservative. Based on its observed effects on health parameters, pathogenic bacteria, antioxidant defence enzymes and immune response, JLE can be regarded as a feed additive that enhances calf health.

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Ethical statement: 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 were conducted according to the guidelines of the Committee.

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