Chilean J. Agric. Anim. Sci. (2026) 42(2):331-344.
https://doi.org/10.29393/CHJAAS42-28CKYS60028

ISSN 0719-3890 online

CHERRY (*Prunus cerasus* L.) KERNEL PULP SUPPLEMENTATION IN PERIPARTUM KURDI EWES: EFFECTS ON DIGESTIBILITY, ANTIOXIDANT STATUS, AND MILK FATTY ACIDS

Yaser Feizdar Barabadi[1*], Seyyed Ehsan Ghiasi[1,2], Mohammad Bagher Montazer Torbati[1,2], Seyed Morteza Vaghar Seyedin[1], Navid Ghavipanje[1,3], and Einar Vargas-Bello-Pérez[4*]

[1] Department of Animal Science, Faculty of Agriculture, University of Birjand, Birjand 97175-331, Iran
[2] Research Group of Environmental Stress in Animal Science, University of Birjand, Iran
[3] South Khorasan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Birjand, Iran
[4] Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Periférico R. Aldama Km 1, 31031 Chihuahua, Mexico
* Corresponding author: y.fazdar.brabadi@birjand.ac.ir; evargasb@uach.mx

ABSTRACT

The periparturient period in ewes is characterized by severe metabolic changes, that may pose risks to animal health and performance. Effective management, particularly nutritional strategies, can enhance pre- and post-partum performance while reducing metabolic disorders. This study determined the effects of supplementation with different levels of cherry kernel pulp (CKP) on colostrum and milk fatty acid (FA) profile, nutrient digestibility, and antioxidant indicators in prepartum ewes. Twenty-eight primiparous Kurdi ewes were randomly divided into four groups (n = 7) supplemented with 0, 4, 8, and 12% CKP on dry matter (DM) basis for 3 weeks pre- and post-partum. Blood samples were collected at multiple time points, and colostrum and milk were sampled post-lambing. CKP-supplemented ewes had lower dry matter intake (DMI; $p = 0.002$) and DM digestibility ($p = 0.0001$), crude protein (CP; $p = 0.0001$), and ether extract (EE; $p = 0.0001$) than control ewes. CKP supplementation did not affect plasma glucose, albumin, creatinine, total protein, high-density lipoprotein, aspartate aminotransferase, or lactate dehydrogenase ($p > 0.05$). However, CKP supplementation increased total antioxidant capacity (TAC; $p = 0.018$) and reduced malondialdehyde (MDA) concentrations ($p = 0.001$) in plasma. Dietary CKP inclusion did not affect colostrum or milk composition or their FA profiles ($p > 0.05$). Overall, our findings indicate that CKP supplementation at levels of up to 8% of dietary DM may improve antioxidant status in transition ewes without adversely affecting postpartum milk yield or composition. However, CKP supplementation was associated with reduced DM and CP digestibility.

Keywords: antioxidants, cherry kernel pulp, digestibility, liver activity, milk composition, transition period.

INTRODUCTION

During the transition from late gestation to early lactation, dairy sheep undergo profound metabolic and endocrine changes related to the partitioning of nutrients toward milk production (Cabiddu et al., 2020). Dairy ewes experience a negative energy balance (NEB) during early lactation (Cabiddu et al., 2020), similar to dairy cows (McCabe and Boerman, 2020) and goats (Ghavipanje et al., 2021), because energy intake is insufficient to meet the increased demand associated with milk production. Additionally, periparturient dairy ewes may experience an

Received: September 10, 2025 Accepted: June 30, 2026

imbalance in oxidative status driven, among other factors, by increased metabolic activity as a result of elevated energy demands and the formation of reactive oxygen species (ROS) (Mavrommatis et al., 2021).

Increased lipid peroxidation and decreased plasma enzymatic and/or non-enzymatic antioxidants are common blood indicators in transition dairy ewes and have been linked to a higher risk of postpartum pathological conditions (Mavrommatis et al., 2021). Therefore, implementing effective animal management strategies, particularly nutritional management, may improve both pre- and post-partum performance, while reducing the occurrence of metabolic disorders in ewes.

The use of natural feed additives from various classes, including alkaloeids, pholyphenols, and tannins, particularly those derived from fruit waste, is gaining increasing attention in ruminant nutrition (Ghavipanje et al., 2021; Omer et al., 2019; Maxiselly et al., 2022; Vaghar Seyedin et al., 2022; Shwerab et al., 2023). These additives not only improve animal health (Ghavipanje et al., 2021) and product quality (Vaghar Seyedin et al., 2022) but also promote sustainable animal production (Abdeltawab and Khattab, 2018). These compounds have been shown to participate in carbohydrate and lipid metabolism, modulate mitochondrial electron transport and energy generation, and alter ruminal fermentation patterns (Lambo et al., 2024). Furthermore, they exhibit a broad spectrum of health-promoting effects, including enhanced antioxidant status, improved immune system responses, reduced inflammation, and decreased concentrations of pro-inflammatory cytokines (Ramdani et al., 2023). However, responses to these compounds may vary significantly depending on the specific compound used, and the underlying mechanisms responsible for this variability remain to be fully elucidated (Lambo et al., 2024; Ramdani et al., 2023).

Substantial amounts of fruit kernel waste are currently generated, posing environmental concerns due to microbial decomposition (Maxiselly et al., 2022). Cherry (*Prunus cerasus* L.) is a stone fruit of the Rosaceae family (Hussain et al., 2021), with global annual production reaching 4.3–5 million metric tons in 2023 (FAO, 2023). The kernel constitutes a substantial proportion of cherry dry matter (DM), accounting for approximately 10% of the fruit weight and representing a significant by-product of cherry processing (Hussain et al., 2021). These kernels have potential as functional feed additive for livestock due to their rich content in bioactive compounds (Gungor and Erener, 2020; Afonso et al., 2020). Nutritionally, cherry kernels contain 29.3% crude protein (CP), 17.0% fat, and 30.3% crude fiber on a DM basis (Gungor and Erener, 2020; Yılmaz and Gökmen, 2013). However, they also contain antinutritional compounds, primarily prunasin glycosides, a cyanogenic glycoside belonging to the amygdalin group (Shamsi, 2017). Cherry kernels are rich in bioactive compounds such as polyphenols, flavonoids, alkaloids, and tannins, which act as immune modulators, antioxidants, and growth stimulants in livestock (Gungor and Erener, 2020). It has been reported that the most important polyphenolic compounds in cherry kernel pulp (CKP) include anthocyanins (cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside), flavonoids, procyanidins, hydroxycinnamates, and melatonin (Shamsi, 2017; Capanoglu et al., 2011). Nevertheless, the high cellulose content and cyanogenic diglycosides (e.g. amygdalin, prunasin, and vicianin) present in cherry kernels may act as antinutritional factors in livestock (Senica et al., 2016; Altop, 2019).

Despite the favorable chemical composition and bioactive properties of CKP, limited research has investigated its use as a feed additive in livestock nutrition. However, the existing literature highlights its potential benefits as a feed additive. For instance, tart cherries (*P. cerasus* L.) supplementation has been shown to reduce inflammatory symptoms in rats (Tall et al., 2004). Furthermore, Altop (2019) revealed that the dietary supplementation with fermented cherry (*P. avium* L.) kernel at 10 g kg^{-1} had a positive impact on performance, intestinal health, meat color, and the cecum microbiota of broilers. It has been also reported that the raw and fermented sour cherry (*P. cerasus* L.) kernel at levels up to 1% and 2% DM of diet, respectively, could be used in broiler chickens without any detrimental effect on growth (Gungor and Erener, 2020).

As previously noted, the transition from late pregnancy to early lactation in dairy ruminants, including ewes, is characterized by significant metabolic challenges, inflammation, and oxidative stress (Cabiddu et al., 2020; McCabe and Boerman, 2020; Ghavipanje et al., 2021). Consequently, developing effective strategies to mitigate these challenges during the transition period is a priority for nutritionists. While CKP shows promise as a functional feed additive for enhancing antioxidant status and promoting animal health (Gungor and Erener, 2020; Altop, 2019), effects of its dietary supplementation for alleviating oxidative stress in ruminant remain understudied. Therefore, the objective of this study was to evaluate the effects of dietary CKP supplementation at 0, 4, 8, and 12% of dietary

DM on oxidative status, metabolic profile, and productivity of transition dairy Kurdi ewes. We hypothesized that CKP supplementation during late pregnancy and early lactation would reduce oxidative stress and support liver function without negatively affecting animal performance.

MATERIAL AND METHODS

Study location, animals, and synchronization protocol

The study was conducted at the Research Station of the Faculty of Agriculture, University of Birjand, Iran (37.42° N, 57.31° E; 1491 m.a.s.l.). The region has an average annual temperature of 12 °C and average annual rainfall of 171 mm.

All experimental procedures and animal care were reviewed by the Animal Welfare and Ethical Review Board of the Department of Animal Science, University of Birjand (project ID 1398/D/45838), which granted the clearance for the study in compliance with the standards of the Iranian Council of Animal Care (protocol no. 19293). To synchronize estrus outside the natural breeding season, 40 ewes were treated with Eazi-Breed CIDR sheep devices (Zoetis Inc, Auckland 1010, New Zealand) containing 330 mg of progesterone. After 14 days, the CIDRs were removed, and each ewe received an intramuscular injection of 500 IU of PMSG (Pregnant Mare Serum Gonadotropin). The ewes were then divided into four groups (n=10/group), with one ram introduced per group for natural mating. Pregnancy was confirmed 42 days post-mating via transabdominal ultrasonography equipped with a sector probe (Honda-HS200, Toyohashi, Japan). Only ewes carrying a single fetus with a similar body condition score (BCS) were selected for the study. Finally, 28 healthy primiparous Kurdi ewes, with an average body weight (BW) of 47.49 ± 3.09 kg and a BCS of 2.9 ± 0. were included in the experiment.

Diets, husbandry, and feeding

CKP is a by-product of cherry juice/concentrate processing industry. During processing, cherries are washed, crushed, and pressed to extract the juice, leaving the solid residue consisting primarily of cherry kernels and pulp, which is typically discarded as waste. In the present study, the CKP was obtained from a cherry processing factory (Sha hiran factory, Mashhad, Iran). Upon arrival, the material was spread in thin layers and oven-dried (Lit50, Labtron & Behdad, Tehran, Iran) at 65°C for 48 h until a constant weight was achieved. The pre- and post-partum diets, with forage-to-concentrate ratios of 41:59 and 46:54, respectively, were formulated to be isocaloric and isonitrogenous and to meet nutritional requirements of the ewes during each period using the Small Ruminant Nutrition System (SRNS version, 1.7), a mechanistic model that predicts nutrient requirements for small ruminants based on animal characteristics, physiological stage, and feedstuff composition (Tedeschi et al., 2010). At the beginning of the study, five weeks before anticipated lambing date, the ewes were transferred to individual pens (2.0 m × 1.2 m) and allowed a two-week adaptation period. Ewe were fed total mixed rations (TMR) *ad libitum*, with 5 to 10% orts, twice daily at 0700 and 1,600 h for 3 weeks before and 3 weeks after parturition, and had free access to water. The experimental diets were formulated to contain 0, 4, 8, and 12% CKP, designated as Ctrl, CKP4, CKP8, and CKP12, respectively, with CKP at the expense of wheat bran. The ingredients and chemical compositions of the prepartum and postpartum diets are presented in Table 1.

Measurements and Laboratory Analyses

Chemical composition, nutrient intake, and digestibility

The amounts of DM intake and refusals were recorded daily before morning feeding. Feed and feces samples were collected for three days before and after parturition and stored at -20 °C until further analysis.

The CKP, TMR, and orts samples were combined and ground separately using a hammer mill fitted with a 1-mm screen (TS1500, Toos Shekan Co., Khorasan, Iran). DM (methods no. 934.01), ash, ether extract (EE, methods no. 945.38), and CP (methods no. 997.06) contents were analyzed in triplicate following AOAC (2019) guidelines. Ash-free neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using a Fibertec 1010 (Tecator, Höganäs, Sweden) according to Van Soest et al. (1991). Apparent nutrient digestibility in the entire digestive tract was determined using acid-insoluble ash (AIA) as an internal marker.

Blood sampling and biochemical indicators

Blood samples (10 mL ewe^{-1}) were collected via jugular venipuncture into EDTA-containing tubes 2 h before morning feeding on days -21, -14, -7, 0, 7, 14, and 21 relative to lambing. Following centrifugation (3,000 ×g, 15 min, 4 °C), plasma was aliquoted and stored at -20 °C until subsequent biochemical analyses. The activity of lactate dehydrogenase (LDH; IU L^{-1}), alanine aminotransferase (ALT; IU L^{-1}), and aspartate aminotransferase (AST; IU L^{-1}) were analyzed by standard laboratory kits (Darman Faraz

Table 1. Ingredients and nutrient composition (DM basis) of pre- and post-partum diets.

Item	Prepartum[5]				Postpartum[6]			
	Ctrl	CKP4	CKP8	CKP12	Ctrl	CKP4	CKP8	CKP12
Ingredient (%)								
Alfalfa hay	18.85	18.85	20.78	20.78	32.02	31.90	32.19	32.14
Wheat straw	22.45	22.45	20.14	20.14	15.30	15.24	14.31	14.29
Corn grain, ground	32.08	32.08	32.23	32.23	13.52	13.47	14.31	14.29
Barley grain, ground	7.22	7.22	8.05	8.05	17.79	18.08	18.10	17.93
Wheat bran	12.03	8.02	4.03	0.00	12.01	7.99	4.01	0.00
CKP[1]	0.00	4.01	8.05	12.08	0.00	4.01	8.01	12.06
Soybean Meal	0.00	0.00	0.00	0.00	3.01	3.41	3.77	4.35
Oil	4.01	4.01	3.62	3.62	3.06	2.64	2.15	1.79
Salt	0.40	0.40	0.40	0.40	0.36	0.35	0.36	0.36
Dicalcium phosphate	0.48	0.48	0.29	0.29	0.36	0.35	0.36	0.36
Calcium carbonate	0.72	0.72	0.64	0.64	1.57	1.56	1.43	1.43
Urea	0.64	0.64	0.64	0.64	0.00	0.00	0.00	0.00
Minerals and vitamins premix[2]	1.12	1.12	1.13	1.13	1.00	1.00	1.00	1.00
Chemical composition								
ME (Mcal/kg of DM)	2.431	2.447	2.453	2.447	2.406	2.382	2.366	2.341
CP (% DM)	11.90	11.70	11.80	11.60	13.00	13.00	13.10	13.10
Ash (% DM)	6.80	6.60	6.20	6.00	8.30	8.10	7.70	7.60
NDF[3] (% DM)	36.70	37.50	37.50	38.30	37.00	37.80	38.20	39.10
NFC[4] (% DM)	41.00	40.00	40.10	39.20	38.30	37.50	37.30	36.40

[1] Cherrykernel pulp (CKP) contain 95.55% DM, 11.96% CP, 14.53% EE, 3.95 ash, 71.5 NDF, 45.8% ADF, 4.99 total phenols, and 8.12 total lignin (DM basis). [2] Each kg of supplement contained 100 mg of vitamin E, 400000 IU of vitamin A, 100000 IU of vitamin D, 10 mg of vitamin B_1, 20 mg of vitamin B_2, 30 g calcium, 12 g phosphorus, 40 g sodium, 2000 mg manganese, 2000 mg zinc, 3000 mg iron, 1000 mg copper, 60 mg iodine, 60 mg cobalt, and 11 g Magnesium. 3 Neutral detergent fiber (NDF). [4] Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC = 100 − (NDF + CP + EE + Ash) (NRC 2007). [5] From 21 days before parturition until lambing. [6] From lambing until 21 days of lactation.

ME, Metaboliszble energy; CP, crude protein.

Kave, Tehran, Iran). Additionally, concentration of triglyceride (mg dL^{-1}), cholesterol (mg dL^{-1}), high and low-density lipoprotein (HDL and LDL; mg dL^{-1}), urea (mg dL^{-1}), creatinine (mg dL^{-1}), bilirubin (mg dL^{-1}), glucose (mg dL^{-1}), total protein (TP, mg dL^{-1}), and albumin (g dL^{-1}) were assessed using an autoanalyzer (ChemGesan 200 PRO VET, Campobello di Mazara TP, Italy). Plasma total antioxidant capacity (TAC) was determined by ferric reducing antioxidant power (FRAP) assay using a commercial kit (Navand Salamat Company, Urmia, Iran). Plasma malondialdehyde (MDA) concentrations were measured by the thiobarbituric acid colorimetric method with a commercial Lipid Peroxidation (MDA) Assay Kit (Nalondi™, Urumiyeh, Iran).

Colostrum and milk sampling and analysis

Colostrum and milk samples (50 mL ewe^{-1}) were collected in plastic bottles containing potassium dichromate as a preservative and stored at 4 °C until analysis. Colostrum samples were collected immediately after lambing (day 0), while milk was sampled on days 7, 14, and 21 postpartum. Individual samples were analyzed for protein, lactose, fat, and solids-not-fat (SNF) contents using a Milko Scan instrument (FT 200, Foss Electric, Hillerod, Denmark).

Samples for milk FA analysis were immediately sent to a certified laboratory (Rojan Alvand Milk Analyzing Laboratory, Karaj, Iran). Milk fatty acid (FA) profiles were determined using a Fourier ransform infrared (FTIR) spectrophotometer (CombiScope model 600 HP, Delta Instruments, Drachten, Netherlands). De novo and preformed milk FAs were estimated using FTIR spectroscopy and partial least squares (PLS) prediction models, as described by Woolpert et al. (2016). Gas-liquid chromatography reference chemistry was used to calibrate the prediction models for milk FA parameters.

Calculations and Statistical Analysis

The data analysis followed a completely randomized design using SAS version 9.4 (SAS/STAT 9.4, 2014, SAS Institute Inc., Cary, NC) with the MIXED procedure. All data were screened for normality using the UNIVARIATE procedure and the REPEATED statement of SAS was used for dependent variables measured over time. The fixed effects in the model were as follows: dietary treatment (CKP supplementation), sampling time (time), and their interaction (diet × time), while individual ewes were included as a random factor as follows:

$$y_{ijkl} = \mu + D_i + A_{j(i)} + T_k + D_i \times T_k + \varepsilon_{ijkl}$$

Where y_{ijkl} is the dependent variable, μ is the overall mean, D_i is the fixed effect of diet, $A_{j(i)}$ is the random effect of the *j*th ewe within the *i*th diet, T_k is the fixed effect of time (repeated measure), $D_i \times T_k$ is the interaction effect between diet and time, and ε_{ijkl} is the residual error.

Repeated measures were included with the three covariance structures (compound symmetry, autoregressive, or unstructured) and selection was based on the smallest Akaike information criterion for a finite sample size (AICC). Least square means (LSM) separation between time points was performed using the PDIFF statement by Tukey's test at $\alpha = 0.05$ and is presented as LSM ± standard error of means (SEM). Significance was declared at $p \leq 0.05$, and trends were declared at $p < 0.10$ and $p > 0.05$. The linear and quadratic effects of the treatment were analyzed for all data using orthogonal polynomial contrasts.

RESULTS

Dry matter intake and nutrient digestibility

There was a significant effect of treatment (p = 0.002), periods (p = 0.032), and their interactions (p = 0.050) for DMI (Table 2). Dietary inclusion of CKP linearly (p = 0.007) and quadratically (p = 0.009) decreased the DMI of ewes. Furthermore, the highest and lowest DM digestibility values were observed in the Ctrl and CKP12 groups, respectively (p = 0.001). Similarly, the apparent digestibility values of CP (p = 0.001) and EE (p = 0.001) were higher in the Ctrl group than in the CKP8 and CKP12 groups, while CKP4 did not differ significantly from Ctrl for CP digestibility. In contrast, ewes fed CKP4 had greater NDF digestibility (p = 0.001) than those fed the Ctrl diet. Moreover, the supplementation of CKP4 and CKP8 led to the highest and lowest ADF digestibility, respectively (p = 0.001), with a significant period effect (time p = 0.037).

Blood parameters and antioxidant status

Albumin, direct bilirubin, total bilirubin, creatinine, glucose, total protein, urea, and uric acid levels were not affected by CKP ingestion throughout the transition period (Table 3). Blood glucose (p = 0.001), albumin (p = 0.035), total bilirubin (p = 0.001), creatinine (p = 0.007), total protein (p = 0.001), and urea (p = 0.001) levels were all affected by the period, indicating physiological adaptations during the transition from pregnancy to lactation. Plasma triglyceride (p = 0.2128), HDL (p = 0.1020), and LDL (p = 0.1600) were not altered by diets. In addition, the blood cholesterol concentration in CKP8 group was higher (p = 0.0193) than Ctrl, with intermediate values observed in CKP4 and CKP12 groups, exhibiting a quadratic response (p = 0.026).

Plasma levels of AST (p = 0.448) and LDH (p = 0.6967) remained unaffected by CKP supplementation (Fig. 1). Additionally, the CKP8 group had the highest concentration of ALT, whereas Ctrl had the lowest (p = 0.001). However,

Table 2. Effect of graded levels of cherry kernel pulp (CKP) supplementation (0, 4, 8, and 12% of DM) on nutrient intake and digestibility of peripartum Kurdi ewes.

	Treatment[2]				Trend *p-value*		*p-value*			
Item[1]	Ctrl	CKP4	CKP8	CKP12	Linear	Quadratic	Treat	Period	T×P	SEM
DMI, Kg	2.477a	2.053b	2.029b	1.980b	0.007	0.009	0.002	0.032	0.050	0.097
Nutrient digestibility (%)										
DM	68.51a	66.18b	64.26c	59.31d	0.189	0.001	0.001	0.003	0.012	0.341
CP	80.50a	79.96a	75.68b	76.20b	0.108	0.001	0.001	0.003	0.208	0.292
EE	92.68a	89.21c	88.51c	90.68b	0.001	0.001	0.001	0.006	0.831	0.212
NDF	42.10b	50.84a	43.50b	41.60b	0.007	0.001	0.001	0.423	0.402	0.811
ADF	27.17b	35.65a	15.48c	34.80a	0.0001	0.001	0.0001	0.0377	0.082	0.793

[a–d] Values within a row with different superscripts differ significantly at $p \leq 0.05$. [1]DMI, dry matter intake (kg); DM, dry matter; CP, crude protein; EE, ether extract; NDF, natural detergent fiber; ADF, acid detergent fiber. [2] The Ctrl, CKP4, CKP8, and CKP12 were supplemented with 0, 4, 8, and 12% CKP (DM basis), respectively.

Table 3. Effect of graded levels of cherry kernel pulp (CKP) supplementation (0, 4, 8, and 12% of DM) on blood parameters of peripartum Kurdi ewes.

	Treatment[1]				Trend *p-value*		Effects *p-value*			
Item	**Ctrl**	**CKP4**	**CKP8**	**CKP12**	**Linear**	**Quadratic**	**Treat**	**Period**	**T×P**	**SEM**
Albumin (g dL^{-1})	5.623	5.894	6.080	6.167	0.111	0.710	0.435	0.035	0.155	0.254
Direct bilirubin (mg dL^{-1})	0.253	0.239	0.231	0.241	0.695	0.631	0.946	0.067	0.845	0.269
Total bilirubin (mg dL^{-1})	0.553	0.575	0.549	0.583	0.584	0.814	0.729	0.001	0.028	0.026
Creatinine (mg dL^{-1})	1.010	0.887	0.941	0.883	0.102	0.464	0.168	0.007	0.296	0.047
Glucose (mg dL^{-1})	61.56	63.70	70.72	56.03	0.659	0.100	0.231	0.001	0.401	5.079
Total protein (mg dL^{-1})	6.113	6.012	5.529	5.591	0.200	0.951	0.478	0.001	0.373	0.340
Urea (mg dL^{-1})	37.16	34.22	31.90	31.78	0.031	0.443	0.164	0.001	0.242	1.891
Uric acid (mg dL^{-1})	0.778	0.434	0.410	0.373	0.580	0.124	0.101	0.408	0.203	0.126
Cholesterol (mg dL^{-1})	100.2b	107.9ab	140.5a	106.3ab	0.715	0.026	0.019	0.001	0.030	8.778
Triglyceride (mg dL^{-1})	32.09	26.16	25.67	27.58	0.154	0.091	0.212	0.001	0.600	2.407
High-density lipoprotein (mg dL^{-1})	23.73	24.56	31.85	24.64	0.362	0.113	0.102	0.239	0.005	2.436
Low-density lipoprotein (mg dL^{-1})	28.59	29.01	38.49	29.95	0.408	0.232	0.160	0.019	0.014	3.538

[a–b] Values within a row with different superscripts differ significantly at $p \leq 0.05$. [1] The Ctrl, CKP4, CKP8, and CKP12 were supplemented with 0, 4, 8, and 12% CKP (DM basis), respectively.

as CKP percentages increased, serum MDA levels quadratically decreased ($p < 0.001$). Specifically, plasma MDA levels were higher in the Ctrl and CKP4 groups ($p < 0.001$). Ewes receiving CKP12 had higher TAC than those in all other groups ($p = 0.018$).

Colostrum and milk composition and fatty acids profile

Dietary CKP inclusion levels did not affect the composition of milk or colostrum (Table 4). The effects of dietary treatments on the FA profile of milk and colostrum are presented in Table 5.

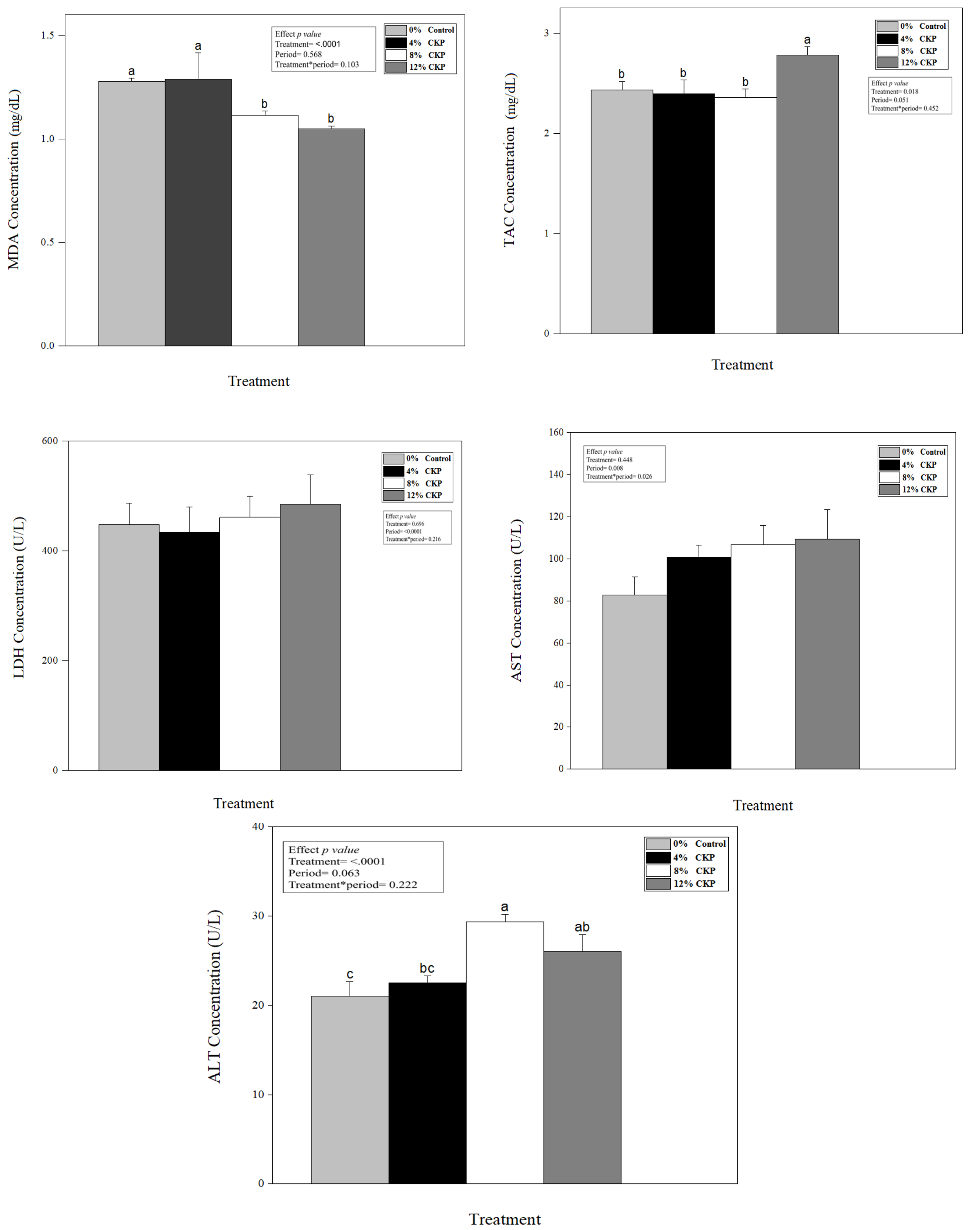

Fig. 1. Effect of graded levels of cherry kernel pulp (CKP) supplementation (0, 4, 8, and 12% of DM) on plasma malondialdehyde (MDA), total antioxidant capacity (TAC), lactate dehydrogenase (LDH), aspartate aminotransferase enzyme (AST), and alanine aminotransferase (ALT) of peripartum Kurdi ewes. a–c Values with different superscript letters are significantly different ($p \leq 0.05$).

Table 4. Effect of graded levels of cherry kernel pulp (CKP) supplementation (0, 4, 8, and 12% of DM) on colostrum and milk composition of peripartum Kurdi ewes.

Item	Treatment[1]				Trend *p-value*		Effects *p-value*			SEM
	Ctrl	CKP4	CKP8	CKP12	Linear	Quadratic	Treat	Period	T×P	
Colostrum										
Fat, %	7.21	7.25	10.14	5.28	0.820	0.393	0.688	-	-	2.99
Protein, %	14.7	12.9	12.8	12.9	0.590	0.236	0.4317	-	-	1.65
Fat/protein	0.667	0.696	0.986	0.446	0.780	0.389	0.710	-	-	0.344
Lactos,e %	3.54	3.96	3.12	3.52	0.424	0.962	0.134	-	-	0.260
Total solids, %	26.1	23.6	26.9	22.8	0.478	0.708	0.458	-	-	2.20
Solids- non-fat, %	18.6	15.8	16.4	17.1	0.5970	0.323	0.694	-	-	1.82
Milk urea nitrogen, %	14.6	15.2	14.8	16.5	0.473	0.710	0.812	-	-	1.74
Milk										
Fat, %	4.66	4.83	4.83	5.15	0.908	0.557	0.790	0.031	0.226	1.29
Protein, %	5.09	5.11	5.16	5.55	0.293	0.539	0.604	0.009	0.066	0.313
Fat/protein, %	0.932	0.892	0.937	0.949	0.800	0.334	0.597	0.106	0.328	0.180
Lactose, %	4.53	4.61	4.56	4.50	0.899	0.709	0.974	0.026	0.611	0.210
Total solids, %	15.6	15.7	14.8	16.7	0.575	0.364	0.569	0.001	0.074	1.01
Solids- non-fat, %	10.5	10.6	10.7	10.9	0.053	0.303	0.160	0.052	0.128	0.147
Milk urea nitrogen, %	15.8	13.7	15.4	14.9	0.862	0.404	0.481	0.073	0.988	0.098

[1] The Ctrl, CKP4, CKP8, and CKP12 were supplemented with 0, 4, 8, and 12% CKP (DM basis), respectively.

Different amounts of CKP did not affect the FAs profile of either milk or colostrum. Among the measured parameters, only colostrum acetone concentration differed significantly ($p < 0.05$), with a higher concentration in the CKP8 group compared to the CKP4 group.

DISCUSSION

To date, the effect of CKP supplementation on ruminant physiology, productivity, and health has not been documented. Therefore, to the best of our knowledge, this is the first study to investigate the effects of dietary CKP supplementation on milk FA profile and oxidative status of ewes during the periparturient period. Dietary CKP is not commonly used in ruminant feeding, probably because its bioactive compounds, including anthocyanins, procyanidins, alkaloids, and polyphenols, may negatively affect diet palatability and acceptability. Our findings demonstrate that DMI declined with increasing levels of CKP in ewe diets, which may be partly attributed to the progressive increase in dietary NDF and ADF content in CKP-containing diets. Consistent with our findings, previous studies investigating the inclusion of fruit kernels in ruminant have reported a reduction in DMI with grape seed kernel (Manso et al., 2016), mango seed kernel (Omer et al., 2019), and coffee cherry kernel (Maxiselly et al., 2022). It is well known that higher dietary NDF and ADF concentrations can limit DMI in ruminants (NRC, 2017).

In the present study, increasing CKP in the diet tended to reduce CP digestibility by 0.7%, 5.9%, and 5.3% in CKP4, CKP8, and CKP12, respectively. Increasing dietary CKP levels also reduced plasma urea concentrations by 7.9%, 14.2%, and 14.5% in the CKP4, CKP8, and CKP12 groups, respectively, and total protein concentrations by 1.6%, 13.4%, and 8.7% in CKP4, CKP8, and CKP12 groups, respectively. These findings support the inhibitory effects of high tannin concentrations on protein digestion. These findings agree with those of Besharati et al. (2008), who reported that dietary supplementation with raisin by-products, a tannin-rich feed source, reduced ruminal CP digestibility in Ghezel sheep . It has also been reported that dietary supplementation with mango seed kernel (Omer et al., 2019) and coffee cherry kernel (Maxiselly et al., 2022) negatively affected nutrient digestibility in sheep, an effect that was mainly associated with the presence of tannins, saponins, and phenolic compounds in these fruit kernels. This pattern of reduced digestibility may be attributed to the formation of tannin-nutrient complexes that are resistant to enzymatic degradation, as well as the potential antimicrobial effects of phenolic compounds on ruminal microbiota, thereby limiting the extent of

Table 5. Effect of graded levels of cherry kernel pulp (CKP) supplementation (0, 4, 8, and 12% of DM) on colostrum and milk fatty acid profile of peripartum Kurdi ewes.

Parameter[1]	Treatment[2]				Trend *p*-value		Effects *p*-value			
	Ctrl	CKP4	CKP8	CKP12	Linear	Quadratic	Treat	Period	T×P	SEM
Colostrum										
De novo fatty acids, %	2.14	2.33	2.90	1.37	0.595	0.245	0.489	-	-	0.768
Mixed fatty acids, %	1.22	1.49	1.55	0.908	0.803	0.559	0.914	-	-	0.844
Preformed fatty acids, %	3.23	3.42	5.25	2.64	0.993	0.308	0.566	-	-	1.44
FFA, MEq/100g fat	1.40	1.35	0.697	2.36	0.373	0.720	0.099	-	-	0.478
NEFA, %	400.1	658.7	921.9	592.4	0.511	0.307	0.662	-	-	251.7
BHB, %	0.258	0.142	0.325	0.141	0.861	0.817	0.172	-	-	0.062
Acetone, %	0.299ab	0.253b	0.495a	0.329ab	0.173	0.265	0.036	-	-	0.055
SFA, %	4.44	4.761	6.07	3.14	0.729	0.337	0.646	-	-	1.78
UFA, %	1.79	2.02	3.17	1.70	0.867	0.472	0.801	-	-	0.967
MUFA, %	1.34	1.54	2.37	1.30	0.879	0.544	0.875	-	-	0.864
PUFA, %	0.466	0.488	0.793	0.438	0.774	0.289	0.469	-	-	0.185
C16:0, %	1.23	1.47	1.47	0.887	0.753	0.572	0.909	-	-	0.606
C18:0, %	1.44	1.19	1.82	1.03	0.740	0.518	0.569	-	-	0.442
Milk										
De novo fatty acids, %	1.46	1.48	1.12	1.72	0.791	0.390	0.623	0.004	0.266	0.348
Mixed fatty acids, %	1.24	1.31	0.943	1.11	0.520	0.864	0.746	0.063	0.351	0.261
Preformed fatty acids, %	1.87	1.99	1.56	2.25	0.714	0.522	0.723	0.267	0.078	0.456
FFA, mEq/100g fat	1.25	2.53	0.892	2.37	0.534	0.874	0.211	0.697	0.523	0.613
NEFA, %	570.1	715.4	848.5	826.4	0.130	0.507	0.416	0.007	0.754	129.7
BHB, %	0.117	0.143	0.209	0.110	0.695	0.031	0.753	0.145	0.044	0.026
Acetone, %	0.225	0.265	0.336	0.265	0.232	0.139	0.230	0.216	0.087	0.036
SFA, %	3.54	2.38	2.39	3.54	0.869	0.496	0.611	0.020	0.252	0.671
UFA, %	1.08	1.04	1.12	1.28	0.630	0.766	0.937	0.043	0.154	0.333
MUFA, %	0.828	0.813	0.913	0.952	0.689	0.918	0.971	0.059	0.216	0.275
PUFA %	0.227	0.218	0.205	0.316	0.317	0.300	0.443	0.026	0.020	0.058
C16:0, %	1.22	1.170	0.899	1.05	0.485	0.713	0.815	0.067	0.368	0.277
C18:0, %	0.654	0.561	0.455	0.794	0.686	0.241	0.543	0.048	0.261	0.181

[a–b] Values within a row with different superscripts differ significantly at $p \leq 0.05$. [1] BHBA, beta hydroxyl butyrate; FFA, free fatty acids; NEFA, None esterified fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; mEq/100g fat = milliequivalents of a substance per 100 grams of fat.[2] The Ctrl, CKP4, CKP8, and CKP12 were supplemented with 0, 4, 8, and 12% CKP (DM basis), respectively.

fermentation and nutrient utilization (Maxiselly et al., 2022). Similarly, the proanthocyanidins present in CKP can bind to proteins from the diet, saliva, and microbes, forming proanthocyanidin–protein complexes that are highly resistant to ruminal digestion (Makkar, 2003). Consistently, inclusion of yeast-fermented rubber seed kernel at 250 g kg^{-1} in the diets of dairy heifers reduced DMI and nutrient intake, including OM, NDF, ADF, and EE, and resulted in a linear decrease in EE digestibility. However, the inclusion level was not associated with any adverse effects on DM, OM, or CP digestibility (Achour et al., 2019). Moreover, the higher acid detergent lignin (ADL) content in CKP might reduce fiber degradability, thereby reducing DM digestibility (Yansari et al., 2007). Lignification of plant cell walls creates physical barriers that prevent microbial attachment and enzymatic hydrolysis, resulting in reduced ruminal degradation of structural carbohydrates (Makkar, 2003). Similarly, Vaghar Seyedin et al., (2023) reported a decrease in DM, OM, and ADL digestibility following the inclusion of *Berberis vulgaris* leaf, which contained 14.6 mg g^{-1} phenolic compounds and 42.6 mg equivalents of quercetin (EQU) g^{-1} flavonoids, in the finishing diets of *Baluchi* lambs, accompanied by deceased body weight in the lambs.

The lower DM intake observed in prepartum ewes receiving high levels of CKP may also be related to the negative effects of phenolic compounds on diet palatability. In ruminants, phenolic compounds, especially tannins, can reduce DM intake by negatively affecting sensory characteristics, ruminal turnover, and digestion (Makkar, 2003). Phenolic compounds typically impart an astringent taste that reduces diet palatability, while simultaneously slowing ruminal digestion kinetics, which collectively contribute to decreased feed intake (Makkar, 2003). CKP is known to have high levels of phenolic compounds, particularly anthocyanins and procyanidins (Capanoglu et al., 2011). The presence of these compounds in plant cells resulted in a lower digestibility by limiting the attachment of microorganisms to the plant material (Jonker and Yu, 2017). In the present study, the dietary inclusion of CKP linearly decreased the apparent digestibility of DM, CP, and EE in Kurdi ewes. Consistently, a previous report in monogastrics animals showed that the cyanogenic glycosides were metabolized into hydrogen cyanide in the intestinal epithelium, negatively affecting growth performance and nutrient digestibility in broiler chickens (Rodriguez et al., 2001). Similarly, Achour et al. (2019) reported that the inclusion of apricot kernels, a fruit by-product of the Rosaceae family rich in cyanogenic glycosides similar to those found in cherry kernels, impaired growth performance in broilers.

In the present study, nearly all measured blood metabolites were within the normal ranges reported for prepartum ewes (Cabiddu et al., 2020). Dietary inclusion of CKP in prepartum ewe diets did not negatively affect blood parameters including albumin, bilirubin, creatinine, glucose, HDL, and LDL. Blood glucose concentration was not significantly affected by dietary treatment under the present experimental conditions, although ewes fed CKP8 showed numerically higher glucose levels. Previous studies have reported inconsistent effects of tannin-containing feed additives on blood glucose concentrations. For example, Maxiselly et al. (2022) observed enhanced blood glucose levels in sheep fed coffee cherry kernel at a rate of 200, g/day. Dietary tannins may increase the molar ratio of propionate in the rumen, thereby contributing to increased blood glucose levels (Makkar, 2003). In contrast, Sundod et al. (2023) reported no effect of tannin source on blood glucose concentrations in lactating Saanen goats. Similarly, Vaghar Seyedin et al. (2022) found that feeding lambs with *Berberis vulgaris* leaf, a rich source of tannins and phenols, reduced blood glucose concentrations. These discrepancies may be attributed to differences in the composition of the bioactive compounds, inclusion level, physiological status of the animals, and environmental factors (Vaghar Seyedin et al., 2022).

Plasma cholesterol concentrations showed a significant quadratic response to dietary CKP inclusion, with the CKP8 group exhibiting significantly higher levels than the control, while CKP4 and CKP12 groups showed intermediate values. The initial increase observed at the 8% inclusion level may be partially related to the FA profile of CKP; oleic acid is the predominant FA in CKP and can be converted to stearic acid through biohydrogenation (Chandra and Nair, 1993). Consequently, it is expected that in CKP8 group the levels of stearic acid will rise, leading to an increase in blood cholesterol levels (Oteng and Kersten, 2020). However, the observed quadratic response suggests that, at higher CKP inclusion rates, the increased intake of bioactive compounds, particularly tannins and phenolics, may inhibit ruminal biohydrogenation and counteract the lipid-supplying effect of CKP (Makmur et al., 2022).

There were no differences among diets in terms of plasma triglyceride concentrations, which is in line with Mohammadabadi et al. (2020), who found that dietary inclusion of oak kernels did not alter blood triglyceride concentrations in buffaloes. Additionally, Lewis (1997) suggested that ruminants maintain tight homeostatic control of plasma triglyceride concentrations, primarily through hepatic regulation of very-low-density lipoprotein (VLDL) secretion, which appears to be less susceptible to dietary phenolic compounds than cholesterol metabolism. This regulatory resilience helps explain why plasma triglyceride levels remained stable across the dietary treatments despite changes in other parameters.

Small ruminants, such as sheep and goats, are particularly susceptible to oxidative stress around parturition (Ghavipanje et al., 2021). In the present study, ewes receiving the CKP12 diet showed an enhanced TAC, while there were no differences among CKP4, CKP8, and Ctrl groups. This suggests that a higher intake of natural antioxidants led to higher transfer of these compounds to tissues, thereby increasing TAC. Higher plasma TAC in CKP12-fed ewes may be attributed to higher transmission of some CKP bioactive compounds, like anthocyanins, flavonoids, and phenols to blood. These results underscore the potential antioxidant properties of CKP, which may be mediated by its rich content of bioactive compounds, such as anthocyanins, flavonoids, and phenolic acids (Afonso et al., 2020). Fruit kernels are known to be rich in antioxidative phytochemicals, including vitamins C and E, carotenoids, polyphenols, and other bioactive compounds (Yılmaz and Gökmen, 2013).

The antioxidant activity of CKP has been confirmed using the 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and β-carotene-linoleic acid bleaching assays (Afonso et al., 2020). Dietary ingestion of anthocyanins has also been reported to increase plasma TAC in monogastric animals (Kay et al., 2010). Notably, the bioavailability of dietary phenolics in ruminants differs from monogastric animals, as extensive ruminal biotransformation generates unique metabolites with superior ability compared to their parent compounds, suggesting that ruminants may derive distinct antioxidant benefits from these compounds (Gladine, 2007). Although the mechanisms by which dietary anthocyanins enhance antioxidant capacity are not fully understood, they may contribute by neutralizing oxidants and reducing lipid peroxidation (Vaghar Seyedin et al., 2022; Farhadi et al., 2022).

Plasma MDA levels were also reduced with CKP12 feeding. MDA serves as an indicator of oxidative stress in ruminants (Ghavipanje et al., 2021; Vaghar Seyedin et al., 2022), and its reduction in CKP12 suggests that the animals experienced less oxidative stress. Overall, our data demonstrate the potential of CKP bioactive compounds to mitigate lipid peroxidation, which is particularly important during the periparturient period, when oxidative stress is typically elevated due to metabolic and physiological adaptations associated with late pregnancy and early lactation (Ghavipanje et al., 2021).

Dietary supplementation with CKP did not affect colostrum or milk composition or the milk FA profile. Although a numerical, non-significant increase in fat content was observed in both milk and colostrum following CKP supplementation, the overall FA profile remained unchanged. This stability suggests that the FAs present in CKP, which are primarily esterified as triglycerides, underwent extensive ruminal lipolysis and biohydrogenation. Previous studies have reported that supplementation with pomegranate seed pulp (Jonker and Yu, 2017) and palm kernel cake (Abdeltawab and Khattab, 2018), both phenolic-rich feed by-products like CKP, enhanced milk fat content in dairy goats and dairy cows, respectively. Shwerab et al. (2023) reported that the dietary inclusion of mango kernel at 10% of DM did not alter the milk FA profile in goats. These findings are comparable to those observed in the present study. To the best of our knowledge, this is the first study to investigate the effects of dietary CKP supplementation on dairy sheep. Therefore, further research is necessary to elucidate the specific metabolic pathways underlying the effects of CKP on milk fat content.

CONCLUSION

Dietary supplementation with cherry kernel pulp (CKP) during the prepartum period reduced DMI and the digestibility of DM, EE, and CP in transition Kurdi ewes, without affecting milk or colostrum yield or composition. However, CKP supplementation reduced MDA concentrations and increased TAC, suggesting potential antioxidant and health-promoting properties. Further research is required to evaluate the effectiveness of CKP as a feed ingredient for ruminants, particularly by investigating strategies to mitigate the potential adverse effects of tannins and cyanogenic glycosides. Additionally, long-term feeding studies involving larger animal cohorts are needed to evaluate the potential effects of CKP compounds on the ruminal microbiota.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the University of Birjand (Iran) for its financial support (grant number: 25828).

Author Contributions

Conceptualization, Y.F.B. and S.E.G.; methodology, Y.F.B., S.E.G., and S.M.V.S.; validation, Y.F.B., S.E.G., M.B.M.T., and S.M.V.S.; formal analysis, Y.F.B., S.M.V.S., and N.G.; investigation, Y.F.B., and S.M.V.S.; data curation, Y.F.B., S.E.G., M.B.M.T., and S.M.V.S.; writing—original draft preparation, Y.F.B., S.M.V.S., and N.G.; writing—review and editing, N.G., and E.V.-B.-P.; visualization, Y.F.B.; supervision, S.E.G., and M.B.M.T. All authors have read and agreed to the published version of the manuscript.

Funding statement

The financial support for this research was partially provided by University of Birjand (Grant number: 25828). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest and that no financial support was received for this work that could have influenced its outcome.

Data Availability

All the required data have been presented in our article.

Ethical standards

The Animal Welfare and Ethical Review Board of the Department of Animal Science, University of Birjand, granted clearance for the study (project ID 1398/D/45838), which complied with the standards of the Iranian Council of Animal Care's protocol no. 19293.

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